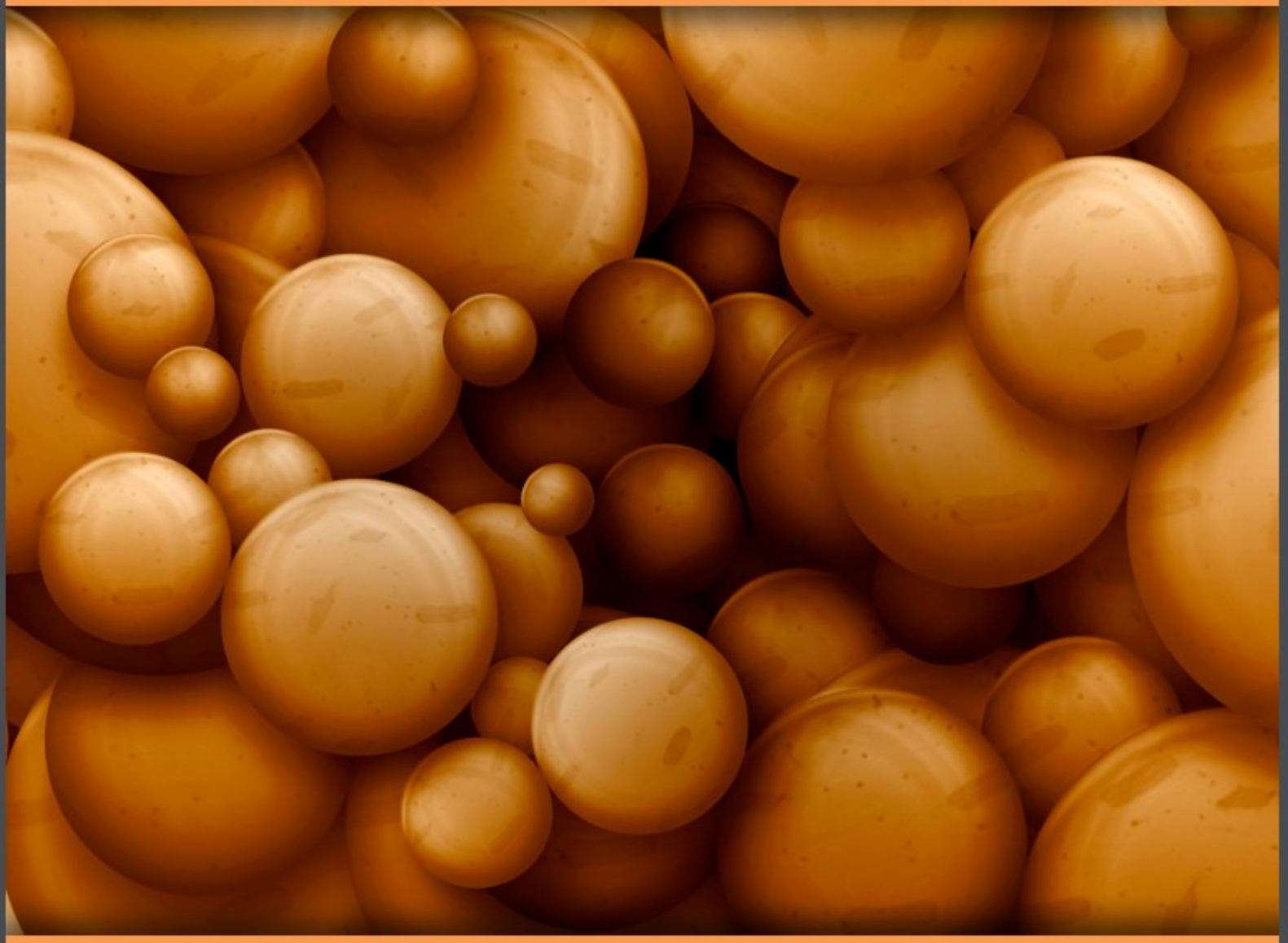
Kinetics for Bioscientist

Peter Klappa



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Contents

Preface

In general, the subject of reaction kinetics includes analysis of how fast reactions occur, the predictions of concentrations of reactants and products and how reactions can be altered by changing conditions. The study of kinetics is fundamental in any modern Bioscience and Pharmacology degree programme worldwide, however, in my experience undergraduate students often find this topic rather difficult. Reasons for this are i) the subject requires some mathematical skills in the interpretation of data and solving of problems and ii) often kinetics is taught in a fairly conceptual style, which does not provide students with an understanding as to why and what they should learn. For example, the principles of reaction kinetics and the methodology to solve problems in this area are not restricted to chemical reactions, but can be transferred to population growth, predator-prey systems, physiology and toxicology. However, traditionally reaction kinetics is taught mainly with reference to chemical concepts, which leaves many students (Biochemistry, Biology, Forensic Biology, Biomedical Sciences, Pharmacy, Pharmacology, Forensic Sciences, etc.) unable to transfer their knowledge to the areas they are interested in.

In addition to the question of relevance there is another very important issue often neglected by many textbooks, namely the way students learn. In my experience very often students acquire knowledge through 'problem-centered' learning. They use problem questions and model answers to understand underlying concepts. For many students, especially in the early stages of their academic careers, this approach is more natural than the 'academic' approach, which is focused on a theoretical conceptualization of a topic. Although it would not be possible to explain complex concepts like reaction kinetics without some theoretical discussion, this should be kept to a minimum with emphasis being placed on conveying concepts through problem-questions.

Also, for many undergraduate students it is important to get the information required in an easy and effective way. In other words, students are usually not interested in reading a whole textbook to obtain the knowledge they require for the problem-solving test next week. What they are really interested in is to see, what they need to know in the test and how to get this knowledge in the most effective way. In my experience students want a very brief(!) summary of the most important equations and concepts (perhaps in form of a bullet point list) and how to apply them to a problem. Students also appreciate if this information is provided in a concise form as a separate section in such a way that it can be easily identified.

Many textbooks are written in a very 'academic' manner, i.e. they often ignore the way undergraduate students learn. In this book I aimed to adopt a problem-centered approach by:

- Presenting reaction kinetics with relevance to biological problems in a student-centred language, which makes this book suitable for undergraduate students, without prior knowledge of the subject.
- Focussing on problem solving and data analysis through worked examples and model calculations.

The general concept of the book has been designed to make it useful for in-class teaching as well as self-study. The chapters of the book are arrange in so that they follow a logic flow, however, most chapters are self-contained and do not necessarily require knowledge from previous chapters.

In this book I have tried to give as many life science-related examples as possible and although the general concepts of the problems are correct, often the parameters of reactions are fictional. For example, it is well known that HIV reverse transcriptase forms a dimer; however the rate constant of this process, as used in chapter 5, has been set more or less arbitrarily. This book is therefore NOT intended as a reference for accurate numbers, but should rather exemplify concepts of reaction kinetics in a Bioscience-context.

I have also attempted to provide help with mathematical concepts and equations relevant to reaction kinetics. In my experience students often find the mathematical nature of data analysis and interpretation challenging – another reason why students might find reaction kinetics difficult. I therefore deliberately incorporated various mathematical concepts in this book, wherever it seemed reasonable from a pedagogical point of view. From many years of teaching experience I know that students very much appreciate this step-by-step approach when it comes to solving problem questions.

1.Why a Bioscientist should care about kinetics

1.1. Introduction and learning outcomes

Reaction kinetics is an important topic in many areas of biosciences and related areas like pharmacology, but many people are quite scared of it. Often they think it is too difficult; it is relevant only for chemists or it has nothing to do with biosciences whatsoever, so why bother?

In this chapter I want to demonstrate that actually many aspects of biosciences are directly related to and can be explained with kinetics. By the end of this chapter you should be able to appreciate that kinetics is relevant to numerous problems in a variety of life science-related topics.

1.2. Problem: Elimination of alcohol from the body

Let's have a look at an example from pharmacology – the elimination of a drug from the body. This could be a drug like Aspirin or Nurofen or even ethanol. In fact, the latter is probably something we all wanted to know at one point in our lives: if we had 4 pints of lager in the pub, how long does it take to be under the limit for safe driving again?

Clearly this is a question that is taken from everyday life – yet it is a very good example to explain kinetics.

Maybe we take a conservative approach and say that our metabolism requires approximately 3 hours for each pint we drink to eliminate the alcohol contained in it. How long would it take to get rid of the alcohol from 4 pints to be ok to drive again?

It should not be too difficult to find the answer to this problem: the elimination rate is 3 hours per pint and we drink 4 pints, hence:

3 hours / pint *x* 4 pints = 12 hours.

Answer: After 12 hours our body should have eliminated the alcohol to a level that is safe to drive again.

For some people it might be quicker, for others it might take longer to reach a safe level. This obviously depends on our metabolism. And this is where kinetics comes into play: the way we calculated how long it takes to reach a safe level is a typical equation in the study of kinetics.

1.3. Problem: Growth of an *E.coli* culture

Here is another example for kinetics:

For many applications in molecular biology, like transformation of a plasmid or the expression of a recombinant protein, we need to work with an *E.coli* culture that has reached mid-log phase. We can determine the growth of our *E.coli* culture by measuring the absorbance of the culture in a spectrophotometer at a wavelength of 600 nm. From experience we know that mid-log phase is reached, when we obtain an absorbance of 0.35. We also know that the doubling time of our culture is roughly 25 minutes. This means that every 25 minutes the number of *E.coli* cells in the medium and hence the absorbance doubles. If we start with an absorbance of 0.0025, how long do we have to grow the culture for it to reach an absorbance of 0.35? Is there enough time to go for a decent cup of coffee in the cafeteria or do we have to drink the horrible stuff from the vending machine?

The easiest way to approach this problem is to do the following calculation:

1. After 25 minutes the absorbance goes from 0.0025 to 0.005 (first doubling);
2. After another 25 minutes the absorbance goes from 0.005 to 0.01 (second doubling);
3. After yet another 25 minutes the absorbance goes from 0.01 to 0.02 (third doubling);
4. After another 25 minutes the absorbance goes from 0.02 to 0.04 (fourth doubling);

Obviously we could continue with this and simply count the doubling times. We would find that after the seventh doubling we have reached an absorbance of 0.32, which is close to the 0.35 we need for our experiment.

Our calculation therefore would be:

7 doublings *x* 25 minutes per doubling = 175 minutes

Answer: After roughly 175 minutes we should have reached the mid-log phase that is required for our experiments - plenty of time to have a decent cup of coffee in the cafeteria.

Comparison with a graph showing some experimental data demonstrates the correctness of our calculation (Figure 1-1)

The method we used to reach this conclusion relied very much on an understanding of reaction kinetics. In fact, we used the concept of ‘doubling-times’ – something we will explore further when we talk about first-order reactions.

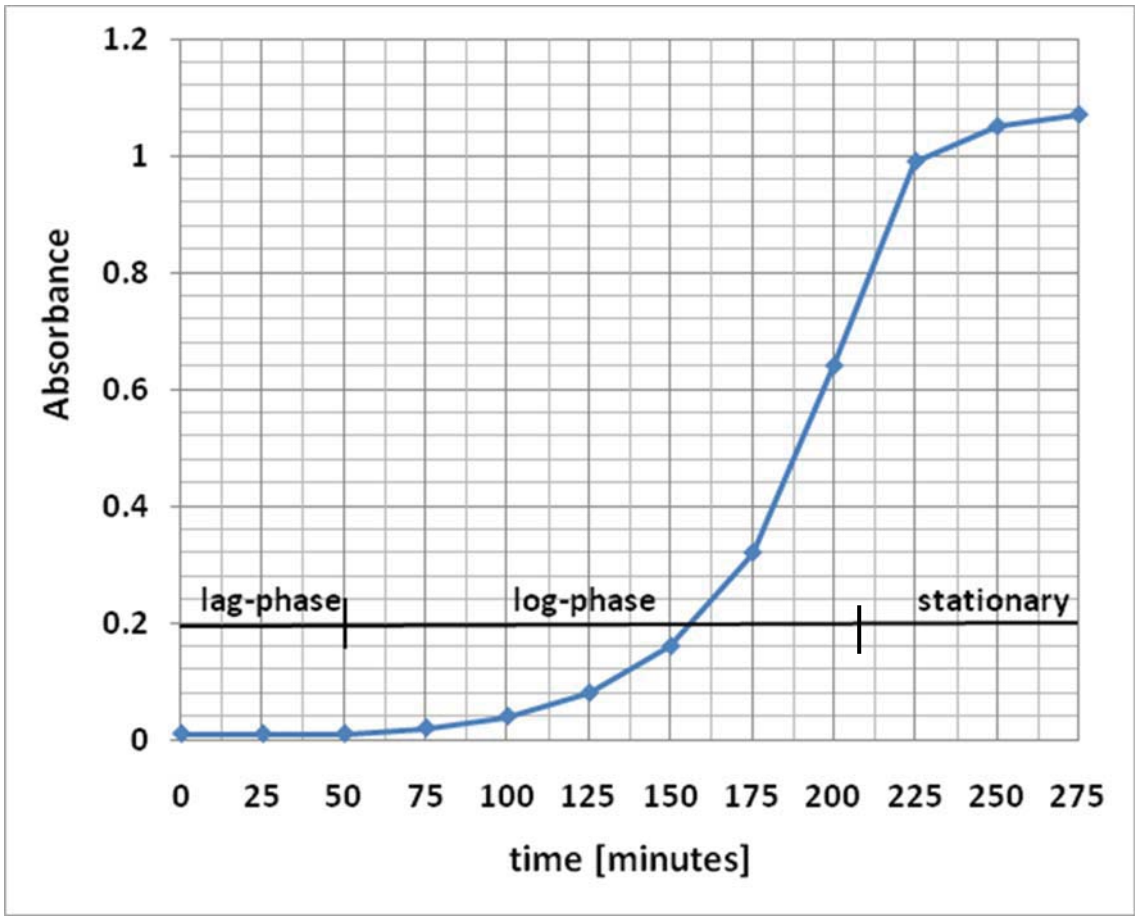


Figure 1-1 Growth curve of *E.coli* in LB-medium at 37°C.

1.4. Problem: Cancer

Let's have a look at another rather complex 'biological' case – cancer!

In general, cancer starts when a cell begins to grow and divide although it is not supposed to do so. Very often this happens if there is no longer an orchestrated response to specific signals. This is a rather simplified view, but for the moment it will do.

First let's have a look at what happens in a 'normal' cell:

A receptor (R) for a growth hormone is synthesised in the cell and then transported to the cell membrane.

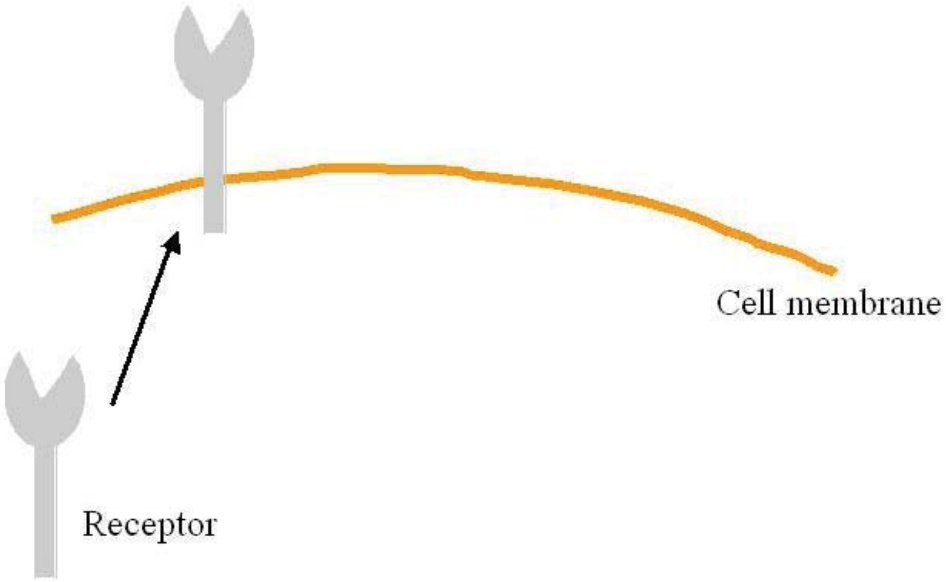


Figure 1-2 Synthesis of a receptor and its transport to the cell membrane.

At the cell membrane this receptor then interacts with a ligand, usually produced by a different cell, to form a receptor-ligand complex (RL).

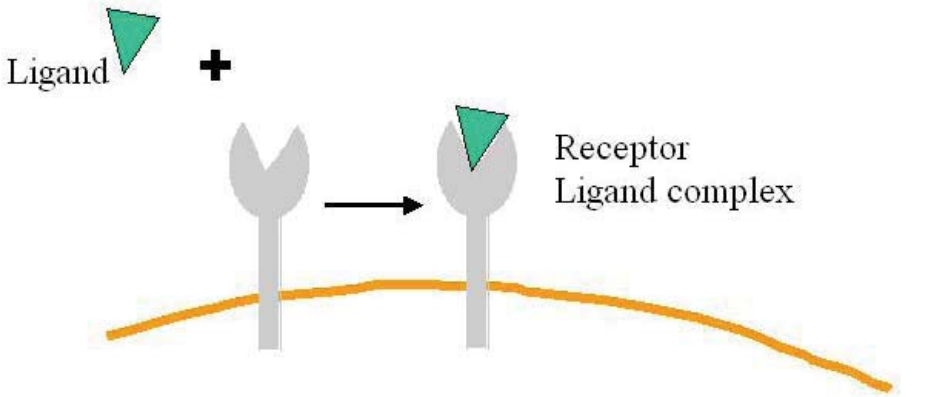


Figure 1-3 Interaction of a receptor with a ligand.

This receptor-ligand complex can interact with a second receptor-ligand complex to form a receptor-ligand dimer (D).

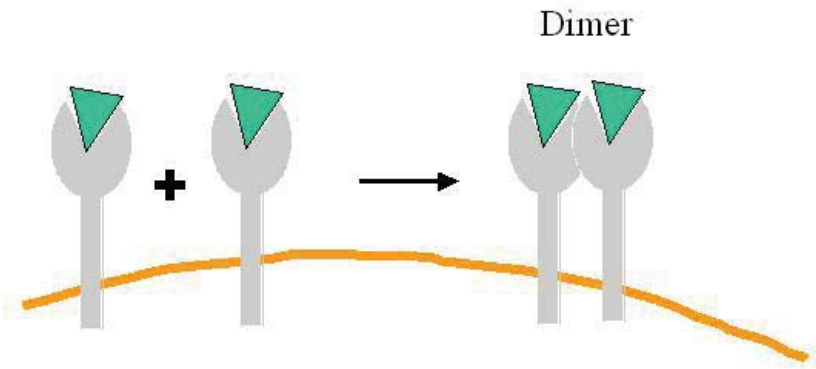


Figure 1-4 Dimerisation of a receptor-ligand complex to form a receptor-ligand pair.

It is this dimer that produces a signal, which stimulates the cell to grow and divide.

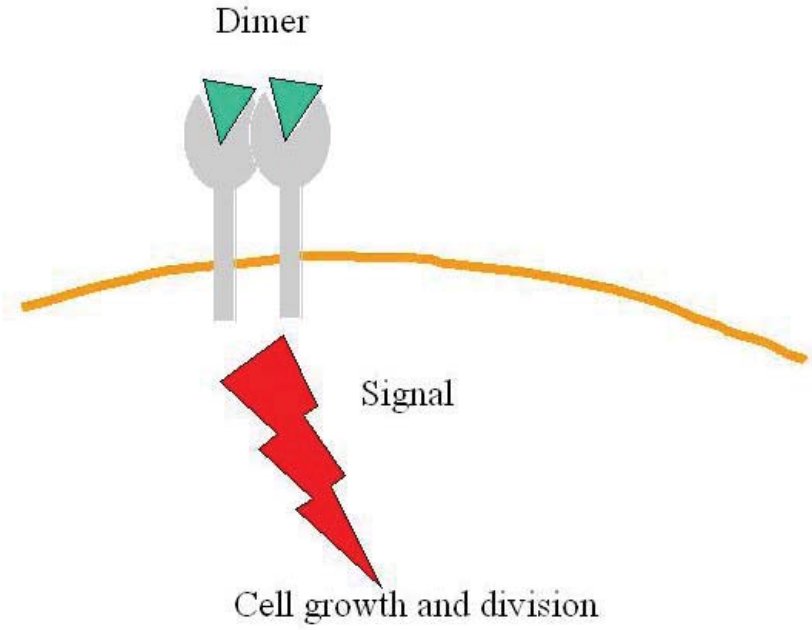


Figure 1-5 The receptor-ligand pair produces a signal that stimulates cell growth.

However, a 'normal' cell will stop dividing sooner or later. To achieve this, the receptor-ligand dimer is degraded and the signal disappears. The cell stops growing and dividing.

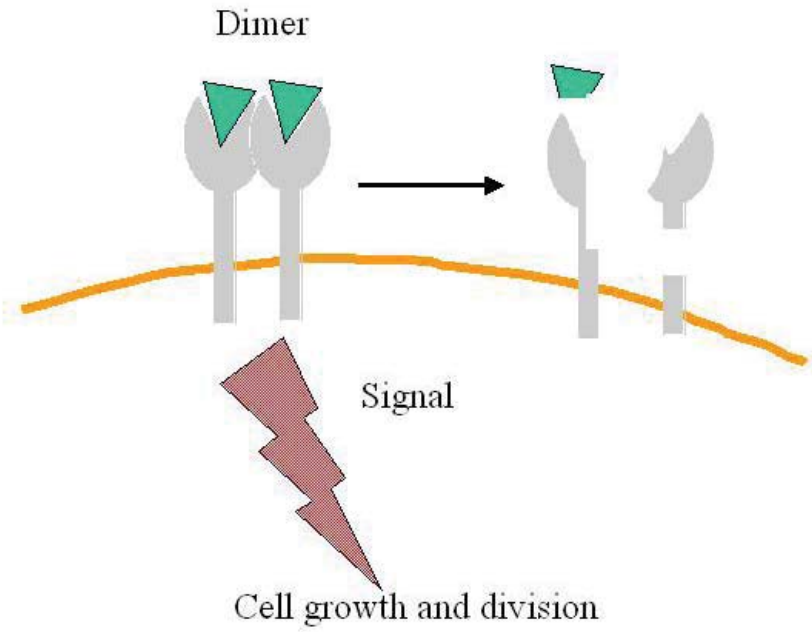


Figure 1-6 Degradation of the receptor-ligand pair stops the signal for cell growth.

In a cancer cell, however, there is a problem:

The signal persists for too long and therefore causes the cell to grow and divide and grow and divide and grow and divide. Eventually a tumour develops which can easily spread and form metastases. These can invade other tissues and lead to a breakdown of vital functions.

What is going wrong?

Very often the signal persists, because the amount of the receptor-ligand dimer is too high (actually, it is not the amount that is the problem; it is the concentration of the dimer). Why?

The concentration of the receptor-ligand dimer depends on two factors, namely how much dimer is produced and how much dimer is degraded.

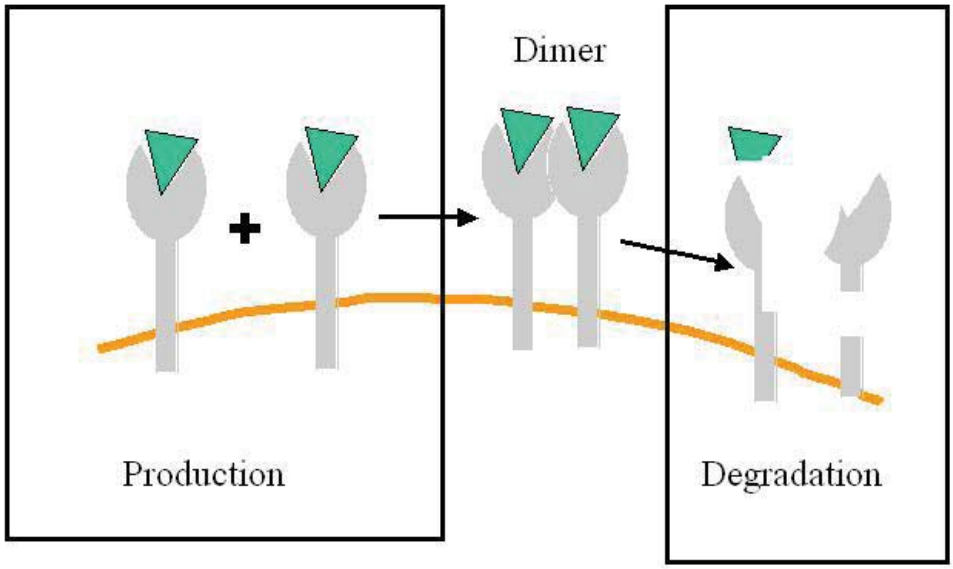


Figure 1-7 The combination of production and degradation dictates the concentration of the receptor-ligand pair.

It is similar to your bank account:

The account is in balance if your monthly pay in is as much as your monthly withdrawal. If you withdraw more than you pay in, sooner or later you will be in debt. If you pay in more than you withdraw, you make savings. In a way, the onset of cancer could be compared to a decrease in withdrawal from the bank account. If you still pay in the same amount of money every month, but you withdraw less, your savings will grow bigger and bigger. This obviously is similar to what happens in the cancerous cell – it produces the same amount of receptor, but reduces the degradation of the receptor-ligand pair.

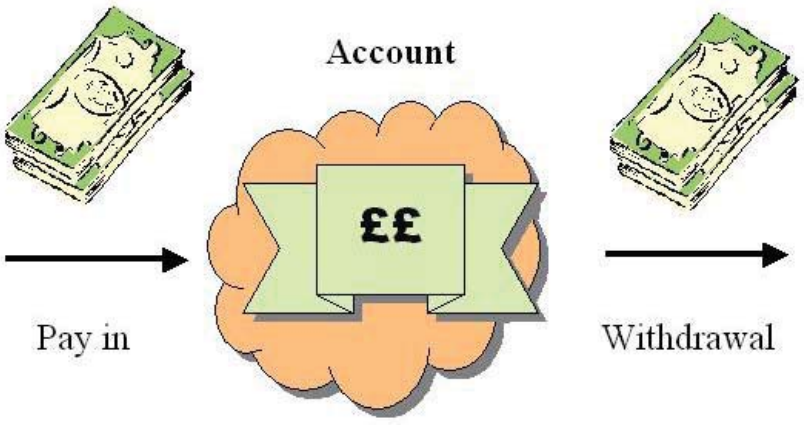


Figure 1-8 Analogy to bank account – the account is in balance if the amount paid in is the same as the withdrawal.

If we want to understand, how cancer actually is brought about, we therefore need to understand, which factors affect the production and degradation of the receptor-ligand dimer, as indicated in Figure 1-7. And this is where kinetics comes into play.

We need to ask how fast the production of the dimer is and how quickly it is degraded. We therefore will have to investigate how the 'speed' of the reactions is influenced by various factors. And this is what kinetics is all about – speed, velocities and rates.

1.5. Summary

Many processes, like drug metabolism, growth of a population or the development of a disease are linked to kinetics. If we want to be able to understand these processes, we need to look further into kinetics and define in more detail, what we actually mean by rates, speed and velocities.

2. Rates, speeds and velocities

2.1. Introduction and learning outcomes

In the previous chapter we looked at some examples from biology and pharmacology, which all have some relevance to kinetics. This chapter we will define what we mean when we talk about rates, speed and velocity. By the end of this chapter you should be able to tell the difference between these three expressions.

2.2. Definitions

When we talk about kinetics, we use the word 'rate' a lot. But what does it mean? And what is the difference between rate, speed and velocity?

In general a rate can be defined as *anything per anything*. Sounds strange!?

A rate could be:

Miles per litre petrol
ff£s per day into your account
Output per employee
Miles per hour
A change in concentration per minute

A rate simply tells you how much one thing changes compared to another. A rate does NOT have to be anything per time (as many people think).

Velocity is a special case of a rate. It is defined as *anything per time*.

A velocity could be:
Miles per hour
ff£s per day payed into your account
A change in concentration per minute
Distance travelled per time interval

When we (or chemists) are talking about rates, we are really talking about velocities. We say, "the rate of a reaction is…." and we usually mean, "the change in concentration per *time* is….".

And what is speed?

Speed, like velocity, is defined as *anything per time*. However, a very important difference between 'speed' and 'velocity' is that speed does not take into account the direction of a reaction, whereas velocity does.

Let me explain:

When you are driving a car at 50 miles per hour, this is a rate, a velocity and a speed. In terms of speed you are driving 50 miles per hour - it does not matter whether you are driving from Canterbury to London or London to Canterbury. Your velocity, however, would be very different, depending on your point of observation, because velocity (and rate) depends on you starting point and your end point. If you start in Canterbury and you are driving towards London, someone in Canterbury would say that your velocity is **–** 50 miles per hour, whereas someone in London might say that your velocity is + 50 miles per hour. As a convention - **going away from your starting point is indicated by a negative sign**, whereas going towards your destination is indicated by a positive sign. This concept might sound strange at the beginning, but it is very useful when we for example look at the different reactions that may or may not lead to cancer.

2.3. Average and instantaneous rates

When we talk about rates (or speeds or velocities) we often distinguish between two different forms of rates (or speeds or velocities). Most people are probably familiar with the concept of an average rate. For example the distance between Canterbury and London is approximately 60 miles. If it takes us to drive this distance in 1 ½ hours we can easily calculate our average speed to

$$\text{Average speed} = \frac{60 miles}{1.5 hours} = 40 \ \frac{miles}{hour}$$

Now the interesting (and important) point here is that the 'average' speed just gives us information on a 'global' scale. In fact we could use this average speed to make some educated guesses about our journey. For example we could predict that, given we drive at an average speed of 40 mph, after 30 minutes into our travel we might have travelled 20 miles. We can also predict that after 1 hour, we travelled 40 miles.

The way we calculate an average rate (or speed or velocity) is that we look on a 'global' scale at the change of something divided by the time (again on a 'global' sale). 'Global' scale in this case indicates that the observation span is over a reasonably long period of time.

In mathematical terms the average rate is given by the equation:

$$\text{Average rate} = \frac{x_{final} - x_{initial}}{t_{final} - t_{initia}l}$$

or

$$\text{Average rate} = \frac{\Delta x}{\Delta t}$$

x_{final} = amount of something at the end of the process,
$x_{initial}$ = amount of something at the beginning of the process
t_{final} = endpoint of the process
$t_{initial}$ = starting point of the process.
The "Δ"symbolises the difference between x_{final} and $x_{initial}$ or t_{final} and $t_{initial}$.

Although knowing the average rate (or speed or velocity) allows us to determine, on a very coarse scale, where we are on our travels, it is sometimes not particularly useful. For example, we might need to know, how fast we are going at a specific moment in time, which is particularly important for areas with speed restrictions and speed cameras. It could very well be that **on average** our speed is 40 mph, but at 15:35 we drove through a 40 mph–restricted zone at a speed of 60 mph. When we receive a speeding ticket it doesn't help us that our average speed was 40 mph. What counts is the speed at the very moment in time, i.e. when we got caught. This is also called the **instantaneous** speed.

So what's the difference between the average rate (or speed or velocity) and the instantaneous equivalent?

The average rate uses a long observation period (in this case 1 ½ hours), whereas in the case of the instantaneous rate the observation period is much, much shorter. In fact, it could be so short, that we do not really recognize it as an observation period. It could very well be that our observation period is only a second during which we measure the distance travelled. Our measurements are no longer on a global scale, they are now on a much finer scale.

Mathematically we can express this as:

$$\text{Rate} = \frac{x_{final} - x_{initial}}{t_{final} - t_{initial}}$$

whereby the differences between x_{final} and $x_{initial}$ or t_{final} and $t_{initial}$ are now extremely small.

In short:

$$\text{Rate} = \frac{\delta x}{\delta t}$$

The "δ" indicates that the differences are now much smaller than our previous "Δ". In our example we go from an hour to a second. But we can make it even smaller. We can ask how far we have travelled in a millisecond or in a microsecond. Actually we can make the change as small as we like. Taking this even further and making the duration of observation close to zero it can be written as:

$$\text{Instantaneous rate} = \frac{dx}{dt}$$

The "d" indicates that our differences in time and distance are infinitesimally small, they are approximating zero.

2.4. Working with instantaneous rates

We can use our previous example of travelling from Canterbury to London to explore the use of instantaneous rates a little bit further. For example we could attach some kind of recording device to our speedometer and record our speed at every moment in time. We could even go one step further and use a GPS (global positioning system) to detect our exact whereabouts at any moment in time. And of course, we could combine the two systems to determine our actual velocity. Why am I now talking of speeds and velocities? Remember, we said earlier that speed does not take into account the direction, whereas velocity does, which is quite important in our example.

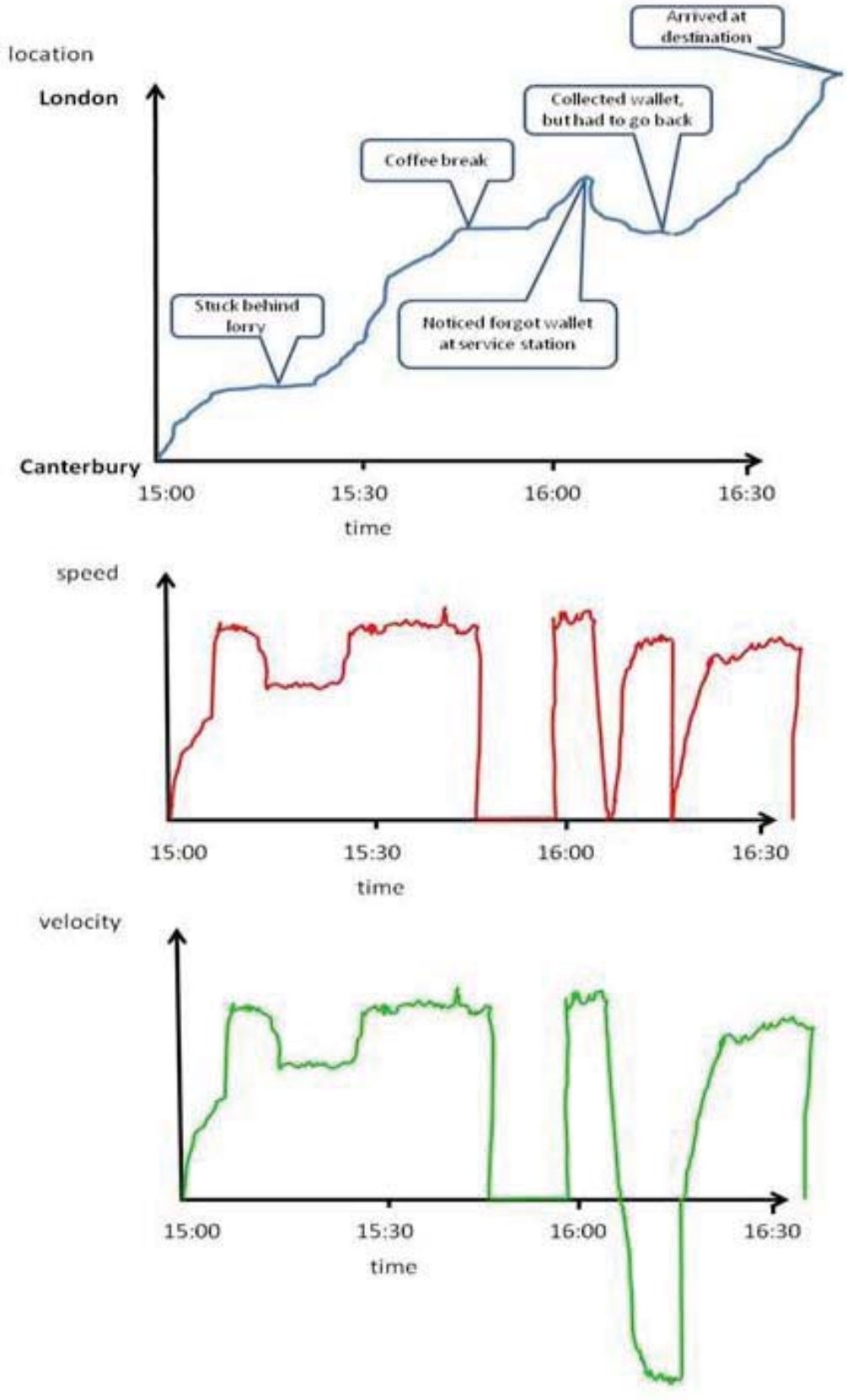


Figure 2-1 Location, speed and velocity – time diagrams

We can see that the graphs for 'speed' and 'velocities' are pretty much the same, with one important exception: When we have to go back to collect the wallet from the service station, where we left it, our speed is still positive, whereas the velocity is negative. The reason for this is that the velocity takes into account the actual position of where we are, whereas the speed doesn't.

In our example we used a GPS to determine our actual position, but you might argue that this is a rather laborious way and you perhaps wonder, if there isn't any easier way to find out where we are and how far we have travelled. And yes – there is a fairly easy way.

The definition of velocity is, as we stated earlier:

Velocity = $\frac{\delta x}{\delta t}$ or in other words - distance travelled per time interval.

We can rearrange the equation to:

δx = velocity x δt

and calculate where we are by just looking at our velocity.

For example, we know that at the beginning of our journey we drove for 2 minutes at 30 miles per hour. The distance that we travelled in these two minutes therefore would be:

δx = 30 mph x 2 minutes.

Of course we have to be careful with the units – best is probably to convert the velocity from mph into miles per minute:

$$30 \text{ mph} = 30\ \frac{miles}{hour} = \frac{30 miles}{60 \text{ min}} = 0.5 \text{ miles per minute.}$$

With this conversion we now can calculate the distance travelled within the first two minutes as:

$\delta x = 0.5$ miles/min x 2 minutes = 1 mile.

In the first minute we therefore travel 1 mile.

Let's say in the next minute we drive at 60 mph – how far have we gone now?

Again, we convert 60 mph into $\frac{60 miles}{60 \text{ min}}$ = 1 mile per minute.

$\delta x = 1$ mile/min x 1 minute = 1 mile.

Now we can ask the question, how far have we travelled in the first 3 minutes?

To answer this we simply add the distance travelled in the first 2 minutes plus the distance travelled in the third minute:

$\delta x = \delta x_{(0\text{-}2 \text{ min})} + \delta x_{(2\text{-}3 \text{ min})} = 1$ mile + 1 mile = 2 miles.

We could carry on like that for the rest of our journey thus determining every minute how far we have travelled, given that we know the exact velocity during this time span. For this we have to:

a) Multiply our velocity with the time of observation
b) Add up all the individual steps.

Of course a minute is a rather long time and it is fairly unlikely that we are able to drive at exactly the same speed for a whole minute. But this actually does not matter too much – we could shorten our observation time to a much smaller interval. We don't even have to stick to a regular observation interval, like a minute or a second. We might find that we need to use different intervals, for example if we drove for 3 minutes at 45 miles per hour, then for 5 seconds at 60 miles per hour and then slowed down to drive for another 2 minutes at 30 miles per hour.

Our distance travelled would be:

$\delta x = 45$ mph x 3 minutes + 60 mph x 5 seconds + 30 mph x 2 minutes.

We need to convert this all to the same unit, e.g. seconds:

$$\delta x = \frac{45 miles}{3600 \sec} x\ 3\ x\ 60\ \sec + \frac{60 miles}{3600 \sec} x\ 5\ \text{seconds} + \frac{30 miles}{3600 \sec} x\ 2\ x\ 60\ \sec$$

$$\delta x = \frac{45 miles x 3 x 60 \sec}{3600 \sec} + \frac{60 miles x 5 \sec}{3600 \sec} + \frac{30 miles x 2 x 60 \sec}{3600 \sec}$$

δx = 2.25 miles + 0.083 miles + 1 mile = 4.08 miles.

Mathematically we can write:

Distance travelled = $\sum \delta x$, which means nothing else but the sum of all the sub-sections of our travel.

Graphically we can show this procedure as well.

First we draw a velocity-time diagram:

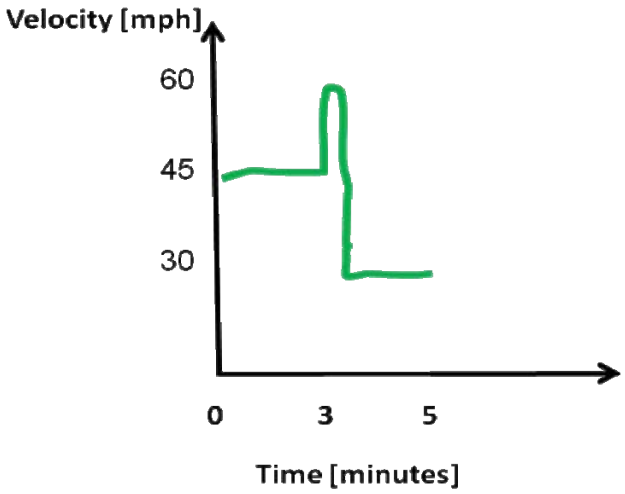


Figure 2-2 Velocity-time diagram

Next we 'graphically' determine the product of velocity *x* time for smaller subsections of the velocity-time diagram. This 'product of velocity *x* time' can be mathematically represented by a square with side length of time and velocity, respectively.

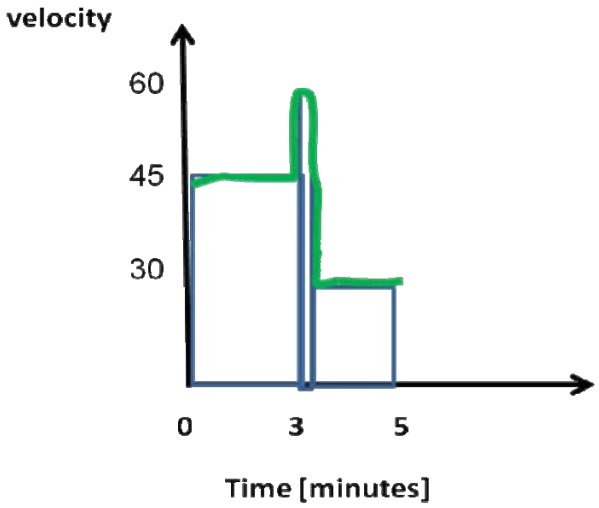


Figure 2-1 Breakdown of velocity-time diagram into sub-sections

By adding up the different squares we get the distance travelled.

Obviously, the smaller we make the intervals of observation, the more accurate our calculations are. However, it also means that we have to add (and calculate) more individual squares.

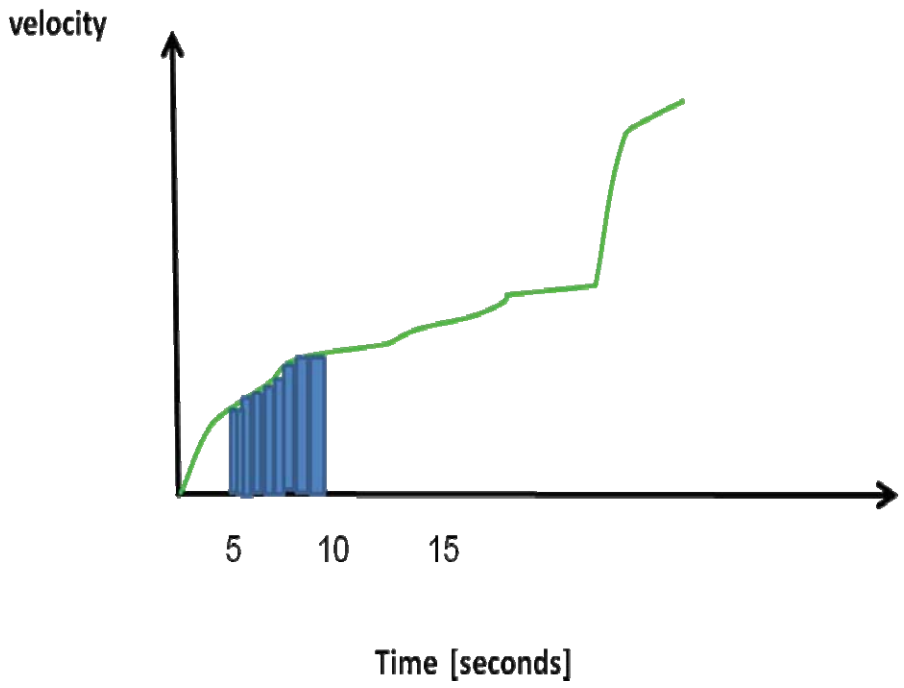


Figure 2-2 Division of the area under the velocity-time curve into smaller sub-sections

Eventually, to achieve the maximum accuracy, we could make our observation intervals infinitesimally small, but then we would need to add up an infinitesimally large amount of squares. However, in mathematical terms this is fairly easy to do – what we have done so far is nothing else but determined the area under the velocity-time curve.

This method is the integration of the instantaneous rate function $\frac{dx}{dt}$. We calculate the product of dx *x* dt and add up all the different squares. Remember, we said that the "d" indicates that our differences in time and distance are approximating zero.

To symbolise that we are adding up an almost infinitesimally large number of squares we no longer use the symbol $\sum \delta x$, instead we write:

Distance travelled = $\int_{t_{initial}}^{t_{final}} dx$.

The "$\int dx$" indicates that we sum up all the different 'dx *x* dt' squares, while t_{final} and $t_{initial}$ tell us for which time span we do this mathematical operation.

As long as we can express our velocity in the form of a mathematical function, like $\frac{dx}{dt}$, we can also integrate it.

Which expression should we use – should we use speed or velocity for the integration?

From Figure 2-1 we noticed that the difference between the speed-time and velocity-time diagram is that velocity can also assume negative values. The interpretation is basically that by using velocity, we take the direction of the reaction (or travel) into consideration. But the question is still – shall we integrate a speed-time or a velocity-time function?

Let's assume we drive for 1 hour at 30 mph. We then stop and drive back to our starting point, driving for 2 hours at 15 mph. We can draw a location-time, speed-time and velocity-time diagram as we have done before:

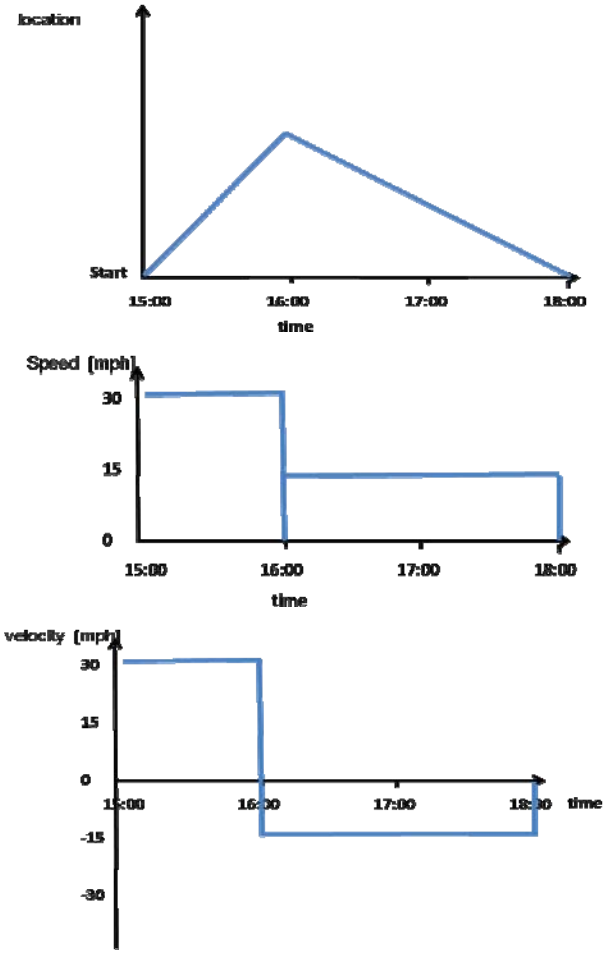


Figure 2-3 Location, speed and velocity – time diagrams

We can now ask two different questions:

1. How many miles have we travelled in total?
2. Where are we in relationship to our starting point?

Both questions require a different approach. To answer the first question we just calculate the sum of the products of velocity *x* time from the speed-time diagram:

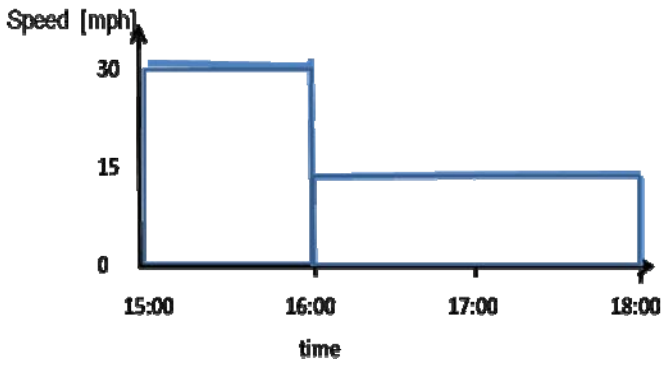


Figure 2-4 Speed-time diagram with breakdown of sections

$\delta x = 30 \text{ mph } x \text{ 1 hour} + 15 \text{ mph } x \text{ 2 hours} = 30 \text{ miles} + 30 \text{ miles} = 60 \text{ miles}.$

In total we travelled 60 miles, however, it does not tell us anything about where we are after we drove for 3 hours. To calculate this, we have to use the velocity-time diagram:

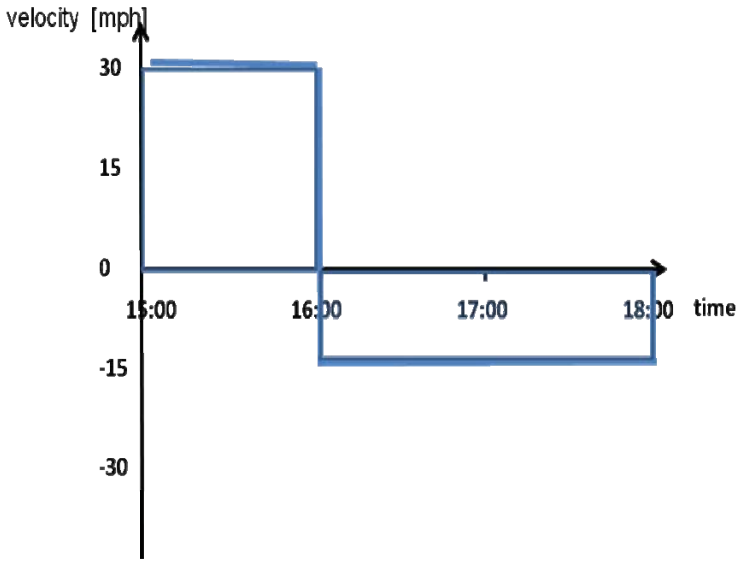


Figure 2-5 Velocity-time diagram with breakdown of sections

Again,we have to calculate the sum of the velocity *x* time squares from the diagram, but the important point here is that in the second part of our journey the square is in the **negative** range. We can take this into account in our equation for δx by using the negative velocity:

δx = 30 mph *x* 1 hour + - 15 mph *x* 2 hours = 30 miles - 30 miles = 0 miles.

The result of our calculation is that we have travelled exactly 0 miles away from our starting point. And this is exactly, what our location-time diagram shows.

The important point that I am trying to make here is that, depending on the question we want to address, we need to either look at the speed-time diagram or at the velocity-time diagram.

The good news, though, is that this is usually only the case, if we indeed undertake some travelling. When we look at what happens to reactions we usually only want to know how far we have gone with respect to our starting point. It therefore would be appropriate to use predominantly velocity-time equations for our kinetics calculations.

2.5. Summary

Speed, rate and velocity mean different things – while rate can basically mean ‘change in something per anything’, speed and velocity indicate a ‘change in something per time interval’. A distinction between speed and velocity is that the latter takes into consideration the direction or an action. By definition a reaction, which goes away from the starting point will be expressed by a negative velocity.

Velocities can be expressed as average or instantaneous velocities. The difference from the starting point of a reaction to a certain (final) point of the reaction can be obtained by measuring the area under the velocity-time curve of this reaction. Mathematically this is achieved by integration of the respective velocity-time function.

3. Zero Order Reactions

3.1. Introduction and learning outcomes

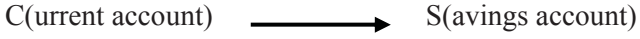
Zero order reactions are a common feature in a variety of disciplines. For example the elimination of a certain drug from the metabolism might follow a zero order reaction. Another example is related to (again) a bank account – standing orders with money paid in or withdrawn often can be described by zero order kinetics.

In this chapter I will show, how we can formulate the typical rate equation for a zero order reaction. Using examples I will develop the equations for this kind of reaction. By the end of this chapter you should be able to recognize a zero order reaction and calculate various components of a zero order equation.

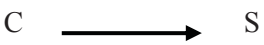
3.2. Some definitions for zero order reactions

Let's go back to our first bank account example (I will use biological examples soon, I promise). At the beginning of the year you have £ 300 in your 'current' account and £ 0 in the savings account. How much money will there be in your current account and your savings account at the end of the year? We could do it by using the average rate (where we simply calculate the transfers on a monthly basis, as we have done before). But we could also use an instantaneous rate equation, in which we look at a daily, or hourly or even smaller basis.

First of all, let's define the flow of money. If we set up a standing order to transfer money from the current account into the savings account, we can write:



Or short:



We always want to transfer the same amount of money in a given period of time, e.g. a fixed sum per month, week, day or any other period of time, and hence we can write the general form of the instantaneous rate equation for this transaction:

Instantaneous rate = a constant payment

This constant payment is usually symbolized by the letter k. k is called the **rate constant** for this particular reaction.

Depending on which account we look at, our instantaneous rates will be very different. If we look at the current account we withdraw money from it. Taking something away is usually indicated by a negative sign. Looking at the current account, we therefore can write:
Instantaneous rate (for current account) = - k

Or short:

$$\frac{dC}{dt} = - k.$$

(It doesn't matter which symbols we use (S or C or E or whatever), as long as we are consistent.)

Now what happens to our savings account?

Clearly, the rate by which the savings account changes is the same rate the current account changes. Well, almost. Remember – in the previous chapter we said that the rate of a reaction (or bank transfer) can be either positive or negative, depending on the direction of the transfer.

We therefore can write:

$$\frac{dS}{dt} = - \frac{dC}{dt}$$

Or, since $\frac{dC}{dt} = - k.$

$$\frac{dS}{dt} = + k.$$

It is essential that we pay attention to the sign of k – it indicates whether money goes into an account or is taken away from it. In the following section we will see, how we can calculate 'S' and 'C' at any given time point.

3.3. Developing the tools for a zero order reaction

3.3.1. Equations

Assuming we have the following zero order reaction:

$$C \xrightarrow{k} S$$

we can write:

$$\frac{dC}{dt} = -k \text{ and } \frac{dS}{dt} = +k.$$

Let's try to solve this differential equation, first for 'C':

The easiest way to solve a differential equation is to 'separate the variables'. This means that everything that contains a 'C' goes to one side of the equation, whereas everything that contains a 't' goes to the other side.

Rearranging our equation therefore gives:

$dC = -k \, x \, dt$

We now have to perform the reverse mathematical operation to differentiation, which is integration. We therefore 'integrate' both sides of the equation, which gets rid of the dC and dt.

$$\int_{C_{initial}}^{C_{(t)}} dC = \int_{t_{initial}}^{t_{(t)}} -kdt$$

The subscript (t) simply stands for 'at a given time' and the subscript 'initial' indicates the status of the 'current' account at the beginning of the transfers ($C_{initial}$ at $t_{initial}$).

The solution of an integral like $\int_{x_{initial}}^{x_{(t)}} dx$ integral is simply $x_{(t)} - x_{initial}$.

The right hand side of the equation $\int_{t_{initial}}^{t_{(t)}} -kdt$ can also be written as $- k\,x \int_{t_{initial}}^{t_{(t)}} dt$ and the solution for this is - $k\,x\,(t_{(t)} - t_{initial})$.

Taken together we can combine the two sides of the equation and write:

$$C_{(t)} - C_{initial} = - k\,x\,(t_{(t)} - t_{initial})$$

In a zero order reaction, the unit for k is the same as the unit for the rate. If we look at the change in the amount of money we have in our current account per time, then our rate constant would be ££s/month (or something to that effect, like ££s/day).

Units for k in a zero order reaction can be:
mM/min
££s/day
or in general **change of something *per time difference***

Now let's look at what happens to our savings account. Every £ that is taken out of the current account goes straight into the savings account. If we know how much money we started with in the current account and if we can calculate how much money is still left in the current account at a given time we should be able to tell, how much money is currently available in the savings account. For example if we started with £ 300 in the current account and transferred £ 10 every month we have in our current account after 3 months:

£ 300 – 3 *x* £ 10 = £ 270.

In our savings account is obviously the difference between £ 300 and £ 270, ie. £ 30. In general we can write:

$$C_{(t)} + S_{(t)} = C_{initial} + S_{initial}$$

By including the expression '$S_{initial}$' into this equation we take into consideration that there might have been already some money in the savings account from a previous transaction, which came from a different account.

Although in this particular case we could also develop a similar equation for $S_{(t)}$ starting from our original differential equation $\frac{dS}{dt} = +k$ it sometimes can cause some problems. It is therefore always best to start by investigating the consumption rate of a reaction, but we will discuss this in more detail a little bit later.

First, we have to look at a limitation of the equation $C_{(t)} - C_{initial} = -k\,x\,(t_{(t)} - t_{initial})$.

3.3.2. Constraints in a zero order reaction

Looking at our example of transferring money out of a current account into a savings account we notice that we probably have to be a bit careful: If there is no money being transferred into the current account, but every month a fixed sum is withdrawn, we sooner or later will end up with no money in the current account left. Even worse – if we don't give detailed instructions to the bank, we might find ourselves in a situation where money has been transferred out of the current account, although there was no money left – we are overdrawn (and the bank will charge us interest). Or in other words, the amount of money left in the current account would be negative – we are in debt. Obviously, this situation can easily happen, when we look at an example where a negative amount of something is possible, like bank accounts. However, if we look at other examples, where there is no such thing as a negative entity, we have to define, what should happen, if we reach a point where we cannot take more of this entity away. For example let's assume we look at the elimination of a drug from the metabolism, which is following zero order kinetics (see next problem). At the beginning of the elimination process we have a certain amount of the drug present. This amount is reduced by a constant level per time unit, until at one point we don't have any drug left in the metabolism. Our drug concentration therefore has dropped to zero. However, by just applying our equation it would be mathematically possible to make the drug concentration even negative, although physiologically this does not make sense. We simply cannot have a negative drug concentration in the blood. In this case we need to explicitly state that our $C_{(t)}$ must be positive or at least equal to zero.

We therefore should write:

$C_{(t)} = C_{(t)}$ if $C_{initial} - k\,x\,(t_{(t)} - t_{initial}) \geq 0$.

In all other cases we define that $C_{(t)} = 0$.

3.3.3. Graphic representation of a zero order reaction

Often we are interested in how much money is left in our current account (or drug in the metabolism) at a given time and therefore can rearrange above equation to

$C_{(t)} = - k \ x \ (t_{(t)} - t_{initial}) + C_{initial}$

This equation looks very similar to the equation for a straight line if we assume that $t_{initial} = 0$ (which makes sense, if we say that the starting point of our experiment is time zero).

$C_{(t)} = - k \ x \ t_{(t)} + C_{initial}$
$y = m \ X + C$

We therefore plot $C_{(t)}$ (amount of money in the current account) versus $t_{(t)}$ (months) and get a straight line with the gradient -k and the intercept with the y-axis of $C_{initial}$:

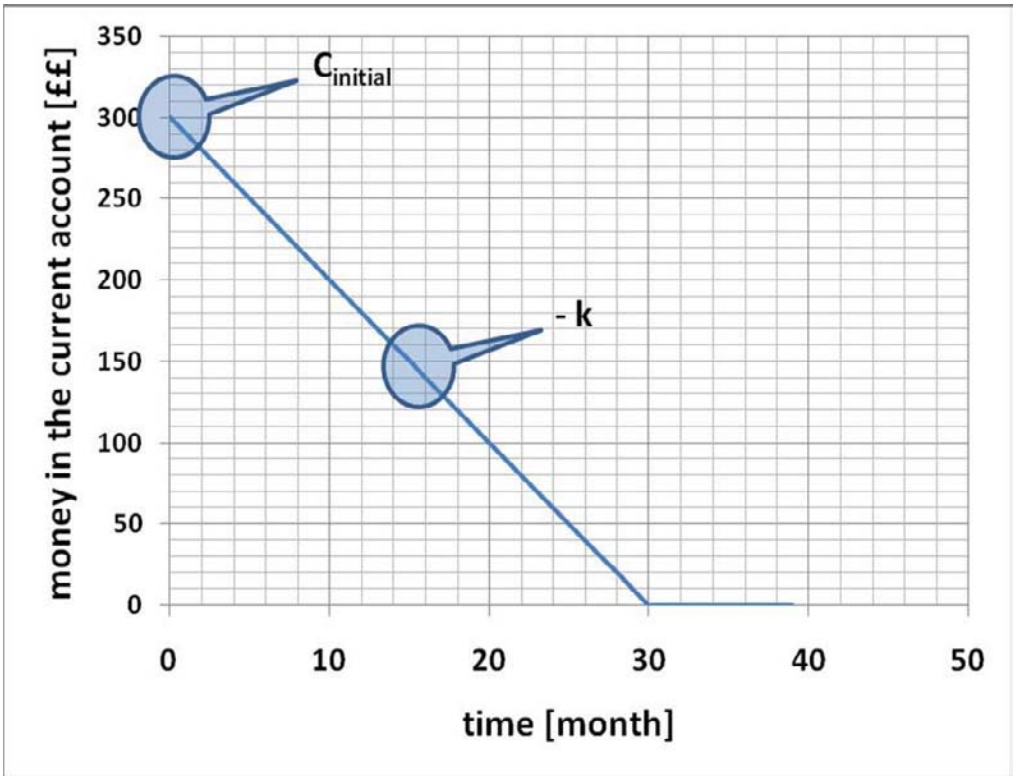


Figure 3-1 Graphic representation of a zero order reaction – consumption of C – money in the current account over time.

We can also look at what happens in the savings account:

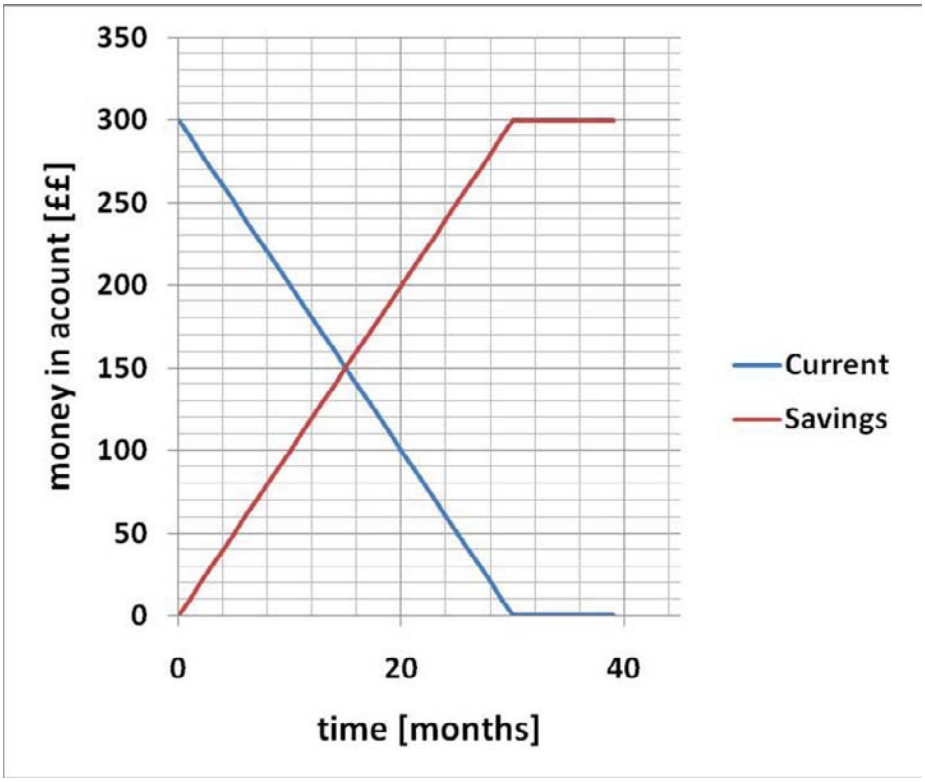


Figure 3-2 Graphic representation of zero order reaction – consumption of C (money in the current account) and production of S (money in the savings account) over time.

How do we know that a reaction follows a zero order scheme?

Usually we do not know, whether a particular reaction indeed follows a zero order scheme. It could very well be that it is a different order, as we will see in subsequent chapters. So how can we find out of what order a reaction is?

The solution is fairly simple:

From the graphic representation in Figure 3-1 we know that by plotting "$C_{(t)}$" versus time we get a straight line, if the transfer of money out of the current account follows a zero order kinetics. Turning this argument around, we can say that if we plot "$C_{(t)}$" versus time and we get a straight line, then the reaction scheme must follow a zero order kinetics.

For some applications we are also interested in a graphical representation of the rate of the reaction. In a zero order reaction this is really easy to graph. All we have to use is the equations we used before:

$$\frac{dC}{dt} = -k \text{ and } \frac{dS}{dt} = +k.$$

Do NOT confuse the different graphical representations! In Figures 3-1 and 3-2 we plotted money in the account versus time whereas in Figure 3-3 we plotted **rate of the reaction** versus $C_{(t)}$.

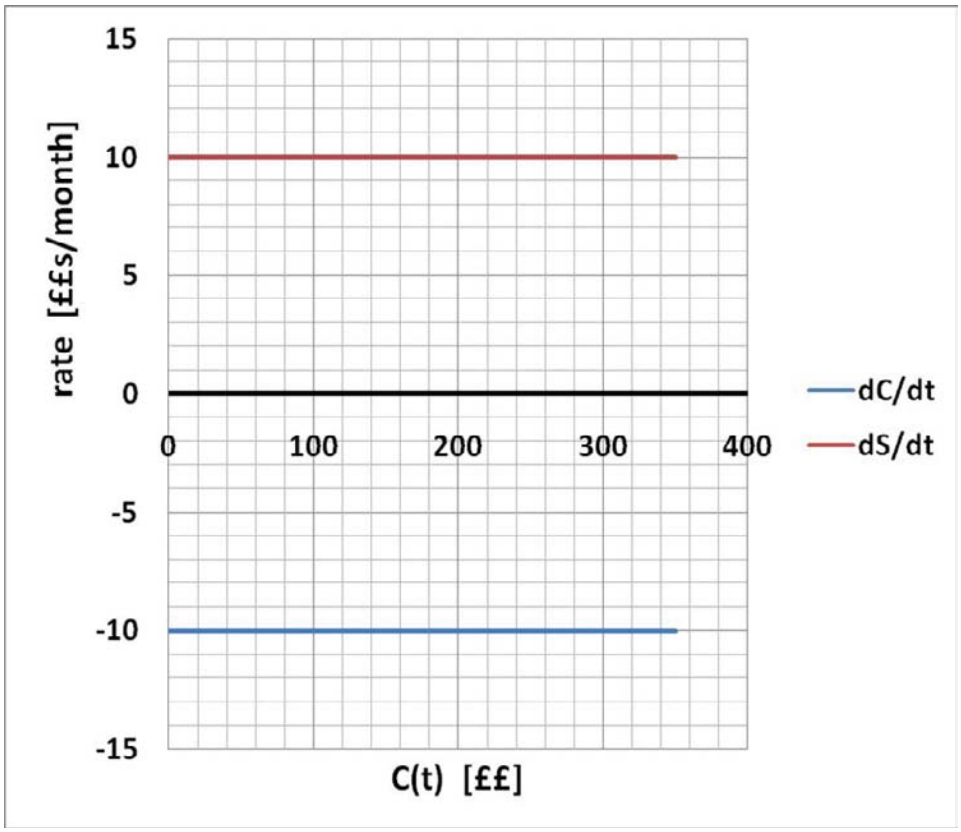


Figure 6-3 Graphic representation of zero order reaction – **rate** of changes in C (current account) and S (savings account), respectively, in relation to amount C at a given time ($C_{(t)}$)

3.4. Problem: Elimination of a drug from the metabolism

The elimination of a drug like Acetylsalicylic acid (aka aspirin) from the metabolism might follow a zero order reaction. You take 200 mg of the drug and every 10 minutes a blood sample is tested for the remaining amount of the drug.

The following table shows your results:

Time [min]	Drug [mg]
0	200
10	190
20	180
30	170
40	160
50	150
60	140
70	130
125	?

How much drug is left after 125 min?

Solution:

First let's consider the reaction scheme:

We are looking at the **decrease** in the amount of the drug like Aspirin and therefore can write:

A(spirin) $\xrightarrow{k}$

Of course we could draw a graph from our blood sample data and 'extrapolate' the line to 125 minutes…….

but this method might not be very accurate!

Instead, let's write the rate equation for this reaction, bearing in mind that because we are looking at a decrease in A the rate must be negative:

$$\frac{dA}{dt} = -k.$$

Separation of variables and integration of the equation gives:

$$A_{(t)} - A_{initial} = -k\,x\,(t_{(t)} - t_{initial})$$

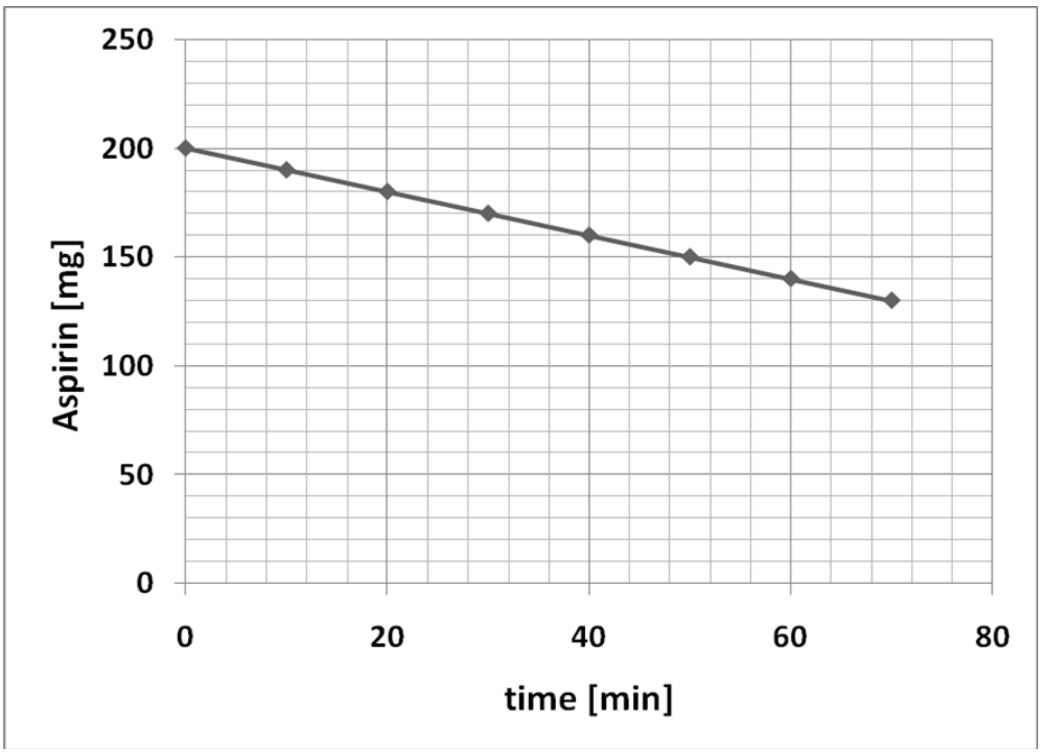


Figure 3-4 Elimination of Aspirin from the metabolism.

But we now have a problem – we do not know k!!!!!!!!!

However, we can calculate k from the data in the table. First let's rearrange the equation and solve for k:

$$k = - \frac{A_{(t)} - A_{initial}}{(t_{(t)} - t_{initial})}$$

By selecting an appropriate $A_{(t)}$ and the corresponding $t_{(t)}$ and also an $A_{initial}$ with corresponding $t_{initial}$ from our data table, we can calculate k.

Time [min]	drug [mg]
0	200
10	190
20	180
30	170
40	160
50	150
60	140
70	130
125	?

With this we get:

$A_{initial}$ = 200 mg	$t_{initial}$ = 0 minutes
$A_{(t)}$ = 140 mg	$t_{(t)}$ = 60 minutes
k = ??	

It does not matter, which dataset we use, but it is a good idea to use two sets that are reasonably well apart from each other.

Using these numbers we now can calculate k:

$$k = -\frac{A_{(t)} - A_{initial}}{(t_{(t)} - t_{initial})} = -\frac{140mg - 200mg}{60\,min - 0\,min} = -\frac{-60mg}{60\,min} = 1\ mg/min.$$

$\underline{k = 1\ mg/min}$

Answer: The rate constant for the elimination of a drug like Aspirin from the metabolism is 1 mg/min.
Note: the rate constant k is always positive.

We can now use this rate constant to calculate the amount of drug left after 125 minutes.
First we use the equation:

$A_{(t)} - A_{initial} = -k\ x\ (t_{(t)} - t_{initial})$

and solve for $A_{(t)}$ (this time for $t_{(t)}$ = 125 minutes).

$A_{(t)} = -\ k\ x\ (t_{(t)} - t_{initial}) + A_{initial}$

Using our data table, we get:

$A_{initial}$ = 200 mg	$t_{initial}$ = 0 minutes
$A_{(t)}$ = ?	$t_{(t)}$ = 125 minutes
k = 1 mg/min	

$A_{(t)} = -\ k\ x\ (t_{(t)} - t_{initial}) + A_{initial}$
$A_{final} = -\ 1\ mg/min\ x\ (125\ min - 0\ min) + 200\ mg$

$A_{(t)} = -\ 125\ mg + 200\ mg$

$\underline{A_{(t)} = 75\ mg.}$

Answer: The amount of drug after 125 minutes is 75 mg.

3.5. Problem: Transport of growth hormone receptor to cell membrane

Now let's look at a biological example, which will confront us with a slightly different problem – the transport of a receptor to the cell membrane.

Let's assume that a 'normal' cell produces a receptor for a growth hormone, which is transported to the cell membrane, and that this follows a zero order reaction. What is the concentration of the receptor after 12 hours, if the rate constant k is 0.5 nM per hour and at the beginning of the reaction the receptor concentration at the cell membrane is already 1 nM?

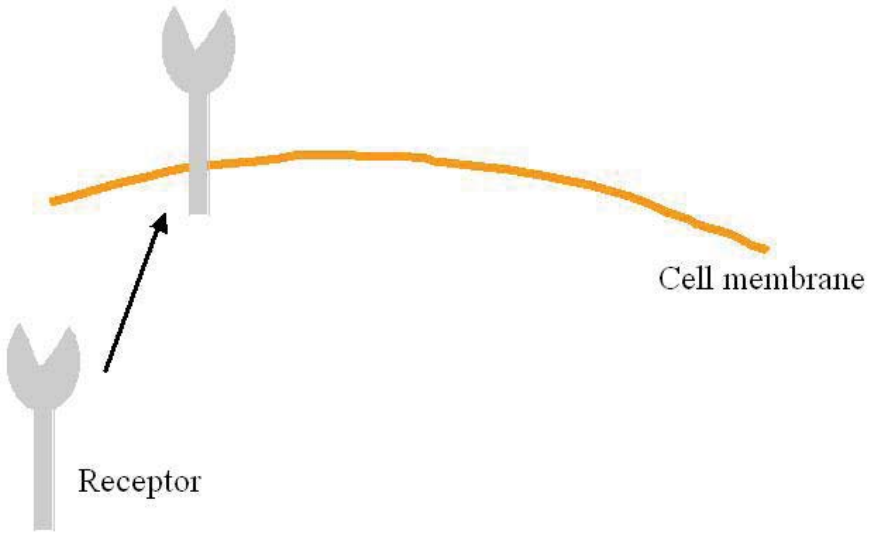


Figure 3-7 Transport of a receptor molecule to the cell membrane.

Solution**:**

The transport of the receptor to the membrane can be represented by the scheme:

$$\xrightarrow{k} \quad \text{R(eceptor)}$$

This scheme is similar to our previous example with the bank account, where we wrote:

$$C \xrightarrow{k} S$$

However, the main difference now is that we do not have a 'C' that we can use to calculate 'S' and hence the previous equation:

$$C_{(t)} + S_{(t)} = C_{initial} + S_{initial}$$

is therefore fairly useless.

What we can do is we can write our rate equation as:

$\frac{dR}{dt} = +k$ because we are looking at an increase of R (and therefore the rate must be positive).

As before we separate the variables of this differential equation so that everything that contains a 'R' goes to one side of the equation, whereas everything that contains a 't' goes to the other side.

$dR = +k \; x \; dt$

Next we integrate both sides of the equation, which gets rid of the dR and dt.

$$\int_{R_{iinitial}}^{R_{(t)}} dR = \int_{t_{initial}}^{t_{(t)}} + kdt$$

Again, the subscripts '(t)' and 'initial' indicate the status of the receptor at the beginning of the reaction ($R_{initial}$ at $t_{initial}$) and at a specific time point that interests us ($R_{(t)}$ at $t_{(t)}$).

The solution to the integral $\int_{R_{iinitial}}^{R_{(t)}} dR$ we have already seen. It is $R_{(t)} - R_{initial}$.

As before the right hand side of the equation $\int_{t_{initial}}^{t_{(t)}} + kdt$ can be written as $+k\ x \int_{t_{initial}}^{t(t)} dt$ and the solution for this integral is k *x* ($t_{(t)} - t_{initial}$).

Taken together we can combine the two sides of the equation and write:

$$R_{(t)} - R_{initial} = k\ x\ (t_{(t)} - t_{initial})$$

Or, if we rearrange the equation and solve for $R_{(t)}$:
$R_{(t)} = k\ x\ (t_{(t)} - t_{initial}) + R_{initial}$

With this equation we can now very easily calculate our receptor concentration with the data provided at the beginning of this section. It is always a good idea to have all the data in a table:

$R_{initial}$ = 1 nM	t_{inital} = 0 hours
$R_{(t)}$ = ???	$t_{(t)}$ = 12 hours
k = 0.5 nM / hours	

We use these data in our equation and get:

$R_{(t)} = k\ x\ (t_{(t)} - t_{initial}) + R_{initial}$
$R_{final} = 0.5$ nM/hr *x* (12 hours – 0 hours) + 1 nM

$R_{(t)} =$ 6 nM + 1 nM

$R_{(t)} =$ 7 nM

Answer: The receptor concentration at the cell membrane will be 7 nM after 12 hours.

3.6. Which equation should I use to calculate the product of a reaction?

The two previous examples highlight an interesting problem: Which equation should one use for the different problems, especially when we look at the production of something, like the receptor at the cell membrane. If we look at a reaction scheme like:

$$A \xrightarrow{k} B$$

Then we can calculate the **consumption of A** very easily with the differential equation:

$$\frac{dA}{dt} = - k$$

And its integrated form:

$A_{(t)} - A_{initial} = - k \; x \; (t_{(t)} - t_{initial})$ for $A_{(t)} \geq 0$ and $A_{(t)} = 0$ in all other events.

Looking at what happens to B we notice that the amount of B that is produced depends on the amount of A that is consumed. We can say that the production of B is strictly **dependent** on the consumption of A. In this case we can use the equation:

$A_{(t)} + B_{(t)} = A_{initial} + B_{initial}$

However, if we have a reaction scheme like:

$$\xrightarrow{k} B$$

there is no obvious reactant consumed in the production of B and the production of B is **independent** from any reactants. In this case we can write the differential equation as:

$$\frac{dB}{dt} = + k$$

And its integrated form:

$B_{(t)} - B_{initial} = + k \; x \; (t_{(t)} - t_{initial})$.

With this latter equation there are two important things to note. Firstly the sign in front of the rate constant k is **positive**. The reason for this is that we are dealing with a **production** of B and not a consumption of anything else. Secondly, we (usually) do not have to put constraints on the integrated form, i.e. we do not have to explicitly state that this equation is only valid if $B_{(t)} \geq 0$ and that $B_{(t)} = 0$ in all other events. Again, this is because we have **production of B** – and B should not be negative in this case anyway.

3.7. Problem solving strategies

Obviously any problem solving strategies have to be tailored towards the problem we are dealing with and no general rule can be given here. However, there are a few strategies that can help in addressing several problems related to a zero order reaction. For example we might be given some experimental data and asked to determine whether this reaction follows a zero order reaction. Another question could be related to calculating the concentration of a reactant (or a product) at a given time, the starting concentration of a reactant, the rate constant etc.

- It is always useful to write down a reaction scheme, for example:

  i) $A \xrightarrow{k}$ or

  ii) $\xrightarrow{k} B$

- Write down the rate equations for this scheme:

$\frac{dA}{dt} = -k$ for consumption of A (case (i))

$\frac{dB}{dt} = +k$, if we are looking at the growth of B, independent of A (case (ii))

- If asked to demonstrate graphically that the data are following a zero order reaction, make a plot of A versus time. This should give a straight line with –k being the gradient and the intersect of the line with the y-axis being the initial concentration of A (see Figure 3-1).
- If asked to calculate concentrations of A, make a table according to the following scheme:

$A_{(t)}$ =	$t_{(t)}$ =
$A_{initial}$ =	$t_{initial}$ =
k =	

- Rearrange the equation:

$A_{(t)} - A_{initial} = -k\,(t_{(t)} - t_{initial})$

for what you are looking for. Put in the numbers from the table and solve. Be aware that sometime there are constraints on $A_{(t)}$, for example $A_{(t)}$ must be larger or equal zero.

- If you haven't got the rate constant k, you can use data and the following equation:

$$k = \frac{(A_{(t)} - A_{initial})}{(t_{(t)} - t_{initial})}$$

Again, use a table with data points like:

$A_{(t)}$ =	$t_{(t)}$ =
$A_{initial}$ =	$t_{initial}$ =

Put in the numbers and calculate k.

- If you need to calculate concentrations of the product B, you need to find out whether B depends on A or not. If you can write a reaction scheme like:

$$A \xrightarrow{k} B$$

Then B is most likely dependent on A. In this case you need to calculate $A_{(t)}$ as shown in the previous steps and from there calculate $B_{(t)}$, using the equation:

$A_{(t)} + B_{(t)} = A_{initial} + B_{initial}$

- If your reaction scheme can be written like this:

$$\xrightarrow{k} B$$

Then it is most likely a growth reaction and B is independent from any reactants. In this case you can use the general rate equation:

$$\frac{dB}{dt} = +k$$

And its integrated form:

$B_{(t)} - B_{initial} = + k\ x\ (t_{(t)} - t_{initial})$.

3.8. Equations for zero order reactions

Reaction scheme:

$$A \xrightarrow{k} B$$

For the consumption of A:

$$\frac{dA}{dt} = - k$$

Integrated form:

$A_{(t)} - A_{initial} = -k\ x\ (t_{(t)} - t_{initial})$ for $A_{(t)} \geq 0$ and $A_{(t)} = 0$ in all other events.

For the production of B:

$A_{(t)} + B_{(t)} = A_{initial} + B_{initial}$

The unit for the k is **Unit of B (or A) / time**

Reaction scheme:

$$\xrightarrow{k} B$$

Production of B:

$$\frac{dB}{dt} = +k$$

Integrated form:

$B_{(t)} - B_{initial} = + k\ x\ (t_{(t)} - t_{initial})$

3.9. More practice questions

1. A new drug has been developed to treat erectile problems in men. The liver rapidly inactivates this drug, but the mechanism is unknown. To be active the drug has to have a blood concentration of at least 40 nM. After administration of the drug, its blood concentration is measured at certain time intervals and these are the data:

time [min]	concentration [μM]
0	0.1
1	0.0988
2	0.0976
3	0.0964
4	0.0952
5	0.094
6	0.0928

a) Demonstrate graphically that the elimination of this drug from the metabolism follows a zero order reaction.
b) *Calculate* the rate constant for the elimination of this drug.
c) *Calculate* how long the drug is active, i.e. has a concentration of at least 40 nM.
d) How long does it take to completely remove the drug from the body?

Answers:

When concentration of drug versus time is plotted, a straight line is obtained, hence it must be a zero order reaction.
k = 1.2 nM / min
50 min
83.3 min

2. The receptor for a growth factor is produced in the cell with a rate constant of 24 pM per 12 minutes. Assuming that the starting concentration of the receptor is 20 pM and the cell becomes responsive to growth factors if the receptor concentration is at least 0.1 nM, calculate how long it takes to reach this level. How do you know that this is a zero order reaction?

Answer 40 minutes; can tell by the unit of the rate constant

4.First order reaction

4.1. Introduction and learning outcomes

In the previous chapter we discussed zero order kinetics; however, many reactions in nature follow a different reaction scheme, namely a first order reaction. For example the elimination of a certain drug from the metabolism might follow a first order reaction. The decay of a radioactive substance, often used as a 'tracer' in biological experiments is always described by a first order reaction. Another example for a first order kinetics is the growth of a bacterial culture, at least during certain phases of the growth.

In this chapter I will show, how we can formulate the typical rate equation for a first order reaction. Using examples I will develop the equations for this kind of kinetics. By the end of this chapter you should be able to:

- Identify reactions that follow this scheme,
- Draw graphical representations for this reaction scheme,
- Use relevant equations to calculate amounts (or concentrations) of reactants and products,
- Use relevant equations to calculate a first order rate constant and its corresponding half life.

4.2. Some definitions for first order reactions

In the previous chapter we transferred money from a 'current' bank account into a savings account by making constant payments each month. However, what happens, if one month our current account was zero and we forgot to cancel the standing order? We soon would find ourselves in debt, since money is still taken out, regardless of how much money is in the account (unless we gave specific orders to the bank NOT to transfer any money, unless the current account was in balance). Wouldn't it be better to link the amount of money we transfer every month to the amount of money that is actually present in the current account? For example, we could negotiate with the bank to make payments from the current account at a variable rate, depending on how much money is in the account. We could, for example, say that instead of transferring £ 10 per month, we might want to transfer 4 % per month of the money in the current account.

The flow of money is:

C(urrent account) $\xrightarrow{k}$ S(avings account)

Let's make a table for this:

Month	In the account	4% transferred into savings account
January	£300	£12
February	£288	£11.52
March	£276.48	£11.06
April	£265.42	£10.62

The monthly payment is no longer constant: It now depends on the amount of money that is actually present in the current account at the time we want to make the payment.

We can express this kind of transfer in a similar way we did with a zero order reaction.
The general form of the instantaneous rate equation for this kind of 'reaction' can be written as:

Instantaneous rate = a constant *x* **the amount in the current account**

Note: When we wrote *Instantaneous rate = a constant* for a zero order reaction, we did not care about how much money was in the current account. Now, in a first order reaction, we DO take into consideration, how much money is in the account.

Using the same conventions we agreed on in the previous chapter, we can write for the transfers out of the current account:

$$\frac{dC}{dt} = - k \, x \, C.$$

Again the rate is **negative** (similar to a zero order reaction), since we are looking at a decrease.

So why is it called a first order reaction?
In the previous chapter I promised to reveal why reaction have ‘orders’, like ‘zero order’ or ‘first order’. Here is the explanation:
Our general rate equation is written as:

$$\frac{dC}{dt} = -k \, x \, C, \text{ which can also be written as}$$

$$\frac{dC}{dt} = -k \, x \, C^1$$

This last term - C^1, with the exponent “1” - gives the reaction its name. As simple as that!

In the case of a zero order reaction

$$\frac{dC}{dt} = - k \quad \text{we can also write}$$

$$\frac{dC}{dt} = -k \, x \, C^0, \text{ because } C^0 = 1.$$

Again, the last term - C^0 with the exponent “0” - gives the reaction its name.

In general, the exponent of the reactant in a rate equation determines the order of the reaction.

$$\frac{dC}{dt} = -k \, x \, C^m$$

is therefore a **m-th** order reaction.

4.3. Developing the tools for a first order reaction

4.3.1. Equations

For the first order reaction

$$C \xrightarrow{k} S$$

We can write:

$$\frac{dC}{dt} = - k \, x \, C \text{ and}$$
$$\frac{dS}{dt} = + k \, x \, C.$$

Let's try to solve this differential equation, first for 'C' (remember, everything with an 'C' goes to one side, everything with a 't' goes to the other):

$$\frac{dC}{C} = - k \, dt$$

We now need to integrate both sides of the equation:

$$\int_{C_{initial}}^{C_{(t)}} \frac{dC}{C} = \int_{t_{initial}}^{t_{(t)}} - k dt$$

Again, the subscript (t) stands for 'at a given time' and the subscript 'initial' indicates the status of the current account at the beginning of the reaction ($C_{initial}$ at $t_{initial}$).

The solution to the right hand side of the equation is simple – it is the same as for the zero order reaction:

$$\int_{t_{initial}}^{t_{(t)}} - k dt = - k \, x \, (t_{(t)} - t_{initial})$$

The solution to the left hand side, however, is different.

$$\int_{C_{initial}}^{C_{(t)}} \frac{dC}{C} = \ln (C_{(t)}) - \ln (C_{initial})$$

Here **ln** stands for natural logarithm (or Briggs' logarithm). On a scientific calculator this is usually the 'ln' key.

It is important to remember that:

$\ln (C_{(t)}) - \ln (C_{initial})$ can also be written as $\ln \left(\frac{C_{(t)}}{C_{initial}}\right)$.

Put together, the equation for a first order reaction is:

$$\ln (C_{(t)}) - \ln (C_{initial}) = - k \, x \, (t_{(t)} - t_{initial}).$$

Or

$$\ln \left(\frac{C_{(t)}}{C_{initial}}\right) = - k \, x \, (t_{(t)} - t_{initial}).$$

This probably doesn't help us a lot, because we still have this strange 'ln' in the equation, and what we really want to know is $C_{(t)}$ (and not **ln** $(C_{(t)})$).

In order to get rid of this ln, we need to use the reverse mathematical operation. For logarithms it is 'to the power'. For example when we use the common log (which is to the base 10) of something, the reverse operation is to put this 'something' to the power of 10 ($10^{something}$). For ln, which has a rather strange base, we need to put this 'something' to the power of 2.71828. This number is called 'e' (in commemoration of the famous German mathematician Leonhard Euler (1707-1783)).

We therefore need to 'e' both sides of the equation:

$$\ln\left(\frac{C_{(t)}}{C_{initial}}\right) = -k\,x\,(t_{(t)} - t_{initial}). \qquad | \; e$$

(this indicates that both sides are taken 'e')

$$e^{\ln\left(\frac{C_{(t)}}{C_{initial}}\right)} = e^{-(k\,x\,(t_{(t)} - t_{initial}))}$$

e and ln cancel each other out and we get:

$$\boxed{\frac{C_{(t)}}{C_{initial}} = e^{-(k\,x\,(t_{(t)} - t_{initial}))}}$$

If we want to know $C_{(t)}$, we can rearrange this equation to:

$$C_{(t)} = C_{initial}\ x\ e^{-(k\,x\,(t_{(t)} - t_{initial}))}.$$

Let's have a look at our bank account example:

If we transfer 4 % of what is in the 'current' account every month, how much money is left after 12 months?

Using the equation $C_{(t)} = C_{initial}\ x\ e^{-(k\,x\,(t_{(t)} - t_{initial}))}$

we now have to put in some numbers. We make a table with the data:

$C_{(t)}$ = ?	$t_{(t)}$ = 12 months
$C_{initial}$ = £ 300	$t_{initial}$ = 0 months
k = 4 % = $\frac{4}{100}$ per month	

$t_{(t)} - t_{initial}) = 12$ months

$$C_{(t)} = £\ 300\ x\ e^{-(4/100\ x\ 12)}$$

$$C_{(t)} = £\ 300\ x\ e^{-(0.48)}$$

$C_{(t)}$ = £ 300 *x* 0.6188 = £ 185.64

Answer: After 12 months there will be £ 185.64 remaining in the current account.

So far we have only looked at the decrease in the current account:

$$\mathbf{C} \xrightarrow{k} S$$

If we want to look at the savings account, all we need to do is change the sign in the equation (as we have done for the zero order reaction).

We can therefore write:

$$\frac{dS}{dt} = - \frac{dC}{dt} = + k \, x \, C$$

But - we cannot easily integrate the equation $\frac{dS}{dt} = + k \, x \, C$, because we do not have the same components in the equation, i.e. the right hand side of the equation does not contain 'S'.

However, we can determine the amount of S at any time by saying that the amount of S at a given time and the amount of C at the same time must always be the same as our starting C, assuming that no S was present at the start point. As we have done before with the zero order reaction, we can write:

$$C_{(t)} + S_{(t)} = C_{initial} + S_{initial}$$

For example, we start with £ 300.- and transfer 4 % per month. How much money is in the savings account after 12 months?

Solution:

We just calculated the amount of money left in the current account, which is $C_{(t)}$ = £ 185.64.

The difference between our starting balance (£ 300) and what's left after 12 months (£ 185.64) must be in the savings account (assuming that nobody has taken or added any money and that at the beginning there was no money in the savings account).

We therefore can write:

$S_{(12months)} = C_{initial} + S_{initial} - C_{(12months)}$

$S_{(12months)}$ = £ 300 + £ 0 - £ 185.64 = £ 114.36.

Answer: After 12 months of saving, we expect to have £ 114.36 in our savings account.

The unit for the rate constant k of a first order reaction is:

Units of k = *per time* or *time* $^{-1}$.
Units of a first order reaction could be:

Minute $^{-1}$,
Per second,
Month^{-1}.

4.3.2. Graphic representation of a first order reaction

How can we graphically represent a first order reaction?

If we tried to graph a first order reaction in the same way we did it for a zero order reaction, i.e. plot $C_{(t)}$ versus time, we would get a rather strange graph, which is certainly not a straight line:

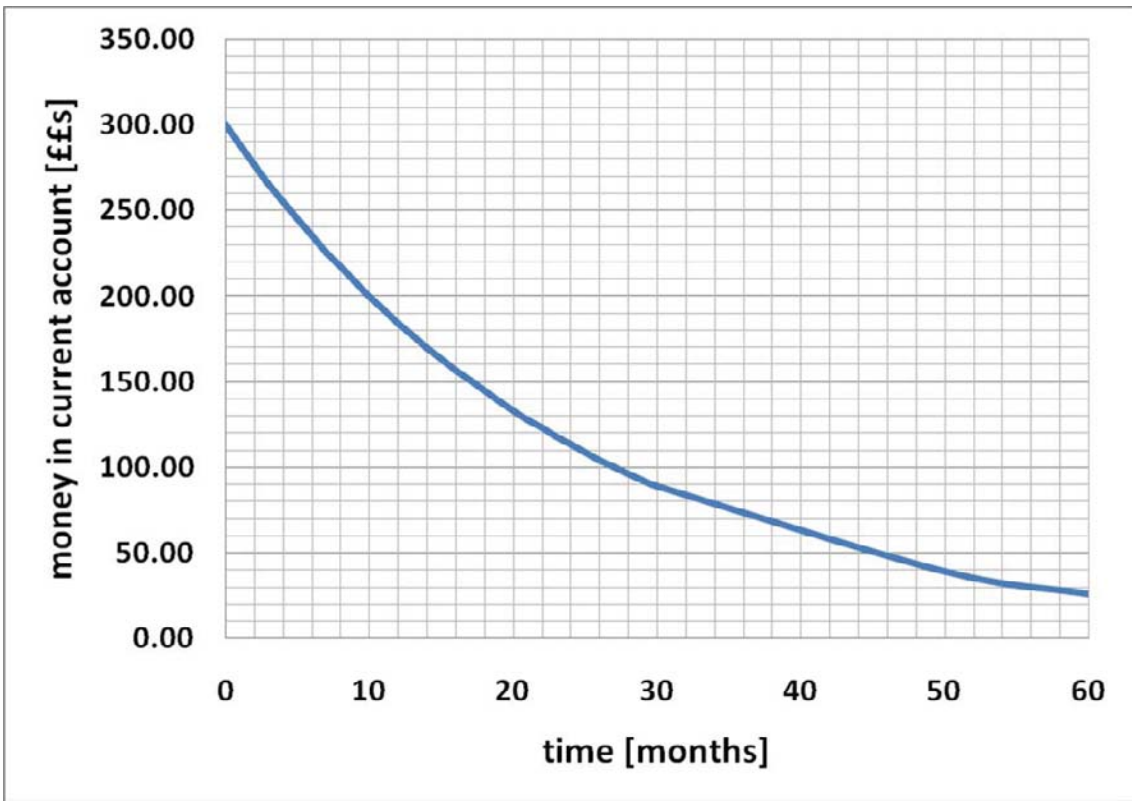


Figure 4-8 Graphic representation of a first order reaction – consumption of C – money in the current account over time.

However, if we go back to one of the equations:

$$\ln (C_{(t)}) - \ln (C_{initial}) = - k \, x \, (t_{(t)} - t_{initial})$$

and rearrange it for

$$\ln (C_{(t)}) = - k \, x \, (t_{(t)} - t_{initial}) + \ln (C_{initial})$$

and also assume that our starting time point $t_{initial} = 0$, we can write:

$$\ln (C_{(t)}) = - k \, x \, t_{(t)} + \ln (C_{initial})$$

Again, this looks very similar to the equation for a straight line

$$y = m \, X + C$$

We therefore can plot $\ln (C_{(t)})$ versus time and get a straight line, with - k being the gradient and $\ln (C_{initial})$ being the intersect with the y-axis.

First of all, we need a data table in which we calculate the ln's for the money in the current account:

Month	Current account	ln (money in current account)
January	300	5.704
February	288	5.663
March	276.48	5.622
April	265.4208	5.581
May	254.804	5.540
June	244.6118	5.500
July	234.8273	5.459
August	225.4342	5.418
September	216.4169	5.377
October	207.7602	5.336
November	199.4498	5.296
December	191.4718	5.255

The graph in Figure 4-2 now shows a straight line! This is actually a very important point, which can help us to discriminate between a zero order and first order reaction:

We can use our experimental data and plot concentration or money in an account or whatever we are interested in versus time. If this gives a straight line, we know that our experiment is based on a zero order reaction. If, however, it does not give a straight line, we can try to plot ln of what we are interested in versus time. If this now gives a straight line, it must be a first order reaction.

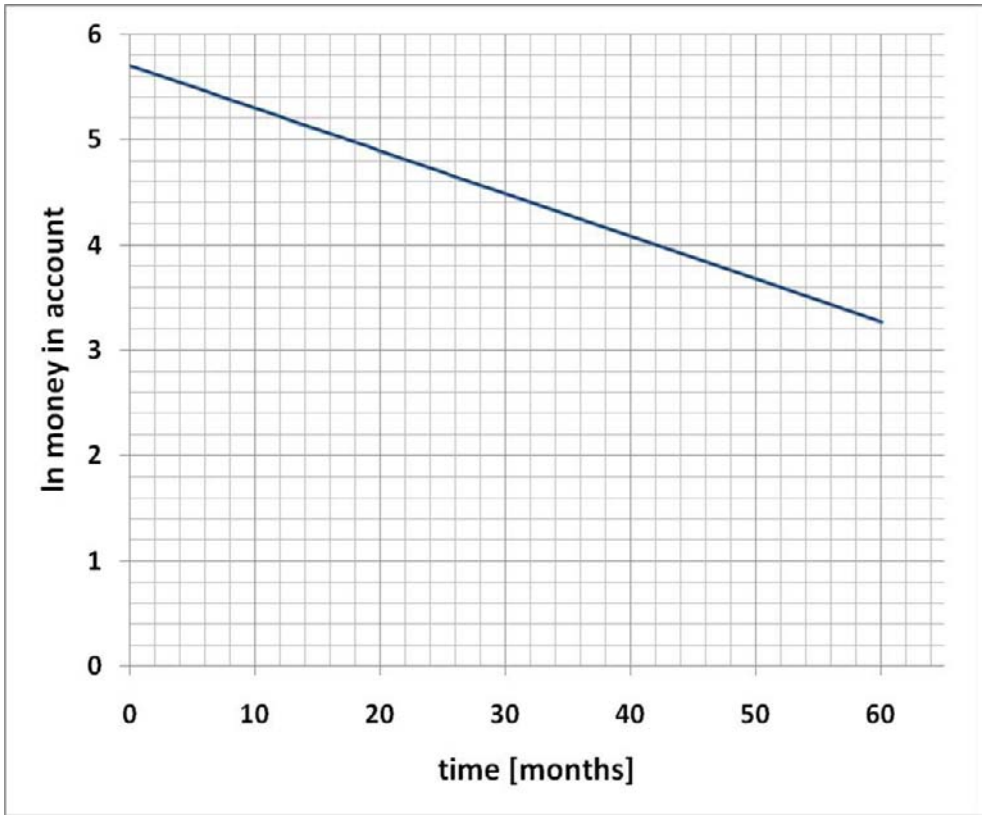


Figure 4-9 Graphic representation of a first order reaction – consumption of C – with a plot of ln (money in the current account) over time.

Now what about the savings account?

We can use the equation $C_{(t)} + S_{(t)} = C_{initial} + S_{initial}$ and solve for $S_{(t)}$. In a graphic representation this would look like the graph in Figure 4-3.

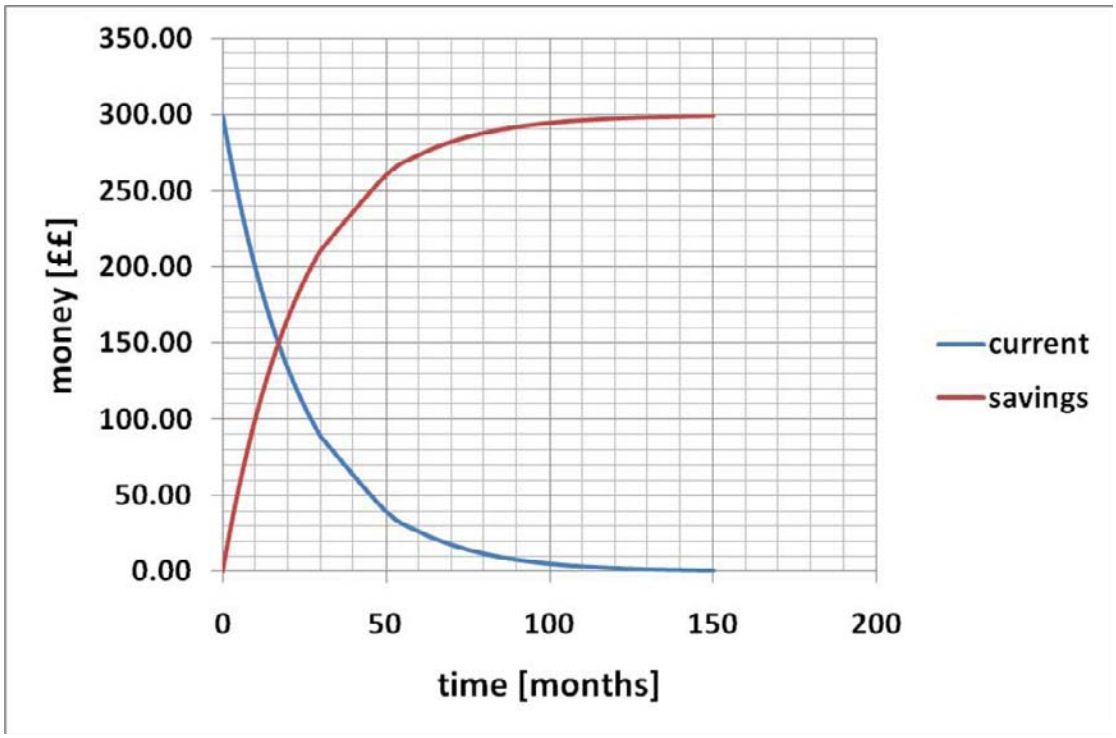


Figure 4-10 Graphic representation of first order reaction – consumption of C (money in the current account) and production of S (money in the savings account) over time.

As with the zero order reactions we also might be interested in a graphical representation of the rate of the reaction. All we have to use is the equations we used before:

$$\frac{dC}{dt} = -\,k\,x\,C^1 \quad \text{and} \quad \frac{dS}{dt} = +\,k\,x\,C^1.$$

The combined graph for the rates $\frac{dC}{dt}$ and $\frac{dS}{dt}$, respectively, looks like the graphs in Figure 4-4.

The interesting bit here is that the lines for the rates $\frac{dC}{dt}$ and $\frac{dS}{dt}$ are symmetric. It also tells us that the rate now depends very much on the amount of money in the current account ($C_{(t)}$). This is clearly different to what we have seen when we did a similar plot for the rate of a zero order reaction.

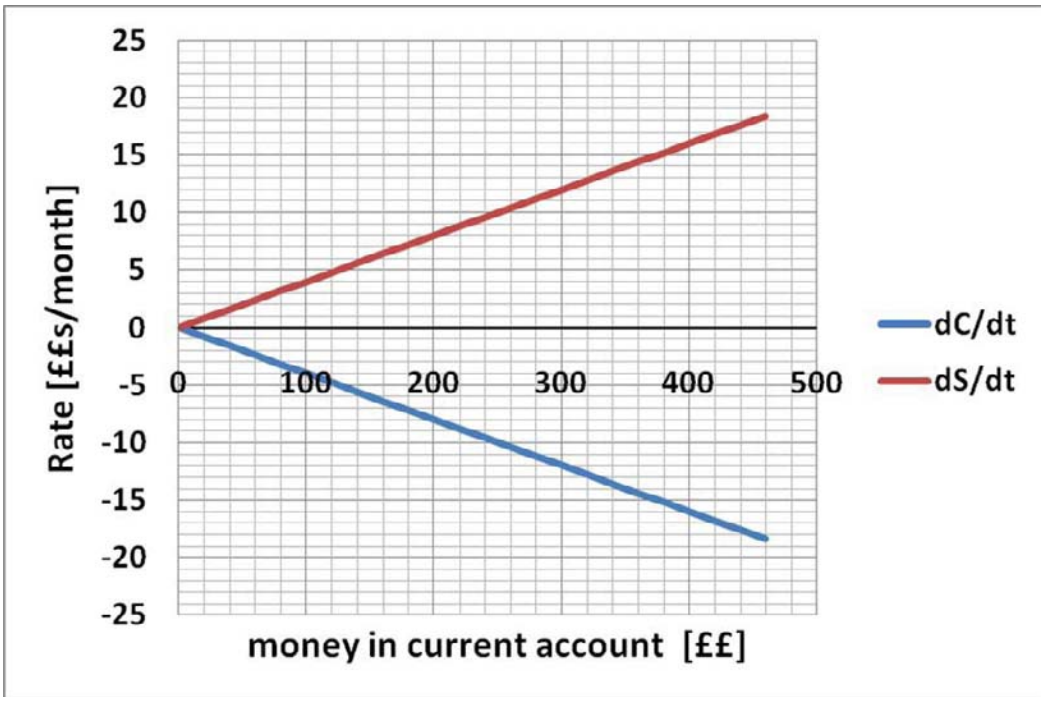


Figure 4-11 Graphic representation of a first order reaction – **rate** of changes in C (current account) and S (savings account), respectively, in relation to amount of money in the current account at a given time ($C_{(t)}$)

A rather important issue with a first order reaction is that there usually are no constraints, unlike we saw in the zero order scheme. Remember – we had to define that in a zero order the amount of the reactant ($C_{(t)}$) could not be smaller than zero. This is not something we have to worry about in a first order reaction. As we can see in Figures 4-1 and 4-3, the amount of $C_{(t)}$ never reaches a value below zero. Strictly speaking, it will never even reach zero. This is because if we have $C_{(t)} = 0$ our rate of reaction would also be zero (see Figure 4-4). We (usually) don't have to define any constraints in a first order reaction.

4.3.3. Half life of a reaction

Here is another important feature of a first order reaction - something called 'half life'.

To illustrate this let's say that we wanted to transfer 4 % per month of the money in the current account into the savings account. At the beginning of the year we have £ 300 in the current account and we want to know when we have exactly half of the original £ 300 left.

Solution:

$$\ln\left(\frac{C_{(t)}}{C_{initial}}\right) = -k \; x \; (t_{(t)} - t_{initial})$$

If we set our starting point $t_{initial} = 0$, the equation can be written as

$$\ln\left(\frac{C_{(t)}}{C_{initial}}\right) = -k \times t_{(t)}$$

We need to rearrange this equation and solve for $t_{(t)}$

$$t_{(t)} = \ln\left(\frac{C_{(t)}}{C_{initial}}\right) / -k$$

Let's put in some numbers, again using a table:

$C_{(t)} = £\ 150$	$t_{(t)} = ??$
$C_{initial} = £\ 300$	$t_{initial} = 0$
$k = 4/100\ month^{-1}$	

With these numbers the equation gives us:

$$t_{(t)} = \ln\left(\frac{£150}{£300}\right) / -0.04\ month^{-1}$$

$t_{(t)} = \ln (0.5) / -0.04 \text{ month}^{-1}$

With ln (0.5) = -0.693 we can write:

$$t_{(t)} = \frac{-0.693}{-0.04 month^{-1}} = \underline{17.33 \text{ month}^{1}}$$

Answer: After 17.33 month only half of the original £ 300 is left in the current account.

We now might wonder how long it would take for this money to be reduced to half the amount, ie from £ 150 to £ 75.

Solution:

We do exactly the same calculation, using the equation

$$t_{(t)} = \ln \left(\frac{C_{(t)}}{C_{initial}}\right) / -k$$

The numbers are now:

$C_{(t)}$ = £ 75	$t_{(t)}$ = ??
$C_{initial}$ = £ 150	$t_{initial}$ = 0
k = 4/100 month^{-1}	

With these numbers the equation gives us:

$$t_{(t)} = \ln \left(\frac{£75}{£150}\right) / -0.04 \text{ month}^{-1}$$

$t_{(t)} = \ln 0.5 / -0.04 \text{ month}^{-1}$

With ln (0.5) = -0.693 we can write:

$$t_{(t)} = \frac{-0.693}{-0.04 month^{-1}} = \underline{17.33 \text{ month}^{1}}$$

After 17.33 month only half of the £ 150 are left in the current account.

Again, it takes 17.33 month to reduce the amount to half of the starting amount. We can now predict that it will take another 17.33 months for the £ 75 to go down to £ 37.5. And another 17.33 months for the £ 37.5 to go down to £ 18.75.

Every 17.33 months our money in the current account is halved. Interestingly, it does not matter, how much money there was in the first place, whether it was £ 300 or £ 37.5 – it is always 17.33 months. Because it is quite important, this particular $t_{(t)}$ was given the name 'half life' and is usually written as $t_{1/2}$.

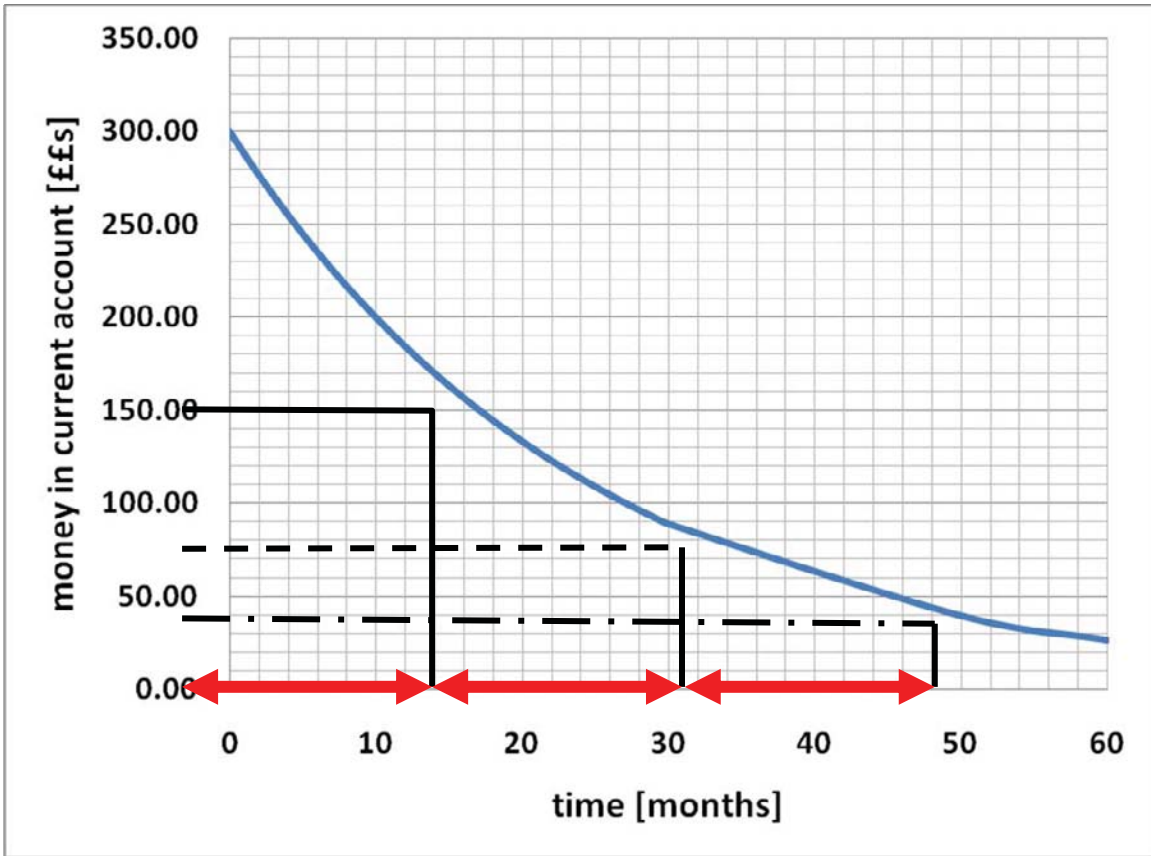


Figure 4-12 Half lives in a first order reaction. The red bars represent half lives of 17.33 months.

The equation for the half life therefore is:

$$t_{1/2} = \frac{\ln(0.5)}{-k}$$ or, if you don't like the – sign in the denominator

$$t_{1/2} = \frac{\ln(2)}{k}$$

Since ln (2) = 0.693, one can also write:

$$t_{1/2} = \frac{0.693}{k}$$

Why didn't I tell you about half lives in the zero order reaction? For a very simple reason: Half lives only work with first order reactions. In all other reactions the half life is dependent on the initial amount and therefore is not a constant, like it is in a first order reaction.

Half lives can be quite useful for calculations. Also half lives are usually used when we talk about radioactivity. In this case people hardly ever use the rate constant for calculations, they rather use half lives. But with the equation $t_{1/2} = \frac{0.693}{k}$ it is not difficult to convert $t_{1/2}$ into k and *vice versa*.

4.4. Problem: Elimination of a drug from the metabolism

a) Very often drugs are eliminated from the body in a first order reaction. The elimination of Diazepam, which is used in veterinary medicine as a sedative and pre-anaesthetic agent, has a first order rate constant of k = 0.07 per hour. If a horse was given an injection of 50 mg Diazepam, how much Diazepam is left after 24 hours?

Solution:

We write down the general rate equation for a first order reaction with the scheme:

D(iazepam) $\xrightarrow{k}$

$\frac{dD}{dt}$ = -k *x* D. We are looking at a decrease and therefore the rate is negative!

After separation of the variables we get

$\frac{dD}{D}$ = -k *x* dt

Integration of both sides gives

$$\int_{D_{initial}}^{D_{(t)}} \frac{dD}{D} = \int_{t_{initial}}^{t_{(t)}} -k\,dt$$

For which the solution is

$\frac{D_{(t)}}{D_{initial}} = e^{-(k\,x\,(t_{(t)} - t_{initial}))}$ or

$D_{(t)} = D_{initial}\ x\ e^{-(k\,x\,(t_{(t)} - t_{initial}))}$

Let's put some numbers in (using a table):

$D_{(t)}$ = ??	$t_{(t)}$ = 24 hours
$D_{initial}$ = 50 mg	$t_{initial}$ = 0
k = 0.07 hour^{-1}	

With these numbers the equation is now written as:

$D_{(t)} = 50 \text{ mg } x \text{ } e^{-0.07 \, x \, 24}$

$D_{(t)} = 50 \text{ mg } x \text{ } e^{-1.68}$

$D_{(t)} = 50 \text{ mg } x \text{ } 0.186$

$\underline{D_{(t)} = 9.32 \text{ mg}}$

Answer: After 24 hours there are 9.32 mg of Diazepam left.

c) For our sedated horse from the previous example to safely participate in a race, the Diazepam level must be lower than 0.5 mg. How long does it take to reach this level (assuming a 50 mg injection, first order rate constant k = 0.07 $hour^{-1}$)?

Solution:

We use the equation:

$$\ln\left(\frac{D_{(t)}}{D_{initial}}\right) = -k \; x \; (t_{(t)} - t_{initial}) \text{ and solve for } t_{(t)}$$

$$(t_{(t)} - t_{initial}) = \ln\left(\frac{D_{(t)}}{D_{initial}}\right) / -k$$

Putting in numbers gives (again using a table):

$D_{(t)} = 0.5$ mg	$t_{(t)} = ??$
$D_{initial} = 50$ mg	$t_{initial} = 0$ (starting point when injection is given)
$k = 0.07\ hour^{-1}$	

$$(t_{(t)} - 0) = \ln\left(\frac{0.5mg}{50mg}\right) / -0.07\ hour^{-1}$$

$$t_{(t)} = \ln(0.01) / -0.07\ hour^{-1}$$

$$t_{(t)} = \frac{-4.605}{-0.07} \text{ hours} = \underline{65.79 \text{ hours}}.$$

Answer: The Diazepam levels dropped from 50 mg to 0.5 mg after 65.79 hours.

d) What is the half life of Diazepam in an organism, assuming that the original injection was 50 mg and the first order rate constant is 0.07 $hour^{-1}$?

Solution:

All we need to do is take the equation for the half life of a first order reaction:

$$t_{1/2} = \frac{0.693}{k}$$

and put in the number for k:

$$t_{1/2} = \frac{0.693}{0.07 hour^{-1}} = \underline{9.9 \text{ hours}}$$

Answer: The half life for Diazepam (in a horse) is close to 10 hours.

This answer actually gives us a way to double check our answer from b):

In this question we asked how long it takes for the Diazepam to drop from 50 mg to 0.5 mg. We now can say that after 10 hours the level of Diazepam dropped from 50 mg to half of it, i.e. 25 mg.
After another 10 hours (now 20 hours in total), the level of Diazepam dropped from 25 mg to 12.5 mg.
After another 10 hours (now 30 in total) the level of Diazepam is down to half of 12.5 mg, i.e. 6.25 mg.
After 10 hours (now 40 in total), the level is down to 3.13 mg and after another 10 hours (now 50 in total) it is down to 1.56 mg. After a total of 60 hours (another 10 hours) the level has dropped to half of 1.56 mg = 0.78 mg. And yet another 10 hours and the Diazepam level is down to 0.39 mg. We therefore have to wait between 60 and 70 hours for the Diazepam level to drop from 50 mg to 0.5 mg. This result is in good agreement with our calculation (65.79 hours).

4.5. Problem: Radioactive decay

In a 'Northern-blot' experiment you are trying to detect a specific mRNA by using a radiolabelled probe. This probe contains the radioactive element ^{32}P(hosphorous), which has a half life of 14.5 days. You know that all radioactive decay follows a first order reaction. From previous experiments you also know that you need at least 10 ng of radiolabelled probe. 3 weeks ago (21 days) you prepared 36 ng of radiolabelled probe. Is there still enough probe left to be used in the experiment, or do you have to make a new probe?

Solution:

We use the equation for a first order reaction:

$$\frac{P_{(t)}}{P_{initial}} = e^{-(k\,x\,(t_{(t)} - t_{initial}))}$$

Rearranging this equation gives:

$$P_{(t)} = P_{initial}\ x\ e^{-(k\,x\,(t_{(t)} - t_{initial}))}$$

And we can easily solve for $P_{(t)}$. The only problem is that we only have the half life, but not the rate constant k.

However, we can convert the half life into the rate constant by using the equation:

$$t_{1/2} = \frac{0.693}{k}$$

Solving for k gives:

$$k = \frac{0.693}{t_{1/2}}$$

$$k = \frac{0.693}{14.5day} = 0.0478 \text{ days}^{-1}$$

We can now put this number into our equation (using a table):

$P_{(t)} =$??	$t_{(t)} = 21$ days
$P_{initial} = 36$ ng	$t_{initial} = 0$
$k = 0.0478 \text{ days}^{-1}$	

$$P_{(t)} = P_{initial} \, x \, e^{-(k \, x \, (t_{(t)} - t_{initial}))}$$

$$P_{(t)} = 36 \text{ ng} \, x \, e^{-(0.0478 \, x \, 21)}$$

$$P_{(t)} = 36 \text{ ng} \, x \, e^{-(1.004)}$$

$$P_{(t)} = 36 \text{ ng} \, x \, 0.367$$

$$\underline{P_{(t)} = 13.2 \text{ ng}}$$

Answer: After 21 days there are 13.2 ng of probe left, which should be enough to do the experiment.

4.6. Problem: Degradation of a receptor/ligand dimer

As shown in the introduction the receptor for a growth hormone dimerizes after binding of the growth hormone to form a receptor/ligand dimer (RD). This complex then triggers a signal, which induces cell growth and division. At the same time this receptor/ligand dimer is degraded.

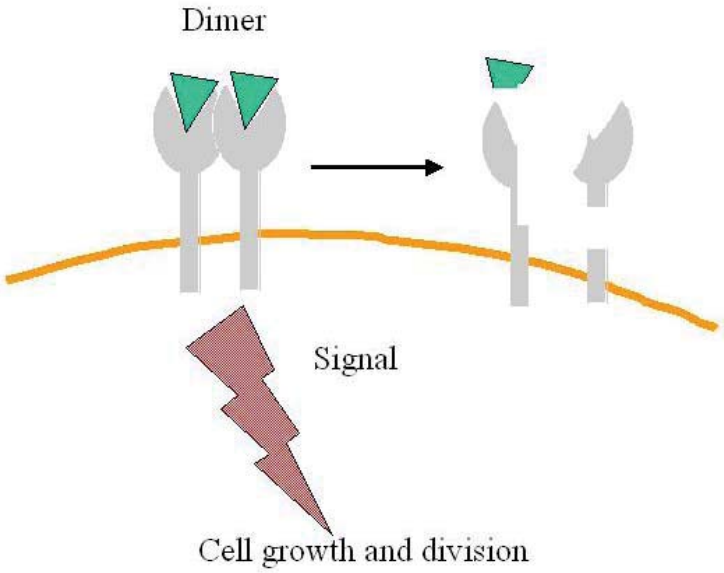


Figure 4-13 Degradation of receptor/ligand dimers following a first order kinetics

In an experiment you measure the concentration of this receptor/ligand dimer (often this is done by measuring the signal that is triggered by the receptor/ligand dimer). The experimental conditions are such that no new receptor/ligand dimer can be formed. These are your data:

Time [min]	Dimer [nM]
0	10.000
10	8.187
20	6.703
30	5.488
40	4.493
50	3.679
120	?

a) Does the degradation of the receptor/ligand dimer follow a first order reaction?
b) Calculate from the data provided how much receptor/ligand dimer is left after 120 minutes.
c) Calculate the concentration of a receptor/ligand dimer breakdown product after 120 minutes.

Solution:

a) In order to find out whether this is a zero order or a first order reaction, we simply have to do the graphs for these reactions. Remember – for a zero order reaction the plot concentration versus time gives a straight line.

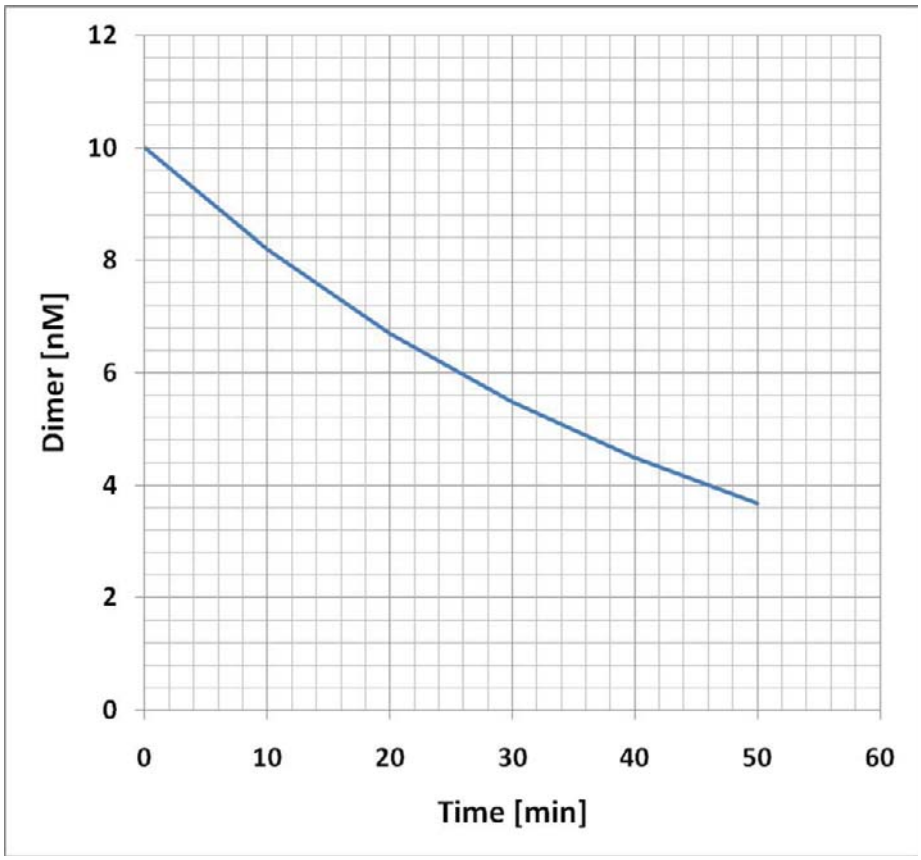


Figure 4-7 Degradation of the receptor dimer shown as a zero order representation

It certainly does not look like a straight line, therefore it is NOT a zero order reaction.

Let's try the plot for a first order reaction. First we calculate ln (dimer concentration) and then plot ln (dimer concentration) versus time:

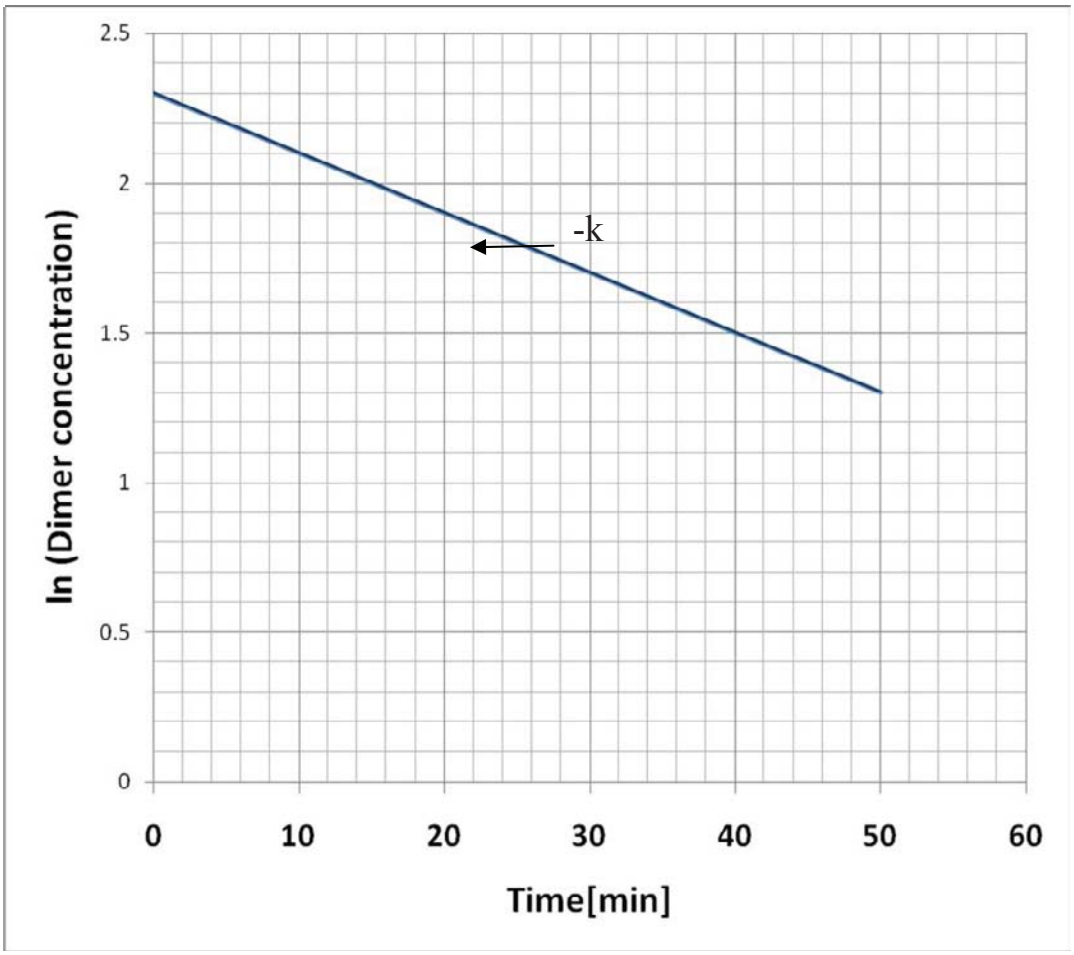


Figure 4-8 Degradation of the receptor dimer shown as a first order representation

This is indeed a straight line, and therefore we can conclude that the reaction follows a first order reaction.

b) To answer the second part of the question we write the general first order rate equation:

$$\frac{dRD}{dt} = -k \; x \; RD.$$

We are looking at a decrease and therefore the rate is negative!

After separation of the variables, integration and rearrangement we get the familiar equation:

$$\ln\left(\frac{RD_{(t)}}{RD_{initial}}\right) = -k \; x \; (t_{(t)} - t_{initial})$$

As in the example in the previous chapter, we now have the problem to determine k. We could use the graph and determine k graphically (remember that k is the gradient in the ln (concentration) versus time plot). Alternatively, we can determine the rate constant from our data set.

We rearrange our equation and solve for k:

$$k = \ln\left(\frac{RD_{(t)}}{RD_{initial}}\right) / -(t_{(t)} - t_{initial})$$

By picking from our data table an appropriate $RD_{(t)}$ and the corresponding $t_{(t)}$ and also an $RD_{initial}$ with corresponding $t_{initial}$, we can now calculate k.

Time [min]	Receptor/ligand dimer [nM]
0	10.000
10	8.187
20	6.703
30	5.488
40	4.493
50	3.679
120	?

$RD_{(t)}$ = 3.679 nM	$t_{(t)}$ = 50 min
$RD_{initial}$ = 10 nM	$t_{initial}$ = 0 min
k = ??	

With these numbers we get:

$$k = \ln\left(\frac{3.679}{10}\right) / -(50\text{min} - 0\text{min})$$

$$k = \frac{\ln(0.3679)}{-50\,\text{min}} = \frac{-1}{-50\,\text{min}} = 0.02\ \text{min}^{-1}$$

Answer: The rate constant for the degradation of the receptor dimer is 0.02 min^{-1}.

We can now use this number to calculate how much receptor/ligand dimer is left after 120 min. For this we use the equation:

$$RD_{(t)} = RD_{initial}\ x\ e^{-(k\ x\ (t_{(t)} - t_{initial}))}$$

$RD_{(t)}$ = ??	$t_{(t)}$ = 120 min
$RD_{initial}$ = 10 nM	$t_{initial}$ = 0 min
k = 0.02 min^{-1}	

Put these numbers into the equation we get:

$$RD_{(t)} = 10\ \text{nM}\ x\ e^{-(0.02\ x\ 120)}$$

$$RD_{(t)} = 10\ \text{nM}\ x\ e^{-(2.4)}$$

$$RD_{(t)} = 10\ \text{nM}\ x\ 0.09$$

$$\underline{\underline{RD_{(t)} = 0.9\ \text{nM}}}$$

Answer: After 120 minutes the receptor/ligand dimer concentration is 0.9 nM.

c) To find out how much receptor/ligand dimer breakdown product has been generated after 120 minutes, we first write down our reaction scheme:

RD (receptor/ligand dimer) $\xrightarrow{k}$ B(reakdown products)

Clearly the concentration of B is **dependent** on the concentration of RD and therefore we can apply the equation $C_{(t)} + S_{(t)} = C_{initial} + S_{initial}$ from our bank account example, we just have to slightly change the symbols.

$$RD_{(t)} + B_{(t)} = RD_{initial} + B_{initial}$$

rearranged for $B_{(t)}$:

$$B_{(t)} = RD_{initial} + B_{initial} - RD_{(t)}$$

$RD_{initial}$ = 10 nM	$B_{initial}$ = 0 nM
$RD_{(120\ minutes)}$ = 0.9 nM	$B_{(t)}$ = ??

$B_{(t)}$ = 10 nM + 0 nM – 0.9 nM.

$\underline{B_{(t)} = 9.1\ nM.}$

Answer: The concentration of a breakdown product is 9.1 nM after 120 minutes.

4.7. Problem: Growth of a population

At the beginning of 2008 the population of the country of Bellestan has reached 10 million people. The growth rate in this country has been determined to be 1 % per year.

a) Calculate how many people will live in Bellestan at the beginning of the year 2018.
b) How long does it take for the population of Bellestan to grow to 15 million people?
c) Calculate in which year the population of Bellestan has doubled.

Solution:

Often the growth of a population follows a first order reaction, at least in some stages of the growth phase.

a) Let's formulate a 'reaction' scheme:

$\xrightarrow{k}$ P(opulation)

P does not seem to be dependent on anything else but P. We therefore can write the general rate equation as:

$$\frac{dP}{dt} = + k\ x\ P^1.$$

Note - We are looking at an increase in population and therefore the rate is positive!

After separation of the variables we get

$$\frac{dP}{P} = + k\ dt$$

Integration of both sides gives

$$\int_{P_{initial}}^{P_{(t)}} \frac{dP}{P} = \int_{t_{initial}}^{t_{(t)}} + kdt$$

For which the solution is

$$\ln\left(\frac{P_{(t)}}{P_{initial}}\right) = + k \; x \; (t_{(t)} - t_{initial})$$

or, if we want to get rid of the ln (and 'e' both sides of the equation):

$$\frac{P_{(t)}}{P_{initial}} = e^{+ (k \; x \; (t_{(t)} - t_{initial}))}$$

Rearranged for $P_{(t)}$:

$$P_{(t)} = P_{initial} \; x \; e^{+ (k \; x \; (t_{(t)} - t_{initial}))}$$

Note - The only difference to the equations previously used in this chapter is the sign in front of k.

$P_{(t)}$ = ??	$t_{(t)}$ = 2018
$P_{initial}$ = 10 x 10^6 people	$t_{initial}$ = 2008
k = 1 % $year^{-1}$ = 1/100 $year^{-1}$	

With these numbers we get:

$$P_{(t)} = 10 \; x \; 10^6 \; x \; e^{+ (1/100 \; x \; (2018 - 2008))}$$

$$P_{(t)} = 10 \; x \; 10^6 \; x \; e^{+ (1/100 \; x \; 10)}$$

$$P_{(t)} = 10 \; x \; 10^6 \; x \; e^{0.1} = 10 \; x \; 10^6 \; x \; 1.105$$

$\underline{P_{(t)} = 11.05 \; x \; 10^6 \text{ people}}$.

Answer: In the year 2018 the population of Bellestan would have risen to 11 million people.

b) To calculate when the population of Bellestan has reached 15 million people we use the equation:

$$\ln\left(\frac{P_{(t)}}{P_{initial}}\right) = + k \; x \; (t_{(t)} - t_{initial})$$

and solve for $(t_{(t)} - t_{initial})$:

$$(t_{(t)} - t_{initial}) = \ln\left(\frac{P_{(t)}}{P_{initial}}\right) / k$$

$P_{(t)} = 15 \times 10^6$ people	$t_{(t)} = ??$
$P_{initial} = 10 \times 10^6$ people	$t_{initial} = 2008$
$k = 1\ \%\ year^{-1} = 1/100\ year^{-1}$	

$$(t_{(t)} - t_{initial}) = \ln\left(\frac{15x10^6}{10x10^6}\right) / 0.01$$

$$(t_{(t)} - t_{initial}) = \frac{0.405}{0.01 year^{-1}} = 40.56 \text{ years}$$

Answer: It would take roughly 40.5 years for the population of Bellestan to reach 15 millions. If the start of the 'reaction' was the beginning of 2008, then this would be around middle of June 2048.

c) To find out, how long it will take to double the population of Bellestan, we could use a very similar approach and write:

$$(t_{(t)} - t_{initial}) = \ln\left(\frac{P_{(t)}}{P_{initial}}\right) / k$$

$P_{(t)} = 20 \times 10^6$ people	$t_{(t)} = ??$
$P_{initial} = 10 \times 10^6$ people	$t_{initial} = 2008$
$k = 1\ \%\ year^{-1} = 1/100\ year^{-1}$	

With these numbers we get:

$$(t_{(t)} - t_{initial}) = \ln\left(\frac{20x10^6}{10x10^6}\right) / 0.01\ year^{-1}$$

$$(t_{(t)} - t_{initial}) = \frac{0.693}{0.01 year^{-1}} = 69.3 \text{ years}$$

This should look rather familiar – we did a very similar calculation when we introduced the concept of half lives. Instead of going through this kind of calculation, we could use our equation for half lives:

$$t_{1/2} = \frac{0.693}{k},$$

only with the difference that now we are not talking about a half live, but a doubling time. Mathematically (and conceptually) there is no difference between a half life and a doubling time and therefore we can use the same equation. Some people don't like the notation of $t_{1/2}$ when talking about a doubling time. They rather prefer $t_{2/1}$, but the equation is the same.

We can even predict, when we get another 'doubling' of the population of Bellestan, namely from 20 million people to 40 million people. Again – this would take another 69.3 years.

We have now calculated a number of data points for the growth of the population in Bellestan and we can try to do a graphic representation of these data:

Year	People living in Bellestan (million)
2008	10
2018	11.05
2048.5	15
2069.3	20
2138.6	40

With these data our graph looks like:

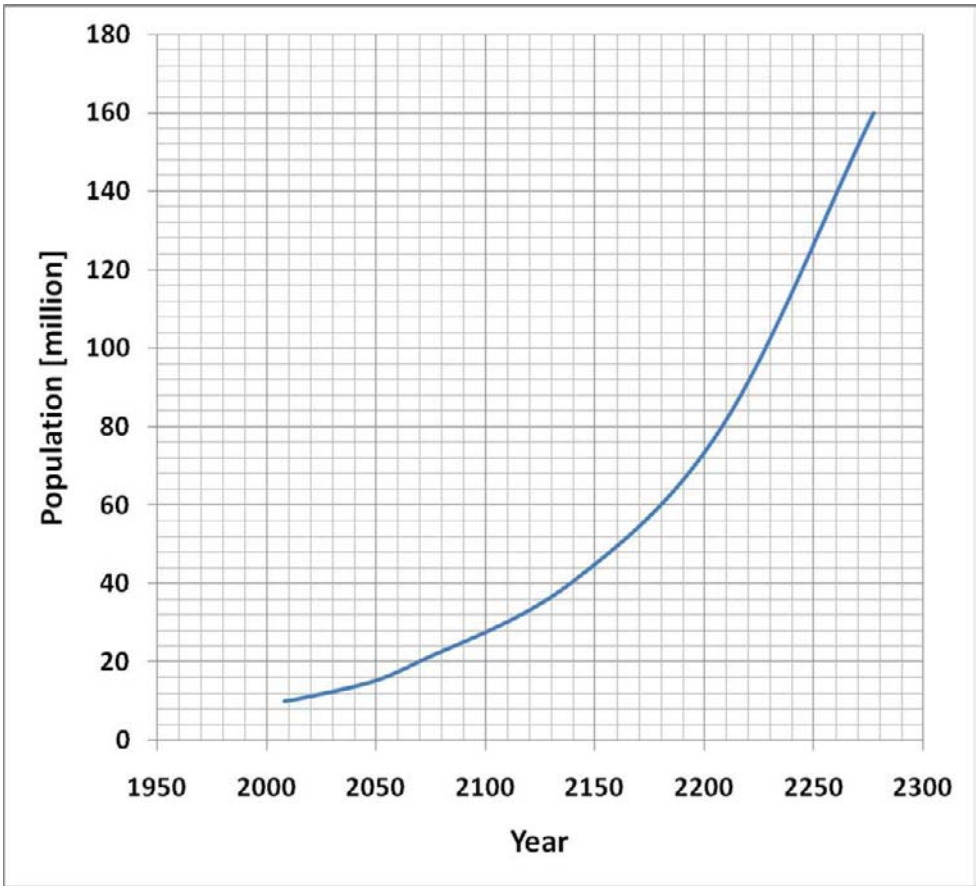


Figure 4-9 Growth curve for the population of Bellestan

This type of growth curve is called a 'logistic' curve – because it follows a logarithmic (or rather exponential) growth. In real life, however, we would never observe a logistic growth, because there would always be some limiting factors that prevent such a growth, for example competition for resources, war etc.

4.8. Problem Solving Strategies

As discussed in the previous chapter there might be a number of different problem styles that require different approaches. For example we might be given some experimental data and asked to determine whether this reaction follows a first order reaction. Another question could be related to calculating the concentration of a reactant (or a product) at a given time, the starting concentration of a reactant, the rate constant etc.

- It is always useful to write down a reaction scheme, for example:

  i) $A \xrightarrow{k} B$ or

ii) $\xrightarrow{k}$ B

- Write down the rate equations for this scheme:

$\frac{dA}{dt}$ = - k *x* A for consumption of A (case (i))

$\frac{dB}{dt}$ = + k *x* B, if we are looking at the growth of B, **independent** of A (case (ii))

- If asked to demonstrate graphically that the data are following a first order reaction, calculate **ln of A** and show a plot of ln (A) versus time. This should give a straight line with –k being the gradient and the intersect of the line with the y-axis being ln ($A_{initial}$).

- If asked to calculate concentrations of A, make a table according to the following scheme:

$A_{(t)}$ =	$t_{(t)}$ =
$A_{initial}$ =	$t_{initial}$ =
k =	

- Rearrange the equation:

$$\frac{A_{(t)}}{A_{initial}} = e^{-(k\,x\,(t_{(t)} - t_{initial}))}$$

Or $\ln(A_{(t)}) - \ln(A_{initial}) = -k\,x\,(t_{(t)} - t_{initial})$

for what you are looking for. Put in the numbers from the table and solve.

- If you haven't got the rate constant k, you can use data and the following equation:

$$k = -\frac{(\ln(A_{(t)}) - \ln(A_{initial}))}{(t_{(t)} - t_{initial})}$$

Again, use a table with data points like:

$A_{(t)}$ =	$t_{(t)}$ =
$A_{initial}$ =	$t_{initial}$ =

Put in the numbers and calculate k.

- k and $t_{1/2}$ can be converted into each other with the equation:

$$t_{1/2} = \frac{0.693}{k}$$

- If you need to calculate concentrations of the product B, you need to find out whether B depends on A or not. If you can write a reaction scheme like:

$$A \xrightarrow{k} B$$

Then B is most likely dependent on A. In this case you need to calculate $A_{(t)}$ as shown in the previous steps and from there calculate $B_{(t)}$, using the equation:

$$A_{(t)} + B_{(t)} = A_{initial} + B_{initial}$$

- If your reaction scheme can be written like this:

$$\xrightarrow{k} B$$

Then it is most likely a growth reaction and B is independent from any reactants. In this case you can use the general rate equation:

$$\frac{dB}{dt} = + k \, x \, B$$

And its integrated form:

$\ln (B_{(t)}) - \ln (B_{initial}) = k \, x \, (t_{(t)} - t_{initial})$ or

$$\frac{B_{(t)}}{B_{initial}} = e^{+ (k \, x \, (t_{(t)} - t_{initial}))}$$

Make a table according to the following scheme:

$B_{(t)}$ =	$t_{(t)}$ =
$B_{initial}$ =	$t_{initial}$ =
k =	

Put in numbers and calculate.

4.9. Equations for a first order reaction

For the reaction

$$A \xrightarrow{k} B$$

The rate equation is

$$\frac{dA}{dt} = -k \, x \, A^1$$

In the integrated form this gives:

$\ln (A_{(t)}) - \ln (A_{initial}) = -k \, x \, (t_{(t)} - t_{initial})$

This can also be written as:

$$\frac{A_{(t)}}{A_{initial}} = e^{- (k \, x \, (t_{(t)} - t_{initial}))}$$

The half life of a first order reaction is given as:

$$t_{1/2} = \frac{0.693}{k}$$

To determine $B_{(t)}$, the following equation should be used:

$A_{(t)} + B_{(t)} = A_{initial} + B_{initial}$

For the reaction:

$\xrightarrow{k}$ B The rate equation is:

$$\frac{dB}{dt} = +k \, x \, B^1$$

In the integrated form this gives:

$\ln (B_{(t)}) - \ln (B_{initial}) = + k \, x \, (t_{(t)} - t_{initial})$

This can also be written as:

$$\frac{B_{(t)}}{B_{initial}} = e^{+(k \, x \, (t_{(t)} - t_{initial}))}$$

The unit for the k is time^{-1}

4.10. More practice questions

1. An excavated bone of a prehistoric elephant contains 10 % of the $^{14}_{6}C$ of a living animal. Calculate, how old this fossil is. The half-life of carbon-14 is 5,700 years.

The radioactive element $^{14}_{6}C$ is used in a technique called “carbon dating”. It is a way of determining the age of certain archaeological artefacts of a biological origin up to about 50,000 years old. It is also used in dating bones, cloth, wood and plant fibers that were created in the relatively recent past by human activities. The carbon-14 atoms that cosmic rays create combine with oxygen to form carbon dioxide, which plants absorb naturally and incorporate into plant fibres by photosynthesis. Animals and people eat plants and take in carbon-14 as well. The ratio of normal carbon (carbon-12) to carbon-14 in the air and in all living things at any given time is nearly constant. Maybe one in a trillion carbon atoms are carbon-14. The carbon-14 atoms are always decaying, but they are being replaced by new carbon-14 atoms at a constant rate. At this moment, your body has a certain percentage of carbon-14 atoms in it, and all living plants and animals have the same percentage. As soon as a living organism dies, it stops taking in new carbon. The ratio of carbon-12 to carbon-14 at the moment of death is the same as every other living thing, but the carbon-14 decays and is not replaced. The carbon-14 decays with its half-life of 5,700 years, while the amount of carbon-12 remains constant in the sample. By looking at the ratio of carbon-12 to carbon-14 in the sample and comparing it to the ratio in a living organism, it is possible to determine the age of a formerly living thing fairly precisely.

Answer: ~ 19000 years.

2. Positron Emission Tomography (PET Imaging) is a diagnostic examination technique that involves the acquisition of physiologic images based on the detection of radiation from the emission of positrons. Positrons are particles emitted from a radioactive substance, fluorine-18, administered to the patient. The subsequent images of the human body developed with this technique are used predominantly in determining the presence and severity of cancers, neurological conditions, and cardiovascular disease.

You measure the decay of a sample of fluorine-18, which has just been delivered, and these are your data:

time [min]	fluorine-18 [μg]
20	0.0882
40	0.0777
60	0.0685
240	0.0220

a) Demonstrate graphically that this is a first order reaction.
b) Calculate the rate constant and half-life of fluorine-18.
c) How much fluorine-18 was delivered?
d) How much fluorine-18 is left after 3 hrs and 12 hrs, respectively?

Answers:

Graph ln (fluorine) versus time gives a straight line
Half life = 110 minutes, k = 6.3 x 10^{-3} min^{-1}
0.1 μg
0.032 μg and 1.1 ng, respectively.

3. An important reaction in the cell to restore energy levels is the conversion of glucose-6-phosphate to glucose, thus generating ATP:

Glucose-6-phosphate + ADP $\rightarrow$ Glucose + ATP

Assuming that under certain conditions this reaction is irreversible *in vitro*, you measure the decrease in the concentration of glucose-6-phosphate and get the following data:

Time [min]	Glucose-6-phosphate [mM]
1	8.2
2	6.7
4	4.5
5	3.7
6	3.0

a) Demonstrate graphically that this is a first order reaction.
b) Calculate the rate constant of this reaction and its half life.

c) How much Glucose-6-phosphate was present at the beginning of the reaction?
d) How much ATP is produced after 3 minutes?
e) How much ATP is produced after 10 minutes?

Answers:

Graph ln (glucose-6-phosphate) versus time gives a straight line
$k = 0.2\ min^{-1}$, half life = 3.465 min.
10 mM
4.5 mM
8.65 mM

4. A census showed that at the beginning of the year 2000 the population of Euphorbia was 10 million people. The average growth rate was determined to be 0.5 % per annum. In contrast, in the state of Brummlidge at the same time there lived only 5 million people, however, the growth rate was found to be close to 2.5 % per annum. The population growth follows a first order mechanism.

a) How long would it take for the population of Euphorbia and Brummlidge, respectively, to double?
b) Calculate how many people would live in the two states in the year 2100, assuming that there are no limiting factors, like war or famine?

Answers:

Euphorbia – 138.6 years, Brum – 27.72 years
Euphorbia – 16.5 x 10^6, Brum – 60.9 x 10^6

5. The radioisotope [$^{32}_{15}$] P is used for labelling of nucleic acids, like DNA and RNA. The isotope shows the following decay:

Time [days]	Amount [mg]
0	1.00
2	0.90
6	0.74
10	0.61
14	0.50
18	0.41
22	0.33

a) Calculate the decay constant k, and check your results graphically.
b) Calculate how much of this radioisotope is left after 50 days?
c) After what time is the radioactivity gone down to 1/1000th of the original amount?

Answers:

k= 0.05 day^{-1}
0.082 mg
138.2 days

6. You measure the absorbance at 600 nm of an *E.coli* culture in the spectrophotometer and get the following data:

time [min]	Absorbance
10	0.03
20	0.04
30	0.05
40	0.07
50	0.09
60	0.12

a) Demonstrate graphically that this is a first order reaction.
b) Calculate the doubling time of *E.coli*
c) Calculate the initial absorbance of the culture (t = 0 min).
d) To make competent cells (cell that can take up DNA), the absorbance should be 0.4. How long do you have to wait before you can harvest the cells?

Answers:

Graph ln (absorbance) versus time shows a straight line
25 minutes
0.023
103 minutes.

5. Second order reaction – a special case

5.1. Introduction and learning outcomes

In the previous chapters we developed equations for zero and first order reactions. In this chapter we will look at a special case of a 'second' order reaction, which involves dimerization reactions. We will define what a second order reaction is and how we can address it mathematically. The aim of this chapter is to give an introduction to second order reactions, in which both reactants are the same chemical. By the end of this chapter you should be able to:

- Identify reactions that follow this scheme,
- Draw graphical representations for this reaction scheme,
- Use relevant equations to calculate amounts (or concentrations) of reactants and products,
- Use relevant equations to calculate a second order rate constant.

5.2. Some general definitions of a second order reaction

A zero order reaction with the reaction scheme

$$A \xrightarrow{k} \text{products}$$

can be written as:

$$\frac{dA}{dt} = -k \; x \; A^0 \text{ which is the same as } \frac{dA}{dt} = -k$$

(k is the rate constant of this reaction and A indicates the concentration of compound A).

For a first order reaction we wrote the general rate equation as:

$$\frac{dA}{dt} = -k \; x \; A^1$$

Again, k is the rate constant for this reaction and A indicates the concentration of compound A.

We now will look at a reaction where not only one component reacts, but two – for example two receptor molecules that have bound a ligand.

In a reaction scheme we can represent this in a very general way as

$$A + B \xrightarrow{k} \text{products}$$

(Again, k is the rate constant of this reaction and A and B are the different compounds that react with each other).

The rate of this reaction clearly depends now on the concentrations of A **and** B and we can write the general rate equation as:

$$\frac{dA}{dt} = - k \, x \, A \, x \, B,$$

where A and B are the respective concentrations for compounds A and B. Since we are looking at a decrease in concentration of A (and B), the rate must be negative.

If 1 molecule of A interacts with 1 molecule of B we assume that we can write (this assumption is not necessarily true, as we will discuss later, but for the time being it will do):

$$\frac{dA}{dt} = - k \, x \, A^1 \, x \, B^1$$

Of course the same principal can be applied to B:

$$\frac{dB}{dt} = - k \, x \, A^1 \, x \, B^1$$

There are two reactants on the right hand side of the equation, namely A and B, and hence this kind of reaction is called a **second order reaction**. We can come to this conclusion by simply adding up the exponents for A and B: 1 + 1 = 2.

This kind of reaction scheme is actually very difficult to solve (we will discuss some solutions in the next chapter) and hence we will use a different scenario for second order reactions to develop the relevant tools.

Instead of looking at the scheme

$$A + B \xrightarrow{k} \text{products}$$

We will look at:

$$A + A \xrightarrow{k} \text{products}$$

In this case two identical reactants A interact with each other to form a product.

5.3. Developing the tools for a second order reaction

5.3.1. Equations

For the reaction scheme:

$$A + A \xrightarrow{k} P(roducts)$$

We can write the general rate equation as:

$$\frac{dA}{dt} = - k \, x \, A \, x \, A$$

Or

$$\boxed{\frac{dA}{dt} = - k \, x \, A^2}$$

which is a differential equation that can be solved easily.

As before we separate the variables and get

$$\frac{dA}{A^2} = - \text{k dt}$$

Integration of this equation gives:

$$\int_{A_{initial}}^{A_{(t)}} \frac{dA}{A^2} = \int_{t_{initial}}^{t_{(t)}} - kdt$$

The solution for the integral on the right hand side of the equation gives:

$$\int_{t_{initial}}^{t_{(t)}} - kdt = -\text{k } x \text{ } (t_{(t)} - t_{initial}).$$

The solution for the left hand side of the equation is:

$$\int_{A_{initial}}^{A_{(t)}} \frac{dA}{A^2} = - \left(\frac{1}{A_{(t)}} - \frac{1}{A_{initial}}\right)$$

Put together, our equation now looks like

$$- \left(\frac{1}{A_{(t)}} - \frac{1}{A_{initial}}\right) = -\text{k } x \text{ } (t_{(t)} - t_{initial})$$

or, if we want to get rid of the – sign and tidy up the whole equation it reads:

$$\frac{1}{A_{(t)}} - \frac{1}{A_{initial}} = + \text{k } x \text{ } (t_{(t)} - t_{initial})$$

Let's look at what happens to the product when we use the scheme:

$$A + A \xrightarrow{k} P(roducts)$$

Now this is a little bit more complicated than in previous examples, because in this case it is 2 components A that form 1 product P. This means that for example, if we started with 100 A the most we could get would be 50 P. If we started with 100 A and after a certain time we had 20 A left then we know that 80 A have been converted into the product. However, these 80 A would only give us 40 P. We therefore can write the more general equation:

$$P_{(t)} = P_{initial} + ½ \, x \, (A_{initial} - A_{(t)})$$

The unit for k is concentration^{-1} time^{-1}.

Units of a second order rate constants could be: **mM^{-1} min^{-1}** or **nM^{-1} s^{-1}**

5.3.2. Graphic representation of a second order reaction

How can we represent this kind of reaction kinetics in a graph?

If we tried to graph this kind of second order reaction in the way we did before with a zero order reaction, i.e. $A_{(t)}$ versus time, or even in the way we did with a first order, i.e. ln ($A_{(t)}$) versus time, we would not get straight lines.

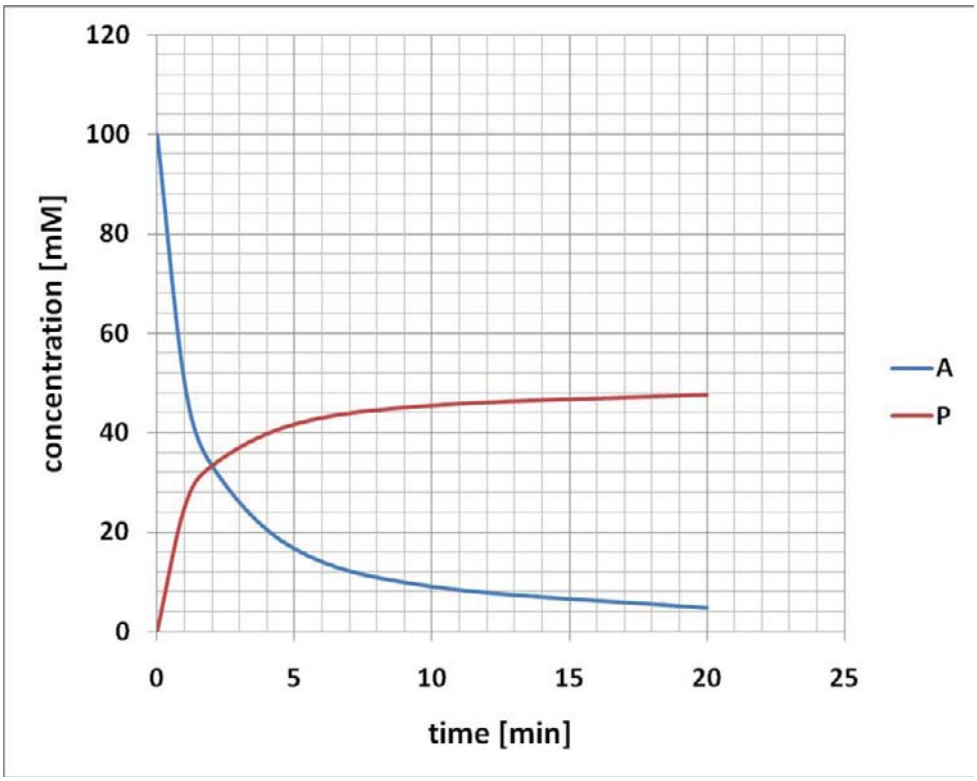


Figure 5-1 Graphic representation of a second order reaction as a zero order plot

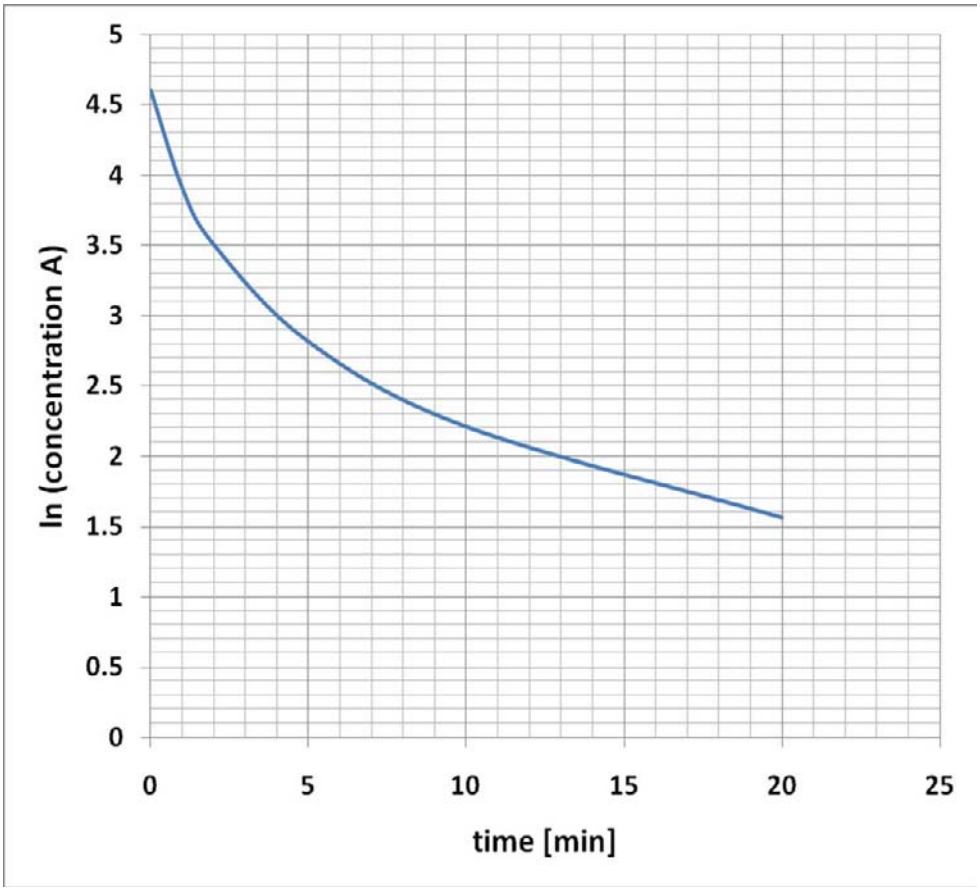


Figure 5-2 Graphic representation of a second order reaction as a first order plot

However, we can rearrange our equation for this type of second order reaction to:

$$\frac{1}{A_{(t)}} = k\ x\ (t_{(t)} - t_{initial}) + \frac{1}{A_{initial}}$$

and again this looks like the equation for a straight line, if we set $t_{initial} = 0$:

y = m X + C

Therefore a plot of $\frac{1}{A_{(t)}}$ versus time gives a straight line with gradient k and an intersect with the y-axis at $\frac{1}{A_{initial}}$.

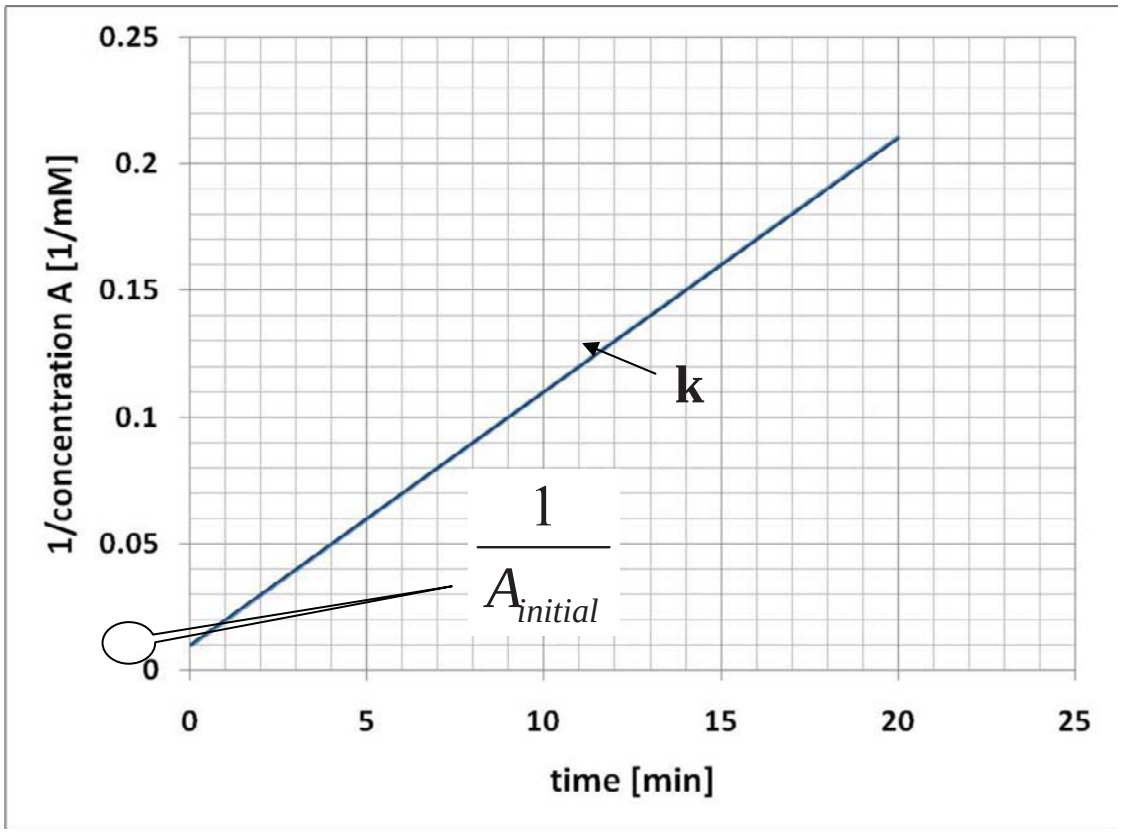


Figure 5-3 Graphic representation of a second order reaction

When we plot this graph we will find that in contrast to a zero order and first order reaction the gradient of the straight line in the second order reaction is **positive**! This is not a mistake but results from the solution of the integral.

We can use this kind of graph for 'diagnostic' purposes – if a plot of $\frac{1}{A_{(t)}}$ versus time results in a straight line, we know it must describe a second order reaction.

5.4. Problem: Dimerization of the receptor for a growth hormone

A receptor for a growth hormone binds its ligand and then dimerizes to form a receptor/ ligand dimer according to the following scheme:

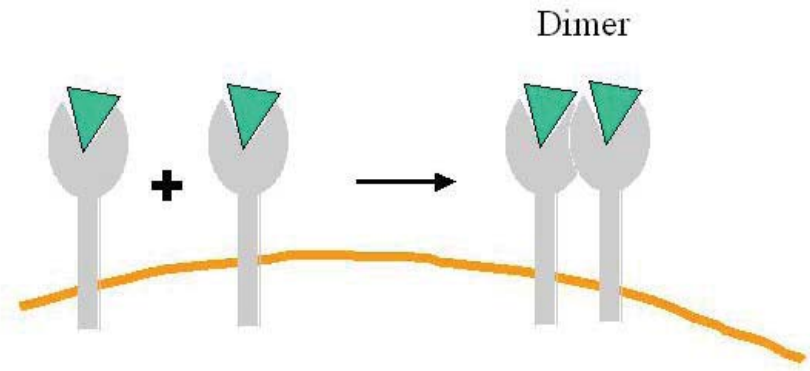


Figure 5-4 Dimerization of a receptor/ligand complex into a receptor/ligand dimer

If the rate constant for this second order reaction was 0.05 nM^{-1} min^{-1} and there is a concentration of 20 nM of receptor/ligand present at the beginning of the experiment, how much receptor/ligand is left after 30 minutes? And how much dimer has formed after 30 minutes?

Solution:

The reaction scheme for the dimerization of the receptor/ligand complex is:

R(eceptor)/L(igand) + R(eceptor)/L(igand) $\xrightarrow{k}$ Dimer

We can therefore write the general rate equation for this reaction as:

$$\frac{dRL}{dt} = -k \, x \, RL^2$$

in which RL indicates the concentration of the receptor/ligand complex.

Separation of the variables and integration gives:

$$\frac{1}{RL_{(t)}} - \frac{1}{RL_{initial}} = k\ x\ (t_{(t)} - t_{initial})$$

We want to know, how much of the receptor/ligand complex is left after 30 minutes and therefore we are looking for $RL_{(t)}$. Rearranging the equation gives:

$$\frac{1}{RL_{(t)}} = k\ x\ (t_{(t)} - t_{initial}) + \frac{1}{[RL]_{initial}}$$

Apart from $RL_{(t)}$ we have all the information we need:

$RL_{initial} = 20$ nM	$t_{initial} = 0$ min
$RL_{(t)} = ???$	$t_{(t)} = 30$ min
$k = 0.05\ nM^{-1}\ min^{-1}$	

Putting in the numbers gives:

$$\frac{1}{RL_{(t)}} = 0.05\ nM^{-1}\ min^{-1}\ x\ (30\ min - 0\ min) + \frac{1}{20nM}$$

$$\frac{1}{RL_{(t)}} = 1.5\ nM^{-1} + 0.05\ nM^{-1} = 1.55\ nM^{-1}$$

We have just calculated $\frac{1}{RL_{(t)}}$, but what we really want to know is $RL_{(t)}$. We therefore need to 'inverse' our result:

$$RL_{(t)} = \frac{1}{1.55nM^{-1}} = \underline{0.645\ nM}$$

Answer**:** After 30 minutes the receptor/ligand concentration is 0.645 nM.

Now for the second part of the questions – how much dimer has been formed after 30 minutes?

We know that 2 receptor/ligand complexes give 1 receptor/ligand dimer, and this stoichiometry we have to take into account when we calculate the dimer concentration.

An easy approach to solving this problem is to calculate, how many receptor/ligand complexes have been used up. From this we then calculate how many dimers have been formed, according to the equation:

$$P_{(t)} = P_{initial} + ½\ x\ (RL_{initial} - RL_{(t)})$$

Let's put the data into a table:

$RL_{initial} = 20$ nM	$P_{initial} = 0$ nM
$RL_{(t)} = 0.645$ nM	$P_{(t)} = ???$

$P_{(t)} = P_{initial} + ½ \, x \, (RL_{initial} - RL_{(t)})$

$P_{(t)} = 0 \quad + ½ \, x \, (20 \text{ nM} - 0.645 \text{ nM}) = ½ \, x \, 19.355 \text{ nM}.$

$$P_{(t)} = \frac{19.355 nM}{2} = \underline{9.678 \text{ nM of dimer}}.$$

Answer: After 30 minutes the dimer concentration is 9.678 nM.

Let's do a quick 'reality check':
Starting with 20 nM of receptor/ligand complex and knowing that 2 of the complexes make 1 dimer, our maximum concentration of the dimer is:

$$\frac{20 nM}{2} = 10 \text{ nM}.$$

That is, if every one of the receptor/ligand complexes would have been used up for dimer formation.

This means that our dimer concentration cannot be larger than 10 nM. Our calculated concentration after 30 min is below this 10 nM maximum and therefore in line with our check.

5.5. Problem: Formation of active reverse transcriptase dimers

Here is another example of a second order reaction in which both reaction partners are the same chemical compound.

The protein reverse transcriptase (RT) is a viral protein essential for the propagation of retroviruses like HIV. RT is initially produced in the cell as a 'monomer', but quickly forms a 'homo-dimer', which is a complex of two RT-monomers. Only this homo-dimer is active, whereas RT monomers are not. Obviously there is a huge interest to find a potent drug against HIV and this can be done by investigating the kinetics of RT dimer formation. If one could stop the formation of RT-dimers then the virus could no longer replicate. To study the formation of RT dimers you use a concentration of 200 nM RT monomers and measure their concentration at certain time intervals:

Time [min]	RT-monomer [nM]
0	200.00
10	40.00
20	22.22
30	15.38
40	11.76
50	9.52

a) Demonstrate that this process follows a second order reaction type.
b) Calculate how much monomer and dimer is present after 60 minutes.

Solution:

To find out what order this reaction is we start by assuming that this is a zero order reaction. In this case we just plot concentration of the RT monomer versus time and check whether this gives a straight line:

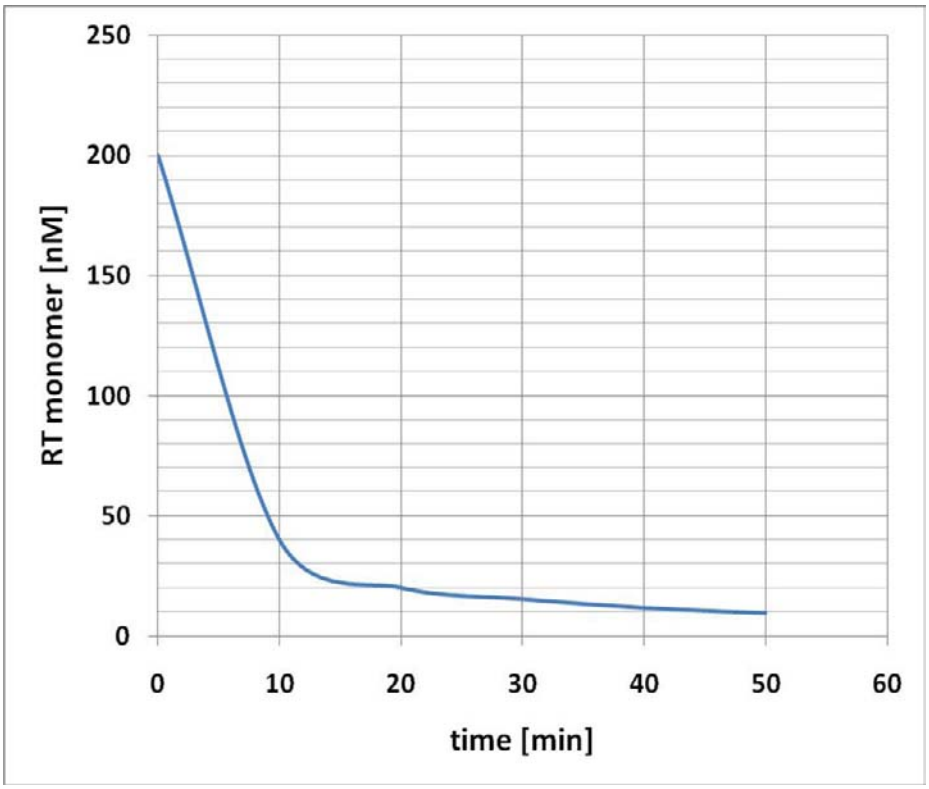


Figure 5-5 Zero order representation of the RT monomer consumption

Obviously this doesn't give a straight line, therefore let's try a first order reaction. In this case we have to calculate ln (concentration of the RT monomer) and plot it versus time:

Time [min]	RT monomer [nM]	ln (RT monomer)
0	200.00	5.298
10	40.00	3.689
20	22.22	3.096
30	15.38	2.733
40	11.76	2.465
50	9.52	2.253

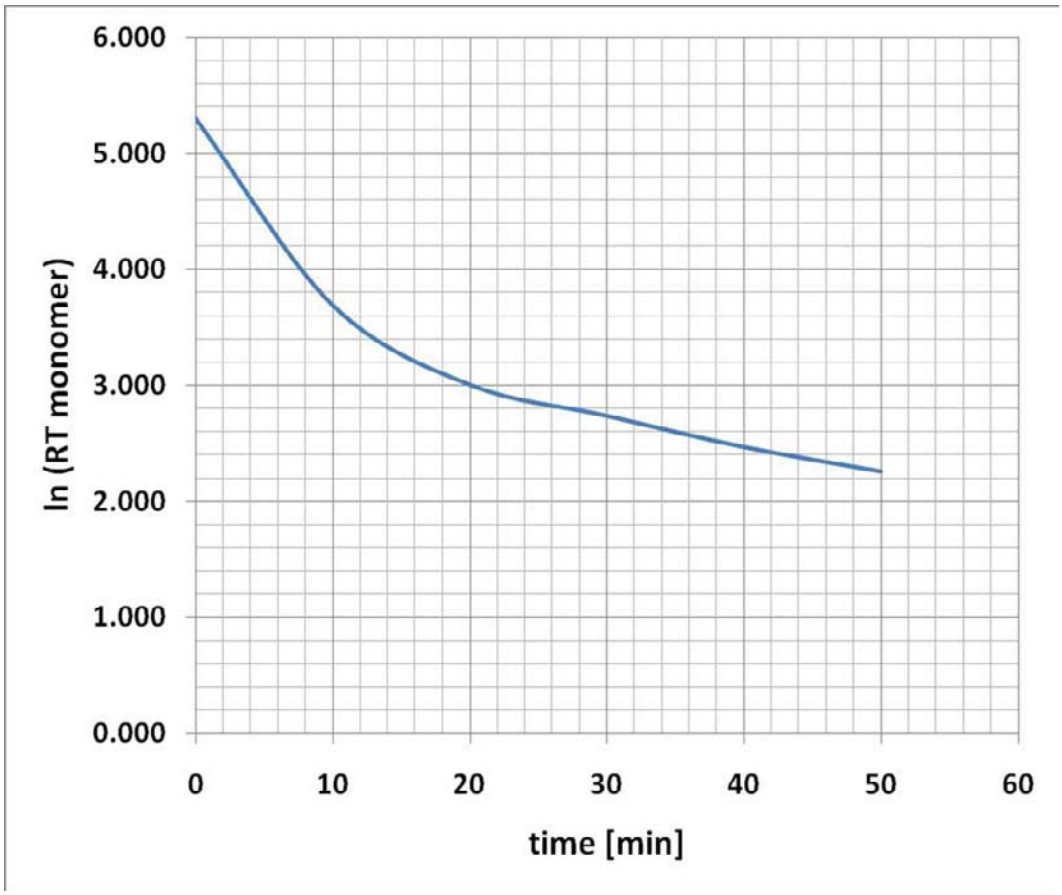


Figure 5-6 First order representation of the RT monomer consumption

It's still not a straight line! Therefore let's try a second order reaction of the type

2 RT monomers $\xrightarrow{k}$ RT dimer

In this case we have to calculate 1/RT monomer concentration and plot it versus time.

Time [min]	RT monomer [nM]	1/RT monomer [nM^{-1}]
0	200.00	0.005
10	40.00	0.025
20	22.22	0.045
30	15.38	0.065
40	11.76	0.085
50	9.52	0.105

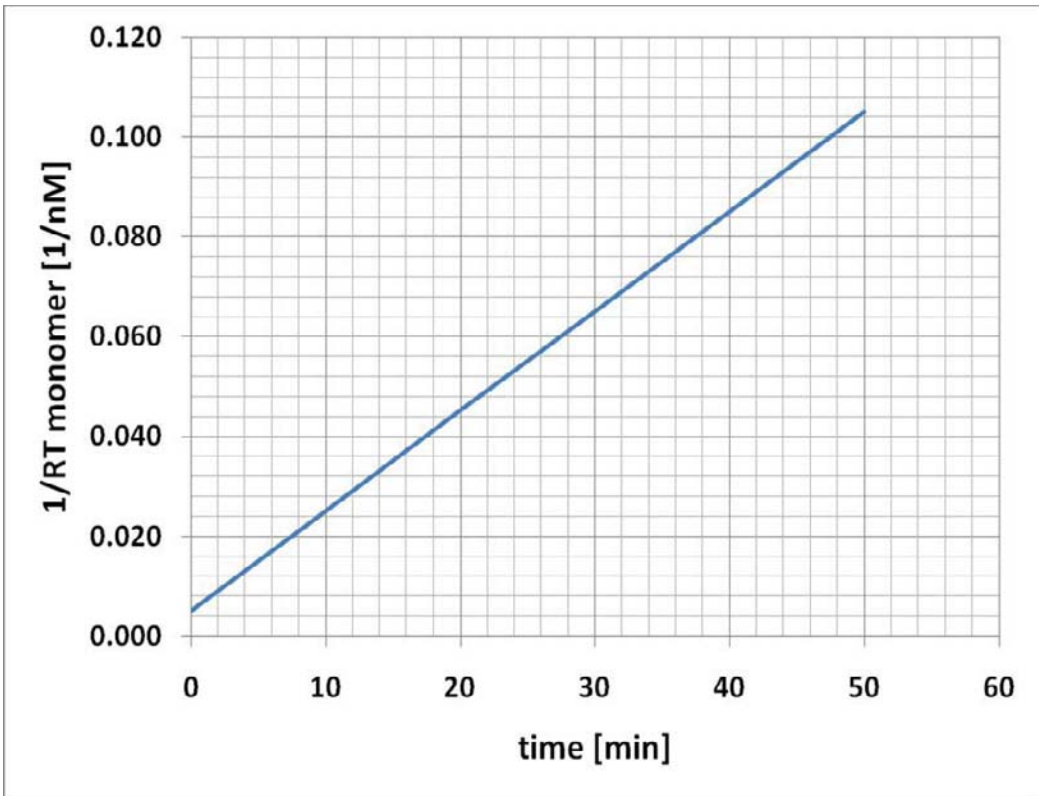


Figure 5-7 Second order representation of the RT monomer consumption

This (finally) gives a straight line, indicating that the reaction follows a second order kinetics.

To calculate the RT monomer concentration after 60 minutes we use the equation:

$$\frac{1}{RT_{(t)}} - \frac{1}{RT_{initial}} = k \; x \; (t_{(t)} - t_{initial})$$

So far so good, but we do not know k!!!
To get k we could look at our graph (remember that k is the gradient), but this might not be accurate enough. We can also calculate k from our experimental data.
To do the latter, we rearrange our equation and solve for k:

$$k = \left(\frac{1}{RT_{(t)}} - \frac{1}{RT_{initial}}\right) / (t_{(t)} - t_{initial})$$

By picking from our table an appropriate $RT_{(t)}$ and the corresponding $t_{(t)}$ and also an $RT_{initial}$ with corresponding $t_{initial}$, we can now calculate k.

Time [min]	RT-monomer [nM]
0	200.00
10	40.00
20	22.22
30	15.38
40	11.76
50	9.52

$RT_{initial}$ = 200 nM	$t_{initial}$ = 0 min
$RT_{(50)}$ = 9.52 nM	$t_{(50)}$ = 50 min
k = ???	

With these numbers we get:

$$k = \left(\frac{1}{RT_{(50)}} - \frac{1}{RT_{initial}}\right) / (t_{(50)} - t_{initial})$$

$$k = \left(\frac{1}{9.52nM} - \frac{1}{200nM}\right) / 50 \text{ min}$$

$$k = (0.105 \text{ nM}^{-1} - 0.005 \text{ nM}^{-1}) / 50 \text{ min}$$

$$k = 0.1 \text{ nM}^{-1} / 50 \text{ min}$$

$$\underline{k = 0.002 \text{ nM}^{-1} \text{ min}^{-1}}$$

With this we can now calculate the concentration of the RT monomer after 60 minutes:

$$\frac{1}{RT_{(60)}} - \frac{1}{RT_{initial}} = k\ x\ (t_{(60)} - t_{initial})$$

Solving for $\frac{1}{RT_{(60)}}$ gives:

$$\frac{1}{RT_{(60)}} = k\ x\ (t_{(60)} - t_{initial}) + \frac{1}{RT_{initial}}$$

With numbers:

$$\frac{1}{RT_{(60)}} = 0.002\ nM^{-1}\ min^{-1}\ x\ 60\ min + \frac{1}{200nM}$$

$$\frac{1}{RT_{(60)}} = 0.12\ nM^{-1} + 0.005\ nM^{-1} = 0.125\ nM^{-1}$$

$$RT_{(60)} = \frac{1}{0.125nM^{-1}} = \underline{8\ nM}$$

Answer: The concentration of the RT monomer is 8 nM after 60 minutes.

To calculate the concentration of the dimer we can again make use of the stoichiometry of the reaction. We know that 2 monomers form 1 dimer, and therefore we can calculate the concentration of monomers that have disappeared after 60 minutes to form the dimer:

Monomers converted to dimers = 200 nM – 8 nM = 192 nM

192 nM of monomers can form $\frac{192nM}{2} = \underline{96\ nM\ dimer}$.

Answer: After 60 minutes the RT dimer concentration is 96 nM.

5.6. Problem solving strategies

- If a reaction has the following scheme:

$A + A \xrightarrow{k}$ products

- Identify what is asked for and what information is available.
- Define whether you are looking for reactants or products. If you want to calculate reactants -
  - Rearrange equation accordingly:

$$\frac{1}{A_{(t)}} - \frac{1}{A_{initial}} = k\ x\ (t_{(t)} - t_{initial})$$

  - Put in numbers and calculate results. Use the correct units.

- If asked for products, use above equation and the information of $A_{initial}$ to calculate the concentration/number of reactants used:

$$A_{consumed} = A_{initial} - A_{(t)}$$

- Divide number/concentration of $A_{consumed}$ by 2 to reflect stoichiometry.
- If there is already some product present, this is taken into consideration with the equation
  $P_{(t)} = P_{initial} + ½ \; x \; (A_{initial} - A_{(t)})$

5.7. Equations for second order reactions in which the reactants are the same chemical

For the reaction $A + A \xrightarrow{k}$ product

The rate equation is

$$\frac{dA}{dt} = -k \; x \; A^2$$

In the integrated form this gives:

$$\frac{1}{A_{(t)}} - \frac{1}{A_{initial}} = +k \; x \; (t_{(t)} - t_{initial})$$

The unit for k is **concentration^{-1} x time^{-1}**

5.8. More practice questions

1. The receptor for a growth hormone shows dimerization according to the scheme:

$A + A \xrightarrow{k} D$

The following data were recorded:

Time [min]	A [nM]
0.0	0.100
10.0	0.083
20.0	0.071
40.0	0.056
50.0	0.050
60.0	0.045
70.0	0.042
80.0	0.038

a) Demonstrate graphically that this is a second order reaction.
b) Calculate the rate constant k.
c) How much A and D are present after 30 minutes?
d) How much D is produced after 100 min?
e) What is the maximum concentration D can reach?

Answers:

Graph of 1/A versus time gives a straight line
k= 0.204 nM^{-1} min^{-1}
0.062 nM A and 0.019 nM D
0.034 nM D
0.05 nM

2. In a spectrophotometric test you measure the disappearance of a coloured compound A:

time [min]	A [mM]
0	
10	2.222
30	1.818
50	1.538
70	1.333
90	1.176
110	1.053

a) Does this follow a zero, first or second order rate?
b) Calculate the rate constant k
c) Calculate, how much A was present at the beginning of the reaction?
d) How much A is present after 80 minutes?

Answers:

Second order reaction, Graph of 1/A versus time gives a straight line
k = 5 *x* 10^{-3} mM^{-1} min^{-1}
2.50 mM
1.25 mM

6. Second order reaction with different reactants

6.1. Introduction and learning outcomes

In the previous chapter we developed the equations for a second order rate kinetics, in which both reactants were identical. This scenario is very common in dimerization reactions. However, in many second order reactions there are two different reactants present. The aim of this chapter is to look at the relevant equations for this case. Additionally, we will look at an experimental way to simplify such general second order kinetics.

The aim of this chapter is to extend the second order kinetics discussed in the previous chapter to a more general concept, in which the two reactants are not identical. Furthermore, the principal of 'pseudo-orders' will be introduced. By the end of the chapter you should be able to:

- Identify second order reactions with different reactants,
- Understand the underlying principle of 'pseudo-orders'

6.2. Extension of the second order rate concept

In the previous chapter we discussed the scheme:

$$A + A \xrightarrow{k} \text{product}$$

in which both reactants are the same. But what if they are not? What would happen, if we looked at the scheme:

$$A + B \xrightarrow{k} \text{product} \quad ?$$

In Biosciences one of the most common applications of a second order kinetics is the interaction of a ligand with its receptor, thus forming a receptor/ligand complex

$$R(\text{eceptor}) + L(\text{igand}) \xrightarrow{k} R(\text{eceptor})L(\text{igand}) \text{ complex}$$

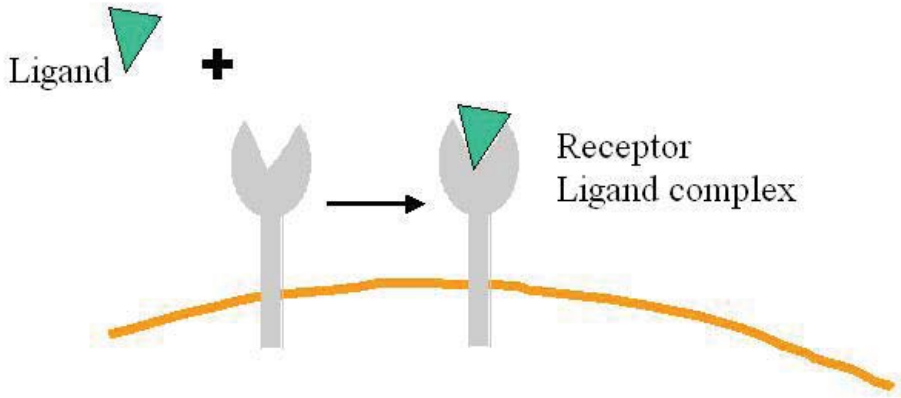


Figure 6-14 Ligand and receptor form a receptor/ligand complex

We can write a general rate equation for this type of reaction (since we look at the disappearance of the reactants the rate is negative):

$$\text{Rate} = - k \, x \, R \, x \, L$$

Each receptor binds 1 ligand and therefore the ratio is 1 : 1 (which makes life a lot easier for us). For each mole of R used one mole of L must be used as well.
We can therefore write:

$$\frac{dR}{dt} = \frac{dL}{dt} = - k \, x \, R^1 \, x \, L^1$$

We assume the rate equation is a first order reaction with respect to R, and also a first order reaction with respect to L. However, since there are the concentrations of **two** components involved, the overall order of the reaction is **second order**.

Let's carry out a receptor-ligand binding experiment and measure the concentration of R, L and RL every 2 minutes:

time [min]	Receptor [nM]	Ligand [nM]	RL-complex [nM]
0	100	80	0
2	47.72	27.72	52.28
4	34.59	14.59	65.41
6	28.83	8.83	71.17
8	25.72	5.72	74.28
10	23.85	3.85	76.15
12	22.66	2.66	77.34
14	21.86	1.86	78.14
16	21.32	1.32	78.68
18	20.94	0.94	79.06
20	20.67	0.67	79.33

The graphic representation of our experiments looks like:

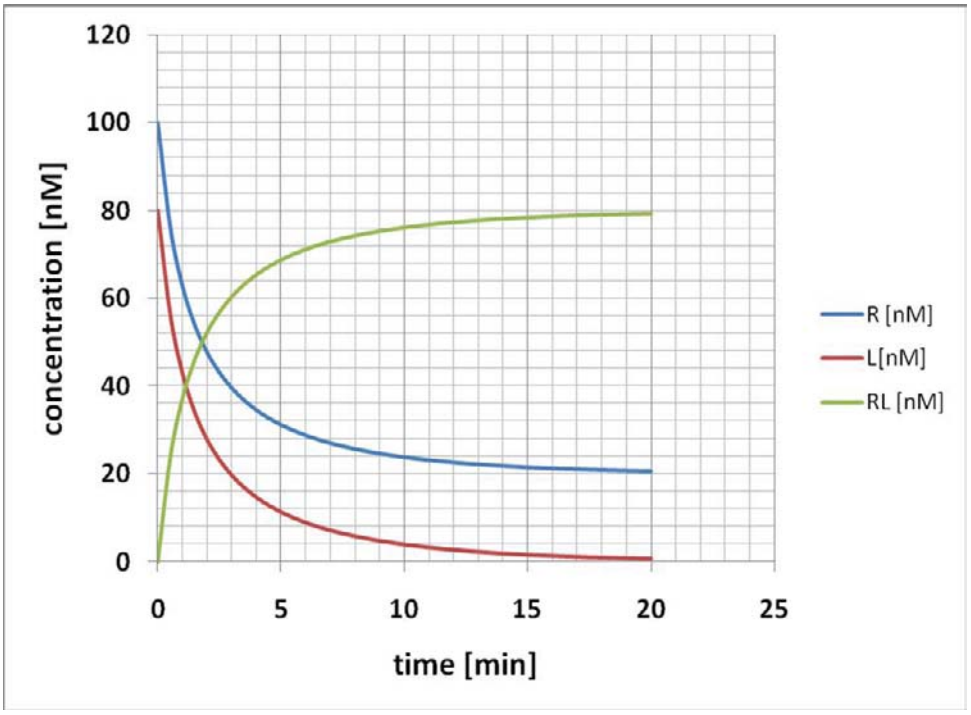


Figure 6-2 Graphic representation of receptor/ligand interaction

This example highlights some important points we need to bear in mind when we look at this kind of reaction. Firstly, it shows that the concentrations of the various reactants can be different. If this is the case then the reactant with the lowest initial concentration, in this case the concentration of L becomes limiting. As a consequence, the concentration of the reactant with the higher concentration, R, will reach a specific level at which it will stay constant. Secondly, the level of the product, here RL is determined by the level of the reactant with the lowest concentration.

Since we can write the rate equation as:

$$\frac{dR}{dt} = \frac{dL}{dt} = - k \, x \, R^1 \, x \, L^1$$

We can try out whether we can use a first order or a second order plot, as we discussed in the previous chapters. For a first order plot we plot ln (receptor concentration) versus time, whereas for a second order (A +A type) reaction we plot 1/(receptor concentration) versus time.

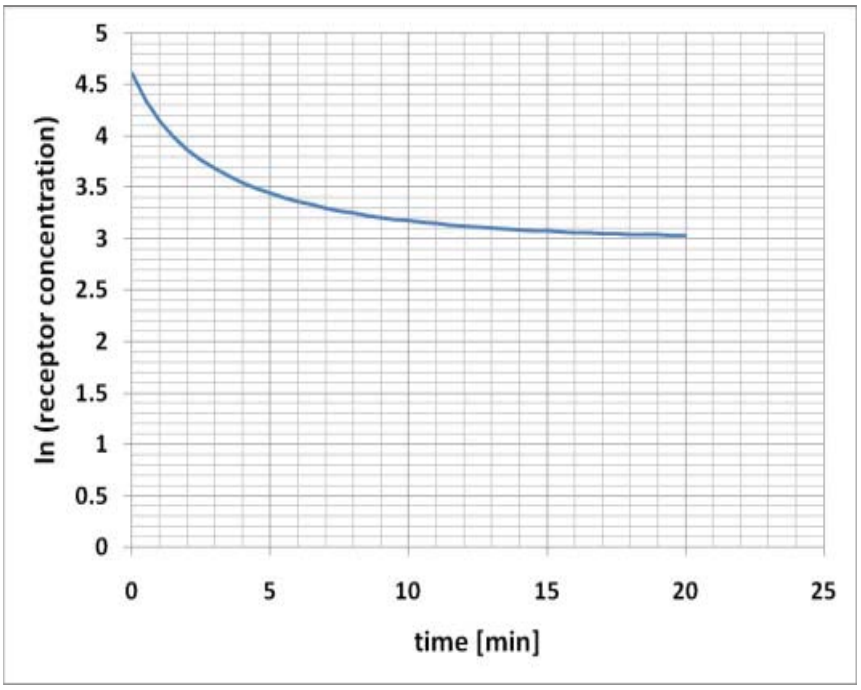


Figure 6-3 First order representation of receptor consumption

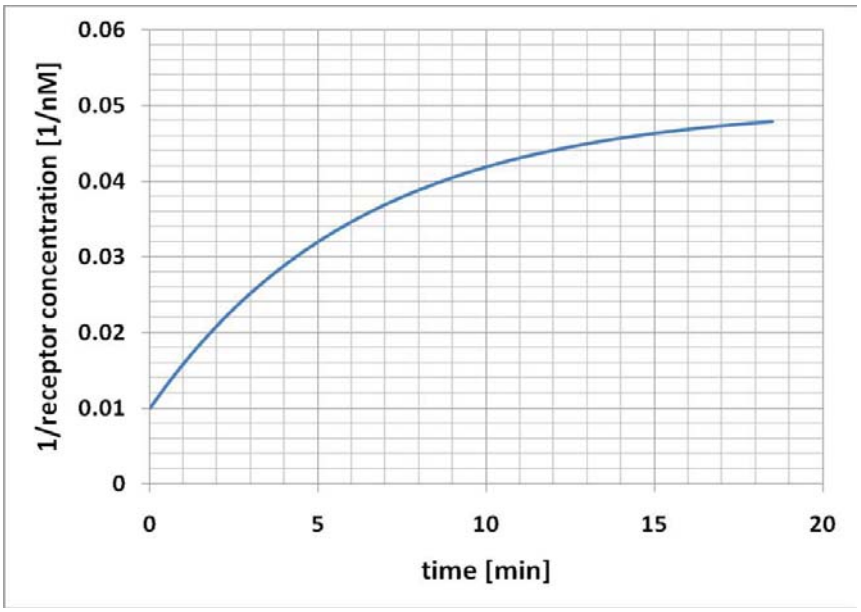


Figure 6-4 Second order (A + A type) representation of receptor consumption

Clearly, none of the plots gives us a straight line, as would be indicative for a true first or second order (A +A type) reaction. We could have done the plots with the ligand concentrations instead of the receptor concentrations, but the results would have been identical – neither of the plots give a straight line.

This example emphasizes the fact that a reaction of the type:

$A + B \xrightarrow{k}$ product

represents an altogether different reaction scheme, which cannot be treated like a first or a second order (A +A type) reaction. To solve this reaction we need to use a different kind of plot.

Integration of this kind of rate equation is rather complex, but not impossible. The general solution is:

$$\frac{1}{A_{initial} - B_{initial}} \; x \ln \left(\frac{A_{(t)} x B_{initial}}{A_{initial} x B_{(t)}} \right) = k \; x \; (t_{(t)} - t_{initial})$$

Solving for $A_{(t)}$ and $B_{(t)}$ gives:

$$\ln \left(\frac{A_{(t)}}{B_{(t)}} \right) = (A_{initial} - B_{initial}) \; x \; k \; x \; \Delta t - \ln \left(\frac{B_{initial}}{A_{initial}} \right)$$

As in previous chapters, this equation is similar to the equation for a straight line with $y = m X + C$

We therefore plot $\ln \left(\frac{A_{(t)}}{B_{(t)}} \right)$ versus time and get a straight line, in which the gradient

$m = (A_{initial} - B_{initial}) \; x \; k$ and the intersect with the y-axis is $\ln \left(\frac{B_{initial}}{A_{initial}} \right)$.

In our example we can substitute A with Receptor concentration and B with Ligand concentration and get:

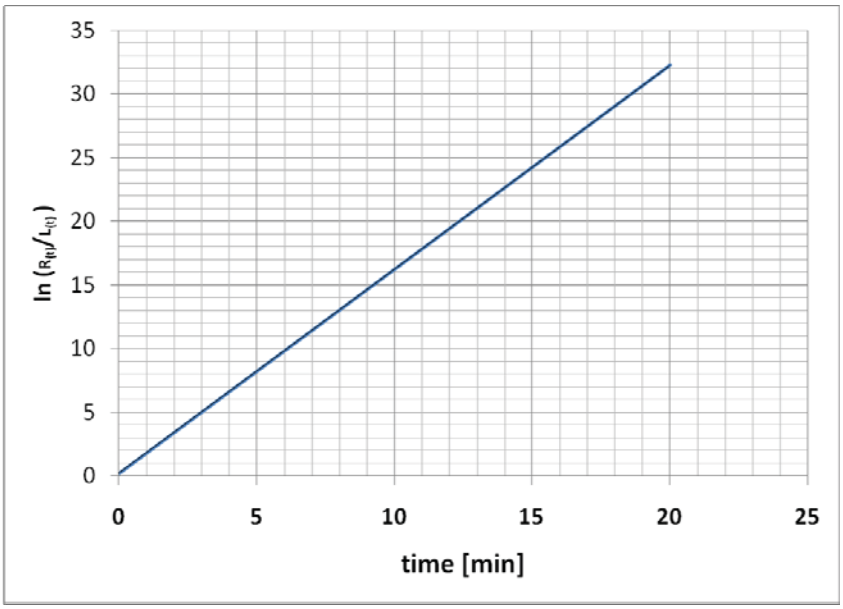


Figure 6-5 Second order representation of a reaction of the A + B type kinetic

6.3. Pseudo-orders

Biologists are not too keen to use such complicated equations (after all, we want to do biology and not maths!) or graphs that are difficult to interpret and therefore came up with a different solution.

Let's assume we keep the concentration of the ligand very, very high compared to the concentration of the receptor. In this case the concentration of the receptor would be limiting and the final concentration of the ligand would not be too different from the initial concentration of the ligand. We can assume that the concentration of the ligand would therefore remain fairly constant and hence we can define a new k for this reaction:

k' (read *k prime*) = k *x* concentration of the ligand.

If we now write our general rate equation we get:

$$\frac{dR}{dt} = - k' \, x \, R^1$$

This equation should look familiar to you – it is the rate equation for a first order reaction. However, it is important to remember that it is only **apparently** a first order reaction – it truly is a second order reaction disguised as a first order reaction. In this case we call this kind of reaction a **'pseudo-first order reaction'**.

What happens, if the concentrations of the two reactants are not different?

If the concentrations of the two reactants cannot be made different (either by the researcher or the cell), we cannot reduce the complicated rate equation to a pseudo-first order reaction. However, if the concentrations of the two reactants are identical, or very similar, we can write:

Concentration of A = concentration of B and for our general rate equation we can simplify the reaction to:

$$\frac{dA}{dt} = - k\, x\, A \times A$$

Again, this should look familiar to you – this is nothing else but the general rate equation for a second order (A +A type) reaction, which follows the scheme:

$A + A \xrightarrow{k}$ products.

6.4. Problem: Hydrolysis of Acetylcholine

Acetylcholine is a neurotransmitter, particularly important in the stimulation of muscle tissue. The transmission of an electric impulse to the end of the nerve causes it to release acetylcholine molecules onto the surface of the next cell, and hence stimulating it. After such release, the acetylcholine is quickly broken into acetate and choline, which is passed back to the first cell to be recycled into acetylcholine again. The poison Curare acts by blocking the transmission of acetylcholine. Some nerve gases operate by preventing the breakdown of acetylcholine, thus causing continual stimulation of the receptor cells, which leads to intense spasms of the muscles, including the heart.

Measuring the disappearance of acetylcholine gives us the following data:

time [min]	Acetylcholine [mM]
0	1.00
0.5	0.76
1	0.57
1.5	0.43
2	0.33
2.5	0.25
3	0.19
3.5	0.14
4	0.11
4.5	0.08
5	0.06

Demonstrate that the hydrolysis of acetylcholine can be treated as a pseudo-first order reaction.

Solution:

The breakdown of acetylcholine can be written as:

$A(cetylcholine) + H_2O \xrightarrow{k}$ acetate + choline.

For the disappearance of A we can write the general second order rate equation:

$$\frac{dA}{dt} = - k \; x \; A \; x \; H_2O$$

The concentration of water, however, is fairly high, compared to the concentration of acetylcholine.

The molecular mass of H_2O is 18. 1 litre of water weighs 1000 g and therefore the concentration of water is:

$$\frac{1000g}{18g/mol} = 55.555 \text{ mol per litre.}$$

This is huge compared to an acetylcholine concentration of 1 mM. Even if all the acetylcholine had reacted the concentration of water would still be:

55.555 M – 0.001 M = 55.554 M

We therefore can write:

$$\frac{dA}{dt} = - k' \; x \; A^1$$

in which k' represents our new rate constant including the concentration of water.

Let's try out whether we can use a first order ln (A) versus time plot to represent our data. First we have to calculate the ln of the acetylcholine concentrations:

t [min]	Acetylcholine [mM]	ln(Acetylcholine)
0	1.00	0
0.5	0.76	-0.27777
1	0.57	-0.55555
1.5	0.43	-0.83332
2	0.33	-1.11109
2.5	0.25	-1.38886
3	0.19	-1.66664
3.5	0.14	-1.94441
4	0.11	-2.22218
4.5	0.08	-2.49995
5	0.06	-2.77772

A plot of ln (acetylcholine concentration) versus time gives:

Figure 6-6 Graphic representation of a pseudo-first order reaction

Clearly the graph shows a straight line, indicative of a first order reaction. However, we need to bear in mind that this is in reality a pseudo-first order reaction in which the concentration of one of the interaction partners is so high that we can treat it as more or less constant during the entire reaction phase.

We can also determine the rate constant from our data set.

To calculate the rate constant from our data, we rearrange our equation for a first order reaction and solve for k:

$$k = \ln\left(\frac{A_{(t)}}{A_{initial}}\right) = -(t_{(t)} - t_{initial})$$

By picking from our data table an appropriate $A_{(t)}$ and the corresponding $t_{(t)}$ and also an $A_{initial}$ with corresponding $t_{initial}$, we can now calculate k:

$A_{(t)} = 0.06$ mM	$t_{(t)} = 5$ min
$A_{initial} = 1.00$ mM	$t_{initial} = 0$ min
k = ??	

With these numbers we get:

$$k = \ln\left(\frac{0.06}{1.00}\right) / -(5\text{min} - 0\text{min})$$

$$k = \frac{\ln(0.06)}{-5\,\text{min}} = \frac{-2.81}{-5\,\text{min}} = 0.56\ \text{min}^{-1}$$

Our rate constant therefore is 0.56 min^{-1}. However, we must not forget that this rate constant in reality represents a pseudo-first order constant k', which we defined as:

k' = k *x* concentration of H_2O.

To find our true second order rate constant we need to rearrange this equation and solve for k:

$$k = \frac{k'}{H_2O} = \frac{0.56\,\text{min}^{-1}}{55.55M} = 0.01\ \text{M}^{-1}\ \text{min}^{-1}.$$

Our second order rate constant therefore is <u>k = 0.01 M^{-1} min^{-1}.</u>

Let's compare this rate constant with the one we get, when we use the equation:

$$\frac{1}{A_{initial} - B_{initial}}\ x \ln\left(\frac{A_{(t)}xB_{initial}}{A_{initial}xB_{(t)}}\right) = k\ x\ (t_{(t)} - t_{initial})$$

We use the following data from the table:

$A_{initial}$ = 0.001 M	$B_{initial}$ = 55.55555 M	$t_{initial}$ = 0 min
$A_{(t)}$ = 0.0006 M	$B_{(t)}$ = 55.55554 M	$t_{(t)}$ = 5 min

Putting these numbers into our equation we get:

$$\frac{1}{0.001M - 55.55555M} \; x \ln \left(\frac{0.0006x55.55555}{0.001x55.55554}\right) = k \; x \; (5min \; - 0 \; min)$$

$$\frac{1}{-55.55455M} \; x \ln (0.06) = k \; x \; 5 \; min$$

$$\frac{1}{-55.55455M} x \; (-2.813) = k \; x \; 5 \; min$$

$0.05064 \; M^{-1} = k \; x \; 5 \; min$

$$k = \frac{0.051M^{-1}}{5\,min} = 0.01 \; M^{-1} \; min^{-1}.$$

Both approaches lead to the same result, but the calculation for the pseudo-first order rate constant was much easier.

6.5. Problem solving strategies

The rate equation for a reaction of the type

$A + B \xrightarrow{k}$ product can be written as:

$$\frac{dA}{dt} = \frac{dB}{dt} = - k \; x \; A^1 \; x \; B^1$$

The overall rate order is a second order with the individual rate orders for A and B being first orders.

The mathematical solution for this type of reaction is somewhat more complicated; however, experimentally these types of reactions can be analyzed by having one of the reactants in vast excess over the other, thus creating a pseudo-first order reaction.

If B >>> A, then the reaction can be expressed as:

$$\frac{dA}{dt} = - k' \; x \; A^1 \text{ with } k' = k \; x \; B.$$

7. Determine the order of a reaction

7.1. Introduction and learning outcomes

So far we have discussed reaction kinetics for rather specific cases, namely zero, first and second order reactions. We investigated how the concentrations of reactants and/or products change depending on the order of a reaction. We also found out how the order of a reaction can be confirmed using experimental data. In this chapter I want to demonstrate how the order of a reaction can be determined by looking at the rates of this reaction (and not looking at the concentrations of reactants and/or products, as we have done before). By the end of the chapter you should be able to:

- Understand the concept of 'initial rates',
- Determine rate orders of a reaction,
- Understand the difference between the order and the molecularity of a reaction.

7.2. The significance of 'initial rates'

Before we start investigating, how the order of a reaction affects the rate, we need to define, which rate we are looking at. Why??

In the last few chapters we always looked at the disappearance of reactants (or the generation of a product) as a function of time. For example we looked at a first order reaction

$$C \xrightarrow{k} P$$

And got the equation:

$$C_{(t)} = C_{initial} \; x \; e^{-(k \, x \, (t_{(t)} - t_{initial}))}.$$

This equation allowed us to calculate the concentration of C at any particular point of time, as long as we knew the initial concentration of C and the rate constant k. We confirmed that this indeed was a first order reaction – we did this by plotting ln (C) versus time, and got a straight line.

Looking at the data of the time course of the consumption of reactant C:

Time [min]	Concentration C [mM]
0	20
1	12.131
2	7.358
4	2.707
6	0.996
8	0.366
10	0.135
12	0.050

We can represent this as a first order reaction:

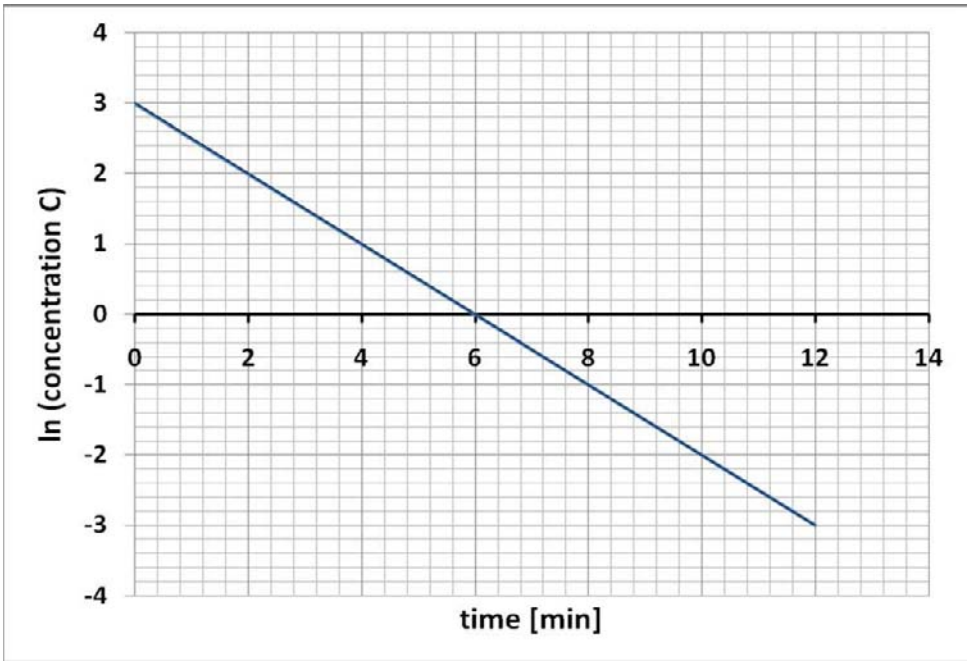


Figure 7-1 Graphic representation of a typical first order reaction

Our starting point for the development of the equation for such a first order reaction was the general rate equation:

$$\text{Rate} = \frac{dC}{dt} = -\,k \cdot C^1 \text{ and also}$$

$$\text{Rate} = \frac{dP}{dt} = +\,k \cdot C^1$$

These latter equations are quite interesting – they tell us, how the rate of the reaction depends on the concentration of C. It basically says:

The rate of the reaction is directly proportional to the concentration of C. The larger C the faster is the reaction. A graphical representation is shown as:

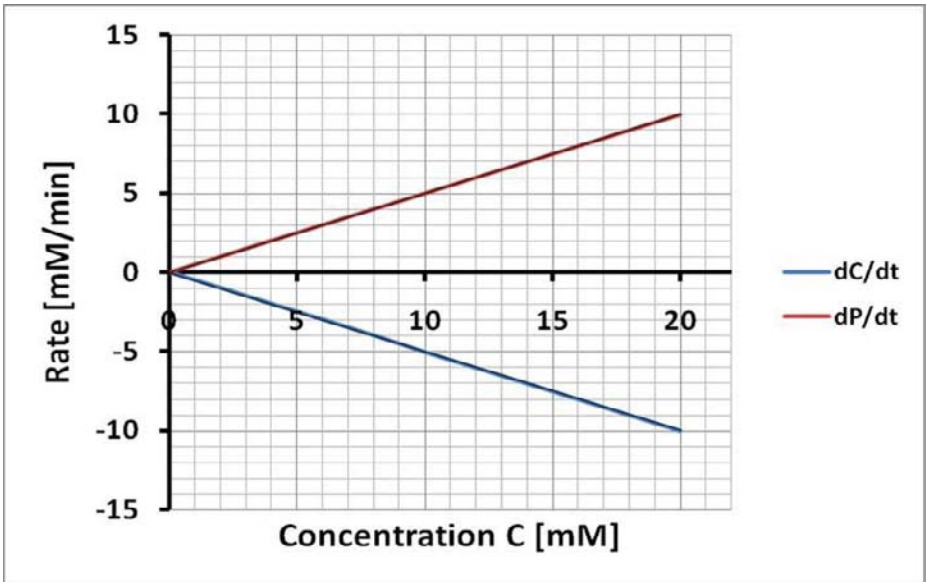


Figure 7-2 Representation of rate of a first order reaction in relation to the concentration of the reactant C

There are some interesting points about this graph. Firstly the rates for $\frac{dC}{dt}$ and $\frac{dP}{dt}$ are mirror images of each other. This is not really surprising, since the only difference between the two rates is the sign in front of k. Secondly both rates go through the origin. Again, not very surprising, if we take into account that without any reactant C present, there would not be a reaction and hence the rate would be zero.

From the first graph in Figure 7-1 we can see that the concentration of C changes all the time; we start with a concentration of 20 mM at $t_{initial}$ = 0 minutes, but after $t_{(t)}$ = 6 minutes our concentration of C is down to 1 mM.

With these data and our rate equation

$$\text{Rate} = \frac{dP}{dt} = + \text{k } x \text{ C}^1$$

We can use the graph in Figure 7-2 to determine the rate of the reaction at different time points:

$t_{initial}$ = 0 min	$C_{initial}$ = 20 mM	$Rate_{(0)}$ = 10 mM/min
$t_{(t)}$ = 6 min	$C_{(6)}$ = 1 mM	$Rate_{(6)}$ = 0.5 mM/min

And of course, we could do this for all the other rates in between $t_{initial}$ = 0 minutes and $t_{(t)}$ = 6 minutes. If we want to compare the rates of different reactions, we need to clearly specify, at which time-point we measured the reaction rate. Although we could do this easily and always state at what time we measured the rate, it is convention that in order to compare rates for different reaction we use the rate at the very beginning of the reaction as our point of reference.

This '$Rate_{(0)}$' is commonly referred to as the **'Initial Rate'** of a reaction. Using this 'initial rate' allows us to easily compare the rates of different reactions.

7.3. Effect of reactant concentrations on rates

Let's start with a simple zero order reaction of the scheme

$$A \xrightarrow{k} P$$

For the general rate equation we can write:

Rate = + k (if we look at the product).

What happens to the rate, if we double the concentration of A?

The answer is **NOTHING**.

The reason is simple – the rate of a zero order reaction is independent of the concentrations of any of the reactants. Although the reaction requires the presence of the reactants (otherwise there would not be a reaction), the rate of the reaction is always constant.

In other words, if we changed the concentration of A in our experiments, the rate would still stay the same.

Why am I telling you this? Well, let's have a look at a first order reaction of the scheme

$$A \xrightarrow{k} P$$

For the general rate equation we can write:

Rate = + k *x* A (if we look at the product).

What happens to the rate, if we double the concentration of A?
Instead of A we now have 2*x* A and the rate would be:

A	$Rate_{(A)} = k \; x \; A$	
2*x* A	$Rate_{(2xA)} = k \; x \; 2x \; A$	$Rate_{(2xA)} = 2x \; Rate_{(A)}$

Comparing $Rate_{(A)}$ with $Rate_{(2xA)}$ shows that by doubling the concentration of A we also double the rate.
In general terms (for a first order reaction):

The rate increases by *n* if we increase the concentration of the reactant by *n*.

What happens to a second order reaction of the scheme?

$$A + A \xrightarrow{k} P$$

We can write the general rate equation as:

$Rate = k \; x \; A^2$

What happens to the rate, if we double the concentration of A?

Instead of A we now have 2*x* A and our new rate would be:

A	$Rate_{(A)} = k \; x \; A^2$	
2*x* A	$Rate_{(2xA)} = k \; x \; (2x \; A)^2$ or $Rate_{(2xA)} = k \; x \; 2^2 x \; A^2$	$Rate_{(2xA)} = 4x \; Rate_{(A)}$

Our rate will quadruple if we double the concentration of A.

In general terms (for a second order reaction (A + A type)):

The rate increases by n^2 if we increased the concentration of the reactant by *n*.

What happens if we go for a 'third' order reaction? We have not discussed this case in previous chapters, but the scheme for it could be:

$A + A + A \xrightarrow{k} P$

We can write the general rate equation as:

$Rate = k \; x \; A^3$

What happens to the rate, if we double the concentration of A?

Instead of A we now have 2*x* A and our new rate would be:

$Rate = k \; x \; (2x \; A)^3$

$Rate = k \; x \; 2^3 x \; A^3$

The rate increases $2^3 =$ 8-fold if we double the concentration of A.

We do not necessarily have to double the concentration of A, we can increase it by whatever factor we want. In a more general form we can express our rate equation as:

$Rate_{(nxA)} = n^m \; x \; Rate_{(A)}$

In words: For a reaction of the *m*-th order the rate increases by n^m if we increase the concentration of the reactant by *n*.

7.4. Equations for the determination of rate orders

7.4.1. Equations for products

In the previous examples we calculated the effect of the rate order on the rate of the reaction. For example, we found that doubling the concentration of a compound in a first order reaction will double the initial rate of this reaction. For a general rate scheme:

$A \xrightarrow{k} P$

We can write:

$$Rate_{(P)} = \frac{dP}{dt} = k \; x \; A^m$$

And take the logarithms of both sides:

$\log (Rate_{(P)}) = \log (k \; x \; A^m)$.

This can be written as:

$\log (Rate_{(P)}) = \log (k) + m \, x \log (A)$.

Rearranging gives:

$\log (Rate_{(P)}) = m \, x \log (A) + \log (k)$

This again has the form of a straight line with:

$y = m X + C$

We therefore can plot $\log (Rate_{(P)})$ versus log (A) and get a straight line for which the gradient represents the rate order and the intersect with the y-axes log (k).

It does not matter, which logarithm we use – we can use the one to the base 10 or we can use ln, or in fact any other one, as long as we are consistent within the equation.

A typical plot for the reaction:

$A \xrightarrow{k} P$

With a data set of:

P [nM]	Rate [nM/min]
1	15
2	30
4	60
6	90
8	120
10	150

We get a graphic representation like:

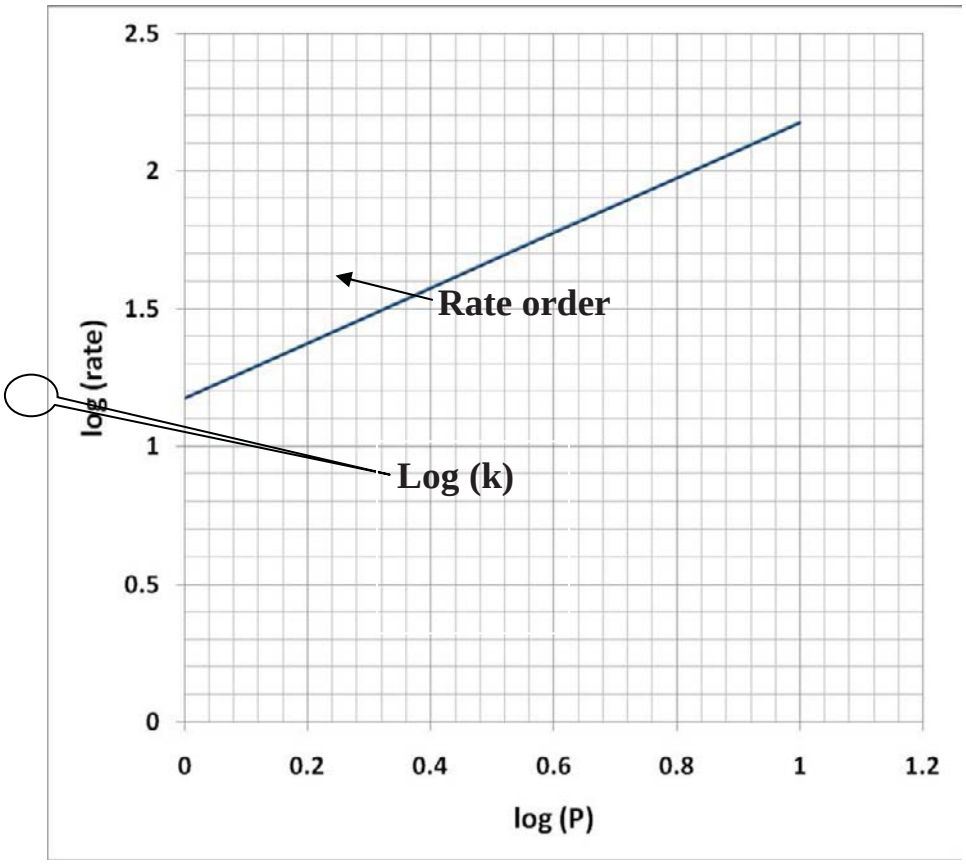


Figure 7-3 Example graph to determine rate order and rate constant of a reaction

7.4.2. Equations for reactants

So far we have looked at the generation of a product and all the rates were positive, but what if we look at the consumption of a reactant????

Since we cannot calculate the logarithm of something smaller than 0, we have to "cheat" a little bit. The best way of dealing with this situation is to 'ignore' the direction of the reaction and write for the reaction:

$$A \xrightarrow{k} P$$

$$Rate_{(A)} = \frac{dA}{dt} = |k \; x \; A^m|$$

The expression $|k \; x \; A^m|$ means that we treat $Rate_{(A)} = \frac{dA}{dt}$ as a **positive** rate. We then can write:

$$\log (Rate_{(A)}) = \log (k \; x \; A^m).$$

This can be written as:

$$\log (Rate_{(A)}) = \log (k) + m \; x \; \log (A).$$

Rearranging gives:

$$\log (Rate_{(A)}) = m \; x \; \log (A) + \log (k)$$

This again has the form of a straight line with:

$$y = m X + C$$

We therefore can plot $\log (Rate_{(A)})$ versus log (A) and get a straight line for which the gradient represents the rate order and the intersect with the y-axes log (k).

7.5. Problem: Determine the rate order of a reaction

Let's assume, we have the following reaction:

$$A \xrightarrow{k} P$$

And measure initial rates for different concentrations of A:

A [mM]	Initial Rate [mM/min]
0.1	-1.2
0.2	-3.394113
0.3	-6.235383
0.5	-13.41641
0.8	-27.1529

Determine the order of this reaction.

7.5.1. Graphic solution

Solution:

First calculate log (A) and log ($Rate_{(A)}$) - to do the latter we simply use the **positive** initial rates:

A [mM]	Rate [mM/min]	log (A)	log (Rate)
0.1	1.2	-1	0.079181
0.2	3.394113	-0.69897	0.530726
0.3	6.235383	-0.52288	0.794863
0.5	13.41641	-0.30103	1.127636
0.8	27.1529	-0.09691	1.433816

We plot a graph of log (A) versus log ($Rate_{(A)}$):

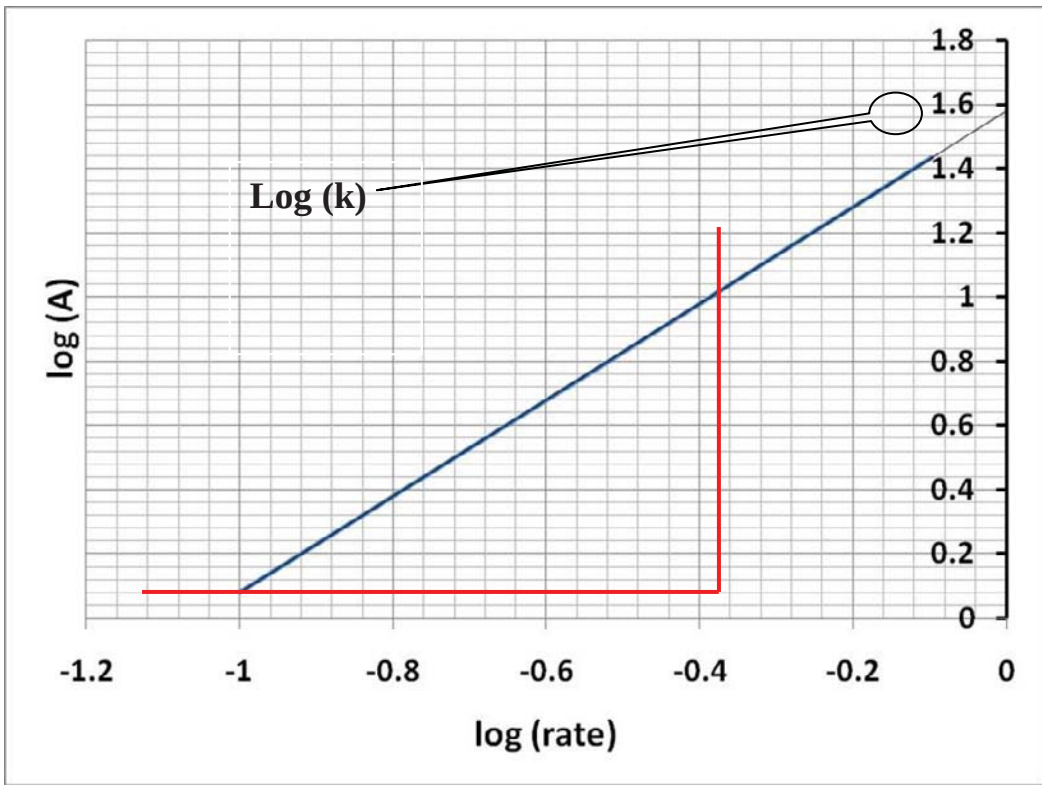


Figure 7-4 Graph to determine rate order and rate constant

The gradient of the graph gives the rate order**;** the intersect of the line with the y-axis shows the logarithm of the rate constant. Note – it is the logarithm of the rate constant and NOT the rate constant itself!

For the rate constant we get:

Log (k) = 1.58 and

$\underline{k = 10^{1.58} = 38.0}$.

The big problem is that we do not get any units for k with this method (because we use log (k)).

7.5.2. Calculating the rate order

As you probably can see from the graph in Figure 7-4, sometimes it is inconvenient to determine the gradient from a graph and hence it would be probably better, if we could calculate the rate order from our experimental data.

For that all we have to do is to pick two points from our data set and "calculate" the gradient.

A [mM]	Rate [mM/min]	log (A)	log (Rate)
0.1	1.2	-1	0.079181
0.2	3.394113	-0.69897	0.530726
0.3	6.235383	-0.52288	0.794863
0.5	13.41641	-0.30103	1.127636
0.8	27.1529	-0.09691	1.433816

We can set:

A1 = 0.1 mM	$Rate_{(A1)}$ = 1.2 mM/min	Log (A1) = -1	Log ($Rate_{(A1)}$) = 0.079
A2 = 0.8 mM	$Rate_{(A2)}$ = 27.15 mM/min	Log (A2) = -0.097	Log ($Rate_{(A2)}$) = 1.434

With these data we can now calculate our rate order by using the general equation for a gradient:

$$m = \frac{\Delta y}{\Delta x}$$

In our case we can write:

$$m = \frac{\Delta \log(Rate_{(A)})}{\Delta \log(A)} = \frac{\log(Rate_{(A2)}) - \log(Rate_{(A1)})}{\log(A2) - \log(A1)}$$

$$m = \frac{1.434 - 0.079}{-0.097 - (-1.0)} = \frac{1.355}{0.903} = 1.5.$$

The rate order for this reaction is:

$\underline{\underline{m = 1.5}}$.

Answer: the rate order for this reaction is 1.5.

With this our rate law expression can be written as:

$$\text{Rate} = \frac{dA}{dt} = k\ x\ A^{1.5}$$

We can also calculate the rate constant – all we have to do is rearrange the rate law expression and solve for k:

$$k = \frac{Rate_{(A1)}}{A1^{1.5}}$$

We can use any A1 and the corresponding $Rate_{(A1)}$, for example:

A1 = 0.1 mM	$Rate_{(A1)}$ = 1.2 mM/min

$$k = \frac{1.2 mM/\min}{(0.1 mM)^{1.5}} = \frac{1.2}{(0.1)^{1.5}} x \frac{mM}{\min x mM^{1.5}} = \frac{1.2}{0.032} x \frac{1}{\min x mM^{0.5}} = 37.9\ mM^{-0.5}\ min^{-1}$$

The rate constant for this reaction is:

$\underline{\underline{k = 37.9\ mM^{-0.5}\ min^{-1}}}$

7.5.3. The 'Unit' equation

The units for this rate constant look rather peculiar, since it contains '$mM^{-0.5}$'. This reflects the fact that the rate order for this reaction is not an integer number. One has to remember that the units for a rate always are concentration/time, e.g. mM^1/min^1. If the rate order is a non-integer number then the units for the concentration of the reactant is a non-integer number as well. As a consequence, to get to the integer units for the rate, we have to adjust the units for the rate constant accordingly.

A simple way of getting the units for the rate constant right is to write down the specific rate late equation, but instead of numbers, put in the units for the various compounds. For example the specific rate law expression for a reaction might look like this:

$$\frac{dA}{dt} = -\ k\ x\ A^{1.5}$$

If the unit of A is given in mM and the time in minutes, the unit equation looks like:

$$\frac{mM}{\min} = \text{k } x \text{ mM}^{1.5}$$

Since we are dealing with the units only, we do not have to worry about the negative sign in front of the rate constant and hence I omitted it. We can rearrange this equation to solve for the unit of k:

$$\text{Unit of k} = \frac{mM}{\min xmM^{1.5}}.$$

With this one 'mM' cancels out and we get:

$$\text{Unit of k} = \frac{1}{\min xmM^{0.5}}, \text{ which can also be written as:}$$

$$\text{Unit of k} = \text{mM}^{-0.5}\ \text{min}^{-1}.$$

A general rule for a reaction like:

$$\frac{dA}{dt} = \text{- k } x \text{ A}^{m}$$

Is that the unit for k can be written as:

Unit of k = concentration $^{m-1}$ time^{-1}

7.6. Rate orders and Molecularity of a reaction

In the previous example we calculated the rate order to be 1.5. This probably looks very strange, since in all examples before the rate orders were whole numbers. But clearly a rate order does not have to be an integer number!

Rate orders are defined as a mathematical way of linking rates to concentrations.

In fact, rate orders can be any number, even negative ones. Also, rate orders can change during a reaction. This might sound really weird, but it happens. For example the rate order of an enzyme reaction actually depends strongly on the concentration of the substrate. Depending on the substrate concentration the rate order of such a reaction shifts from a first order to a zero order reaction. It is therefore always a good idea to graphically examine whether t**he order of the reaction is dependent on the concentration of the reactants**. The easiest way of doing this is to plot log (rate) versus log (reactant), as we have done earlier in this chapter. If this gives a straight line, we can be sure that the rate order is independent of the concentration of the reactant.

Very often people confuse rate orders with what is called the “Molecularity of a Reaction”. This expression makes a statement of how many molecules interact in a reaction during the slowest (rate-limiting) step.

Molecularity is defined as the number of molecules that react in a specific process.

A reaction is said to be mono-molecular, if only 1 molecule is involved, e.g.

A $\xrightarrow{\text{-·-·-·}}$ products
(I have deliberately changed the style of the arrow to indicate that we are now looking at the molecularity and not the rate of this reaction)

In a bi-molecular reaction 2 molecules react with each other, e.g.

A + B $\xrightarrow{\text{-·-·-·}}$ products

Only mono- and bi-molecular reactions are actually known and so far, there is no evidence for a reaction with a higher molecularity. It would be rather surprising, if there was a tri-molecular reaction, because all the different molecules have to bump into each other at the same time with the right energy, which is very unlikely.

However, the polymerisation of prion molecules to form an aggregate is a 12-th order reaction. It does not mean that 12 prion molecules react at the same time, it just tells us that the rate can be expressed mathematically by a 12-th order process.

The following table gives a comparison between order and molecularity of a reaction:

Order	Molecularity
Mathematical expression to link rates and concentrations of reactants or products.	Number of molecules participating in the rate-limiting step of a reaction.
Can be any real number, even negative ones.	Can only be a whole number, either 1 or 2.
Can change in the course of a reaction.	Cannot change in the course of a reaction.
Can be determined mathematically.	Cannot be determined easily.

7.7. Fractal and negative rate orders

I just mentioned that the order of a reaction can be any real number, even a negative one, but what does this actually mean?

7.7.1. Fractal rate orders

In the previous example we determined the rate order to be m = 1.5. How can we interpret this result?

Let's assume, we have the following reaction:

$$A \xrightarrow{k} P$$

With the general rate equation

Rate = $k \, x \, A^m$.

We allow m to be 1.0, 1.5 and 2.0, respectively.

We set the rate constant to 38 (for simplicity sake we do not worry about the units in this example, but you should work out the units of k for each rate order) and get the following data:

A [mM]	Rate (m =1.0) [mM/min]	Rate (m=1.5) [mM/min]	Rate (m=2.0) [mM/min]
0.1	3.8	1.201666	0.38
0.2	7.6	3.398823	1.52
0.3	11.4	6.244037	3.42
0.5	19	13.43503	9.5
0.8	30.4	27.19059	24.32
1	38	38	38
2	76	107.4802	152

Plotting the corresponding graph gives us:

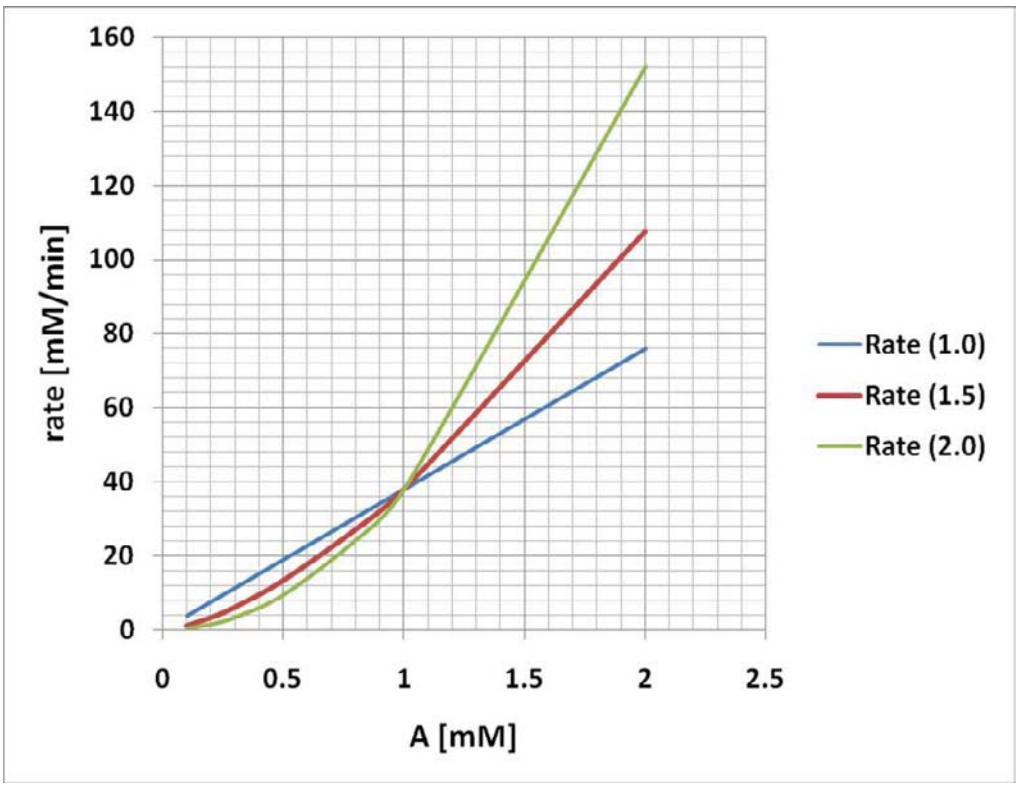


Figure 7-5 Graphic representation of different rate orders

This graph shows that the rate for the reaction with m=1.5 is between the two other rates. There is nothing special about this particular rate order – it just says that at concentrations of A > 1 unit (here mM) the rate for m=1.5 is faster than the rate for m=2.0.

7.7.2. Negative rate orders indicate inhibition of a reaction

What about negative rate orders? How can we interpret those?
Let's assume, we have the following reaction:

$$A \xrightarrow{k} P$$

With the specific rate equation

$$\frac{dA}{dt} = - k \, x \, A^{-1}.$$

We can write this equation also as:

$$\text{Rate} = k\, x\, \frac{1}{A}$$

With a rate constant of $k = 0.1\ mM^2\ min^{-1}$ we get a graph like:

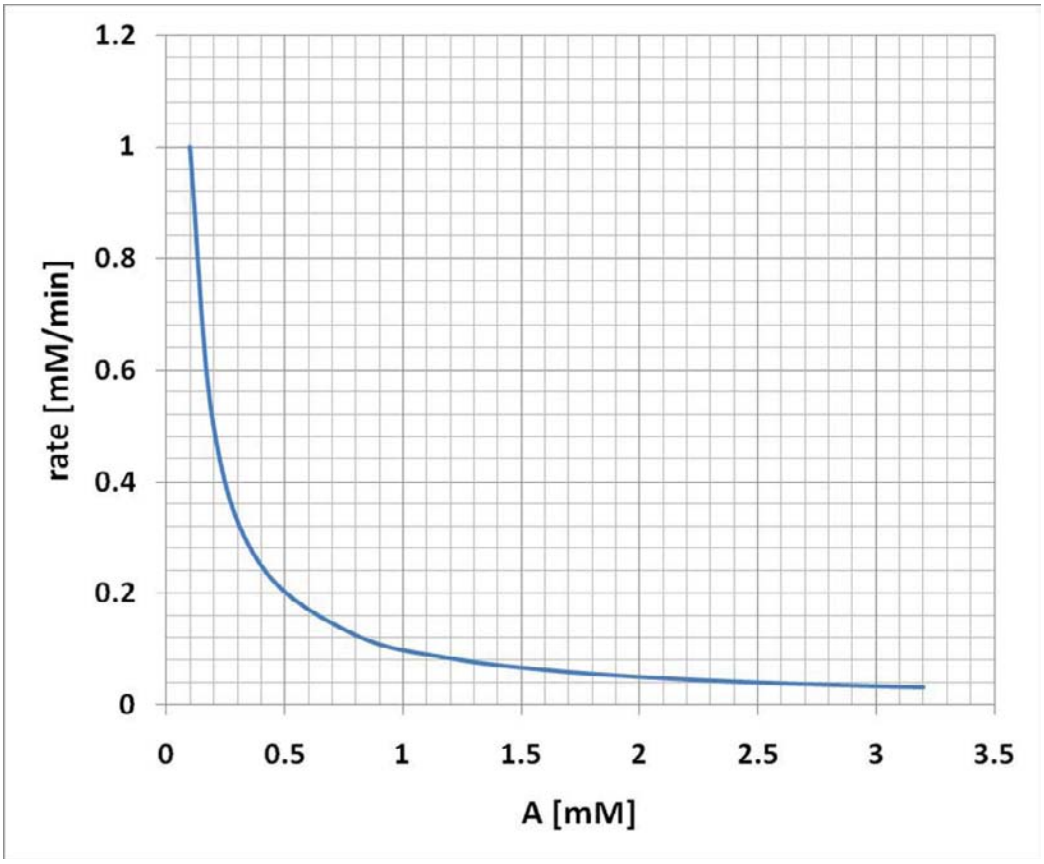


Figure 7-6 Graphic representation of a negative rate order

This graph shows that higher concentrations of A result in a decrease in the rate of the reaction. The most obvious explanation for such a result is that A is in fact an inhibitor of the reaction. Although it is not really relevant for a reaction that follows the scheme of:

$$A \xrightarrow{k} P$$

It becomes more relevant for reactions that contain several reactants, like:

$$A + B + C \xrightarrow{k} P$$

We will discuss these reactions in the next chapter.

7.8. Problem Solving Strategy

To determine the rate order of a reaction, several steps need to be carried out:

- Change the concentration of the reactant, but leave all other reaction conditions (temperature, volume, concentration of any other components, etc) the same.
- Measure the initial rate of the reaction for each concentration of the varied reactant.
- Calculate 'log (rate)' for each 'log (reactant)' and plot the data. If this results in a straight line, then it can be assumed that the rate order of the reaction is constant over the range of reactant concentrations.
- Determine the gradient of the graph.
- Alternatively, if the rate order is independent of the concentration of the reactant, the rate order can be calculated by using the equation:

$$m = \frac{\Delta \log(Rate_{(A)})}{\Delta \log(A)} = \frac{\log(Rate_{(A2)}) - \log(Rate_{(A1)})}{\log(A2) - \log(A1)}$$

7.9. More practice questions

1. A new drug against coronary heart diseases was tested and the elimination of this drug was measured by taking blood samples and analysing them for the production of a fairly stable breakdown product of the drug. The following initial rates for the breakdown product formation were recorded:

Drug [nM]	Rate of product formation [nM/hour]
1	0.10
2	0.48
4	2.26
6	5.63
8	10.76
10	17.78

Determine the rate order and rate constant of this reaction.

Answer:

Rate order = 2.25.
$k = 0.1\ nM^{-1.25}\ h^{-1}$

2. Another drug against coronary heart diseases was tested and, again, the elimination of this drug was measured by taking blood samples and analysing them for the production of a fairly stable breakdown product of the drug. The following initial rates for the breakdown product formation were recorded:

Drug [nM]	Rate [nM/hour]
1	0.20
2	0.67
4	2.26
6	4.60
8	7.61
10	11.25

Determine the rate order and rate constant of this reaction.

Answer:

Rate order = 1.75.
$k = 0.2\ nM^{-0.75}\ h^{-1}$

3. A new drug was developed to increase the uptake of glucose into cells of patients suffering from diabetes. In a large drug trial the initial rates of glucose uptake were measured in response to different drug concentrations. The following data were recorded:

Drug [nM]	Rate of glucose uptake [mM/min]
1	2.0
2	1.4
4	1.0
6	0.8
8	0.7
10	0.6

Determine the rate order of this reaction and interpret your results.

Answer:

Rate order = -0.52. Increasing the drug concentrations leads to a DECREASE in the rate of glucose uptake. Obviously, the drug does not stimulate the uptake reaction, but inhibits it.

4. In an enzyme-catalysed reaction the substrate concentration was varied and the resulting initial rates were recorded. The following data were obtained:

Substrate [mM]	Rate [mM/min]
2	25.0
4	40.0
6	50.0
10	62.5
20	76.9
80	93.0

Determine the order of the reaction.

Answer:

A plot of log (rate) versus log (substrate) shows a non-linear graph, indicating that the order of the reaction depends on the substrate concentration. A unique rate order for all different substrate concentrations cannot be determined.

8. Complex reactions with several reactants

8.1. Introduction and learning outcomes

In the previous chapter we investigated how the order of a reaction can be determined by changing the concentration of a reactant and measuring the resultant change in the rate of the reaction. In this chapter we will build up on this knowledge and investigate how we can analyse reactions with several reactants. In chapter 6 we already discussed a special case of this type of reactions, namely a second order reaction, in which the two reactants are not identical. By the end of this chapter you will be able to:

- Determine rate orders of reactions with several reactants
- Determine the rate constant of reactions with several reactants.

8.2. The general rate law expression for reactions with several reactants

In chapter 6 we discussed a reaction that can be written as:

$$A + B \xrightarrow{k} P$$

Although we found that the equations to express the different concentrations of A and B at various time points were rather complex, we could very easily write the general rate equation for this type of reaction as:

$$\text{Rate} = \frac{dP}{dt} = k\, x\, A^1\, x\, B^1$$

We can now expand this knowledge for more complex reactions, in which even more reactants participate. For example we can formulate the general rate law expression for a reaction like:

$$A + B + C + D \xrightarrow{k} P \quad \text{as}$$

$$\text{Rate} = \frac{dP}{dt} = k\, x\, A^m\, x\, B^n\, x\, C^q\, x\, D^r$$

I am using $\frac{dP}{dt}$ as the rate, since it is easier to look at the generation of the product in this case, but in principal I could use any of the other compounds to formulate the rate. By using $\frac{dP}{dt}$ as the rate I also avoid the use of a negative sign.

The above format is widely known as the ‘general rate law expression’. The term ‘general’ is used to indicate that we have not yet specified the various coefficients, which represent the individual rate orders. Once we have determined these coefficients the general rate law expression is turned into the ‘specific rate law expression’.

But how can we determine these individual rate orders?

8.3. Determining individual rate orders from experimental data

To determine the individual rate orders and thus convert our general rate law expression into the specific rate law expression, we apply the approach from the previous chapter. There we said that to determine the order of a reaction we need to:

- Change the concentration of the reactant, but leave all other reaction conditions (temperature, volume, concentration of any other components, etc) the same.
- Measure the initial rate of the reaction for each concentration of the varied reactant.
- Calculate 'log (rate)' for each 'log (reactant)' and plot the data. If this results in a straight line, then it can be assumed that the rate order of the reaction is constant over the range of reactant concentrations.
- Determine the gradient of the graph.
- Alternatively, if the rate order is independent of the concentration of the reactant, the rate order can be calculated by using the equation:

$$\text{Rate order} = \frac{\Delta \log(Rate_{(A)})}{\Delta \log(A)} = \frac{\log(Rate_{(A2)}) - \log(Rate_{(A1)})}{\log(A2) - \log(A1)}$$

By applying this scheme we will be able to define the various rate orders.

8.3.1. Problem: Experimental approach to determine individual rate orders of a reaction

Let's assume we have a reaction that contains three different reactants:

$$A + B + C \xrightarrow{k} P$$

The general rate law expression can be written as:

$$\text{Rate} = \frac{dP}{dt} = k\ x\ A^m\ x\ B^n\ x\ C^q$$

This is reaction is *m*-th order with respect to A, *n*-th order with respect to B and *q*-th order with respect to C.

The overall order of a reaction is the sum of all individual rate orders

In this case the overall rate order is m + n + q.

We carry out a number of experiments in which we change the concentrations of individual reactants (but only one at the time) and measure the initial rate of the reaction.

Let's say we start with all our concentrations at 0.1 mM and we determine the initial rate to be 10 mM/min. We can write this in the form of a table:

Experiment	A [mM]	B [mM]	C [mM]	Rate [mM/min]
1	0.1	0.1	0.1	10

In the next experiment, we change the concentration of A, but leave all other conditions, including the concentrations of B and C, constant and again measure the initial rate:

Experiment	A [mM]	B [mM]	C [mM]	Rate [mM/min]
2	0.2	0.1	0.1	40

We now can determine the specific rate order for A by using the equation:

$$\text{Rate order} = \frac{\Delta \log(Rate_{(A)})}{\Delta \log(A)} = \frac{\log(Rate_{(A2)}) - \log(Rate_{(A1)})}{\log(A2) - \log(A1)}$$

Putting in the numbers for the various concentrations of A and the resulting initial rates gives us:

$$\text{Rate order} = \frac{\log(40) - \log(10)}{\log(0.2) - \log(0.1)} = \frac{1.602 - 1.0}{-0.699 - (-1.0)} = \frac{0.602}{0.301} = 2.$$

The specific rate order for A is:

m = 2.

To determine the specific rate order for B, we use the same approach. In our first experiment we already measured the rate of the reaction at a concentration of B = 0.1 mM:

Experiment	A [mM]	B [mM]	C [mM]	Rate [mM/min]
1	0.1	0.1	0.1	10

In the next experiment, we change the concentration of B, but leave all other conditions, including the concentrations of A and C, constant and again measure the initial rate:

Experiment	A [mM]	B [mM]	C [mM]	Rate [mM/min]
3	0.1	0.2	0.1	40

We now can determine the specific rate order for B by using the equation:

$$\text{Rate order} = \frac{\Delta \log(Rate_{(B)})}{\Delta \log(B)} = \frac{\log(Rate_{(B2)}) - \log(Rate_{(B1)})}{\log(B2) - \log(B1)}$$

Putting in the numbers for the various concentrations of B and the resulting initial rates gives us:

$$\text{Rate order} = \frac{\log(40) - \log(10)}{\log(0.2) - \log(0.1)} = \frac{1.602 - 1.0}{-0.699 - (-1.0)} = \frac{0.602}{0.301} = 2.$$

The specific rate order for B is:
n = 2.

Finally to get the specific rate order of C, we carry out a fourth experiment, in which we change the concentration of C, but leave all other conditions, including the concentrations of A and B, constant and again measure the initial rate:

Experiment	A [mM]	B [mM]	C [mM]	Rate [mM/min]
1	0.1	0.1	0.1	10
4	0.1	0.1	0.2	20

Putting in the numbers for the various concentrations of C and the resulting initial rates gives us:

$$\text{Rate order} = \frac{\log(20) - \log(10)}{\log(0.2) - \log(0.1)} = \frac{1.301 - 1.0}{-0.699 - (-1.0)} = \frac{0.301}{0.301} = 1.$$

The specific rate order for C is:
q = 1.

With the specific rate orders we now can write our 'specific rate law expression':

$$\text{Rate} = \frac{dP}{dt} = k \, x \, A^2 \, x \, B^2 \, x \, C^1$$

The reaction is second order with respect to A and B and a first order for C.
The overall order of the reaction is:

$m + n + q = 2 + 2 + 1 + 5$.

Overall the reaction is of a fifth-order.

In a real experiment we certainly would test more concentrations of the individual reactants, just to be on the safe side. We would do something like:

Experiment	A [mM]	B [mM]	C [mM]	Rate [mM/min]
1	0.1	0.1	0.1	10
2	0.2	0.1	0.1	40
3	0.4	0.1	0.1	160
4	0.6	0.1	0.1	360
5	0.8	0.1	0.1	640
6	1.0	0.1	0.1	1000
7	0.1	0.2	0.1	40
8	0.1	0.4	0.1	160
9	0.1	0.6	0.1	360

8.3.2. Determine the rate orders of a reaction from a data set

Quite frequently the experiments have already been done and we only get a set of data from which we have to determine the various rate orders. This is in principal the same as what we did in the previous example. The trick here is to choose those experimental data that give us the 'right information.

Let's take the previous example and let's assume that we have done the experiments and determined the resulting rates:

Experiment	A [mM]	B [mM]	C [mM]	Rate [mM/min]
1	0.1	0.1	0.1	10
2	0.2	0.1	0.1	40
3	0.1	0.2	0.1	40
4	0.1	0.1	0.2	20

If we want to determine the individual rate order for A, we have to pick the two experiments in which the concentrations for A were changed, but the concentrations for B and C were unaltered.

This is experiment 1 and 2:

Experiment	A [mM]	B [mM]	C [mM]	Rate [mM/min]
1	0.1	0.1	0.1	10
2	0.2	0.1	0.1	40
3	0.1	0.2	0.1	40
4	0.1	0.1	0.2	20

Now let's determine the individual rate order for B. We have to pick the two experiments in which the concentrations for B, but not the concentrations for A and C were changed.

This is experiment 1 and 3:

Experiment	A [mM]	B [mM]	C [mM]	Rate [mM/min]
1	0.1	0.1	0.1	10
2	0.2	0.1	0.1	40
3	0.1	0.2	0.1	40
4	0.1	0.1	0.2	20

Last but not least, to determine the rate order for C we have to pick the two experiments in which the concentrations for C, but not the concentrations for A and B were cahnged.

This is experiment 1 and 4:

Experiment	A [mM]	B [mM]	C [mM]	Rate [mM/min]
1	0.1	0.1	0.1	10
2	0.2	0.1	0.1	40
3	0.1	0.2	0.1	40
4	0.1	0.1	0.2	20

From these data sets we are able to determine all the individual specific rate orders. This data set is therefore sufficient for our purposes and we do not have to add any further experiments.

8.4. Determine the rate constant for a reactions with several reactants

The experimental data we gathered (or used) even allow us to determine the rate constant k for this particular reaction. A prerequisite for this, however, is that we can formulate our specific rate law expression with individual rate orders. For example the reaction:

$$A + B + C + D \xrightarrow{k} P$$

with the general rate law expression:

$$\text{Rate} = \frac{dP}{dt} = k \, x \, A^m \, x \, B^n \, x \, C^q$$

And the specific rate law expression:

$$\text{Rate} = \frac{dP}{dt} = k \, x \, A^2 \, x \, B^2 \, x \, C^1$$

can be used to determine the rate constant k. All we need to do is to use our experimental data and rearrange the equation to:

$$k = \frac{Rate}{A^2 x B^2 x C^1}$$

We can choose any of the experimental data sets to calculate the rate constant. For example:

Experiment	A [mM]	B [mM]	C [mM]	Rate [mM/min]
1	0.1	0.1	0.1	10

With the numbers added:

$$k = \frac{10mM/\min}{(0.1mM)^2 x (0.1mM)^2 x (0.1mM)^1}$$

If we chose our data set such that all the concentrations of the reactants are the same, we can make life much easier for us. In this case we can combine all the concentrations and simple add up the individual rate orders:

$$k = \frac{10mM/\min}{(0.1mM)^{2+2+1}} = \frac{10mMx\min^{-1}}{0.1^5 mM^5}$$

Our rate constant therefore would be:

$$\underline{k = 10^6\ mM^{-4}\ min^{-1}}$$

With a different data set, we would obtain the same result, but it would be a bit more complicated:

Experiment	A [mM]	B [mM]	C [mM]	Rate [mM/min]
2	0.2	0.1	0.1	40

$$k = \frac{40mM/\min}{(0.2mM)^2 x (0.1mM)^2 x (0.1mM)^1}$$

$$k = \frac{40mM/\min}{(0.2^2 mM^2 x 0.1^2 mM^2 x 0.1^1 mM^1}$$

$$k = \frac{40mMx\min^{-1}}{0.04x0.01x0.1mM^5}$$

$\underline{k = 10^6\ mM^{-4}\ min^{-1}}$

It's the same result, it just requires a bit more calculations.

8.5. Problem: Calculate rates and concentrations of reactants from data sets

Quite often, we would like to make predictions about rates and concentrations of reactants, once we have determined the individual rate orders and rate constants of a specific experiment. This is fairly easy and needs only a little bit of re-arranging of equations.

8.5.1. Calculate rates from varied concentrations

Let's assume we want to predict, how the rate of the previous reaction changes, if we increase the concentration of A.

Experiment	A [mM]	B [mM]	C [mM]	Rate [mM/min]
1	0.1	0.1	0.1	10
2	0.2	0.1	0.1	40
3	0.1	0.2	0.1	40
4	0.1	0.1	0.2	20
5	0.3	0.1	0.1	?????

Determine the rate for experiment 5.

Solution:

The specific rate law expression for this reaction, with the rate constant and the specific rate orders calculated as before, can be written as:

Rate = $10^6\ mM^{-4}\ min^{-1}\ x\ A^2\ x\ B^2\ x\ C^1$

All we need to do is put in the concentrations from experiment 5 and get:

$Rate_{ex5} = 10^6\ mM^{-4}\ min^{-1} \times (0.3mM)^2 \times (0.1mM)^2 \times (0.1mM)^1$

$Rate_{ex5} = 10^6\ mM^{-4}\ min^{-1} \times 0.09mM^2 \times 0.01mM^2 \times 0.1mM$

$Rate_{ex5} = 10^6\ mM^{-4}\ min^{-1} \times 9x10^{-5}\ mM^5$

$\underline{Rate_{ex5} = 90\ mM\ min^{-1}}$

We can do a quick check, to see whether this result is realistic:
Compared to experiment 1 we increased the concentration of A in experiment 5 by 3-fold. Since the specific rate order of A is 2, we can write:

$Rate_{ex5} = 3^2 \times Rate_{ex1}$

$Rate_{ex5} = 9 \times 10\ mM\ min^{-1}$

8.5.2. Calculate the concentration of a reactant from a data set

Let's assume we measured the rate of the previous reaction, but were not sure at which concentration of B the reaction was measured. This actually happens quite frequently, if we use our experiments as an assay to determine unknown concentrations. In this case we use experiments 1 – 5 as a 'standard curve' from which we then calculate unknown concentrations.

Let's try and calculate the concentration of B, which gives us a rate of 160 mM/min, at 0.1 mM of A and C, respectively.

Experiment	A [mM]	B [mM]	C [mM]	Rate [mM/min]
1	0.1	0.1	0.1	10
2	0.2	0.1	0.1	40
3	0.1	0.2	0.1	40
4	0.1	0.1	0.2	20
5	0.3	0.1	0.1	90
6	0.1	????	0.1	160

Solution:

As in the example above we can write the specific rate law expression, in which we include the individual rate orders for A, B and C and also the rate constant. We then re-arrange the equation and solve for B. However, there is an easier way to deal with this kind of problem.

The specific rate law expressions for experiment 1 and experiment 6 are:

$Rate_{ex6} = k \times (0.1mM)^2 \times (B_{ex6})^2 \times (0.1mM)^1$ and

$Rate_{ex1} = k\ x\ (0.1mM)^2\ x\ (B_{ex1})^2\ x\ (0.1mM)^1$

We now divide the two equations and get:

$$\frac{Rate_{ex6}}{Rate_{ex1}} = \frac{kx(0.1mM)^2 x(B_{ex6})^2 x(0.1mM)^1}{kx(0.1mM)^2 x(B_{ex1})^2 x(0.1mM)^1}$$

Although it looks rather convoluted, we can cancel out most of the terms and are left with:

$$\frac{Rate_{ex6}}{Rate_{ex1}} = \frac{(B_{ex6})^2}{(B_{ex1})^2}$$

Since both parts of the right-hand side of the equation have the same exponents, this can also be written as:

$$\frac{Rate_{ex6}}{Rate_{ex1}} = (\frac{B_{ex6}}{B_{ex1}})^2$$

To find B_{ex6} we need to rearrange the equation to:

$$B_{ex6} = B_{ex1} \; x \; \sqrt{\frac{Rate_{ex6}}{Rate_{ex1}}}$$

$$B_{ex6} = 0.1\text{mM} \; x \; \sqrt{\frac{160 mM \; \text{min}^{-1}}{10 mM \; \text{min}^{-1}}} = 0.1\text{mM} \; x \; \sqrt{16} = 0.1\text{mM} \; x \; 4$$

$\underline{B_{ex6} = 0.4\text{mM}}$

In this case the solution was fairly easy, however, sometimes we have non-integer rate orders, e.g. we get an equation like:

$$\frac{Rate_{ex6}}{Rate_{ex1}} = \left(\frac{B_{ex6}}{B_{ex1}}\right)^m$$

In this case the solution is:

$$B_{ex6} = B_{ex1} \; x \; \sqrt[m]{\frac{Rate_{ex6}}{Rate_{ex1}}}$$

Since not all calculators have a function to calculate $\sqrt[m]{\;}$ we have to calculate this equation using logarithms. For this we need to take the logarithms of both sides of the equation and get:

$$\text{Log}\left(\frac{Rate_{ex6}}{Rate_{ex1}}\right) = \text{m} \; x \; \log\left(\frac{B_{ex6}}{B_{ex1}}\right)$$

This equation can also be written as:

$$\text{Log}\left(\frac{Rate_{ex6}}{Rate_{ex1}}\right) = \text{m} \; x \; (\log(B_{ex6}) - \log(B_{ex1}))$$

Rearranging of this equation and solving for log (B_{ex6}) gives:

$$\text{Log}(B_{ex6}) = \log(B_{ex1}) + \left(\log\left(\frac{Rate_{ex6}}{Rate_{ex1}}\right)\right) / \text{m}$$

Putting in numbers:

$$\text{Log}(B_{ex6}) = \log(0.1) + \left(\log\left(\frac{160}{10}\right)\right) / 2$$

$\log(B_{ex6}) = -1 + (\log(16))/2$

$\log(B_{ex6}) = -1 + \frac{1.2}{2} = -1 + 0.6 = -0.4.$

$\log(B_{ex6}) = -0.4.$

To get the concentration of B_{ex6} and not just $\log(B_{ex6})$, we have to do the reverse operation of log:

$B_{ex6} = 10^{\log(Bex6)} = 10^{-0.4}$

$\underline{B_{ex6} = 0.4 \text{ mM}}$

Admittedly, this calculation is fairly long-winded, but it is arguably the best way to calculate concentrations for reactants with fractal, i.e. non-integer rate orders.

8.6. Problem Solving Strategy

For a reaction that follows the scheme:

$A + B + C \xrightarrow{k} P$

And for which a complete data set is available, we first write the general rate law expression as:

$\text{Rate} = k \times A^m \times B^n \times C^q$

To determine the specific rate order m we need to:

- Choose two experiments from the data set, in which the concentrations of A, but not the concentrations of B and C have been varied.

- Calculate 'log (rate)' and 'log (A)'
- Calculate the rate order using the equation:

$$\text{Rate order} = \frac{\Delta \log(Rate_{(A)})}{\Delta \log(A)} = \frac{\log(Rate_{(A2)}) - \log(Rate_{(A1)})}{\log(A2) - \log(A1)}$$

Use the same approach to determine n and q.

To determine the rate constant k:

- Write the specific rate law expression

  Rate = k x A^m x B^n x C^q

  In which the individual rate orders have been determined.

- Calculate k from one of the data sets, e.g. experiment 1:

$$k = \frac{Rate_1}{A^m x B^n x C^q}$$

- Pay attention to the unit of k.

8.6. More practice questions

1. For the reaction

$$A + B + C \xrightarrow{k} \text{Products}$$

you measure the following initial rates:

A [M]	B [M]	C [M]	Rate [M/s]
0.1	0.1	0.1	0. 14
0.2	0.1	0.1	0. 198
0.1	0.3	0.1	1.26
0.2	0.1	0.15	0. 364

a) Determine rate orders of A, B and C. What is the overall rate order?
b) What is the rate constant?
c) How would the rate constant change when you double the concentration of A, B and C simultaneously?

Answers:

A = 0.5, B = 2, C = 1.5
Overall rate order = 4
k = 1400 M^{-3} s^{-1}
Rate constant does not change.

2. For the reaction

$A + B + C \xrightarrow{k}$ Products

You determine the initial rates at different concentrations of A, B and C, respectively, and get the following results:

A [M]	B [M]	C [M]	Rate [M/s]
0.1	0.1	0.1	0.0012
0.2	0.1	0.1	0.0024
0.1	0.3	0.1	0.0036
0.2	0.1	0.15	0.0054
0.2	0.2	0.2	???

e) Determine the rate orders of A, B and C. What is the overall rate order?
f) What is the rate constant?
g) What is the rate of the reaction if all components were present at 0.2 M?

Answers:

A = 1, B = 1, C = 2, overall order = 4
k = 12 M^{-3} s^{-1}
0.0192 M/s

3. For the reaction

$$A + B + C \xrightarrow{k} \text{Products}$$

You determine the initial rates at different concentrations of A, B and C, respectively, and get the following results:

A [mM]	B [mM]	C [mM]	Rate [mM/min]
0.1	0.1	0.1	0.250
0.2	0.1	0.1	1.000
0.1	0.3	0.1	2.250
0.2	0.1	0.15	3.375
0.2	??	0.2	72.000

a) Determine the rate orders of A, B and C. What is the overall rate order?
b) What is the rate constant?
c) What is the concentration of B in the last experiment?

Answers:

A = 2, B = 2, C = 3, overall rate order = 7
$k = 2.5 \times 10^{6}$ mM^{-6} min^{-1}
B = 0.3 mM

9. Coupled reactions

9.1. Introduction and learning outcomes

In previous chapters we looked at specific reactions and explored the equations for their kinetics. For example, we looked at the reaction

$$A + B + C \xrightarrow{k} \text{products}$$

For which we determined the individual rate orders and rate constant.

However, processes in a biological (or chemical) system are usually far more complex than this. Going back to our example how cancer develops (see chapter 1) we found that the strength of the signal, which determines how much a cell grows, depends on the concentration of the receptor/ligand dimer. The concentration of dimer itself depends not only on how fast the dimer is produced but also how fast the dimer is degraded.

In this case we have a 'coupled' reaction, which we could write as:

$$2\ \text{R(eceptor)L(igand)} \xrightarrow{k1} \text{R(eceptor)D(imer)} \xrightarrow{k2} \text{deg(radation)}$$

Another example of a 'coupled' reaction would be the fate of the receptor/ligand complex.

We said that the formation of the receptor/ligand complex can be written as:

$$\text{R(eceptor)} + \text{L(igand)} \xrightarrow{kf} \text{R(eceptor)L(igand)}$$

But some of the receptor/ligand complexes might react backwards:

$$\text{R(eceptor)L(igand} \xrightarrow{kr} \text{R(eceptor)} + \text{L(igand)}$$

and we can write:

$$\text{R(eceptor)} + \text{L(igand)} \underset{k}{\longleftrightarrow} \text{R(eceptor)L(igand)}$$

In this case we have a reversible reaction. It is very important to mention that most reactions in biological systems tend to be reversible (at least to a certain extent), and therefore we need to include these examples in our discussion of reaction kinetics.

The aim of this chapter is to introduce coupled reactions and to provide mathematical concepts to understand the underlying kinetic principles. By the end of this chapter you will be able to:

- Write complex reactions using the *General Mass Action* (GMA) representation
- Calculate equilibrium constants from kinetic data
- Understand the concept of 'steady state'
- Calculate steady state concentrations of intermediates in linear pathways.

9.2. The *General Mass Action* (GMA) representation for complex reactions

Let's have a look at the first example in which we combine a second order reaction with a first order reaction: Two receptor/ligand complexes dimerize to form a receptor/ligand dimer. This dimer then undergoes degradation. We can represent this reaction in the following way:

$$2\ \mathrm{R(eceptor)L(igand)} \xrightarrow{k1} \mathrm{R(eceptor)D(imer)} \xrightarrow{k2} \mathrm{deg(radation)}$$

To analyse this reaction we can split up the 'coupled' reaction and assume that it is composed of a first 'elementary' reaction E1:

$$2\ \mathrm{R(eceptor)L(igand)} \xrightarrow{k1} \mathrm{R(eceptor)D(imer)}$$

We assume that E1 can be represented by a second order reaction of the type A + A, in which the reactants are the same (we have discussed this special case in chapter 5).

The second reaction E2 can be defined as:

$$\mathrm{R(eceptor)D(imer)} \xrightarrow{k2} \mathrm{deg(radation)}$$

For simplicity sake, we assume that E2 can be represented by a first order reaction.

If we want to analyse the different components of this reaction we must look at three different rates, namely $\frac{dRL}{dt}$, $\frac{dRD}{dt}$ and $\frac{d\,\mathrm{deg}}{dt}$.

For the analysis of the first component, RL, we can write:

$$\frac{dRL}{dt} = -\,\mathrm{E1} = -\,\mathrm{k1}\ x\ \mathrm{RL}^2$$

A similar approach is used for the third component, deg:

$$\frac{d\,\mathrm{deg}}{dt} = +\,\mathrm{E2} = +\,\mathrm{k2}\ x\ \mathrm{RD}^1$$

The really interesting part is the analysis of the RD-complex. Clearly this reaction is composed of a combination of the two 'elementary reactions' E1 and E2.

For the **production** of the dimer we can write:

$$\frac{dRD1}{dt} = + E1 = k1 \; x \; RL^2$$

And for the subsequent **consumption** we write:

$$\frac{dRD2}{dt} = - E2 = - k2 \; x \; RD^1$$

$\frac{dRD1}{dt}$ and $\frac{dRD2}{dt}$ are the rates of the different elementary reactions.

To combine the two reactions into one we combine the two elementary reactions and write:

$$\frac{dRD}{dt} = \frac{dRD1}{dt} + \frac{dRD2}{dt}$$

$$\frac{dRD}{dt} = k1 \; x \; RL^2 - k2 \; x \; RD^1$$

This differential equation can be solved, but very often things get a bit messy with these equations. Bioscientists therefore use computer programmes designed to 'model' such interactions. One of these programmes is called Jdesigner and can be downloaded for free from http://www.cds.caltech.edu/~hsauro/JDesigner.htm.

A typical graphical representation might look like:

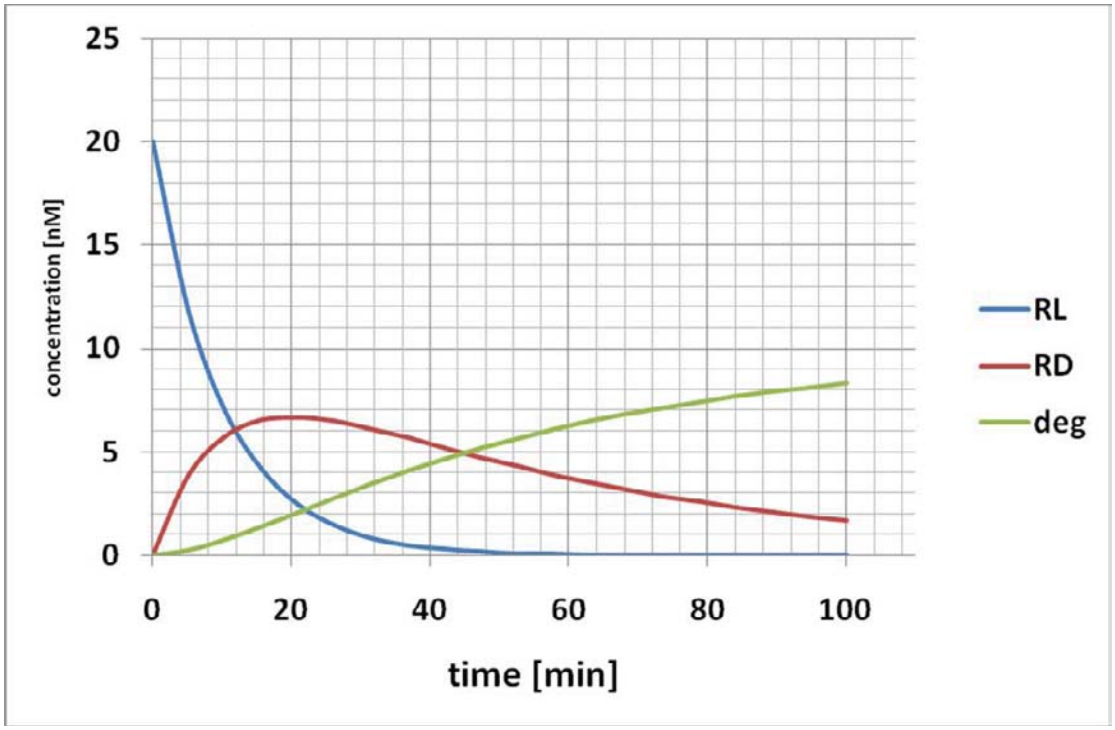


Figure 9-1 Graphic representation of a coupled reactions. For illustration purposes the following initial values have been used: **RL = 20 nM; RD = 0; deg = 0; k1 = 0.05 nM^{-1} min^{-1}; k2 = 0.02 min^{-1}.**

In general, for a reaction of the scheme:

$$A \xrightarrow{k1} B \xrightarrow{k2} C$$

In which the consumption of A follows an *m-th* order and the consumption of B follows an *n-th* order we can analyse the three components A, B and C with the following equations:

$$\frac{dA}{dt} = - k1 \; x \; A^m$$

$$\frac{dB}{dt} = +k1 \; x \; A^m - k2 \; x \; B^n$$

$$\frac{dC}{dt} = + k2 \; x \; B^n$$

It is important to note that the rate of production of B must be the same as the rate of consumption of A. This is a direct consequence of the reaction scheme $A \xrightarrow{k1} B$. Likewise, the rate of production of C must be the same as the rate of consumption of B. Whenever we carry out a kinetic analysis of a system, we have to ensure that this fundamental rule is obeyed.

This way of writing the different rate law expressions is commonly known as ***General Mass Action* (GMA) representation**.

9.2.1. The steady state of a reaction

The aforementioned example allows us to explore another very important point, which is relevant to many biological systems. We can ask what happens, if in the equation for the rate of B:

$$\frac{dB}{dt} = k1\ x\ A^m - k2\ x\ B^n$$

$k1\ x\ A^m = k2\ x\ B^n$?

In other words, what happens if the production of B is the same as its consumption?

The answer is simple – very clearly, in this case:

$$\frac{dB}{dt} = 0.$$

Note: The **rate of B is zero**, but the **concentration of B is not** – it rather stays constant. This situation is called ***steady state***.

Obviously with a concentration of A being variable and of a limited nature, the concentration of B will not reach such a steady state, however, in certain cases there is an almost constant amount of A present and hence the concentration of B can reach a steady state. In the linear reaction:

$$\xrightarrow{k0} A \xrightarrow{k1} B \xrightarrow{k2} C \xrightarrow{k3}$$

A might be kept at a fairly constant level. As a consequence the concentration of B might be constant as well, if the **production** of B is offset by the **consumption** of B.

9.2.2. Problem: Calculate the concentration of an intermediate at steady state

In in the linear reaction:

$$\xrightarrow{k0} A \xrightarrow{k1} B \xrightarrow{k2} C \xrightarrow{k3}$$

The concentration of A is kept at 50 mM. The rate constants for k1 and k2 are 1.0 min^{-1} and 1.5 min^{-1}. Calculate the resulting steady state concentration for B. What would happen, if the concentration of A was changed to 30 mM?

Solution:

The GMA for B can be written as:

$$\frac{dB}{dt} = k1 \; x \; A^m - k2 \; x \; B^n$$

At steady state

$$\frac{dB}{dt} = 0.$$

And $k1 \; x \; A^m = k2 \; x \; B^n$

The concentration of components like B at the steady state can be calculated by rearranging the equation:

$$B = \sqrt[n]{\frac{k1xA^m}{k2}}$$

From the units fo the rate constants (min^{-1}), we know that both k1 and k2 are the rate constants for first order reactions. We therefore can write:

$$\frac{dB}{dt} = k1 \; x \; A^1 - k2 \; x \; B^1$$

At steady state $\frac{dB}{dt} = 0$ and $k1 \; x \; A^1 = k2 \; x \; B^1$.

$$B = \frac{k1xA}{k2} = \frac{1.0\,\mathrm{min}^{-1}\,x50mM}{1.5\,\mathrm{min}^{-1}} = \frac{50mM}{1.5}$$

B = 33.333 mM.

Answer: The steady state concentration of B is 33.33 mM.

If the concentration of A changed from 50 mM to 30 mM, the concentration of B changes to:

$$B = \frac{k1xA}{k2} = \frac{1.0\,\mathrm{min}^{-1}\,x30mM}{1.5\,\mathrm{min}^{-1}} = \frac{30mM}{1.5}$$

B = 20 mM.

Answer: By changing the concentration of A from 50 mM to 30 mM, the steady state concentration of B changes from 33.333 mM to 20 mM.

9.3. The GMA representation for a reversible reaction

Another, very frequent reaction scheme is the one of a reversible reaction:

$$\text{R(eceptor)} + \text{L(igand)} \underset{kr}{\overset{kf}{\rightleftharpoons}} \text{R(eceptor)L(igand)}$$

kf is often called the association constant, kr is the dissociation constant.

Again, we can split this reaction into its elementary reactions. For E1 we get:

$$\text{R(eceptor)} + \text{L(igand)} \xrightarrow{kf} \text{R(eceptor)L(igand)}$$

And for E2:

$$\text{R(eceptor)L(igand)} \xrightarrow{kr} \text{R(eceptor)} + \text{L(igand)}$$

For simplicity sake, let's assume that E1 is a second order reaction in which both reactants are different (as discussed in chapter 6), whereas E2 is best represented by a first order reaction.
As in the previous example, we can write the rate equations for the different components, namely R, L and RL. Let's start with RL and write the rate equations for the two reactions. Looking at the production of RL we can write for E1:

$$\frac{dRL1}{dt} = \text{kf } x \text{ R } x \text{ L}$$ (rate is positive because we are looking at the production of RL)

and for E2:

$$\frac{dRL2}{dt} = -\text{ kr } x \text{ RL}$$ (rate is negative, because we are looking at the consumption of RL)

Again, $\frac{dRL1}{dt}$ and $\frac{dRL2}{dt}$ indicate the rates of the elementary reactions.

We can combine the two elementary reactions and write for the overall reaction:

$$\frac{dRL}{dt} = \frac{dRL1}{dt} + \frac{dRL2}{dt} = \text{kf } x \text{ R } x \text{ L} - \text{kr } x \text{ RL}$$

Looking at the consumption of R we can write for E1:

$$\frac{dR1}{dt} = - kf\, x\, R\, x\, L$$ (rate is negative because we are looking at the consumption of R)

and for E2:

$$\frac{dR2}{dt} = + kr\, x\, RL$$ (rate is positive, because we are looking at the production of R)

Again, $\frac{dR1}{dt}$ and $\frac{dR2}{dt}$ indicate the rates of the elementary reactions.

We can combine the two elementary reactions and write for the overall reaction:

$$\frac{dR}{dt} = \frac{dR1}{dt} + \frac{dR2}{dt} = - kf\, x\, R\, x\, L + kr\, x\, RL$$

For L we can use the same rate equation as for R and write:

$$\frac{dL}{dt} = - kf \, x \, R \, x \, L + kr \, x \, RL$$

Our three rate equations for the different compounds therefore read:

$$\frac{dRL}{dt} = kf \, x \, R \, x \, L - kr \, x \, RL$$

$$\frac{dR}{dt} = - kf \, x \, R \, x \, L + kr \, x \, RL$$

$$\frac{dL}{dt} = - kf \, x \, R \, x \, L + kr \, x \, RL$$

We could try to solve this differential equation or we can use the 'modelling' programme Jdesigner. A typical graphical representation might look like:

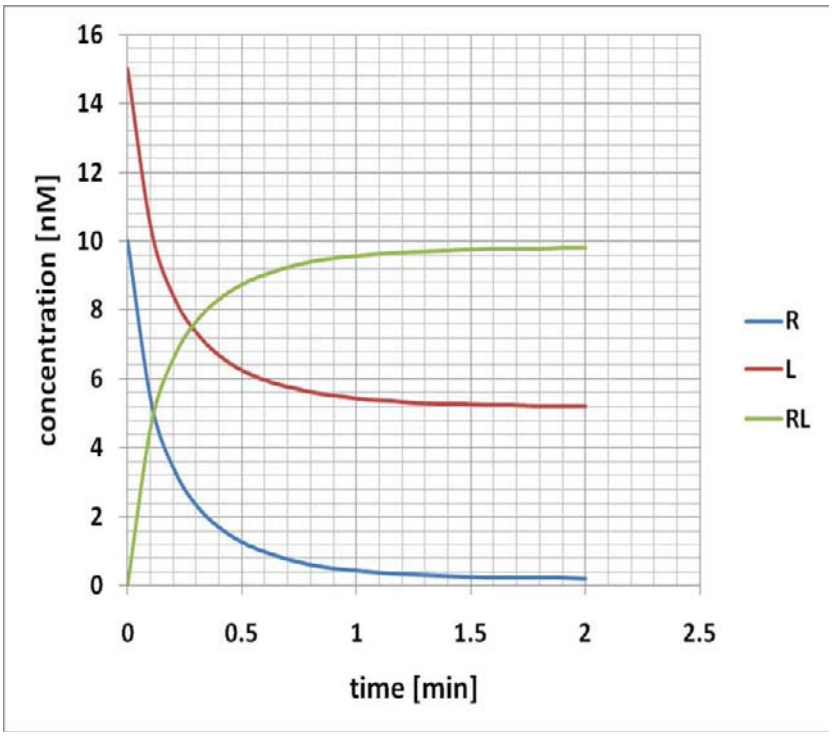


Figure 9-2 Graphic representation of a reversible reaction. For illustration purposes the following initial values have been used: **R = 10 nM; L = 15 nM; RL = 0 nM; kf = 0.5 nM^{-1} min^{-1}; kr = 0.05 min^{-1}**

It is clear that after 1.5 min or so none of the concentrations change any longer. The system has reached its '**equilibrium**'.

We can explain this observation by saying that the production of RL is offset by its decomposition into R and L. Both processes are in balance and hence the change in RL concentration is zero. Note – it is $\frac{dRL}{dt}$ that is zero, not the RL concentration itself.

When the equilibrium has been reached we can write:

$$\frac{dRL}{dt} = kf \; x \; R \; x \; L - kr \; x \; RL = 0.$$

Rearranging this equation gives:

$$kf \; x \; R \; x \; L = kr \; x \; RL$$

Moving all the rate constants on one side of the equation and all the components to the other side gives:

$$\frac{kf}{kr} = \frac{RL}{RxL}$$

The left hand side of this equation is often written as:

$$\frac{kf}{kr} = K_{eq}$$

K_{eq} is called the **equilibrium constant** of this reaction. It is nothing else but the ratio of the rate constants for the forward and the reverse reaction.

In a more general form we can write for a reversible reaction:

$$A + B \underset{kr}{\overset{kf}{\rightleftharpoons}} P$$

$$\frac{P}{AxB} = \frac{kf}{kr} = K_{eq}$$

It looks innocent enough, but a lot of people have problems with it. The main thing to remember is that when we talk about the concentrations of A, B and P, this is **AFTER** we have established the equilibrium.

For the equation it therefore would be better to write:

$$K_{eq} = \frac{P_{eq}}{A_{eq} x L_{eq}}$$

In which the subscript 'eq' indicates that we have reached the equilibrium phase of the reaction.

9.4. Problem: Calculate the equilibrium constant of a complex reversible reaction

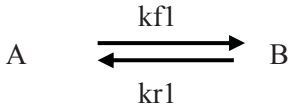
Biological reactions usually consist of a number of consecutive reversible reactions, which can be written as:

$$A \underset{kr1}{\overset{kf1}{\rightleftharpoons}} B \underset{kr2}{\overset{kf2}{\rightleftharpoons}} C$$

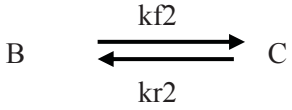
How can we represent this kind of reaction in the GMA-format and what is the equilibrium constant for the overall reaction?

9.4.1. GMA representation of a complex reversible reaction

To address the first question – how we can represent this reaction in the GMA-format – we break down the reaction into two elementary reactions, namely E1:



And E2:



For simplicity sake, let's assume that all reactions follow first order kinetics.

We can write the rate equations for the different components, namely A, B and C. Let's start with A and write the combined rate equation for A:

$$\frac{dA}{dt} = -\,kf1\ x\ A + kr1\ x\ B.$$

The first part of the right hand side of the equation, "- kf1 *x* A", takes into account the consumption of A, while the term "+ kr1 *x* B" looks at the production of A.

A similar approach can be taken for C:

$$\frac{dC}{dt} = kf2\ x\ B - kr2\ x\ C.$$

For B the situation is more complex – again, we have two elementary reactions, each one of which each is a reversible one. For E1 we can write:

$$\frac{dB1}{dt} = kf1\ x\ A - kr1\ x\ B$$

And for E2 it is:

$$\frac{dB2}{dt} = -\,kf2\ x\ B + kr2\ x\ C$$

The overall rate equation for B therefore is the combination of E1 and E2:

$$\frac{dB}{dt} = \frac{dB1}{dt} + \frac{dB2}{dt}$$

$$\frac{dB}{dt} = \text{kf1 } x \text{ A} - \text{kr1 } x \text{ B} - \text{kf2 } x \text{ B} + \text{kr2 } x \text{ C}$$

With this the three rate equations for the three different components A, B and C can be written in the GMA representation as:

$$\frac{dA}{dt} = - \text{kf1 } x \text{ A} + \text{kr1 } x \text{ B}.$$

$$\frac{dC}{dt} = \text{kf2 x B} - \text{kr2 } x \text{ C}.$$

$$\frac{dB}{dt} = \text{kf1 } x \text{ A} - \text{kr1 } x \text{ B} - \text{kf2 } x \text{ B} + \text{kr2 } x \text{ C}$$

9.4.2. Equilibrium constant for a complex reversible reaction

The overall equilibrium constant can be expressed as:

$$K_{eq} = \frac{C_{eq}}{A_{eq}}$$

In which A_{eq} and C_{eq} represent the equilibrium concentrations for A and C, respectively.

For the first elementary reaction

$$\text{A} \underset{\text{kr1}}{\overset{\text{kf1}}{\rightleftharpoons}} \text{B}$$

we can write the equilibrium constant K_{eq1} as:

$$K_{eq1} = \frac{B_{eq}}{A_{eq}} = \frac{kf1}{kr1}$$

For the second elementary reaction

$$B \underset{kr2}{\overset{kf2}{\rightleftharpoons}} C$$

the equilibrium constant K_{eq2} can be written as:

$$K_{eq2} = \frac{C_{eq}}{B_{eq}} = \frac{kf2}{kr2}$$

To get the overall equilibrium constant we multiply the two equilibrium constants for the elementary reactions:

$K_{eq} = K_{eq1} \, x \, K_{eq2}$

Why????

We said that

$$K_{eq1} = \frac{B_{eq}}{A_{eq}} \text{ and } K_{eq2} = \frac{C_{eq}}{B_{eq}}.$$

If we multiply the two equilibrium constants, we get:

$$K_{eq} = K_{eq1}\, x\, K_{eq2} = \frac{B_{eq}}{A_{eq}} x \frac{C_{eq}}{B_{eq}} = \frac{kf1}{kr1} x \frac{kf2}{kr2}$$

From this equation we can cancel out B_{eq} and get:

$$K_{eq} = \frac{C_{eq}}{A_{eq}} = \frac{kf1}{kr1} x \frac{kf2}{kr2}$$

Answer: The overall equilibrium constant can be obtained by multiplying the equilibrium constants for the individual elementary reactions. The overall equilibrium constant can be expressed as the product of the rate constants for the forward reactions divided by the product of the rate constants for the reverse reactions.

Although we developed the overall equilibrium constant for the reaction

$$A \underset{kr1}{\overset{kf1}{\rightleftharpoons}} B \underset{kr2}{\overset{kf2}{\rightleftharpoons}} C$$

We can formulate the overall equilibrium constant for more general reactions, e.g.:

$$A + B \underset{kr1}{\overset{kf1}{\rightleftharpoons}} C \underset{kr2}{\overset{kf2}{\rightleftharpoons}} D + E$$

$$K_{eq} = \frac{D_{eq} x E_{eq}}{A_{eq} x B_{eq}} = \frac{kf1}{kr1} x \frac{kf2}{kr2}$$

9.5. Problem: Calculating equilibrium concentrations from rate constants

In many biological problems we know the overall equilibrium constant AND the starting concentrations of the reactants, but we want to know the final concentrations of products and reactants AFTER the equilibrium has been established. A typical reaction is:

$$R(eceptor) + L(igand) \underset{kr}{\overset{kf}{\rightleftharpoons}} RL \text{ (receptor/ligand complex)}$$

Calculate the equilibrium concentrations of receptor, ligand and receptor/ligand complex with the following data:

$R_{initial}$ = 10 nM
$L_{initial}$ = 15 nM
$RL_{initial}$ = 0 nM
kf = 0.5 nM^{-1} min^{-1}
kr = 0.05 min^{-1}

Assume that all reactions can be described by first order equations.

Solution:

In GMA representation the rates for the individual reactants and the product can be written as:

$$\frac{dR}{dt} = - kf \, x \, R \, x \, L + kr \, x \, RL.$$

$$\frac{dL}{dt} = - kf \, x \, R \, x \, L + kr \, x \, RL.$$

$$\frac{dRL}{dt} = + kf \, x \, R \, x \, L - kr \, x \, RL.$$

The equilibrium is established when the rate of the forward reaction is equal to the rate of the reverse reaction:

kf *x* R *x* L = kr *x* RL

In this case

$$\frac{dR}{dt} = \frac{dL}{dt} = \frac{dRL}{dt} = 0.$$

Again, note that the **rates** are zero, but NOT the concentrations!

For the overall equilibrium constant we can write:

$$K_{eq} = \frac{RL_{eq}}{R_{eq} x L_{eq}} = \frac{kf}{kr}$$

If we want to calculate RL_{eq} we will discovery very quickly that there is a problem – we do not know the concentrations of R_{eq} or L_{eq}!!!

But what we do know is that the receptor can either have a ligand bound (then it is in the RL state) or it hasn't (and therefore is in the R state). The starting concentration of the receptor is the total concentration of the receptor, which is composed of:

$R_{total} = RL + R$ or, rearranged and solved for R:

$R = R_{total} - RL$

This is the same expression once the equilibrium has been reached:

$R_{eq} = R_{total} - RL_{eq}$

For the ligand we can write a similar expression:

$L_{eq} = L_{total} - RL_{eq}$

If we substitute RL_{eq} with y we can write:

$y = RL_{eq}$ and

$$K_{eq} = \frac{kf}{kr} = \frac{RL_{eq}}{R_{eq} x L_{eq}} = \frac{y}{(R_{total} - y)(L_{total} - y)}$$

Putting in our numbers:

$R_{initial} = 10$ nM
$L_{initial} = 15$ nM
$RL_{initial} = 0$ nM
$kf = 0.5\ nM^{-1}\ min^{-1}$
$kr = 0.05\ min^{-1}$

we get:

$$\frac{0.5 nM^{-1}\,\min^{-1}}{0.05\,\min^{-1}} = \frac{y}{(10nM - y)(15nM - y)}$$

$$10 = \frac{y}{150 - 25y + y^2}$$ (for simplicity's sake I omit he units in the following calculations)

$10\ x\ (150 - 25y + y^2) = y$

$1500 - 250y + 10\ y^2 - y = 0$

$1500 - 251y + 10\ y^2 = 0$

This is the standard format for a quadratic equation of the form:

$a\ y^2 + b\ y + c = 0$

for which the solution is:

$$y = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

If we put in our numbers, we get:

$$y = \frac{-(-251) \pm \sqrt{(-251)^2 - 4x10x1500}}{2x10}$$

$$y = \frac{251 \pm \sqrt{63001 - 60000}}{20}$$

$$y = \frac{251 \pm \sqrt{3001}}{20}$$

$$y = \frac{251 \pm 54.8}{20}$$

y1 = 15.29 nM

y2 = 9.81 nM

Since it is a quadratic equation, we get two solutions, however, our total concentration of receptor is only 10 nM and hence the equilibrium concentration of RL concentration must be smaller than 10 nM. We therefore can ignore the first solution for y1 and only consider y2:

$\underline{RL_{eq} = 9.81 \text{ nM.}}$

With this we can calculate R_{eq} and L_{eq}:

$R_{eq} = R_{total} - RL_{eq} = 10 \text{ nM} - 9.81 \text{ nM} = \underline{0.19 \text{ nM}}$

$L_{eq} = L_{total} - RL_{eq} = 15 \text{ nM} - 9.81 \text{ nM} = \underline{5.19 \text{ nM}}$

Answer: The equilibrium concentrations for RL, R and L are 9.81 nM, 0.19 nM and 5.19 nM, respectively.

These are exactly the numbers we got from our simulation in Figure 9-2.!!!

9.6. Problem solving strategies

- For a reaction of the scheme:

  $A \xrightarrow{k1} B \xrightarrow{k2} C$

  In which the consumption of A follows an *m-th* order and the consumption of B follows an *n-th* order the rates for the three components A, B and C can be presented in the GMA format by combining the rates for the individual elementary reactions:

$$\frac{dA}{dt} = - k1 \; x \; A^m$$

$$\frac{dB}{dt} = k1 \; x \; A^m - k2 \; x \; B^n$$

$$\frac{dC}{dt} = + k2 \; x \; B^n$$

- If the concentration of A remains constant throughout the experiment, the concentration of B reaches a steady state, if $\frac{dB}{dt} = 0$.

  This is the case, if $k1 \; x \; A^m = k2 \; x \; B^n$.

  The concentration B at the steady state is:

$$B = \sqrt[n]{\frac{k1 x A^m}{k2}}$$

- In reversible reactions of the scheme:

$$A + B \underset{kr}{\overset{kf}{\rightleftharpoons}} P$$

The rate equations for the three components can be written as:

$$\frac{dA}{dt} = \frac{dB}{dt} = - kf \, x \, A \, x \, B + kr \, x \, P$$

$$\frac{dP}{dt} = + kf \, x \, A \, x \, B - kr \, x \, P$$

The equilibrium constant is expressed as:

$$K_{eq} = \frac{P_{eq}}{A_{eq} x B_{eq}} = \frac{kf}{kr}$$

Note: Equilibrium indicates that the rate of the forward reaction is equal to the rate of the reverse reaction.

- For reactions with the scheme:

$$A + B \underset{kr1}{\overset{kf1}{\rightleftharpoons}} C \underset{kr2}{\overset{kf2}{\rightleftharpoons}} D + E$$

The overall equilibrium constant can be expressed as:

$$K_{eq} = \frac{D_{eq} x E_{eq}}{A_{eq} x B_{eq}} = \frac{kf1}{kr1} \, x \, \frac{kf2}{kr2}$$

9.7. More problem questions

1. Figure 9-3 shows a simplified diagram for the development of cancer.

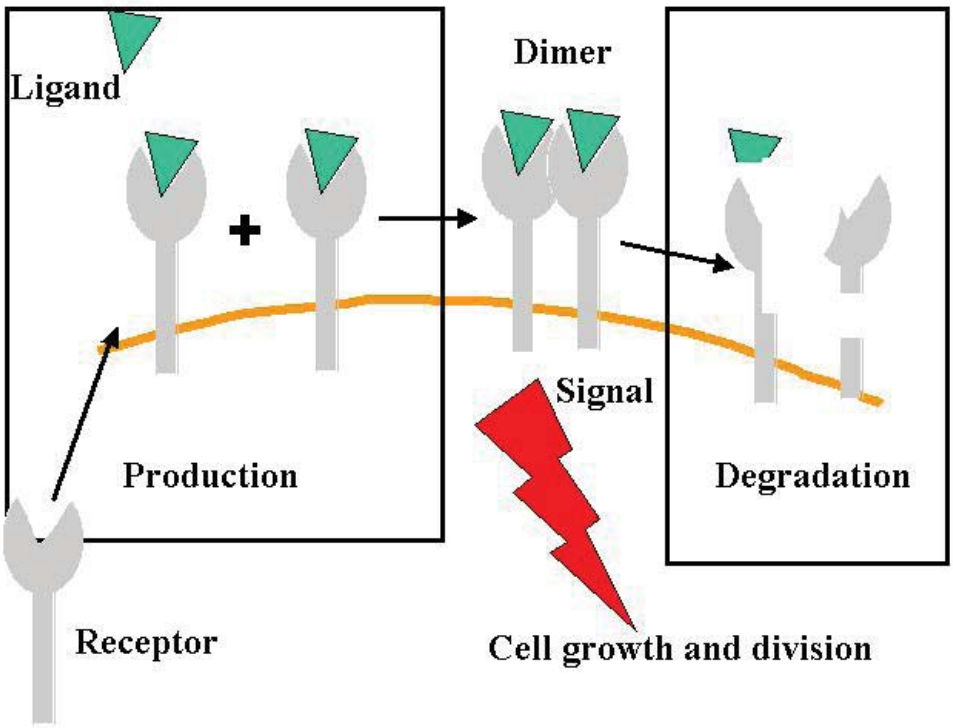


Figure 9-3 Schematic diagram illustrating the onset of cancer

With the following reactions:

Reaction 1:

$$\xrightarrow{k1} R(eceptor)$$

Reaction 2:

$$R(eceptor) + L(igand) \underset{k3}{\overset{k2}{\rightleftharpoons}} R(eceptor)L(igand)$$

Reaction 3:

$$2\ R(eceptor)L(igand) \underset{k5}{\overset{k4}{\rightleftharpoons}} D(imer)$$

Reaction 4:

$$D(imer) \xrightarrow{k6} degrad(ation\ products)$$

Formulate the rate equation for each component in the GMA-representation.

Answer:

$$\frac{dR}{dt} = k1 - k2\ x\ R\ x\ L + k3\ x\ RL$$

$$\frac{dL}{dt} = -\ k2\ x\ R\ x\ L + k3\ x\ RL$$

$$\frac{dRL}{dt} = k2\ x\ R\ x\ L - k3\ x\ RL\ + k5\ x\ D - k4\ x\ RL^2$$

$$\frac{dD}{dt} = k4\ x\ RL^2 - k5\ x\ D - k6\ x\ D$$

2. In a cell line the metabolite S undergoes the following conversion:

$$S \underset{k1r}{\overset{k1f}{\rightleftharpoons}} P \underset{k2r}{\overset{k2f}{\rightleftharpoons}} Q$$

a) The following properties were determined in an experiment:

$S_{initial}$ = 100 mM	k1f = 0.1 min^{-1}
$P_{initial}$ = 0 mM	k1r = 0.04 min^{-1}
$Q_{initial}$ = 0 mM	k2f = 0.4 min^{-1}
	k2r = 0.1 min^{-1}

Formulate the general rate equations for all components in the GMA representation.

Calculate the overall equilibrium constant for $\frac{Q}{S}$

b) A mutant cell line has the following properties:

$S_{initial}$ = 100 mM	k1f = 0.1 min^{-1}
$P_{initial}$ = 0 mM	k1r = 0.04 min^{-1}
$Q_{initial}$ = 0 mM	k2f = 0.0 min^{-1}
	k2r = 0.0 min^{-1}

Calculate the equilibrium concentrations for S, P and Q, respectively, in this mutant

c) Another mutant cell line has the following properties:

$S_{initial}$ = 100 mM	k1f = 0.05 min^{-1}
$P_{initial}$ = 0 mM	k1r = 0.0 min^{-1}
$Q_{initial}$ = 0 mM	k2f = 0.4 min^{-1}
	k2r = 0.0 min^{-1}

In an experiment the concentration of S is kept constant at 100 mM. Calculate the steady state concentration of P. How would the steady state concentration of P change in relation to the concentration of S?

Answers:

a)

$$\frac{dS}{dt} = - k1f \, x \, S + k1r \, x \, P$$

$$\frac{dP}{dt} = k1f \, x \, S - k1r \, x \, P + k2r \, x \, Q - k2f \, x \, P$$

$$\frac{dQ}{dt} = - k2r \; x \; Q + k2f \; x \; P$$

$K_{eq} = 10$ (no units).

b) $Q_{eq} = 0$ mM
$S_{eq} = 28.6$ mM
$P_{eq} = 71.4$ mM

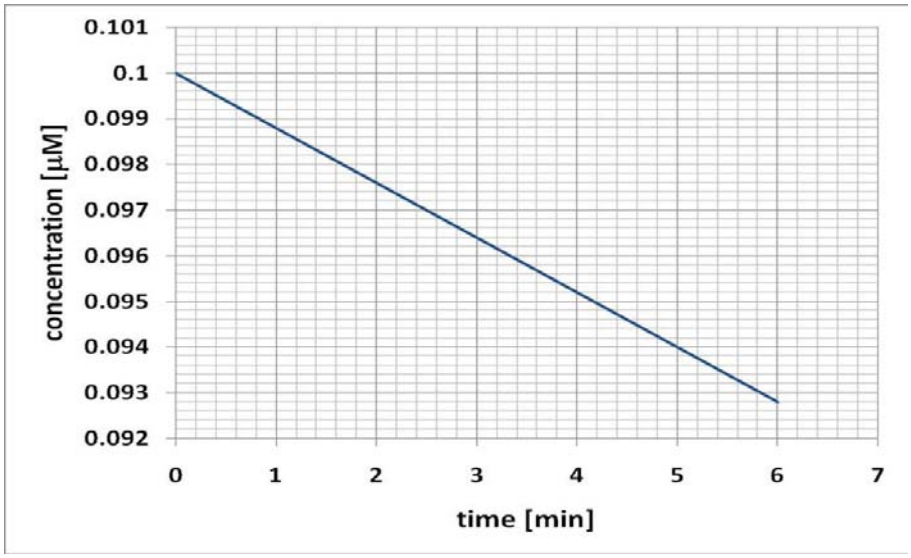
c) $P_{steadystate} = 125$ mM.

10. Model Answers to practice questions

10.1. Answers to Chapter 3

Question 1:

a) Plot concentration of drug versus time gives a straight line, therefore it must be a zero order reaction:



b) Using the equation

$A_{(t)} - A_{initial} = -k \; x \; (t_{(t)} - t_{initial})$ we solve for k:

$$k = -\frac{A_{(t)} - A_{initial}}{t_{(t)} - t_{initial}}$$

From the data table we pick two data sets:

time [min]	concentration [µM]
0	0.1
1	0.0988
2	0.0976
3	0.0964
4	0.0952
5	0.094
6	0.0928

And get:

$$k = -\frac{0.0928 - 0.1}{6 - 0}\ \mu M/min$$

k = 0.0012 μM/min = 1.2 nM/min.

c) Using the equation
$A_{(t)} - A_{initial} = -k\ x\ (t_{(t)} - t_{initial})$ and setting $t_{initial} = 0$ we solve for $t_{(t)}$:

$t_{(t)} = (A_{(t)} - A_{initial}) / -k$

$A_{initial}$ = 0.1 μM = 100 nM	$t_{initial} = 0$
$A_{(t)}$ = 40 nM	$t_{(t)}$ = ???
k = 1.2 nM/min	

$t_{(t)}$ = (40 nM – 100 nM) / -1.2 nM/min = - 60 nM / -1.2 nM/min = 50 min.

d) Using the equation

$A_{(t)} - A_{initial} = -k\ x\ (t_{(t)} - t_{initial})$ and setting $t_{initial} = 0$ we solve for $t_{(t)}$:

$t_{(t)} = (A_{(t)} - A_{initial}) / -k$

$A_{initial}$ = 0.1 μM = 100 nM	$t_{initial} = 0$
$A_{(t)}$ = 0 nM	$t_{(t)}$ = ???
k = 1.2 nM/min	

$t_{(t)}$ = (0 nM – 100 nM) / -1.2 nM/min = - 100 nM / -1.2 nM/min = 83.3 min.

Question 2:

It must be a zero order reaction, because the rate constant k = 24 pM per 12 minutes. The typical unit for a zero order rate constant is concentration per time.

Using the equation
$A_{(t)} - A_{initial} = +k\ x\ (t_{(t)} - t_{initial})$ and setting $t_{initial} = 0$ we solve for $t_{(t)}$:

$t_{(t)} = (A_{(t)} - A_{initial}) / k$

$A_{initial}$ = 20 pM	$t_{initial} = 0$
$A_{(t)}$ = 0.1 nM = 100 pM	$t_{(t)}$ = ???
k = 24 pM/12 min = 2 pM/min	

$t_{(t)}$ = (100 pM – 20 pM) / 2 pM/min = 40 min.

10.2. Answers to Chapter 4

Question 1:

Using the equation

$\ln\left(\frac{A_{(t)}}{A_{initial}}\right) = -k \times (t_{(t)} - t_{initial})$ and setting $t_{(t)} = 0$ (this is today) we solve for $t_{initial}$:

$t_{initial} = \ln\left(\frac{A_{(t)}}{A_{initial}}\right) / k$

$\frac{A_{(t)}}{A_{initial}} = 10\ \% = 0.1$	$t_{initial}$ = ???
$t_{1/2}$ = 5,700 years	

$k = 0.693/ t_{1/2} = 0.693/5700\ y = 1.22 \times 10^{-4}\ y^{-1}$

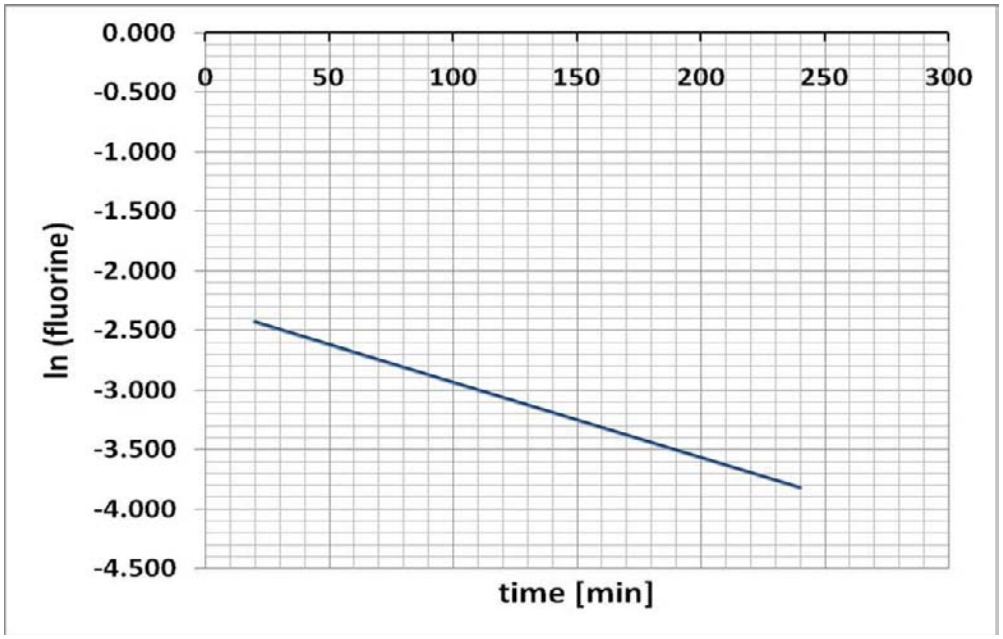
$t_{initial} = \ln(0.1) / 1.22 \times 10^{-4}\ y^{-1} = -18940$ years

Question 2:

a) Calculate ln (fluorine concentration)

time [min]	**fluorine-18 [µg]**	**ln (flourine)**
20	0.0882	-2.428
40	0.0777	-2.555
60	0.0685	-2.681
240	0.022	-3.817

Plot ln (fluorine concentration) versus time:



Since this graph shows a straight line, it must be a first order reaction.

b) Using the equation

$\ln\left(\frac{A_{(t)}}{A_{initial}}\right) = -k \times (t_{(t)} - t_{initial})$ we solve for k:

$k = \ln\left(\frac{A_{(t)}}{A_{initial}}\right) / -(t_{(t)} - t_{initial})$

From the data table we pick two data sets:

time [min]	fluorine-18 [µg]
20	0.0882
40	0.0777
60	0.0685
240	0.022

$k = \ln\left(\frac{0.022}{0.0882}\right) / -(240 \text{ min} - 20 \text{ min}) = \ln(0.25) / -220 \text{ min} = -1.39 / -220 \text{ min} = 6.3 \times 10^{-3} \text{ min}^{-1}$

$t_{1/2} = 0.693/ k = 0.693/ 6.3 \times 10^{-3} \text{ min}^{-1} = 110 \text{ min}.$

c) Using the equation

$\frac{A_{(t)}}{A_{initial}} = e^{-(k \, x \, (t_{(t)} - t_{initial}))}$, setting $t_{initial} = 0$ and solving for $A_{initial}$:

$A_{initial} = A_{(t)} / e^{-k \, x \, t_{(t)}}$

From the data we can pick any $A_{(t)}$ and corresponding $t_{(t)}$.

$A_{initial} = 0.022 \, \mu g / e^{-6.3 \, x \, 10\text{-}3 \, x \, 240} = 0.022 \, \mu g / e^{-1.512} = 0.022 \, \mu g / 0.22 = 0.1 \, \mu g$

d) Using the equation

$\frac{A_{(t)}}{A_{initial}} = e^{-(k \, x \, (t_{(t)} - t_{initial}))}$, setting $t_{initial} = 0$ and solving for $A_{(t)}$:

$A_{(t)} = A_{initial} \, x \, e^{-k \, x \, t_{(t)}}$

For $t_{(t)} = 3$ h $= 3 \, x \, 60$ min, we get:

$A_{(t)} = 0.1 \, \mu g \, x \, e^{-6.3 \, x \, 10\text{-}3 \, x \, 180} = 0.1 \, \mu g \, x \, e^{-1.134} = 0.1 \, \mu g \, x \, 0.32 = 0.032 \, \mu g = 32$ ng.

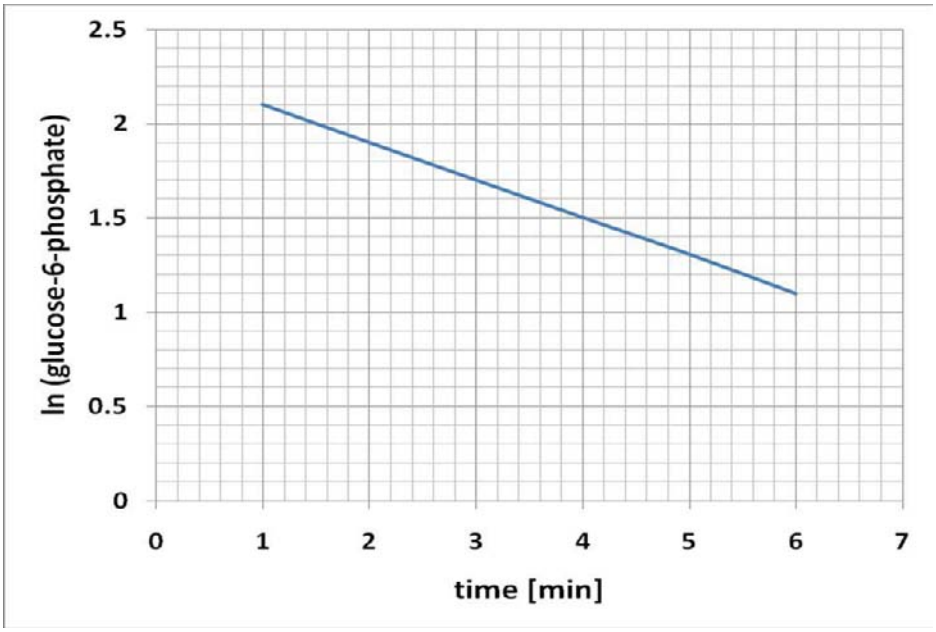
For $t_{(t)}$ = 12 h = 12 *x* 60 min, we get:
$A_{(t)} = 0.1\ \mu g\ x\ e^{-6.3\ x\ 10\text{-}3\ x\ 720} = 0.1\ \mu g\ x\ e^{-4.536} = 0.1\ \mu g\ x\ 0.011 = 0.0011\ \mu g = 1.1\ ng.$

Question 3:

a) Calculate ln (glucose-6-phosphate

Time [min]	Glucose-6-phosphate [mM]	ln (glucose-6-phospate)
1	8.2	2.104134
2	6.7	1.902108
4	4.5	1.504077
5	3.7	1.308333
6	3	1.098612

Plot ln (glucose-6-phospate) versus time



Since this graph shows a straight line, it must be a first order reaction.

b) Using the equation

$\ln\left(\frac{A_{(t)}}{A_{initial}}\right) = -k\ x\ (t_{(t)} - t_{initial})$ we solve for k:

$k = \ln\left(\frac{A_{(t)}}{A_{initial}}\right) / -(t_{(t)} - t_{initial})$

From the data table we pick two data sets:

Time [min]	Glucose-6-phosphate [mM]
1	8.2
2	6.7
4	4.5
5	3.7
6	3.0

$k = \ln(\frac{3.0}{8.2}) / -(6\text{ min} - 1\text{ min}) = \ln(0.366) / -5\text{ min} = -1.0 / -5\text{ min} = 0.2\text{ min}^{-1}$

$t_{1/2} = 0.693/ k = 0.693/ 0.2\text{ min}^{-1} = 3.47\text{ min}.$

c) Using the equation

$\frac{A_{(t)}}{A_{initial}} = e^{-(k\,x\,(t_{(t)} - t_{initial}))}$, setting $t_{initial} = 0$ and solving for $A_{initial}$:

$A_{initial} = A_{(t)} / e^{-k\,x\,t_{(t)}}$

From the data we can pick any $A_{(t)}$ and corresponding $t_{(t)}$.

$A_{initial} = 3.0\text{ mM} / e^{-0.2\,x\,6} = 3.0\text{ mM} / e^{-1.2} = 3.0\text{ mM} / 0.3 = 10\text{ mM}$

d) First we need to calculate, how much Glucose-6-phosphate is left after 3 minutes. Using the equation

$\frac{A_{(t)}}{A_{initial}} = e^{-(k\,x\,(t_{(t)} - t_{initial}))}$, setting $t_{initial} = 0$ and solving for $A_{(t)}$:

$A_{(t)} = A_{initial}\ x\ e^{-k\,x\,t_{(t)}}$

For $t_{(t)} = 3$ min we get:

$A_{(t)} = 10\text{ mM}\ x\ e^{-0.2\,x\,3} = 10\text{ mM}\ x\ e^{-0.6} = 10\text{ mM}\ x\ 0.55 = 5.5\text{ mM}.$

This is the amount of glucose-6-phosphate that is left from the 10 mM after 3 minutes. We therefore used:

10 mM – 5.5 mM = 4.5 mM glucose-6-phosphate, which gives us the same concentration of ATP.

e) First we need to calculate, how much Glucose-6-phosphate is left after 10 minutes. Using the equation

$\frac{A_{(t)}}{A_{initial}} = e^{-(k\,x\,(t_{(t)} - t_{initial}))}$, setting $t_{initial} = 0$ and solving for $A_{(t)}$:

$A_{(t)} = A_{initial}\ x\ e^{-k\,x\,t_{(t)}}$

For $t_{(t)} = 10$ min we get:

$A_{(t)} = 10\ mM\ x\ e^{-0.2\,x\,10} = 10\ mM\ x\ e^{-2.0} = 10\ mM\ x\ 0.135 = 1.35\ mM.$

This is the amount of glucose-6-phosphate that is left from the 10 mM after 10 minutes. We therefore used:

10 mM – 1.35 mM = 8.65 mM glucose-6-phosphate, which gives us the same concentration of ATP.

Question 4:

a) Converting the rate constants into doubling times with the equation

$t_{2/1} = 0.693/ k$ and the growth constant for Euphorbia = 0.5 % y^{-1} = 0.5/100 y^{-1} gives:

$$t_{2/1} = \frac{0.693}{0.005 y^{-1}} = 138.6 \text{ y}$$

For Brumlidge with a growth constant = 2.5 % y^{-1} = 2.5/100 y^{-1} the conversion gives

$$t_{2/1} = \frac{0.693}{0.025 y^{-1}} = 27.7 \text{ y}$$

b) Using the equation

$\frac{A_{(t)}}{A_{initial}} = e^{+(k \, x \, (t_{(t)} - t_{initial}))}$, setting $t_{initial} = 2000$ and $t_{(t)} = 2100$ and solving for $A_{(t)}$:

$A_{(t)} = A_{initial} \, x \, e^{+k \, x \, \Delta t}$

For Euphorbia $A_{initial} = 10 \, x \, 10^6 = 1 \, x \, 10^7$, k = 0.5/100 y^{-1}

$A_{(t)} = 1 \, x \, 10^7 \, x \, e^{+0.5/100 \, x \, 100} = 1 \, x \, 10^7 \, x \, 1.65 = 1.65 \, x \, 10^7 = 16.5 \, x \, 10^6$

For Brummlidge $A_{initial} = 5 \, x \, 10^6$, k = 2.5/100 y^{-1}

$A_{(t)} = 5 \, x \, 10^6 \, x \, e^{+2.5/100 \, x \, 100} = 5 \, x \, 10^6 \, x \, 12.2 = 60.9 \, x \, 10^6$

Question 5:

a) Using the equation

$\ln \left(\frac{A_{(t)}}{A_{initial}} \right) = - k \, x \, (t_{(t)} - t_{initial})$ we solve for k:

$$k = \ln \left(\frac{A_{(t)}}{A_{initial}} \right) / - (t_{(t)} - t_{initial})$$

From the data table we pick two data sets:

Time [days]	Amount [mg]
0	1.00
2	0.90
6	0.74
10	0.61
14	0.50
18	0.41
22	0.33

$k = \ln\left(\frac{0.33}{1.0}\right) / -(22\text{ d} - 0\text{ d}) = \ln(0.33) / -22\text{ d} = -1.1 / -22\text{ d} = 0.05\text{ d}^{-1}$

We can check the answer by looking at the data table. We note that after 14 days exactly ½ of the original amount is left. Therefore the $t_{½} = 14$ d. With the equation:

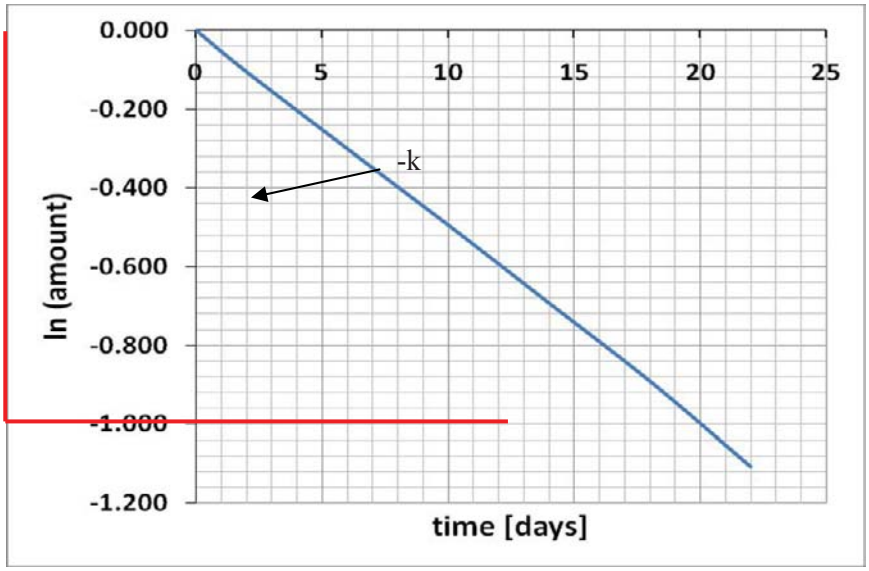
$t_{½} = 0.693/ k$ solved for k:

$k = 0.693 / t_{½} = 0.693 / 14\text{ d} = 0.05\text{ d}^{-1}$

For a graph, calculate ln (amount):

Time [days]	Amount [mg]	ln (amount)
0	1	0.000
2	0.9	-0.105
6	0.74	-0.301
10	0.61	-0.494
14	0.5	-0.693
18	0.41	-0.892
22	0.33	-1.109

Plot ln (amount) versus time:



The gradient (indicated by the red lines) can be determined with the equation:

$$m = \frac{\Delta \ln(amount)}{\Delta time} = \frac{-1-0}{20d-0d} = -1 / 20\ d = -0.05\ d^{-1}$$

m = - k therefore k = 0.05 d^{-1} which is in agreement with the calculations.

b) Using the equation

$\frac{A_{(t)}}{A_{initial}} = e^{-(k\ x\ (t_{(t)} - t_{initial}))}$, setting $t_{initial} = 0$ and solving for $A_{(t)}$:

$A_{(t)} = A_{initial}\ x\ e^{-(k\ x\ (t_{(t)} - t_{initial}))}$

$A_{(t)} = 1\ mg\ x\ e^{-(0.05\ d\text{-}1\ x\ (50\ d))} = 1\ mg\ x\ e^{-2.5} = 1\ mg\ x\ 0.082 = 0.082\ mg$

c) Using the equation

$\ln\left(\frac{A_{(t)}}{A_{initial}}\right) = -k \times (t_{(t)} - t_{initial})$, setting $t_{initial} = 0$ and solving for $t_{(t)}$:

$$t_{(t)} = \ln\left(\frac{A_{(t)}}{A_{initial}}\right) / -k$$

With $\frac{A_{(t)}}{A_{initial}} = 1/1000$ and $k = 0.05\ d^{-1}$

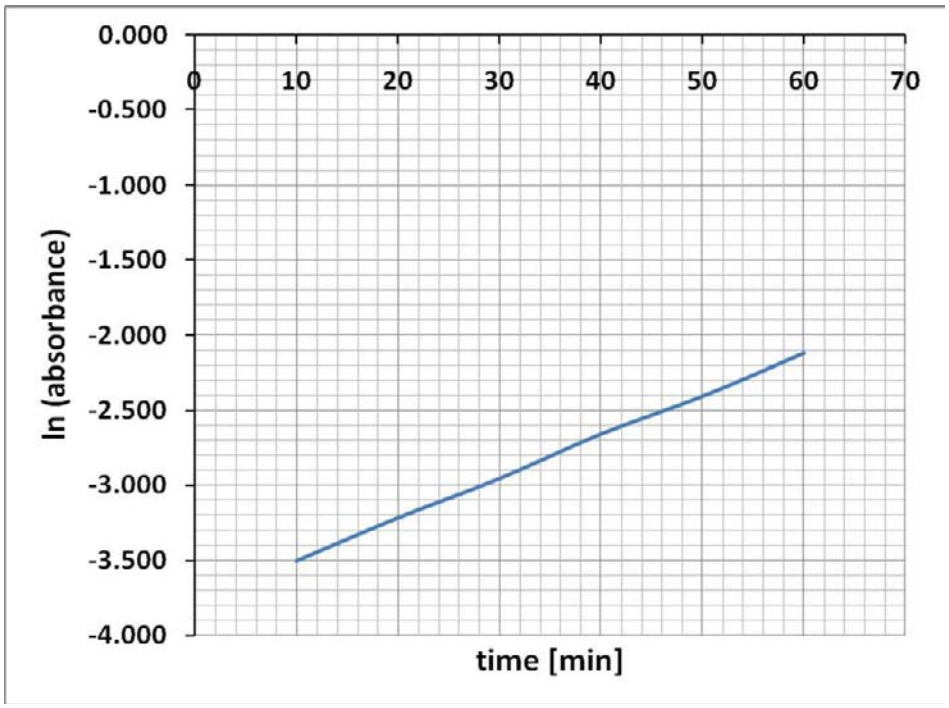
$t_{(t)} = \ln(1/1000) / -0.05\ d^{-1} = -6.9 / -0.05\ d^{-1} = 138.2\ d$

Question 6:

a) Calculate ln (absorbance):

time [min]	Absorbance	ln (absorbance)
10	0.03	-3.507
20	0.04	-3.219
30	0.05	-2.996
40	0.07	-2.659
50	0.09	-2.408
60	0.12	-2.120

Plot ln (absorbance) versus time:



The graph shows a straight line, therefore it must be a first order reaction.

b) Using the equation

$\ln\left(\frac{A_{(t)}}{A_{initial}}\right) = + k \; x \; (t_{(t)} - t_{initial})$ we solve for k:

$k = \ln\left(\frac{A_{(t)}}{A_{initial}}\right) / + (t_{(t)} - t_{initial})$

From the data table we pick two data sets:

time [min]	Absorbance
10	0.03
20	0.04
30	0.05
40	0.07
50	0.09
60	0.12

$k = \ln\left(\frac{0.12}{0.03}\right) / + (60 \text{ min} - 10 \text{ min}) = \ln(4) / 50 \text{ min} = 1.39 / 50 \text{ min} = 0.0277 \text{ min}^{-1}$

To convert the rate constant into $t_{1/2}$ we use:

$t_{1/2} = 0.693 / k = 0.693 / 0.0277 \text{ min}^{-1} = 25 \text{ min}.$

c) Using the equation

$\frac{A_{(t)}}{A_{initial}} = e^{+ (k \, x \, (t_{(t)} - t_{initial}))}$, setting $t_{initial} = 0$ and solving for $A_{initial}$:

$A_{initial} = A_{(t)} / e^{+ (k \, x \, (t_{(t)} - t_{initial}))}$

We can use any $A_{(t)}$ with the corresponding $t_{(t)}$

$A_{initial} = 0.12 / e^{+ (0.0277 \, x \, (60 \text{ min} - 0 \text{ min}))} = 0.12 / e^{+ 1.662} = 0.12/5.27 = 0.023.$

d) Using the equation

$\ln\left(\frac{A_{(t)}}{A_{initial}}\right) = + k \, x \, (t_{(t)} - t_{initial})$, setting $t_{initial} = 0$ and solving for $t_{(t)}$:

$t_{(t)} = \ln\left(\frac{A_{(t)}}{A_{initial}}\right) / + k$

$t_{(t)} = \ln\left(\frac{0.4}{0.023}\right) / 0.0277 \text{ min}^{-1} = \ln(17.4) / 0.0277 \text{ min}^{-1} = 2.86 / 0.0277 \text{ min}^{-1} = 103 \text{ min}.$

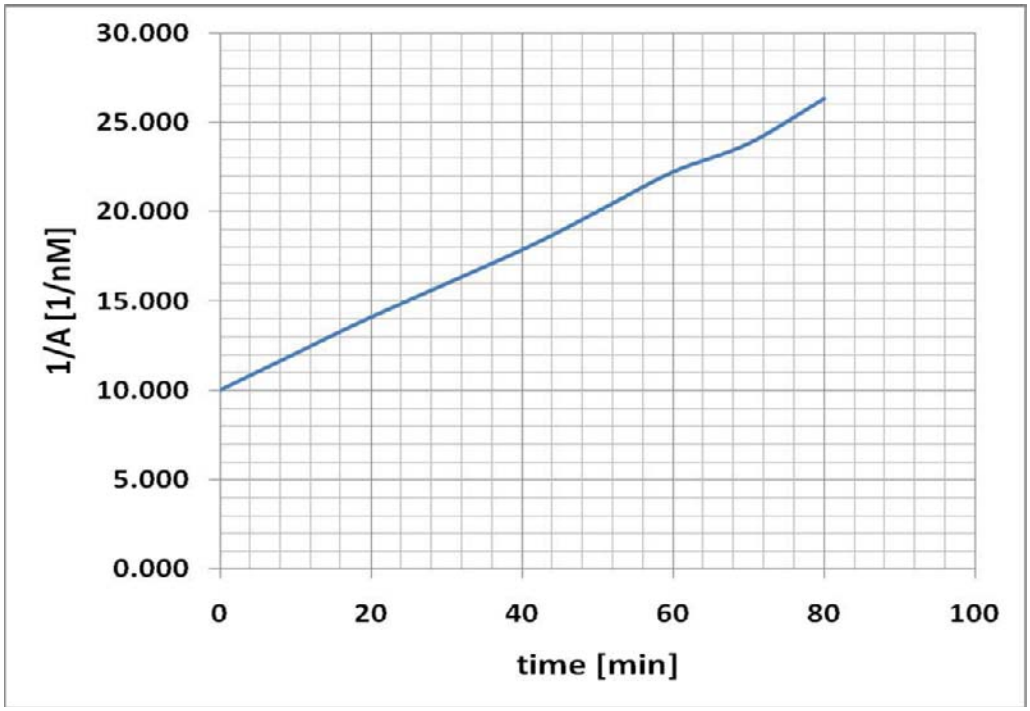
10.3. Answers to Chapter 5

Question1:

a) Calculate 1/A:

Time [min]	A [nM]	1/A [1/nM]
0	0.1	10.000
10	0.083	12.048
20	0.071	14.085
40	0.056	17.857
50	0.05	20.000
60	0.045	22.222
70	0.042	23.810
80	0.038	26.316

Plot 1/A versus time:



Within experimental error limits the graph shows a straight line, therefore it is a second order (A + A type) reaction.

b) Using the equation:

$\frac{1}{A_{(t)}} - \frac{1}{A_{initial}} = +k\ x\ (t_{(t)} - t_{initial})$, setting $t_{initial} = 0$, $A_{initial} = 0.1$ nM and solving for k:

$k = \left(\frac{1}{A_{(t)}} - \frac{1}{A_{initial}}\right) / \ t_{(t)}$

From the data set we can use any $A_{(t)}$ with corresponding $t_{(t)}$:

Time [min]	A [nM]
0.0	0.100
10.0	0.083
20.0	0.071
40.0	0.056
50.0	0.050
60.0	0.045
70.0	0.042
80.0	0.038

$k = \left(\frac{1}{0.038nM} - \frac{1}{0.1nM}\right) / \ 80 \text{ min} = (26.316 \text{ nM}^{-1} - 10 \text{ nM}^{-1}) / 80 \text{ min} = 16.316 \text{ nM}^{-1} / \ 80 \text{ min} = 0.204 \text{ nM}^{-1} \text{ min}^{-1}$.

c) Using the equation:

$\frac{1}{A_{(t)}} - \frac{1}{A_{initial}} = +k \ x \ (t_{(t)} - t_{initial})$, setting $t_{initial} = 0$, $A_{initial} = 0.1$ nM and solving for $\frac{1}{A_{(t)}}$:

$$\frac{1}{A_{(t)}} = +k \ x \ (t_{(t)}) + \frac{1}{A_{initial}}$$

$$\frac{1}{A_{(t)}} = 0.204 \text{ nM}^{-1} \text{ min}^{-1} \ x \ 30 \text{ min} + \frac{1}{0.1nM} = 6.12 \text{ nM}^{-1} + 10 \text{ nM}^{-1} = 16.12 \text{ nM}^{-1}$$

To get $A_{(t)}$ we need to inverse the result:

$$A_{(t)} = 1 / \frac{1}{A_{(t)}} = 1 / 16.12 \text{ nM}^{-1} = 0.062 \text{ nM}$$

To calculate the concentration of D we need to determine, how much A has been consumed:
$A_{initial} = 0.1$ nM and $A_{(t)} = 0.062$ nM therefore 0.1 nM – 0.062 nM = 0.038 nM of A have been consumed.

Since every D is produced by **2** molecules of A we use:

$D = ½ \ x \ A_{consumed}$

$D = ½ \ x \ 0.038 \text{ nM} = 0.019 \text{ nM}$

d) First we calculate how much A is left after 100 minutes, using the equation $\frac{1}{A_{(t)}} - \frac{1}{A_{initial}} = +k\ x\ (t_{(t)} - t_{initial})$, setting $t_{initial} = 0$, $A_{initial} = 0.1$ nM and solving for $\frac{1}{A_{(t)}}$:

$$\frac{1}{A_{(t)}} = +k\ x\ (t_{(t)}) + \frac{1}{A_{initial}}$$

$$\frac{1}{A_{(t)}} = 0.204\ nM^{-1}\ min^{-1}\ x\ 100\ min + \frac{1}{0.1nM} = 20.4\ nM^{-1} + 10\ nM^{-1} = 30.4\ nM^{-1}$$

To get $A_{(t)}$ we need to inverse the result:

$$A_{(t)} = 1 / \frac{1}{A_{(t)}} = 1 / 30.4\ nM^{-1} = 0.033\ nM$$

To calculate the concentration of D we need to determine, how much A has been consumed:
$A_{initial} = 0.1$ nM and $A_{(t)} = 0.033$ nM therefore 0.1 nM – 0.033 nM = 0.067 nM of A have been consumed.

Since every D is produced by **2** A's we use:

$D = ½ \; x \; A_{consumed}$

$D = ½ \; x \; 0.067 \text{ nM} = 0.0335 \text{ nM}$

e) The maximum amount of D is produced if all of $A_{initial}$ has been consumed.

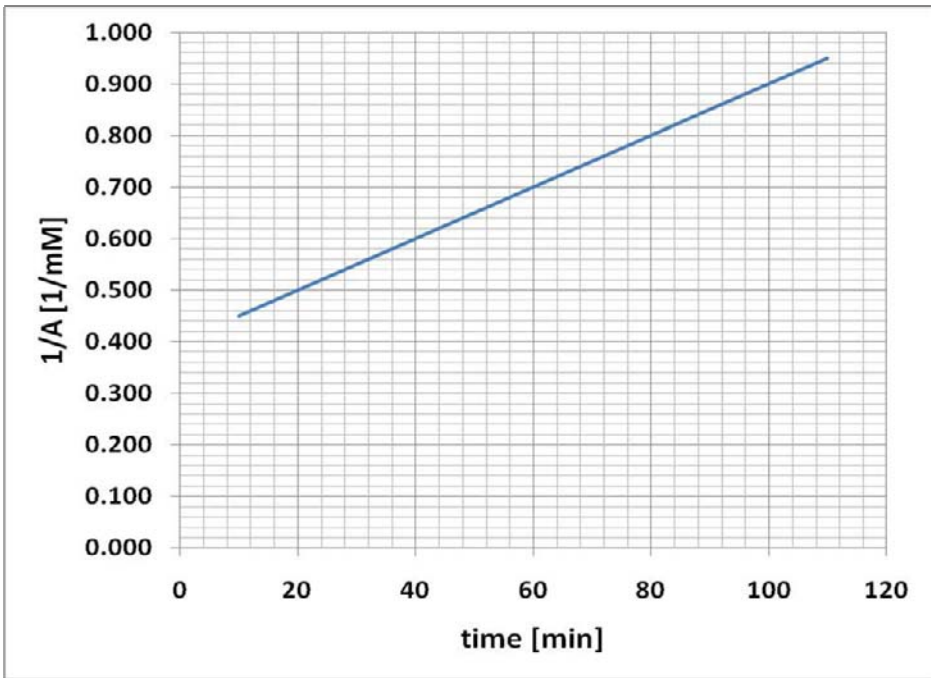
$D = ½ \; x \; A_{initial} = ½ \; x \; 0.1 \text{ nM} = 0.05 \text{ nM}$

Question 2:

a) Calculate 1/A:

time [min]	A [mM]	1/A [1/mM]
0		
10	2.222	0.450
30	1.818	0.550
50	1.538	0.650
70	1.333	0.750
90	1.176	0.850
110	1.053	0.950

Plot 1/A versus time:



Only a 1/A versus time plot gives a straight line, therefore it must be a second order (A + A type) reaction.

b) Using the equation:

$\frac{1}{A_{(t)}} - \frac{1}{A_{initial}} = +k \; x \; (t_{(t)} - t_{initial})$, setting $t_{initial} = 10$, $A_{initial} = 2.222$ mM and solving for k:

$$k = \left(\frac{1}{A_{(t)}} - \frac{1}{A_{initial}}\right) / \; (t_{(t)} - t_{initial})$$

From the data set we can use any $A_{(t)}$ with corresponding $t_{(t)}$:

time [min]	A [mM]
0	
10	2.222
30	1.818
50	1.538
70	1.333
90	1.176
110	1.053

$k = \left(\frac{1}{1.053mM} - \frac{1}{2.222mM}\right) / \; (110 \text{ min} - 10 \text{ min}) = (0.95 \text{ mM}^{-1} - 0.45 \text{ mM}^{-1}) / 100 \text{ min} =$
$= 0.5 \text{ mM}^{-1} / 100 \text{ min} = 5 \; x \; 10^{-3} \text{ mM}^{-1} \text{ min}^{-1}$

c) Using the equation:

$\frac{1}{A_{(t)}} - \frac{1}{A_{initial}} = +k \; x \; (t_{(t)} - t_{initial})$, setting $t_{initial} = 0$, and solving for $\frac{1}{A_{initial}}$:

$$\frac{1}{A_{initial}} = \frac{1}{A_{(t)}} - k \; x \; t_{(t)}$$

We can use any $A_{(t)}$ with the corresponding $t_{(t)}$ from the data set.

$$\frac{1}{A_{initial}} = \frac{1}{1.053mM} - 5 \; x \; 10^{-3} \text{ mM}^{-1} \text{ min}^{-1} \; x \; 110 \text{ min} = 0.95 \text{ mM}^{-1} - 0.55 \text{ mM}^{-1} = 0.4 \text{ mM}^{-1}$$

To get $A_{initial}$ we need to inverse the result:

$A_{initial} = 1 / \frac{1}{A_{initial}} = 1 / 0.4\ mM^{-1} = 2.5\ mM$

d) Using the equation:

$\frac{1}{A_{(t)}} - \frac{1}{A_{initial}} = +k\ x\ (t_{(t)} - t_{initial})$, setting $t_{initial} = 0$, $A_{initial} = 2.5$ mM and solving for $\frac{1}{A_{(t)}}$:

$$\frac{1}{A_{(t)}} = k\ x\ t_{(t)} + \frac{1}{A_{initial}}$$

$$\frac{1}{A_{(t)}} = 5\ x\ 10^{-3}\ mM^{-1}\ min^{-1}\ x\ 80\ min + \frac{1}{2.5mM} = 0.4\ mM^{-1} + 0.4\ mM^{-1} = 0.8\ mM^{-1}$$

To get $A_{(t)}$ we need to inverse the result:

$A_{(t)} = 1 / \frac{1}{A_{(t)}} = 1 / 0.8\ mM^{-1} = 1.25\ mM$

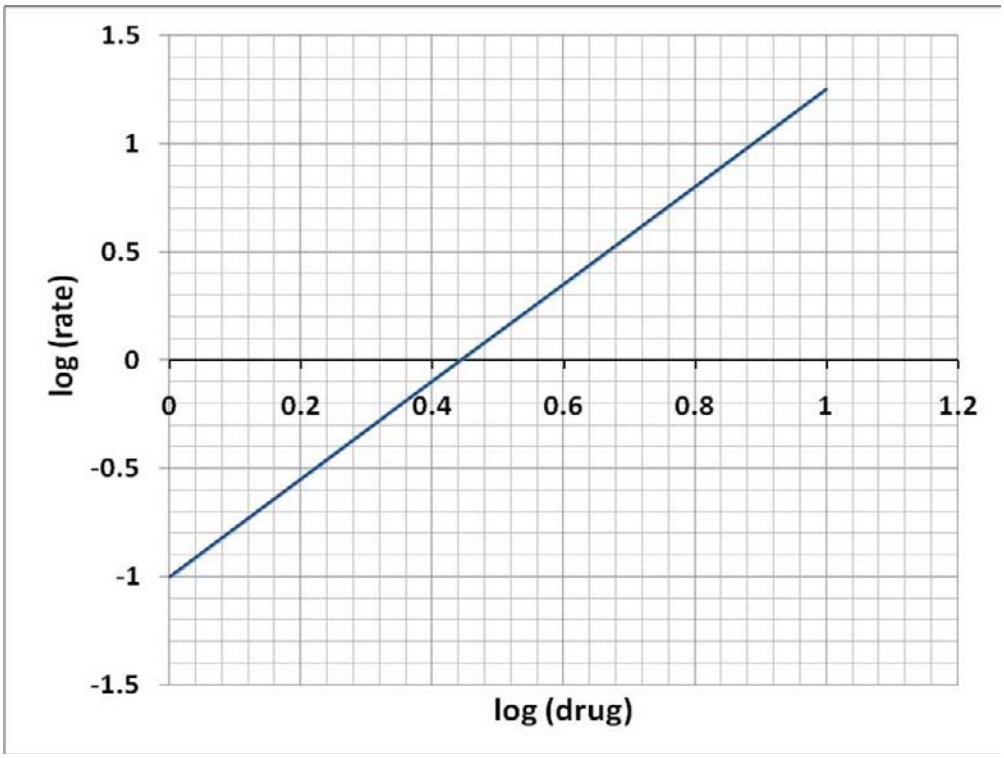
10.4. Answers to Chapter 7

Question 1:

Calculate log (drug concentration) and log (rate):

Drug [nM]	Rate of product formation [nM/hour]	log (drug)	log (rate)
1	0.1	0.00	-1.00
2	0.48	0.30	-0.32
4	2.26	0.60	0.35
6	5.63	0.78	0.75
8	10.76	0.90	1.03
10	17.78	1.00	1.25

Plot a graph with log (drug concentration) versus log (rate):



The graph shows a straight line and hence the rate order of the reaction does not change in the course of the reaction.

Using the equation

$m = \frac{\log(Rate_{(A2)}) - \log(Rate_{(A1)})}{\log(A2) - \log(A1)}$ and picking suitable data from the data set we get:

Drug [nM]	Rate of product formation [nM/hour]
1	0.1
2	0.48
4	2.26
6	5.63
8	10.76
10	17.78

$$m = \frac{\log(Rate_{(A2)}) - \log(Rate_{(A1)})}{\log(A2) - \log(A1)} = \frac{\log(17.78) - \log(0.1)}{\log(10) - \log(1)} = \frac{1.25 - (-1)}{1 - 0} = \frac{2.25}{1} = 2.25$$

To determine k we write the specific rate law expression:

Rate = k *x* $A^{2.25}$ and solve for k:

$$k = \frac{Rate}{A^{2.25}}$$

Any drug concentration and the corresponding rate can be used:

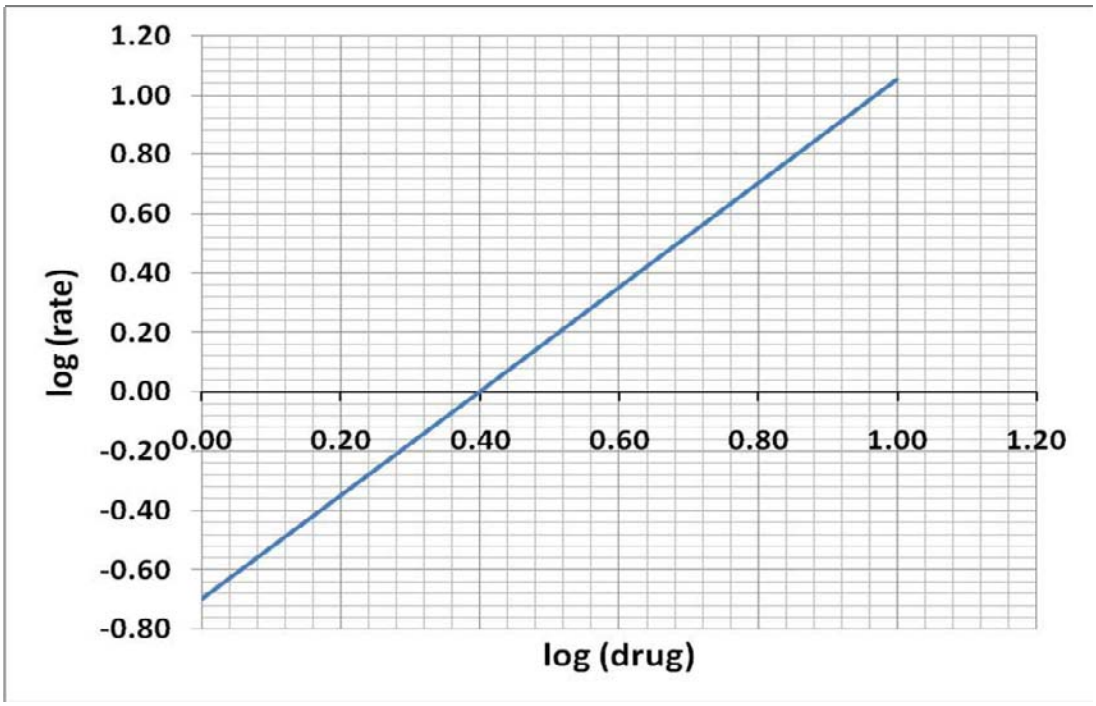
$$k = \frac{0.1nM/h}{(1nM)^{2.25}} = \frac{0.1nM/h}{1^{2.25}nM^{2.25}} = \frac{0.1nM/h}{1nM^{2.25}} = 0.1\ nM^{-1.25}\ h^{-1}$$

Question 2:

Calculate log (drug concentration) and log (rate):

Drug [nM]	Rate [nM/hour]	log (drug)	log (rate)
1	0.2	0.000	-0.699
2	0.67	0.301	-0.174
4	2.26	0.602	0.354
6	4.6	0.778	0.663
8	7.61	0.903	0.881
10	11.25	1.000	1.051

Plot a graph with log (drug concentration) versus log (rate):



The graph shows a straight line and hence the rate order of the reaction does not change in the course of the reaction.

Using the equation

$m = \frac{\log(Rate_{(A2)}) - \log(Rate_{(A1)})}{\log(A2) - \log(A1)}$ and picking suitable data from the data set we get:

Drug [nM]	Rate [nM/hour]
1	0.20
2	0.67
4	2.26
6	4.60
8	7.61
10	11.25

$$m = \frac{\log(Rate_{(A2)}) - \log(Rate_{(A1)})}{\log(A2) - \log(A1)} = \frac{\log(11.25) - \log(0.2)}{\log(10) - \log(1)} = \frac{1.05 - (-0.7)}{1 - 0} = \frac{1.75}{1} = 1.75$$

To determine k we write the specific rate law expression:

Rate = k *x* $A^{1.75}$ and solve for k:

$$k = \frac{Rate}{A^{1.75}}$$

Any drug concentration and the corresponding rate can be used:

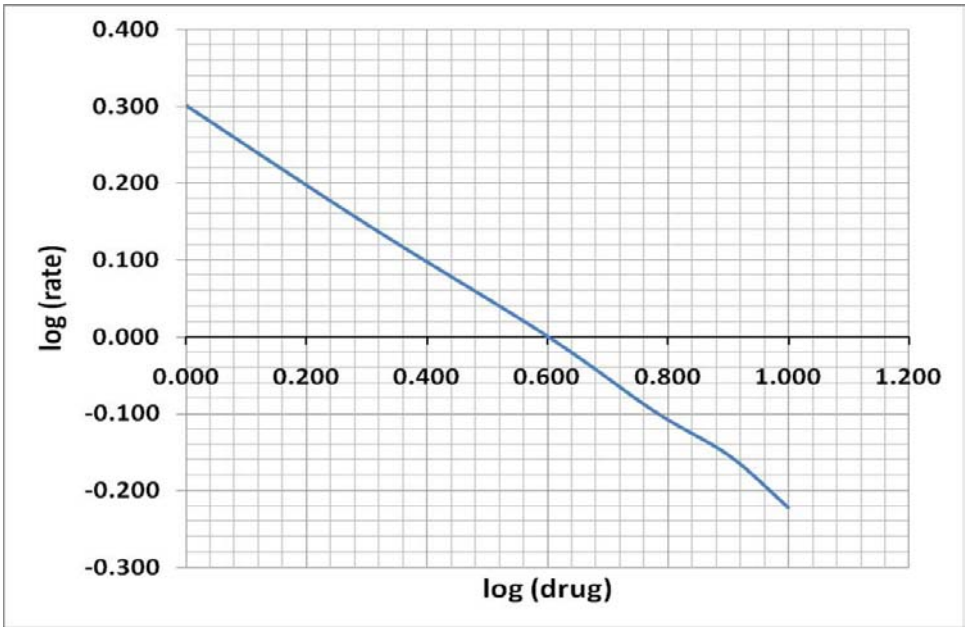
$$k = \frac{0.2nM/h}{(1nM)^{1.75}} = \frac{0.2nM/h}{1^{1.75}nM^{1.725}} = \frac{0.2nM/h}{1nM^{1.75}} = 0.2\ nM^{-0.75}\ h^{-1}$$

Question 3:

Calculate log (drug concentration) and log (rate):

Drug [nM]	Rate of glucose uptake [mM/min]	log(drug)	log (rate)
1	2	0.000	0.301
2	1.4	0.301	0.146
4	1	0.602	0.000
6	0.8	0.778	-0.097
8	0.7	0.903	-0.155
10	0.6	1.000	-0.222

Plot a graph with log (drug concentration) versus log (rate):



The graph shows a straight line and hence the rate order of the reaction does not change in the course of the reaction.

Using the equation

$$m = \frac{\log(Rate_{(A2)}) - \log(Rate_{(A1)})}{\log(A2) - \log(A1)}$$ and picking suitable data from the data set we get:

Drug [nM]	Rate of glucose uptake [mM/min]
1	2.0
2	1.4
4	1.0
6	0.8
8	0.7
10	0.6

$$m = \frac{\log(Rate_{(A2)}) - \log(Rate_{(A1)})}{\log(A2) - \log(A1)} = \frac{\log(0.6) - \log(2)}{\log(10) - \log(1)} = \frac{-0.22 - (0.3)}{1 - 0} = \frac{-0.52}{1} = -0.52$$

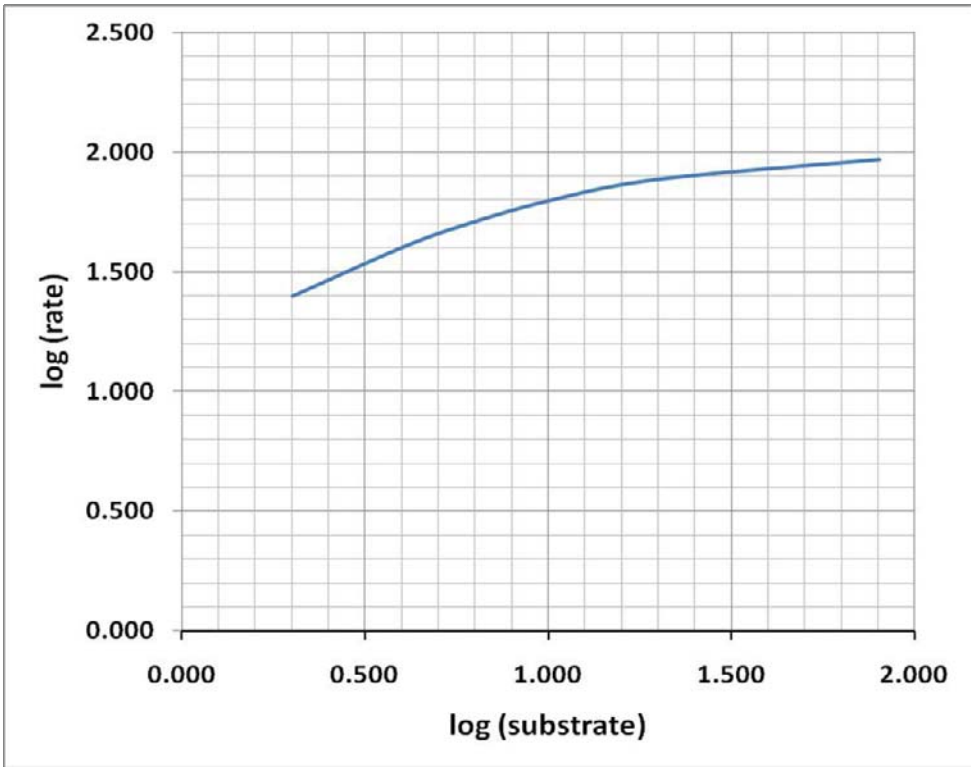
Increasing the drug concentrations leads to a DECREASE in the rate of glucose uptake. Obviously, the drug does not stimulate the uptake reaction, but inhibits it.

Question 4:

Calculate log (substrate) and log (rate):

Substrate [mM]	Rate [mM/min]	log (substrate)	log (rate)
2	25	0.301	1.398
4	40	0.602	1.602
6	50	0.778	1.699
10	62.5	1.000	1.796
20	76.9	1.301	1.886
80	93	1.903	1.968

Plot a graph with log (substrate) versus log (rate):



The graph does NOT give a straight line, indicating that the rate order changes in the course of the reaction. This is typical for enzyme catalyzed reactions.

10.5. Answers to Chapter 8

Question 1:

a) Write the general rate law expression as:

Rate = k *x* A^m *x* B^n *x* C^q

To determine the specific rate order m choose two experiments from the data set, in which the concentrations of A, but not the concentrations of B and C have been varied:

A [M]	B [M]	C [M]	Rate [M/s]
0.1	0.1	0.1	0. 14
0.2	0.1	0.1	0. 198

Calculate the rate order using the equation:

$$m = \frac{\log(Rate_{(A2)}) - \log(Rate_{(A1)})}{\log(A2) - \log(A1)}$$

$$m = \frac{\log(0.198) - \log(0.14)}{\log(0.2) - \log(0.1)} = \frac{-0.7 - (-0.85)}{-0.7 - (-1)} = \frac{0.15}{0.3} = 0.5$$

To determine the specific rate order n choose two experiments from the data set, in which the concentrations of B, but not the concentrations of A and C have been varied:

A [M]	B [M]	C [M]	Rate [M/s]
0.1	0.1	0.1	0. 14
0.1	0.3	0.1	1.26

Calculate the rate order using the equation:

$$n = \frac{\log(Rate_{(B2)}) - \log(Rate_{(B1)})}{\log(B2) - \log(B1)}$$

$$n = \frac{\log(1.26) - \log(0.14)}{\log(0.3) - \log(0.1)} = \frac{0.1 - (-0.85)}{-0.522 - (-1)} = \frac{0.954}{0.477} = 2$$

To determine the specific rate order q choose two experiments from the data set, in which the concentrations of C, but not the concentrations of A and B have been varied:

A [M]	B [M]	C [M]	Rate [M/s]
0.2	0.1	0.1	0. 198
0.2	0.1	0.15	0. 364

$$q = \frac{\log(Rate_{(C2)}) - \log(Rate_{(C1)})}{\log(C2) - \log(C1)}$$

$$q = \frac{\log(0.364) - \log(0.198)}{\log(0.15) - \log(0.1)} = \frac{-0.44 - (-0.7)}{-0.82 - (-1)} = \frac{0.264}{0.176} = 1.5$$

Overall rate order is m + n + p = 0.5 + 2 + 1.5 = 4

b) To determine the rate constant k write the specific rate law expression

Rate = k *x* $A^{0.5}$ *x* B^2 *x* $C^{1.5}$ and solve for k:

$$k = \frac{Rate}{A^{0.5} x B^2 x C^{1.5}}$$

Any rate with the corresponding concentrations of A, B and C can be used, but the easiest approach is to use rate and concentrations in which all the concentrations are the same.

A [M]	B [M]	C [M]	Rate [M/s]
0.1	0.1	0.1	0. 14

$$k = \frac{0.14M/s}{(0.1M)^{0.5}\,x\,(0.1M)^{2}\,x\,(0.1M)^{1.5}} = \frac{0.14M/s}{(0.1M)^{4}} = \frac{0.14M/s}{1x10^{-4}M^{4}} = 1400\ M^{-3}\ s^{-1}$$

c) Rate constant is, as the expression suggests, a constant and does NOT change.

Question 2:

a) Write the general rate law expression as:

Rate = $k\ x\ A^{m}\ x\ B^{n}\ x\ C^{q}$

To determine the specific rate order m choose two experiments from the data set, in which the concentrations of A, but not the concentrations of B and C have been varied:

A [M]	B [M]	C [M]	Rate [M/s]
0.1	0.1	0.1	0.0012
0.2	0.1	0.1	0.0024

Calculate the rate order using the equation:

$$m = \frac{\log(Rate_{(A2)}) - \log(Rate_{(A1)})}{\log(A2) - \log(A1)}$$

$$m = \frac{\log(0.0024) - \log(0.0012)}{\log(0.2) - \log(0.1)} = \frac{-2.62 - (-2.92)}{-0.7 - (-1)} = \frac{0.3}{0.3} = 1$$

To determine the specific rate order n choose two experiments from the data set, in which the concentrations of B, but not the concentrations of A and C have been varied:

A [M]	B [M]	C [M]	Rate [M/s]
0.1	0.1	0.1	0.0012
0.1	0.3	0.1	0.0036

Calculate the rate order using the equation:

$$n = \frac{\log(Rate_{(B2)}) - \log(Rate_{(B1)})}{\log(B2) - \log(B1)}$$

$$n = \frac{\log(0.0036) - \log(0.0012)}{\log(0.3) - \log(0.1)} = \frac{-2.44 - (-2.92)}{-0.522 - (-1)} = \frac{0.477}{0.477} = 1$$

To determine the specific rate order q choose two experiments from the data set, in which the concentrations of C, but not the concentrations of A and B have been varied:

A [M]	B [M]	C [M]	Rate [M/s]
0.2	0.1	0.1	0.0024
0.2	0.1	0.15	0.0054

$$q = \frac{\log(Rate_{(C2)}) - \log(Rate_{(C1)})}{\log(C2) - \log(C1)}$$

$$q = \frac{\log(0.0054) - \log(0.0024)}{\log(0.15) - \log(0.1)} = \frac{-2.27 - (-2.62)}{-0.82 - (-1)} = \frac{0.352}{0.176} = 2$$

Overall rate order is m + n + p = 1 + 1 + 2 = 4

b) To determine the rate constant k write the specific rate law expression

Rate = k *x* A^1 *x* B^1 *x* C^2 and solve for k:

$$k = \frac{Rate}{A^1 x B^1 x C^2}$$

Any rate with the corresponding concentrations of A, B and C can be used, but the easiest approach is to use rate and concentrations in which all the concentrations are the same.

A [M]	B [M]	C [M]	Rate [M/s]
0.1	0.1	0.1	0.0012

$$k = \frac{0.0012M/s}{(0.1M)^1 x (0.1M)^1 x (0.1M)^2} = \frac{0.0012M/s}{(0.1M)^4} = \frac{0.0012M/s}{1x10^{-4}M^4} = 12\ M^{-3}\ s^{-1}$$

c) To calculate the rate of the reaction at

A [M]	B [M]	C [M]	Rate [M/s]
0.2	0.2	0.2	

Use the special rate law expression

Rate = k *x* A^1 *x* B^1 *x* C^2

Rate = 12 M^{-3} s^{-1} *x* 0.2 M *x* 0.2 M *x* $(0.2M)^2$ =12 M^{-3} s^{-1} *x* 0.2^4 M^4 = 12 M^{-3} s^{-1} *x* 1.6 x 10^{-3} M^4 = =0.0192 M s^{-1}

Question 3:

a) Write the general rate law expression as:

Rate = k *x* A^m *x* B^n *x* C^q

To determine the specific rate order m choose two experiments from the data set, in which the concentrations of A, but not the concentrations of B and C have been varied:

A [mM]	B [mM]	C [mM]	Rate [mM/min]
0.1	0.1	0.1	0.250
0.2	0.1	0.1	1.000

Calculate the rate order using the equation:

$$m = \frac{\log(Rate_{(A2)}) - \log(Rate_{(A1)})}{\log(A2) - \log(A1)}$$

$$m = \frac{\log(1) - \log(0.25)}{\log(0.2) - \log(0.1)} = \frac{0 - (-0.6)}{-0.7 - (-1)} = \frac{0.6}{0.3} = 2$$

To determine the specific rate order n choose two experiments from the data set, in which the concentrations of B, but not the concentrations of A and C have been varied:

A [mM]	B [mM]	C [mM]	Rate [mM/min]
0.1	0.1	0.1	0.250
0.1	0.3	0.1	2.250

Calculate the rate order using the equation:

$$n = \frac{\log(Rate_{(B2)}) - \log(Rate_{(B1)})}{\log(B2) - \log(B1)}$$

$$n = \frac{\log(2.25) - \log(0.25)}{\log(0.3) - \log(0.1)} = \frac{0.352 - (-0.6)}{-0.522 - (-1)} = \frac{0.952}{0.477} = 2$$

To determine the specific rate order q choose two experiments from the data set, in which the concentrations of C, but not the concentrations of A and B have been varied:

A [mM]	B [mM]	C [mM]	Rate [mM/min]
0.2	0.1	0.1	1.000
0.2	0.1	0.15	3.375

$$q = \frac{\log(Rate_{(C2)}) - \log(Rate_{(C1)})}{\log(C2) - \log(C1)}$$

$$q = \frac{\log(3.375) - \log(1)}{\log(0.15) - \log(0.1)} = \frac{0.528 - (0)}{-0.82 - (-1)} = \frac{0.528}{0.176} = 3$$

Overall rate order is m + n + p = 2 + 2 + 3 = 7

b) To determine the rate constant k write the specific rate law expression

Rate = k *x* A^2 *x* B^2 *x* C^3 and solve for k:

$$k = \frac{Rate}{A^2 x B^2 x C^3}$$

Any rate with the corresponding concentrations of A, B and C can be used, but the easiest approach is to use rate and concentrations in which all the concentrations are the same.

A [mM]	B [mM]	C [mM]	Rate [mM/min]
0.1	0.1	0.1	0.250

$$k = \frac{0.25mM/\min}{(0.1mM)^2 x (0.1mM)^2 x (0.1mM)^3} = \frac{0.25mM/\min}{(0.1mM)^7} = \frac{0.25mM/\min}{1x10^{-7} mM^7} = 2.5 \times 10^6 \text{ mM}^{-6} \text{ min}^{-1}$$

c) To determine the concentration of B for

A [mM]	B [mM]	C [mM]	Rate [mM/min]
0.2	??	0.2	72.000

d) Write the the specific rate law expression

Rate = k x A^2 x B^2 x C^3 and solve for B:

$$B^2 = \frac{Rate}{kxA^2xC^3}$$

$$B = \sqrt{\frac{Rate}{kxA^2xC^3}}$$

$$B = \sqrt{\frac{72}{2.5x10^6x(0.2)^2x(0.2)^3}}$$

$$B = \sqrt{\frac{72}{2.5x10^6x(0.2)^5}} = \sqrt{\frac{72}{2.5x10^6x3.2x10^{-4}}}$$

$$B = \sqrt{\frac{72}{800}} = \sqrt{0.09} = 0.3$$ units are in mM

10.6. Answers to Chapter 9

Question 1:

$$\frac{dR}{dt} = k1 - k2\ x\ R\ x\ L + k3\ x\ RL$$

$$\frac{dL}{dt} = -k2\ x\ R\ x\ L + k3\ x\ RL$$

$$\frac{dRL}{dt} = k2\ x\ R\ x\ L - k3\ x\ RL + k5\ x\ D - k4\ x\ RL^2$$

$$\frac{dD}{dt} = k4\ x\ RL^2 - k5\ x\ D - k6\ x\ D$$

Question 2:

a) $\frac{dS}{dt} = -\,k1f\,x\,S + k1r\,x\,P$

$\frac{dP}{dt} = k1f\,x\,S - k1r\,x\,P + k2r\,x\,Q - k2f\,x\,P$

$\frac{dQ}{dt} = -\,k2r\,x\,Q + k2f\,x\,P$

To calculate the overall equilibrium constant for $\frac{Q}{S}$ we use the equation

$K_{eq} = \frac{P_{eq}}{S_{eq}}\ x\ \frac{Q_{eq}}{P_{eq}} = \frac{k1f}{k1r}\ x\ \frac{k2f}{k2r}$

$S_{initial}$ = 100 mM	k1f = 0.1 min^{-1}
$P_{initial}$ = 0 mM	k1r = 0.04 min^{-1}
$Q_{initial}$ = 0 mM	k2f = 0.4 min^{-1}
	k2r = 0.1 min^{-1}

$K_{eq} = \frac{Q_{eq}}{S_{eq}} = \frac{0.1\,min^{-1}}{0.04\,min^{-1}}\ x\ \frac{0.4\,min^{-1}}{0.1\,min^{-1}} = 2.5\ x\ 4 =\ 10$ (all units cancelled out)

b) Since k2f and k2r are both zero, the reaction is a simple equilibrium reaction:

$$S \underset{k1r}{\overset{k1f}{\rightleftharpoons}} P$$

$S_{initial}$ = 100 mM	k1f = 0.1 min^{-1}
$P_{initial}$ = 0 mM	k1r = 0.04 min^{-1}
$Q_{initial}$ = 0 mM	k2f = 0.0 min^{-1}
	k2r = 0.0 min^{-1}

$K_{eq} = \frac{P_{eq}}{S_{eq}} = \frac{k1f}{k1r} = \frac{0.1\,min^{-1}}{0.04\,min^{-1}} = 2.5$

Also P + S + Q = $S_{initial}$ = 100 mM

Since k2f (and k2r) = 0 no Q can be formed and hence Q = 0 mM.

$P_{eq} + S_{eq} = 100$ mM and $P_{eq} = 100\ mM - S_{eq}$

Substituting P_{eq} in the equilibrium equation gives:

$$K_{eq} = \frac{P_{eq}}{S_{eq}} = \frac{100mM - S_{eq}}{S_{eq}} = 2.5$$

Solving for S_{eq}:

$100\ mM - S = 2.5\ x\ S_{eq}$
$100\ mM = 2.5\ x\ S_{eq} + S_{eq}$

$$S_{eq} = \frac{100mM}{3.5xS_{eq}} = 28.6\ mM$$

Since $P_{eq} = 100\ mM - S_{eq} = 100\ mM - 28.6\ mM = 71.4\ mM$

c) Since the rate constants for the reverse reactions are zero, the scheme is:

$$S \xrightarrow{k1f} P \xrightarrow{k2f} Q$$

$$\frac{dP}{dt} = k1f\ x\ S - k2f\ x\ P$$

In steady state

$$\frac{dP}{dt} = 0$$ therefore

$k1f\ x\ S - k2f\ x\ P = 0$ or $k1f\ x\ S = k2f\ x\ P$

Solving for P:

$$P_{steadystate} = \frac{k1fxS}{k2f} = \frac{0.05\,\text{min}^{-1}\ x100mM}{0.4\,\text{min}^{-1}} = 125\ mM.$$

The steady state concentration of P is direct proportional to the concentration of S. The higher the concentration of S, the higher the steady state concentration of P.