2 Amount and concentration: making and diluting solutions

2A Rationale

Biological and biochemical investigations rely completely upon being able to detect the concentration of a variety of substances. For example, in diabetics it is important to know the concentration of glucose in the blood and you may also need to be able to calculate how much insulin would need to be dissolved in a certain volume of saline so as to give the right amount in a 1ml injection volume. It is also vitally important that you perform these calculations quickly, reliably and accurately.

This section aims to help you understand how to go about doing these calculations and to give you plenty of practice so that you can do them quickly and reliably.

2B Distinguishing between amount and concentration, g and %w/v

The **amount** of a substance is often measured by how much it weighs – i.e. in g or mg or kg etc.

The **concentration** is the **amount divided by the volume** it is dissolved in.

$$concentration = \frac{amount}{volume}$$

For example you might dissolve

0.9g of sodium chloride in 100mL of water.

Sometimes this is referred to as **0.9% w/v** since it is 0.9g weight divided by 100mL volume and "w/v" stands for weight per volume."

The concentration of this solution could be described as

0.009 g/mL or 9 mg/mL.

Alternatively you could notice that if 0.9g is dissolved in 100mL, then 9g would be dissolved in 1000ml (=1L) and the concentration could also be written as

9g/L

2C Distinguishing between amount and concentration, moles and molar

Another way of representing the amount of a substance is to use the concept of **mole**.
A mole is simply 6.0223×10^{23} molecules (this is "Avogadro's number")

Eggs come in dozens (12), molecules come in moles (6×10^{23})

The mass of a mole of molecules is the formula weight in grams.
This is sometimes called "molar mass" with symbol M.
Sometimes it is referred to as molecular weight.
Sometimes it is called "relative molecular mass" since the mass of a carbon element is designated to be 12 and other molecules or elements are given masses relative to that.

The molar mass of a small molecule such as an amino acid (eg glycine is NH_2CH_2COOH and has molecular mass = 75.07 $g.mol^{-1}$) is much less than the molar mass of a protein (eg haemoglobin has molecular mass ~ 64500 $g.mol^{-1}$).

6.0223×10^{23} glycine molecules (or 1 mole) would weigh 75.07 g whereas
6.0223×10^{23} haemoglobin molecules (or 1 mole) would weigh ~64500g (or 64.5kg)

The important thing to remember for calculations is that the molecular weight/molecular mass/molar mass – call it what you will – has the units of $g.mol^{-1}$

To convert between an amount in g and an amount in moles,

$$\boxed{\text{Amount (in g)} = \text{Amount (in mol)} \times \text{Molar Mass } (g.mol^{-1})}$$

Sometimes the term **"Dalton"** is used for molecular mass.
For example the molecular mass of glycine is 75 Da while the molecular mass of haemoglobin is 64.5kDa (or 64500 Da).

The **concentration** of a solution of 1 mole dissolved in 1 L is 1 M ("one molar")

$$\boxed{1\text{ M} = \frac{1\text{ mole}}{1\text{ litre}}}$$

Don't confuse this symbol "M" for mol/l with "M" for molar mass. It's unfortunate they have been used for two quite different things.

In the case of glucose, a 1 M solution would have 1 mole = 180 g dissolved in 1 L.

So another way of saying that is 1 M glucose solution = 180 g/1000 ml = 0.18 g/ml

Alternatively you could call it 18 % (w/v).

You need to be able to convert easily and accurately between different ways of expressing concentration.

2D Practice converting g/L to M and vice versa

Example 1: Converting g/L to M

You have weighed out 20 g KCl (formula weight of KCl is 74.55 $g.mol^{-1}$) and dissolved it in 500 mL, what is the concentration in M?

Work out how many moles are equivalent to 20 g.

Amount (in g) = Amount (in mol) x Molar Mass ($g.mol^{-1}$)

Rearranging:

$$\text{Amount (in moles)} = \frac{\text{amount (in g)}}{\text{molar mass (in } g.mol^{-1})}$$

$$= \frac{20g}{74.55g.mol^{-1}} \qquad = 0.268 \text{ mol}$$

Now work out the concentration by dividing the amount (in mol) by the volume (in L)

$$\text{Concentration (M)} = \frac{\text{amount (in mol)}}{\text{volume (in L)}}$$

$$= \frac{0.268mol}{0.5L} \qquad = 0.537 \text{ M}$$

Example 2.
Converting M to g/L

You have a 1 M solution of NaCl, what is the concentration in % (w/v)?

1 M = 1 mol / 1 L

1 mol of NaCl = 58.44 $g.mol^{-1}$

$$\text{Concentration (M)} = \frac{1 \text{ mol}}{1 \text{ L}} = \frac{58.44\text{g}}{1 \text{ L}} = \frac{5.844\text{g}}{100 \text{ mL}} = 5.8\% \text{ (w/v)}$$

Example 3.
You are given 580 mg of adrenaline and need to make a 1 mM solution. What volume of water do you need to dissolve it in? The molar mass of adrenaline is 183.2 $g.mol^{-1}$

First you need to convert 580 mg to moles:

$$\text{Amount (in moles)} = \frac{\text{amount (in g)}}{\text{molar mass (in g.mol}^{-1}\text{)}}$$

$$= \frac{0.58g}{183.2g.mol^{-1}} = 0.003166 \text{ mol}$$

Then work out the volume by rearranging the equation:

$$\text{Concentration (M)} = \frac{\text{amount (in mol)}}{\text{volume (in L)}}$$

becomes:

$$\text{Volume (in L)} = \frac{\text{amount (in mol)}}{\text{concentration (in M)}}$$

$$\text{Volume (in L)} = \frac{0.003166 \text{ mol}}{0.001 \text{ mol.L}^{-1}}$$

$$= 3.166 \text{ L}$$

Example 4.

You have a 1 mM solution of adrenaline and wish to dispense a volume which contains 10 mg. What volume do you need to dispense?

First convert 10mg to moles:

$$\text{Amount (in moles)} = \frac{\text{amount (in g)}}{\text{molar mass (in g.mol}^{-1}\text{)}}$$

$$= \frac{0.01g}{183.2g.mol^{-1}} = 5.46 \times 10^{-5} \text{ mol}$$

$$\text{volume} = \frac{\text{amount}}{\text{concentration}}$$

$$\text{volume} = \frac{5.46 \times 10^{-5}\ \text{mol}}{1 \times 10^{-3}\ \text{mol.L}^{-1}}$$

$$\text{volume} = 5.46 \times 10^{-2}\ \text{L} = 0.0546\ \text{L} = 54.6\ \text{mL}$$

Example 5.
You want to make 10 mL of a 5 mM solution of adrenaline, how much should you weigh out?

Amount = volume x concentration
= 0.01 L x 5 x 10^{-3} mol.L^{-1}
= 5 x 10^{-5} mol
= 5 x 10^{-5} mol x 183.2 g.mol^{-1}
= 916 x 10^{-5} g
= 9.16 mg

2E Diluting Solutions

Simple Dilutions
Make a 1:10 dilution (say this as "a one in ten dilution").

Take 1 part of your stock solution and add 9 parts of solvent (usually water but sometimes alcohol or other organic solvent).

If your original sodium chloride solution is 1M, and you dilute it 1:10, then you might

take	10ml of 1M NaCl	and	90ml water,	(10 + 90 = 100)
or you could take	1ml of 1M NaCl	and	9ml water,	(1 + 9 = 10)
or	0.2 ml 1M NaCl	and	1.8ml water	(0.2 + 1.8 = 2)

In all cases you are diluting by the same factor.

The concentration of the resulting solution is 1M /10 = 0.1M where 10 is the *dilution factor*.

[Although it is really quite obvious, you can convince yourself that the resulting concentration is 0.1M by the following:

Take 10 ml of 1M NaCl, how much NaCl is there in that?

Answer:

$$concentration = \frac{amount}{volume} \quad \rightarrow \quad amount = concentration \times volume$$

Amount in 10ml = 10ml x 1mol.L^{-1} x 0.001 L.mL^{-1} = 0.01mol

So imagine that you are taking 10ml which has 0.01mol salt in it, and you put it into a total volume of 100ml.

$$concentration = \frac{amount}{volume} = \frac{0.01mol}{0.100L} = 0.1M \qquad]$$

For example, a
1:5 dilution = 1 unit volume of diluent + 4 unit volumes of solvent;
→ dilution factor = 5

1:2 dilution = 1 unit volume of diluent + 1 unit volume of solvent;
→ dilution factor = 2

1:7.4 dilution = 1 unit volume of diluent + 6.4 unit volumes of volume:
→ dilution factor = 7.4

Serial Dilutions

Serial dilutions are a quick and convenient way to prepare a wide range of concentrations. Typically, they are done in powers of 10 or 100 (but not always).
So for example:

Take a 1mM stock solution of adrenaline, dilute it 1:10 (0.1ml + 0.9ml) to give…
a 0.1mM solution – dilute this 1:10 to give …
a 0.01mM solution – dilute this 1:10 to give …
a 0.001mM solution – dilute this 1:10 to give …
a 0.0001mM solution and so on…

This approach allows you to prepare very dilute solutions from a concentrated stock much more accurately than the alternative.

2F Practice calculating dilutions

The most common task you will be asked to do is to prepare a particular concentration from a stock solution. So say you are asked to prepare 0.32M sucrose from a 1M sucrose stock.
There are two ways of approaching this and these are illustrated below. Think about them both and then choose whichever makes the most sense for you.

Method 1:
Step 1: work out what the dilution factor is ..
1/0.32 = 3.125

Step 2: turn this into volume of diluent and solvent..

1 part of 1M sucrose plus 2.125 parts water gives 3.125 parts of 0.32M sucrose

you could write this as 1ml x 1M sucrose + 2.125ml water → 3.125ml x 0.32M sucrose

or you could scale it up to whatever volume you want, say
32ml x 1M sucrose + 68ml water → 100 ml x 0.32M sucrose

Method 2:
The alternative method is this:
Start by noticing that amount = concentration x volume or A = C.V

Now if you take a volume V1 of your concentrated solution (concentration = C1)
Then the amount you have in your pipette is A1.
Now if you put that amount into a new volume V2, the amount is still constant, but now because you have a bigger volume, the concentration C2 = A/V2.
But from before, A = C1.V1,

so C1.V1 = C2.V2

What this is saying is that if the amount is the same in both solutions, then their concentrations and volumes must be related.

You can use this relationship to calculate the required volume V2 to get a particular concentration. Taking our earlier example,
C1 = 1M, V1 = 1ml, C2 (the desired concentration) = 0.32M what is the resulting volume?

$$1M \times 1ml = 0.32M \times V2.$$

$$V2 = \frac{1}{0.32} ml$$

$$V2 = 3.125ml$$

What about if you were asked to make 100ml of 0.32M sucrose?
Now you have C1=1M, C2 = 0.32M, V2 = 100ml and V1 is the unknown.
1M x V1 = 0.32M x 100ml
V1 = 32ml

Summary of learning objectives

At the end of this section you should be able to:

a. To understand the difference between amount and concentration
b. To be able to calculate the concentration of a solution in a variety of formats including M, g/l, %v/v, %w/v
c. To be able to quickly and reliably convert from one unit to another
d. To be able to quickly and reliably calculate the appropriate way to prepare a dilution