Foundation Mathematics for Biosciences

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Foundation Mathematics for **Biosciences**



Solution

Since 1 ppm is equivalent to 1 mg/L, we need to find out how many moles of NO_2^- there are in 1 mg. We can write a proportion:

$$\frac{46.01\,\mathrm{g}}{1\,\mathrm{mol}} = \frac{1\,\mathrm{mg}}{x}$$

where x is the number of moles of NO_2^- in 1 mg of this compound. From this proportion:

$$x = \frac{1 \text{ mol} \times 1 \text{ mg}}{46.01 \text{ g}} = \frac{1 \text{ mol} \times 10^{-3} \text{ g}}{46.01 \text{ g}} = 2.2 \times 10^{-5} \text{ mol}$$
 (2 s.f.)

Since this is the number of moles present in 1 L of water, then the concentration of NO₂⁻ is:

$$2.2 \times 10^{-5} \, mol/L = 2.2 \times 10^{-5} \, M = 22 \times 10^{-6} \, M = 22 \, \mu M$$

SELF-ASSESSMENT

- 6.1.1 How much magnesium chloride $(MgCl_2)$ do you need to weigh out to make 250 mL of a 1.2 M solution? The molar masses of magnesium and chloride are: M(Mg) = 24.305 g/mol and M(Cl) = 35.453 g/mol.
- 6.1.2 How much adenosine 5'-triphosphate (ATP) do you need to make 2 L of a 150 μM solution? The molar mass of ATP is 507.18 g/mol.
- 6.1.3 How much ferrous sulphate heptahydrate (FeSO₄ · 7H₂O) do you need to prepare 50 cm³ of an 85 mM solution? The molar mass of this compound is 278.01 g/mol.
- **6.1.4** What is the molar concentration of a solution containing 9.6×10^{22} molecules per litre?
- 6.1.5 You have made a solution containing 27 g of ribose ($C_5H_{10}O_5$) in 500 mL. The molar mass of ribose is 150.13 g/mol. What is the

- concentration of this solution expressed in g/L, as molarity and percentage (w/v) concentration?
- 6.1.6 What is the percentage (w/v) concentration of a 6.5 M solution of guanidine hydrochloride (CH₆ClN₃)? The molar mass of this compound is 95.53 g/mol.
- 6.1.7 What is the molar concentration of a 58 % (w/v) solution of ammonium sulphate, (NH₄)₂ SO₄? The molar mass of this compound is 132.14 g/mol.
- 6.1.8 You have a vial containing freeze-dried actin from rabbit muscle with a purity of 85%. The total mass of the powder inside the vial is 5 mg. In what volume should you resuspend the contents of the vial to obtain a 1 mg/mL solution of actin?
- **6.1.9** What is the concentration of 100 μM fluoride (F⁻) in water expressed in ppm if the molar mass of F⁻ is 19.0 g/mol?

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- 6.1.1 The molar mass of sodium chloride (NaCl) is 58.44 g/mol. How much NaCl do you need to weigh out to prepare the following:
 - (a) 0.025 L of 5 M solution
 - (b) 150 mL of 20 mM solution
- 6.1.2 You are making 0.2 M Tris-HCl, pH 6.8 by dissolving Tris base (molar mass 121.14 g/mol) in water and adjusting the pH with HCl. How much Tris base do you need to weigh out to prepare 0.8 dm³ of this buffer?
- 6.1.3 The molar mass of glucose (C₆H₁₂O₆) is 180.16 g/mol. How much glucose do you need to weigh out to prepare the following:
 - (a) 0.25 L of 0.036 M solution
 - (b) 5 L of 670 μM solution
- 6.1.4 EDTA (ethylene diamine tetraacetic acid) solutions are time-consuming to prepare as EDTA does not dissolve easily and the pH of the solution needs to be adjusted as well. Therefore, it is a common practice to prepare a 0.5 M stock solution and dilute small amounts of it as required. How much EDTA (molar mass 292.24 g/mol) would you need to weigh out to make 0.25 L of 0.5 M solution?
- 6.1.5 The molar mass of potassium dihydrogen phosphate (KH₂PO₄) is 136.09 g/mol. How much potassium dihydrogen phosphate would you use to make the following:
 - (a) 0.75 dm³ of 0.4 M solution
 - (b) 2 dm³ of 1 mM solution
- 6.1.6 How much dithiothreitol (DTT) do you need to use to prepare 16 mL of 1 M stock solution? The molar mass of DTT is 154.25 g/mol.
- **6.1.7** The molar mass of glycine (C₂H₅NO₂) is 75.07 g/mol. What amount of glycine is required to make the following:

- (a) $500 \,\mathrm{cm}^3$ of $0.009 \,\mathrm{M}$ solution
- **(b)** 0.8 dm³ of 25 mM solution
- 6.1.8 How much sodium acetate (CH₃COONa, molar mass 82.03 g/mol) do you need to weigh out to prepare the following:
 - (a) 400 cm³ of 5 mM solution
 - (b) 25 cm³ of 1.4 M solution
- 6.1.9 You have to prepare 50 mL of 180 μM lysozyme solution for an experiment. How much lysozyme do you need to weigh out if the molar mass of this protein is 14388 g/mol?
- 6.1.10 You are studying protein folding using urea as a protein denaturant. How much urea (NH₂CONH₂, molar mass 60.06 g/mol) do you need to make 150 mL of 8 M solution?
- **6.1.11** How much Triton X-100 do you need to weigh out to prepare 25 cm³ of 6 % (w/v) solution?
- 6.1.12 Cobalt chloride solution changes colour from clear to blue when heated and can be used as a temperature indicator. You need to have 400 cm³ of 18 % (w/v) cobalt chloride solution to carry out an experiment.
 - (a) How much anhydrous CoCl₂ (molar mass 129.84 g/mol) would you use?
 - (b) If you had to make this solution with cobalt chloride hexahydrate, what amount of this compound would you have to use? The molar mass of CoCl₂·6H₂O is 237.93 g/mol.
- 6.1.13 You need to prepare 150 cm³ of 9% (w/v) MgCl₂ solution. However, you do not have MgCl₂ in the lab and have to use magnesium chloride hexahydrate (MgCl₂·6H₂O) instead. How much MgCl₂·6H₂O do you need to weigh out to prepare this solution? The molar masses of MgCl₂ and MgCl₂·6H₂O are 95.21 and 203.30 g/mol, respectively.

- 6.1.14 You are transfecting cultured cells using a calcium phosphate method in which DNA precipitates are formed by mixing DNA with a highly concentrated solution of calcium chloride. How much calcium chloride dihydrate (CaCl₂·2H₂O, molar mass 147.01 g/mol) do you need to weigh out to make 80 mL of 2.5 M CaCl₂ solution?
- 6.1.15 How many glucose molecules are there in $1 \mu L$ of 4 pM glucose solution? Assume that the Avogadro constant is equal to $6.022 \times 10^{23} \, \text{mol}^{-1}$.
- 6.1.16 How many carbon atoms are there in 1 μ L of 1.3 pM glucose solution? The molecular formula of glucose is $C_6H_{12}O_6$. Assume that the Avogadro constant is equal to $6.022 \times 10^{23} \, \text{mol}^{-1}$.
- 6.1.17 The molar mass of sucrose (C₁₂H₂₂O₁₁) is 342.30 g/mol. You have made a solution containing 153 g/L of sucrose. What is the molar and percentage (w/v) concentration of this solution?
- **6.1.18** The molar mass of sodium hydroxide (NaOH) is 40.00 g/mol. You have dissolved 150 g of NaOH in 250 cm³ of water and added water to the total volume of

- 500 cm³. What is the molar and percentage (w/v) concentration of this solution?
- **6.1.19** What is the percentage (w/v) concentration of a 0.45 M solution of sodium sulphate (Na₂SO₄)? The molar mass of Na₂SO₄ is 142.04 g/mol.
- 6.1.20 What is the percentage (w/v) concentration of 5.2 M solution of ammonium acetate (CH₃CO₂NH₄)? The molar mass of ammonium acetate is 77.08 g/mol.
- **6.1.21** What is the molar concentration of 35 % (w/v) solution of glucose (C₆H₁₂O₆)? The molar mass of glucose is 180.16 g/mol.
- 6.1.22 What is the molar concentration of 6.3 % (w/v) solution of potassium chloride (KCl)? The molar mass of KCl is 74.55 g/mol.
- 6.1.23 You have made a 20 mg/mL solution of bovine serum albumin (BSA) that you intend to use as a standard in Bradford assay. What is the percentage (w/v) concentration of this solution?
- **6.1.24** You have a 127 μM solution of lysozyme. What is the concentration of this solution expressed in mg/mL? The molar mass of lysozyme is 14388 g/mol.

6.2 Standard and serial dilutions

Frequently in laboratory work, a concentrated solution of a substance is prepared (a **stock solution**) which is diluted immediately before use to obtain a 'working solution' of a specific concentration. One of the reasons for this is that complex solutions may take a considerable amount of time to prepare. For example, in order to make a phosphate buffered saline $10\times$ (PBS $10\times$) solution you need to weigh out 80 g of NaCl, 2 g of KCl, 14.4 g of Na₂HPO₄ and 2.4 g of KH₂PO₄. After dissolving them in a small volume of water, you need to adjust the pH with 1 M HCl to pH 7.4 before you can finally adjust the volume to 1 L. Other solutions may take a long time to prepare because they contain compounds with low solubility. For example, in order to make a solution of sodium orthovanadate (Na₃VO₄), you have to adjust the pH to 10, boil the solution and cool it down. This cycle of adjusting the pH, boiling and cooling has to be repeated, usually 3–5 times.

Another reason why stock solutions are made is that weighing out very small amounts of substance results in a large relative error. For example, let us estimate the error made while weighing out

0.005 g and 5 g using a typical laboratory balance with a 0.001 g resolution. The relative error when weighing out 0.005 g is:

$$\frac{0.001 \text{ g}}{0.005 \text{ g}} = 0.2 = 20 \%$$

The relative error when weighing out 5 g is much smaller:

$$\frac{0.001 \text{ g}}{5 \text{ g}} = 0.0002 = 0.02 \%$$

So a solution prepared using 5 g of substance would have a more accurate concentration than the other solution. One might argue that you could simply dissolve 5 g of substance in a larger volume and obtain the concentration you need without having to make a stock solution. However, if you wanted to obtain a solution with a 0.005 g/L concentration using 5 g of substance, you would have to prepare a thousand litres of solution!

If dilution of the stock solution can be accomplished easily and accurately in one step, a **standard dilution** is performed. Where the dilution requires two or more stages to achieve the required concentration, this is referred to as a **serial dilution**. We will consider examples of both standard and serial dilutions in the following sections.

6.2.1 Standard dilutions

The process of dilution is illustrated in Fig. 6.2.1, where the solution with an initial concentration C_i and volume V_i represents the stock solution and the solution with a final concentration C_f and volume V_f represents the diluted solution.

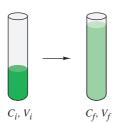


Figure 6.2.1 During the process of dilution, a solution with an initial concentration C_i and volume V_i is diluted to a final concentration C_f and volume V_f .

A quantity that is often used in calculations related to dilutions is a **dilution factor** *DF* (also referred to as **dilution fold**). It is defined as the ratio of the concentrations:

$$DF = \frac{C_i}{C_f} \tag{6.2.1}$$

It is also equal to the ratio of the volumes:

$$DF = \frac{V_f}{V_i} \tag{6.2.2}$$

As dilution factor is a ratio of two quantities with the same units, it is another example of a dimensionless quantity, defined in Section 3.1.3.

For example, if we take 1 cm³ of a 1 M stock solution and dilute it to obtain 10 cm³ of a 0.1 M solution, we have made a dilution with the dilution factor that can be calculated using either Equation 6.2.1 or 6.2.2:

$$DF = \frac{C_i}{C_f} = \frac{1 \text{ M}}{0.1 \text{ M}} = 10$$

$$DF = \frac{V_f}{V_i} = \frac{10 \text{ cm}^3}{1 \text{ cm}^3} = 10$$

We can see that a 10-fold dilution is associated with a 10-fold decrease in concentration and a 10-fold increase in the volume of the solution.

Sometimes the dilution factor is given in the name of the stock solution. For example, the earlier mentioned PBS 10× solution would be diluted 10 times for its normal use.



Worked example 6.2.1

You are preparing a 100-fold dilution from a 50 % sucrose solution to give a final volume of 100 cm³. Calculate the volume of the stock solution and water that you should use and the concentration of sucrose in the diluted solution.

Solution

We can use Equation 6.2.2 to calculate the volume of the stock solution by rearranging it to obtain an expression for the initial volume V_i :

$$V_i = \frac{V_f}{DF}$$

$$V_i = \frac{100 \,\mathrm{cm}^3}{100} = 1 \,\mathrm{cm}^3$$

Having established that 1 cm³ of sucrose solution is required, the volume of the **diluent** will be the difference between the final volume of the diluted solution (V_f) and the initial volume of the solution (V_i) :

volume of diluent =
$$V_f - V_i$$

This is equal to:

$$100 \,\mathrm{cm}^3 - 1 \,\mathrm{cm}^3 = 99 \,\mathrm{cm}^3$$

To calculate the concentration of sucrose in the diluted solution, we can use Equation 6.2.1 by rearranging it to obtain an expression for the final concentration C_f :

$$C_f = \frac{C_i}{DF}$$

$$C_f = \frac{50 \%}{100} = 0.5 \%$$

So a 100-fold dilution of 1 $\rm cm^3$ of a 50 % sucrose solution to 100 $\rm cm^3$ will result in a solution with a 0.5 % concentration.



Worked example 6.2.2

You are preparing a 1 in 5 dilution from a 40 % sodium chloride solution in water to give a final volume of 50 cm³. Calculate the volume of the stock solution and water that you should use and the concentration of NaCl in the diluted solution.

Solution

To perform a 1 in 5 dilution, 1 part of the NaCl stock solution must be mixed with 4 parts of water so that in the diluted solution there is 1 part NaCl stock in a total of 5 parts. Therefore, the dilution factor here is 5. The volume of the stock solution that needs to be diluted is:

$$V_i = \frac{V_f}{DF} = \frac{50 \text{ cm}^3}{5} = 10 \text{ cm}^3$$

The volume of water that has to be added is therefore:

$$50 \text{ cm}^3 - 10 \text{ cm}^3 = 40 \text{ cm}^3$$

The concentration of NaCl in the diluted solution is:

$$C_f = \frac{C_i}{DF} = \frac{40 \%}{5} = 8 \%$$



Worked example 6.2.3

What is the dilution factor for a drug solution that is diluted from a 40 mg/mL stock solution to a 2 mg/mL stock solution using methanol? Calculate the volume of the stock solution and methanol required to prepare the dilution where the final volume will be 70 mL.

Solution

We can use Equation 6.2.1 to calculate the dilution factor:

$$DF = \frac{C_i}{C_f} = \frac{40 \text{ mg/mL}}{2 \text{ mg/mL}} = 20$$

The volume of stock required can be calculated using a rearranged form of Equation 6.2.2:

$$V_i = \frac{V_f}{DF} = \frac{70 \text{ mL}}{20} = 3.5 \text{ mL}$$

The volume of methanol required will be therefore:

$$70 \text{ mL} - 3.5 \text{ mL} = 66.5 \text{ mL}$$



Worked example 6.2.4

You are preparing a sample for sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE), a technique used for the separation of proteins. You are adding a 4× sample loading buffer to 24 µL of protein solution. What volume of the 4× buffer do you need to use to obtain a 1× solution?