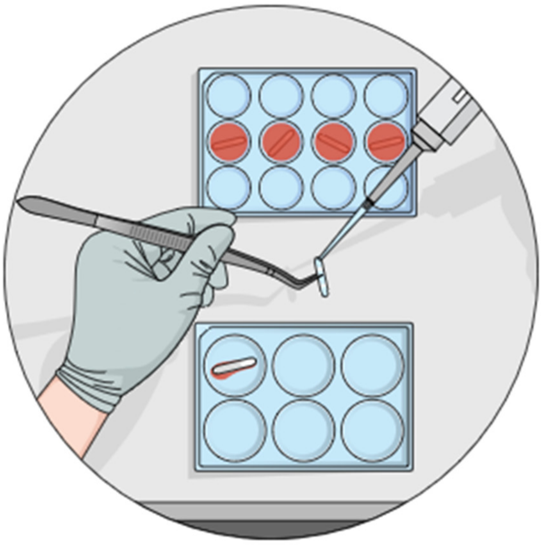
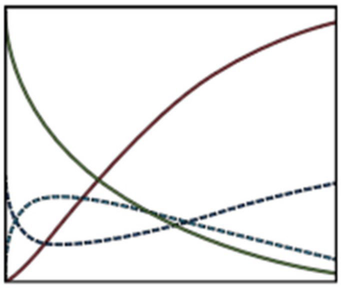
CONCENTRATION, CELL DENSITY AND SATURATION CALCULATIONS IN CELL BIOLOGY





Peter Nagy

Department of Biophysics and Cell Biology

University of Debrecen

Debrecen, Hungary

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https://biophys.med.unideb.hu

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1. Concentration calculations

1.1. Definition of concentration-related terms

- Mole (abbreviation: mol): unit of measurement for the amount of substance, one mole contains $6 \cdot 10^{23}$ elementary entities. This number is called Avogadro number (N_A), or Loschmidt number in German texts.
- Molarity (molar concentration): number of moles of solute in 1 L of solution. Unit is mol/L=mol/dm^3=M. A solution containing 1 mol solute in 1 L of solution is said to be 1 molar (1 M).
- Molality: number of moles of solute in 1 kg of solvent. Unit is mol/kg.
- Molecular mass or molecular weight: the mass of 1 mole of material. Its unit is g/mol. In biology molecular weight is usually given in dalton (Da) or kilodalton (kDa) units. 1 Da=1 g/mol.
- Mass by volume percentage: mass of the solute in 100 mL of solution. The fact that mass is divided by volume is usually denoted by w/v, i.e., weight by volume. A 1% (w/v) solution contains 1 g solute in 100 mL of solution:

$$1\%(\text{w/v}) = \frac{1 \text{ g solute}}{100 \text{ mL solution}} \tag{1}$$

  Mass by volume fraction can also be expressed in other units, e.g., g/L. 1 g/L = 1 mg/mL. The mass by volume percentage of a solution with a concentration of 1 mg/ml is 0.1%:

$$\frac{1 \text{ mg solute}}{1 \text{ mL solution}} = \frac{1 \text{ g solute}}{1 \text{ L solution}} = \frac{0.1 \text{ g solute}}{100 \text{ mL solution}} = 0.1\% \text{ (w/v)} \tag{2}$$

- Mass percentage: mass of the solute in 100 g of solution.

$$1\%(\text{w/w}) = \frac{1 \text{ g solute}}{100 \text{ g solution}} \tag{3}$$

  The label w/w (weight by weight) is commonly replaced by m/m (mass by mass). Mass fraction is the mass of the solute divided by the mass of the solution. If the mass fraction of a solution is 0.01, its mass percentage is 1%:

$$\text{mass fraction} = 0.01 \Rightarrow \frac{0.01 \text{ g solute}}{1 \text{ g solution}} = \frac{1 \text{ g solute}}{100 \text{ g solution}} = 1\% \text{ (w/w)} \tag{4}$$

- Volume percentage: volume of solute in 100 mL of solution.

$$1\%(\text{v/v}) = \frac{1 \text{ mL solute}}{100 \text{ mL solvent}} \tag{5}$$

Volume fraction is the volume of the solute divided by the volume of the solution. If the volume fraction of a solution is 0.01, its volume percentage is 1% (v/v).

$$\text{volume fraction}=0.01 \Rightarrow \frac{0.01\ \text{mL solute}}{1\ \text{mL solution}} = \frac{1\ \text{mL solute}}{100\ \text{mL solution}} = 1\%\ (\text{v/v}) \quad (6)$$

1.2. Metric prefixes

Prefix		Multiplier
Name	Symbol	
quetta	Q	10^{30}
ronna	R	10^{27}
yotta	Y	10^{24}
zetta	Z	10^{21}
exa	E	10^{18}
peta	P	10^{15}
tera	T	10^{12}
giga	G	10^{9}
mega	M	10^{6}
kilo	k	10^{3}
hecto	h	10^{2}
deca	da	10^{1}

Prefix		Multiplier
Name	Symbol	
deci	d	10^{-1}
centi	c	10^{-2}
milli	m	10^{-3}
micro	μ	10^{-6}
nano	n	10^{-9}
pico	p	10^{-12}
femto	f	10^{-15}
atto	a	10^{-18}
zepto	z	10^{-21}
yocto	y	10^{-24}
ronto	r	10^{-27}
quecto	q	10^{-30}

1.3. Conversion of concentrations using metric prefixes

- *Convert 10 μM to M, nM and mM units.*

According to the definition of the micro (μ) prefix:

$$10\ \mu\text{M}=10 \cdot 10^{-6}\ \text{M} = 10^{1} \cdot 10^{-6}\ \text{M} = 10^{1-6}\ \text{M} = 10^{-5}\ \text{M} \quad (7)$$

Let us perform the conversion to nM. nano is multiplication by 10^{-9}, micro is multiplication by 10^{-6}. Consequently, micro is 1,000-times larger than nano:

$$1\ \mu\text{M}=1{,}000\ \text{nM} \quad (8)$$

and therefore

$$10\ \mu\text{M}=10{,}000\ \text{nM}=10^{4}\ \text{nM} \quad (9)$$

We can obtain the same solution by using the result in equation (7), and realizing that according to the definition of nano

$$1\ \text{M} = 10^{9}\ \text{nM} \quad (10)$$

$$10\ \mu\text{M}= 10^{-5}\ \text{M}=10^{-5} \cdot 10^{9}\ \text{nM}=10^{-5+9}\ \text{nM} = 10^{4}\ \text{nM} \quad (11)$$

Let us perform the conversion to mM. micro is multiplication by 10^{-6}, milli is multiplication by 10^{-3}, and micro is consequently 1,000-times smaller than milli:

$$1\ \mu\text{M} = 10^{-3}\ \text{mM} \tag{12}$$

Therefore,

$$10\ \mu\text{M} = 10 \cdot 10^{-3}\ \text{mM} = 10^{1} \cdot 10^{-3}\ \text{mM} = 10^{1-3}\ \text{mM} = 10^{-2}\ \text{mM} = 0.01\ \text{mM} \tag{13}$$

- *Convert 0.01 M to mM, μM and nM units.*

According to the definition of the milli, micro and nano prefixes

$$1\ \text{M} = 10^{3}\ \text{mM} = 10^{6}\ \mu\text{M} = 10^{9}\ \text{nM} \tag{14}$$

Consequently,

$$0.01\ \text{M} = 10^{-2}\ \text{M} = 10^{-2} \cdot 10^{3}\ \text{mM} = 10\ \text{mM} \tag{15}$$

$$0.01\ \text{M} = 10^{-2}\ \text{M} = 10^{-2} \cdot 10^{6}\ \mu\text{M} = 10^{4}\ \mu\text{M} \tag{16}$$

$$0.01\ \text{M} = 10^{-2}\ \text{M} = 10^{-2} \cdot 10^{9}\ \text{nM} = 10^{7}\ \text{nM} \tag{17}$$

- *Convert 5 g/L to mg/mL, μg/mL, ng/mL and ng/μL units.*

Since 1 g is 10^3 mg, and 1 L is 10^3 mL

$$5\ \frac{g}{L} = 5\ \frac{10^{3}\ \text{mg}}{10^{3}\ \text{mL}} = 5\ \frac{\text{mg}}{\text{mL}} \tag{18}$$

Conversion to μg/mL. Since 1 mg=10^3 μg

$$5\ \frac{g}{L} = 5\ \frac{\text{mg}}{\text{mL}} = 5\frac{10^{3}\ \mu\text{g}}{\text{mL}} = 5{,}000\ \frac{\mu\text{g}}{\text{mL}} \tag{19}$$

Conversion to ng/mL. Since 1 μg=10^3 ng

$$5\ \frac{g}{L} = 5{,}000\ \frac{\mu\text{g}}{\text{mL}} = 5{,}000\ \frac{1000\ \text{ng}}{\text{mL}} = 5 \cdot 10^{6}\ \frac{\text{ng}}{\text{mL}} \tag{20}$$

Conversion to ng/μL from ng/mL. Since 1 mL=10^3 μL

$$5\ \frac{g}{L} = 5 \cdot 10^{6}\ \frac{\text{ng}}{\text{mL}} = 5 \cdot 10^{6}\ \frac{\text{ng}}{10^{3}\ \mu\text{L}} = 5 \cdot 10^{6-3}\ \frac{\text{ng}}{\mu\text{L}} = 5 \cdot 10^{3}\ \frac{\text{ng}}{\mu\text{L}} \tag{21}$$

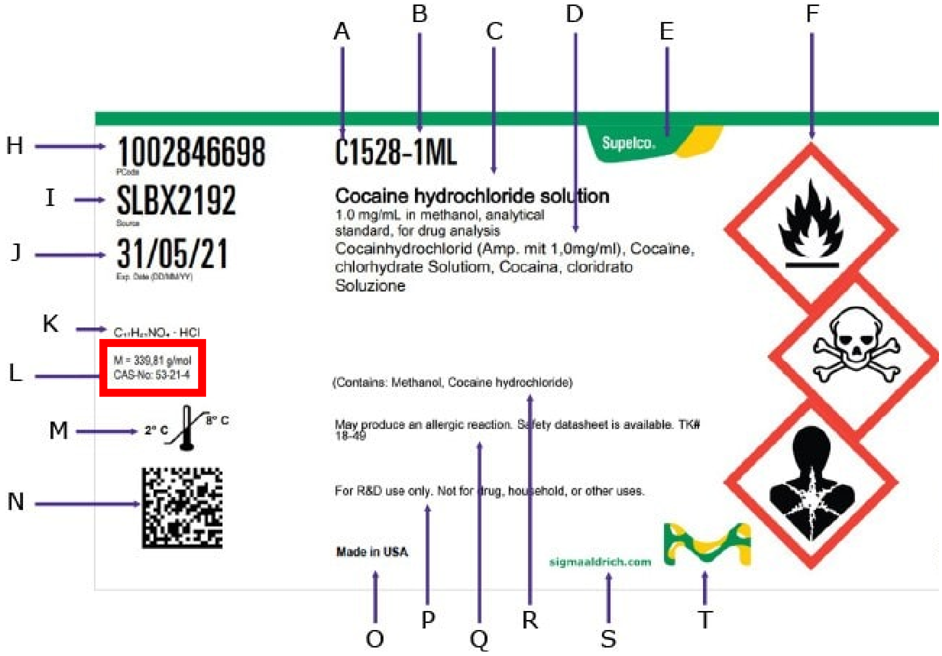
Conversion to ng/μL directly from g/L. Since 1 g=10^9 ng, and 1 L=10^6 μL

$$5\ \frac{g}{L} = 5\ \frac{10^{9}\ \text{ng}}{10^{6}\ \mu\text{L}} = 5 \cdot 10^{9-6}\ \frac{\text{ng}}{\mu\text{L}} = 5 \cdot 10^{3}\ \frac{\text{ng}}{\mu\text{L}} \tag{22}$$

In conclusion, we can state that we have to express the units to be converted from (g and L in the previous equation) in the units to be converted to (ng and μl, red part in the previous equation) in order to perform such unit conversions.

1.4. Determine the amount of solute of known molecular weight to prepare a solution of a given molar concentration

The molecular weight (MW) of cocaine hydrochloride is 339.61 g/mol. How much cocaine hydrochloride is required to prepare 2 mL of solution with a molar concentration of 5 mM?



The molecular weight of a substance is usually written on the label on the vial, albeit typically in very small letters.

The formula we must use in concentration calculations all the time is

$$c = \frac{m}{V} \tag{23}$$

i.e., the concentration (c) of a substance is equal to the amount of material (m) divided by the volume of the solution (V).

A solution with a molar concentration of 5 mM contains 5 mmol substance in 1 L solution. 2 mL of this solution contains

$$\begin{gathered} c = \frac{m}{V} \Rightarrow m = c\,V \\ 5\ \text{mM} \cdot 2 \cdot 10^{-3}\ \text{L} = 5\ \frac{\text{mmol}}{\text{L}} \cdot 2 \cdot 10^{-3}\ \text{L} = 10^{-2}\ \text{mmol} \end{gathered} \tag{24}$$

We can arrive at the same result using a proportion.

$$\begin{gathered} \text{1 L solution contains ....} \quad \text{5 mmol substance} \\ \underline{\text{2 mL} = 2 \cdot 10^{-3}\ \text{L solution contains ..}\quad X\ \text{mmol substance}} \\ X = \frac{2 \cdot 10^{-3}\ \text{L}}{1\ \text{L}}\ 5\ \text{mmol} = 10^{-2}\ \text{mmol} = 0.01\ \text{mmol} \end{gathered} \tag{25}$$

Therefore, you have to dissolve

$$10^{-2}\ \text{mmol} \cdot 339.61\ \frac{\text{g}}{\text{mol}} = 10^{-5}\ \text{mol} \cdot 339.61\ \frac{\text{g}}{\text{mol}} = 0.0033961\ \text{g} = 3.3961\ \text{mg} \qquad (26)$$

cocaine hydrochloride to prepare 2 mL solution with a concentration of 5 mM.

1.5. Prepare a solution with a given concentration knowing the molecular weight and the mass of a substance

The molecular weight of gefitinib, an inhibitor of the kinase domain of epidermal growth factor receptor (EGFR), is 446.9 g/mol. You have 3 mg of gefitinib. In how much volume of solvent do you have to dissolve it to obtain a solution with a molar concentration of 4 mM?

3 mg gefitinib corresponds to

$$\frac{3\ \text{mg}}{446.9\ \frac{\text{g}}{\text{mol}}} = \frac{0.003\ \text{g}}{446.9\ \frac{\text{g}}{\text{mol}}} = 6.71 \cdot 10^{-6}\ \text{mol} \qquad (27)$$

substance. The same result can be obtained using a proportion:

$$\begin{array}{l} 446.9\ \text{g gefitinib is ....}\ 1\ \text{mol} \\ \underline{3\ \text{mg} = 0.003\ \text{g gefitinib is ....}\ X\ \text{mol}} \\ X = \frac{0.003\ \text{g}}{446.9\ \text{g}}\ 1\ \text{mol} = 6.71 \cdot 10^{-6}\ \text{mol} \end{array} \qquad (28)$$

In order to prepare a solution with a concentration of 4 mM, you need to dissolve it in

$$c = \frac{m}{V} \Rightarrow V = \frac{m}{C} = \frac{6.71 \cdot 10^{-6}\ \text{mol}}{4\ \text{mM}} = \frac{6.71 \cdot 10^{-6}\ \text{mol}}{4 \cdot 10^{-3}\ \text{M}} = \frac{6.71 \cdot 10^{-6}\ \text{mol}}{4 \cdot 10^{-3}\ \frac{\text{mol}}{\text{L}}} = \\ 1.678 \cdot 10^{-3}\ \text{L} = 1.678\ \text{mL} = 1{,}678\ \mu\text{L} \qquad (29)$$

The same result using a proportion

$$\begin{array}{l} 1\ \text{L of a 4 mM solution contains}\ 4 \cdot 10^{-3}\ \text{mol substance} \\ \underline{X\ \text{L of a 4 mM solution contains}\ 6.71 \cdot 10^{-6}\ \text{mol}} \\ X = \frac{6.71 \cdot 10^{-6}\ \text{mol}}{4 \cdot 10^{-3}\ \text{mol}}\ 1\ \text{L} = 1.678 \cdot 10^{-3}\ \text{L} \end{array} \qquad (30)$$

1.6. Convert mg/mL concentration to molar concentration

The molecular weight of a pentapeptide is 630.75 Da. We have a solution with a concentration of 8 mg/mL. What is its molar concentration?

Since 8 mg/mL=8 g/L, 1 L of this solution contains 8 g pentapeptide corresponding to a molar concentration of

$$\frac{8}{630.75}\ \text{mol} = 0.0127\ \text{mol} = 12.7\ \text{mmol} \tag{31}$$

Since this is the number of moles present in 1 L of solution, the molar concentration is 12.7 mM.

1.7. Convert molar concentration to mg/mL

The molecular weight of a substance is 8.43 kDa, and the molar concentration of its solution is 10 μM. What is its concentration in mg/mL and how many mg substance does a volume of 5 μL contain?

1 L solution with a concentration of 10 μM contains 10 μmol=$10 \cdot 10^{-6}$ mol=10^{-5} mol substance. Using the known molecular weight of the substance the mass of this amount of substance is

$$10^{-5}\ \text{mol} \cdot 8430\ \frac{\text{g}}{\text{mol}} = 0.0843\ \text{g} \tag{32}$$

Since 0.0843 g is present in 1 L, the mass per volume concentration is

$$0.0843\ \frac{\text{g}}{\text{L}} = 0.0843\ \frac{\text{mg}}{\text{mL}} \tag{33}$$

5 μL of this solution contains

$$5\ \mu\text{L} \cdot 0.0843\ \frac{\text{mg}}{\text{mL}} = 5 \cdot 10^{-3}\ \text{mL} \cdot 0.0843\ \frac{\text{mg}}{\text{mL}} = 4.215 \cdot 10^{-4}\ \text{mg} \tag{34}$$

1.8. Dilution of a stock solution

The concentration of the stock solution of a certain substance is 12 mM. Calculate how to dilute the stock to obtain a 100 μL solution with a concentration of 2 μM.

The fold-difference between the concentrations of the stock and the final solution is

$$\frac{12\ \text{mM}}{2\ \mu\text{M}} = \frac{12{,}000\ \mu\text{M}}{2\ \mu\text{M}} = 6{,}000 \tag{35}$$

Consequently, you need to use

$$\frac{100\ \mu\text{L}}{6{,}000} = 0.0167\ \mu\text{L} \tag{36}$$

of the stock solution, i.e., the recipe for preparing the solution is

$$\begin{array}{l} 0.0167\ \mu\text{L stock} \\ \underline{100\text{-}0.0167\ \mu\text{L} \approx 100\ \mu\text{L solvent}} \\ 100\ \mu\text{L solution} \end{array} \tag{37}$$

It is practically impossible to measure 0.0167 μl with a pipette. Therefore, preparation of the solution should be carried out in two steps, e.g., Step 1: prepare a 60-fold dilution of the stock

$$\begin{array}{l} 1\ \mu\text{L stock} \\ \underline{59\ \mu\text{L solvent}} \\ 60\ \mu\text{L solution} \end{array} \tag{38}$$

Step 2: prepare a 100-fold dilution of the 60-fold diluted stock resulting in the required 6,000-fold dilution:

$$\begin{array}{l} 1\ \mu\text{L 60-fold diluted stock} \\ \underline{99\ \mu\text{L solvent}} \\ 100\ \mu\text{L solution} \end{array} \tag{39}$$

Alternative solution:

Calculation of the required volume of the stock, i.e., 0.0167 μl, can also be calculated using repeated application of the $c=m/V$ formula. The amount of material present in 100 μL solution with a concentration of 2 μM is

$$c_{final} = \frac{m}{V_{solution}} \Rightarrow m = c_{final}\ V_{solution} = 2\ \mu\text{M}\ 100\ \mu\text{L}=2\ \mu\text{M}\ 10^{-4}\ \text{L} = 2\cdot 10^{-4}\ \mu\text{mol} \tag{40}$$

In the second step, let us calculate in how much volume of the stock this amount of substance is present:

$$c_{stock} = \frac{m}{V_{stock}} \Rightarrow V_{stock} = \frac{m}{c_{stock}} = \frac{2\cdot 10^{-4}\ \mu\text{mol}}{12\ \text{mM}} = \frac{2\cdot 10^{-4}\ \mu\text{mol}}{12\cdot 10^{3}\ \mu\text{M}} = \frac{2\cdot 10^{-4}\ \mu\text{mol}}{12\cdot 10^{3}\ \frac{\mu\text{mol}}{\text{L}}} = \tag{41}$$
$$1.67\cdot 10^{-8}\ \text{L} = 1.67\cdot 10^{-8}\cdot 10^{6}\ \mu\text{L} = 1.67\cdot 10^{-2}\ \mu\text{L}$$

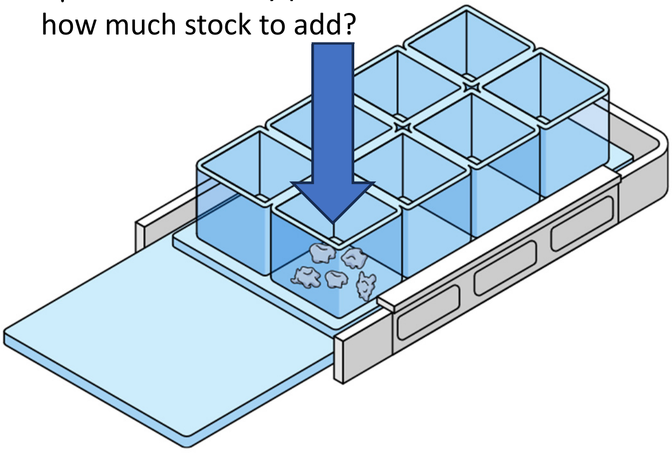
This alternative solution also contains the dilution factor used in the first kind of calculation, which can be shown by substituting $m=c_{\text{final}}\ V_{\text{solution}}$ (from equation (40)) into equation (41):

$$V_{stock} = \frac{m}{c_{stock}} = \frac{c_{final}\ V_{solution}}{c_{stock}} \tag{42}$$

where $c_{\text{final}}\ /\ c_{\text{stock}}$ is the dilution factor.

1.9. Dilution of a stock solution by adding it to solvent already present (large dilution)

This scenario is very commonly encountered in cell biology when cells present in a certain amount of buffer are to be treated with a certain substance, which is available in a concentrated stock solution. *Example: Cells are present in a well of an Ibidi chamber in 200 μL* *buffer. They are to be treated with 5 nM EGF. The concentration of the EGF stock is 250 nM. How much of the stock solution to add to the cells?*



The required fold-dilution of the EGF stock is 250/5=50. Since the volume of the buffer already present in the well is 200 μl,

$$\frac{200\ \mu L}{50} = 4\ \mu L \tag{43}$$

of the EGF stock is to be added to the cells. We can obtain the same result by substituting into the rearranged form of the $c=m/V$ equation:

$$c_{stock} = \frac{m}{V_{stock}} \Rightarrow V_{stock} = \frac{m}{c_{stock}} = \frac{\overbrace{c_{final}\ V_{buffer}}^{\text{amount of material presen in the final solution}}}{c_{stock}} = \frac{5\ \mu M}{250\ \mu M}\ 200\ \mu L = 4\ \mu L \tag{44}$$

<u>This simple solution, according to either equation (43) or (44), is only correct if the volume of the stock added is negligible compared to the volume of the buffer already present, i.e., if the final volume can be assumed to be 200 μL.</u> The error due to neglecting the volume of 4 μL added can be calculated. 4 μL stock solution is sufficient for a solution with a volume of 200 μL, but the final volume of the solution is actually 204 μL, so the actual concentration of EGF in the final solution will be

$$5\ nM\ \frac{200}{204} = 4.90196\ nM \tag{45}$$

Step-by-step calculation of equation (45):

$$c_{final} = \frac{m_{EGF}}{V_{final}} = \frac{c_{stock}\ V_{stock}}{V_{final}} = \frac{250\ nM\ 4\ \mu L}{204\ \mu L} = 4.90196\ nM \tag{46}$$

1.10. Dilution of a stock solution by adding it to solvent already present (small dilution, volume of stock added is not negligible)

Cells are present in a well of an Ibidi chamber in 200 μL buffer. They are to be treated with 1 mM of a substance. The concentration of the stock solution of the substance is 4 mM. How much of the stock solution to add to the cells?

Using the simple "dilution" approach one would determine that a 4-fold dilution of the stock is required, and one would need to add 200/4=50 μL stock. This would, however, result in a large error since the actual final concentration of the substance would be

$$1\text{ mM}\,\frac{200}{250}=0.8\text{ mM} \tag{47}$$

Since this is an unacceptably large error, this approach is not applicable. The correct solution can be obtained by using equation (44) without neglecting the extra volume added. We need to add an unknown volume of V_{stock} to the cells, in which the amount of substance present is $c_{stock}\,V_{stock}$. This amount of material will be present in a final volume of $V_{buffer}+V_{stock}$, and the required final concentration of the substance in the solution is 1 mM (c_{final}):

$$\frac{\overset{\text{amount added}}{c_{stock}\,V_{stock}}}{\underset{\text{final volume}}{V_{buffer}+V_{stock}}}=c_{final} \tag{48}$$

This equation has to be solved for V_{stock}:

$$\begin{aligned}
c_{stock}\,V_{stock} &= c_{final}\left(V_{buffer}+V_{stock}\right)\\
c_{stock}\,V_{stock}-c_{final}\,V_{stock} &= c_{final}\,V_{buffer}\\
V_{stock}\left(c_{stock}-c_{final}\right) &= c_{final}\,V_{buffer}\\
V_{stock} &= \frac{c_{final}\,V_{buffer}}{c_{stock}-c_{final}}
\end{aligned} \tag{49}$$

Substituting into the final equation provides the solution to the problem:

$$V_{stock}=\frac{1\text{ mM }200\text{ μL}}{4\text{ mM}-1\text{ mM}}=66.67\text{ μL} \tag{50}$$

It can be seen that if $c_{final} << c_{stock}$, i.e., when c_{final} is negligible compared to c_{stock}, equation (49) reduces to a simple "dilution" problem (identical to equation (44)):

$$V_{stock}=\frac{c_{final}\,V_{buffer}}{c_{stock}-c_{final}}\text{ if }c_{final}\ll c_{stock}\Rightarrow V_{stock}=\frac{c_{final}\,V_{buffer}}{c_{stock}} \tag{51}$$

where $c_{final}\,/\,c_{stock}$ is the dilution factor.

1.11. Dilution to obtain a certain number of molecules in a given volume

You have a 0.1 μg/mL solution of a substance whose molecular weight is 5 kDa. How many fold dilution is necessary to obtain a solution in which there is 1 molecule in 1 fL?

If the molecular weight is 5 kDa, 0.1 μg/mL corresponds to

$$0.1\ \frac{\mu g}{mL} = 0.1\ \frac{mg}{L} = \frac{0.1}{5000}\ \text{mM} = 2.5\cdot10^{-5}\ \text{mM} = 2.5\cdot10^{-8}\ \text{M} \tag{52}$$

molar concentration. In such a solution there are

$$2.5\cdot10^{-8}\cdot6\cdot10^{23} = 1.5\cdot10^{16} \tag{53}$$

molecules in 1 L. Since 1 fL=10^{-15} L, there are

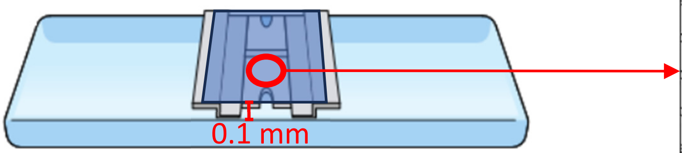
$$1.5\cdot10^{16}\cdot10^{-15} = 15 \tag{54}$$

molecules in 1 fL. Consequently, you have to prepare a 15-fold dilution of the solution.

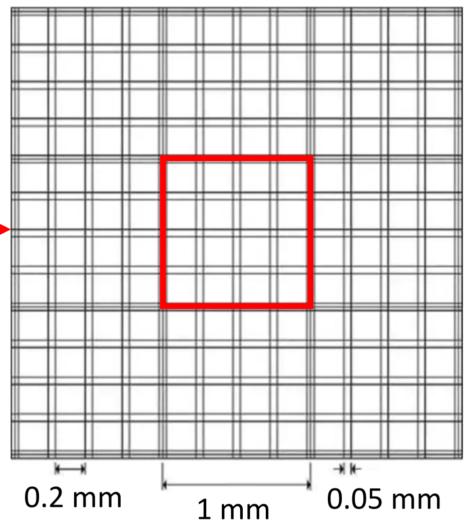
2. Cell density calculations

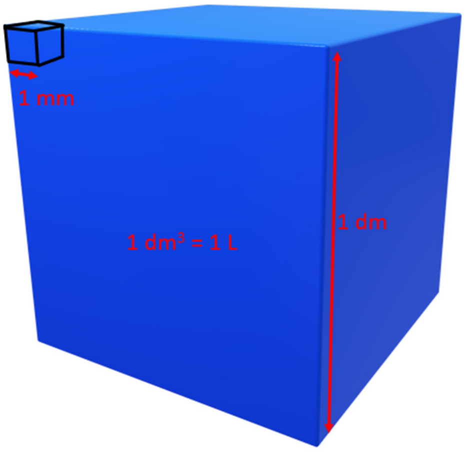
2.1. Counting cells with a Bürker chamber

The volume of your cell suspension is 15 mL, a small aliquot is pipetted into a Bürker chamber, and the cells are counted. The cell count in the displayed area of the Bürker chamber is 120. How many cells do you have in total? After centrifuging the cells, in how many mL buffer do you have to resuspend them to obtain a cell density of 10^6/mL?



Since the thickness of the liquid layer under the coverslip is 0.1 mm, the volume of the red-labeled area is
1 mm · 1 mm · 0.1 mm = 0.1 mm^3





The volume of 1 mm^3 is 10^{-6} L since

1 mm=0.01 dm=10^{-2} dm

1 mm^3 = $(10^{-2}\ dm)^3$ = 10^{-6} dm^3 = 10^{-6} L = 1 μL.

Therefore, the volume under the red square in which the cells are counted is 0.1 mm^3 = 0.1 μL.

There are 120 cells in 0.1 μL, so the number of cells in 1 mL, which is usually used to describe cell density, is

$$\frac{\text{120 cells in 0.1 μL}}{X \text{ cells in 1 mL=1000 μL}} \quad (55)$$

$$X = \frac{1000}{0.1} 120 = 10^4 \cdot 120 = 1.2 \cdot 10^6$$

Since you have 10 mL of cell suspension, the total number of cells is

$$1.2 \cdot 10^6 \frac{1}{\text{mL}}\ 10\ \text{mL} = 1.2 \cdot 10^7 \quad (56)$$

In order to have 10^6 cells/mL after centrifugation, you have to resuspend the pellet in

$$V = \frac{N_{cells}}{c_{cells}} = \frac{1.2 \cdot 10^7}{10^6\ \frac{1}{\text{mL}}} = 12\ \text{mL} \quad (57)$$

buffer.

2.2. Calculation of cell density for seeding cells

You have a total number of 7.2·10^6 cells in suspension, and you have to seed 10^4 cells in a single well of an Ibidi chamber in 200 μL. In how large volume do you have to resuspend the pellet?

If the required number of cells in 200 μL is 10^4, the cell density is

$$c_{cells} = \frac{N_{cells}}{V} = \frac{10^4}{0.2\ \text{mL}} = 5 \cdot 10^4\ \frac{1}{\text{mL}} \quad (58)$$

In order to obtain this cell density, the pellet has to be resuspended in

$$V = \frac{N_{cells,tot}}{c_{cells}} = \frac{7.2 \cdot 10^6}{5 \cdot 10^4 \ \frac{1}{\text{mL}}} = 144 \text{ mL} \tag{59}$$

buffer. If you indeed need to fill one single well of an Ibidi chamber, i.e., if you would need only 200 μL of the 144 mL cell suspension, it would be advisable to discard the majority of the cells, e.g., resuspending the cell pellet in 1 mL buffer, taking 10 μL out of this volume, i.e., 1% of the total cell number, and diluting it to 1.44 mL. 200 μL of this suspension will contain 10^4 cells. Detailed calculation:

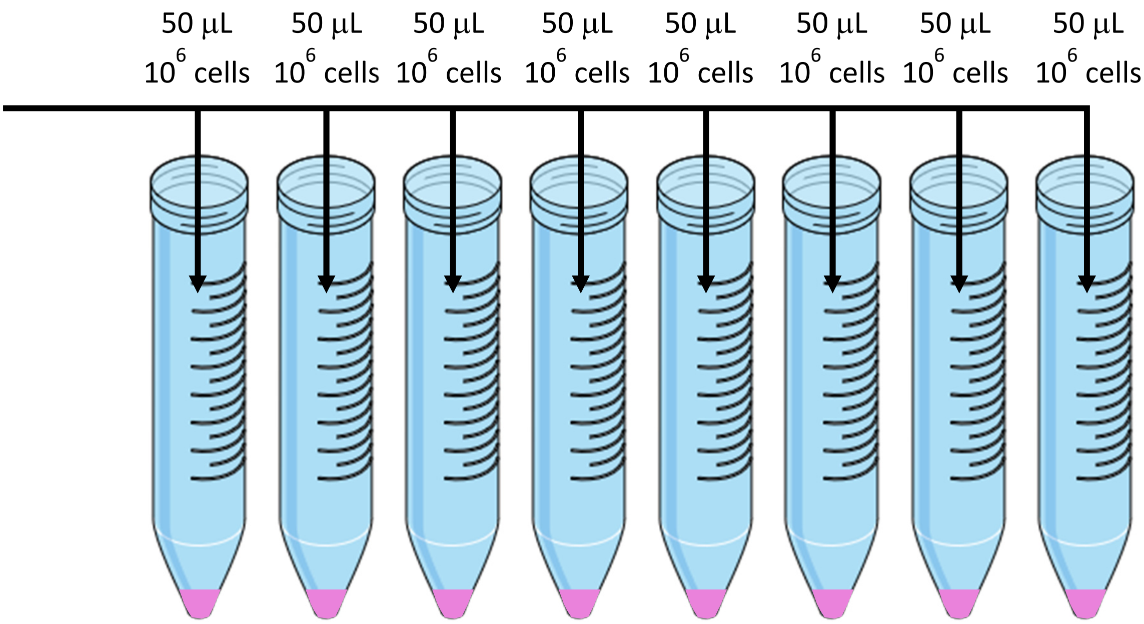
- number of cells in 1 mL buffer is $7.2 \cdot 10^6$
- number of cells in 10 μL buffer is $7.2 \cdot 10^4$
- if this number of cells in diluted to 1.44 mL, the cell concentration will be

$$c_{cells} = \frac{N_{cells}}{V} = \frac{7.2 \cdot 10^4}{1.44 \text{ mL}} = 5 \cdot 10^4 \ \frac{1}{\text{mL}} \tag{60}$$

- If you take 200 μL = 0.2 mL, i.e., 1/5 mL, there will be 10^4 cells in this volume.

2.3. Calculation of cell density for an experiment

You have a total number of $1.3 \cdot 10^7$ cells in suspension, and you have to label them in 8 different tubes with monoclonal antibodies. There should be 1 million cell in each tube in 50 μL buffer. How to perform the resuspension after centrifuging the cells?



If the desired cell concentration is 1 million in 50 μL, you have to resuspend the pellet containing $1.3 \cdot 10^7$ cells in

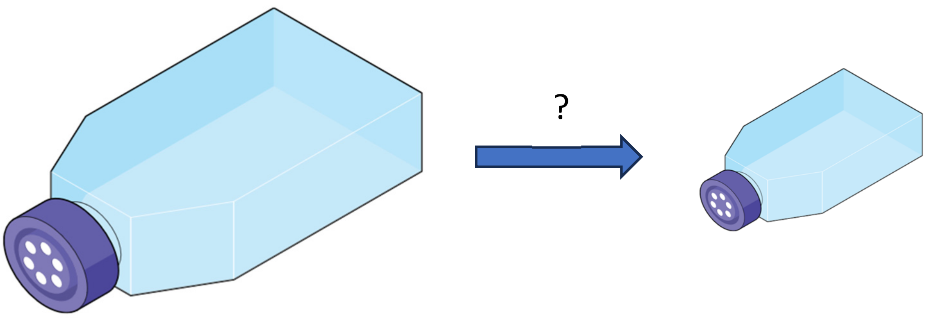
$$\begin{aligned}&10^6 \text{ cells in } 50\ \mu\text{L}\\ &\underline{1.3\cdot 10^7 \text{ cells in } X\ \mu\text{L}}\\ &X=\frac{1.3\cdot 10^7}{10^6}50\ \mu\text{L}=650\ \mu\text{L}\end{aligned} \qquad (61)$$

buffer, and distribute 50 μL of this suspension to every tube for labeling with antibodies.

2.4. Calculation of the dilution of adherent cells for splitting

Adherent cells cultured in a T75 flask are confluent, and you have to split them in a ratio of 1:3 to a T25 flask, i.e., the seeded cells should be 1/3 confluent after seeding in the T25 flask. How to perform the splitting?

Without providing a detailed cell culturing protocol and just concentrating on the math, here is what to do.



T75 flask (area: 75 cm²)   T25 flask (area: 25 cm²)

- Trypsinize cells from the T75 flask using 2 mL trypsin.
- Stop the action of trypsin by adding large excess of medium. The exact amount of medium you add can be determined in different ways, a possible approach is described below.
- Let us determine what fraction of the trypsinized cells you need.

$$\begin{aligned}&100\% \text{ of cells .... confluent on an area of } 75\text{ cm}^2\\ &\underline{X\% \text{ of cells..................... confluent on an area of } 25\text{ cm}^2}\\ &X=100\frac{25}{75}\%=\frac{100}{3}\%\\ \\ &100\% \text{ of cells .... confluent on an area of } 75\text{ cm}^2\\ &\underline{X\% \text{ of cells..................... } \tfrac{1}{3} \text{ confluent on an area of } 25\text{ cm}^2}\\ &X=100\frac{25}{75}\frac{1}{3}\%=\frac{100}{9}\%\end{aligned} \qquad (62)$$

1/3 of the trypsinized cells would be confluent in the T25 flask, and 1/3 of this 1/3, i.e., 1/9 of the total, is required to achieve 1/3 confluency in the T25 flask.

- According to the previous calculation, the easiest way of performing the splitting is to add 7 mL medium to the 2 mL trypsin. In this way the cells will be present in 9 mL, and you have to take 1 mL out of this suspension and transfer it to the T25 flask.
- Add 4 mL medium to the T25 flask, in this way there will be 5 mL medium in the flask, which is the usual amount used for adherent cells in T25 flasks.

3. Saturation calculations

3.1. Calculate the fraction of occupied binding sites

The K_D of an anti-EGF receptor is 8 nM, and a certain cell type expresses $5 \cdot 10^5$ EGF receptors in the plasma membrane. Cells are labeled by the aforementioned antibody whose concentration in 1 μg/mL. How large fraction of the receptors is bound to the antibody? How many receptors/cell are bound to the antibody?

The binding of a ligand is usually described by a Michaelis-Menten type equation in cell biology:

$$c_{RL} = R\frac{c_{L,free}}{c_{L,free} + K_D} \tag{63}$$

where

- c_{RL} is the concentration of the receptor-ligand complex
- $c_{L,free}$ is the concentration of free, unbound ligand
- R is the number of receptors, i.e., the highest possible concentration of the receptor-ligand complex. If binding to a receptor is measured by fluorescence, R is the highest fluorescence intensity corresponding to when all receptors are occupied by the ligand.
- K_D is the dissociation constant of the receptor-ligand complex.

Although this equation is almost universally used to describe binding events in cell biology, its practical applicability is limited to cases when

- binding is monovalent, or it can be reasonably well approximated by this equation. For an IgG, this equation is obviously an approximation since an antibody is bivalent and may bind two antigens at the same time. However, in most practical cases this equation provides a reasonable quantitative description of antibody binding.

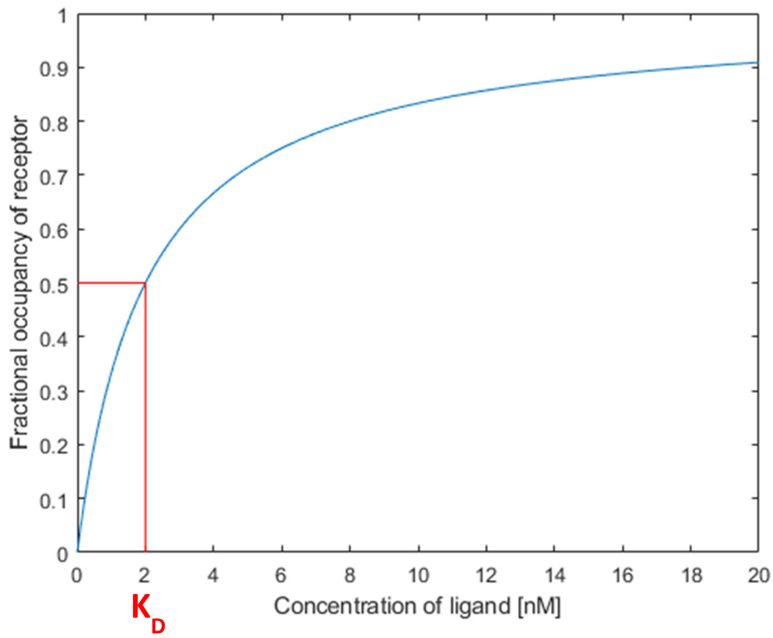
- the fraction of ligand bound to the receptor is negligible compared to the total amount of ligand added to the system, i.e., <u>no ligand depletion takes place</u>:

$$\begin{gathered} c_{L,tot} = c_{L,free} + c_{RL} \\ \text{if } c_{RL} \ll c_{L,free} \Rightarrow c_{L,tot} = c_{L,free} \end{gathered} \tag{64}$$

If this approximation is acceptable, equation (63) can be rewritten:

$$c_{RL} = R \underbrace{\frac{c_{L,tot}}{c_{L,tot} + K_D}}_{\substack{\text{fraction of} \\ \text{occupied receptors}}} \tag{65}$$

Equation (63) is always correct for monovalent binders independent of whether ligand depletion is present or not, however it is not directly usable to calculate binding since $c_{L,free}$ on the right side is an unknown, and therefore c_{RL} cannot be calculated directly from this equation. The slight change converting equation (63) to equation (65), i.e., replacement of $c_{L,free}$ with $c_{L,tot}$, may seem almost unnoticeable, it actually makes the equation practically usable. For further considerations about ligand depletion read section 3.2.



The graph on the left shows a plot of equation (65) assuming a K_D of 2 nM. It can be seen that the K_D is numerically equal to the <u>ligand concentration that leads to 50% receptor occupancy.</u>

We can conclude that equation (65) is a reasonable approximation of antibody binding and assuming that no ligand depletion takes place, let us proceed to using it to solve the problem. Before substituting into the equation, we have to determine the molar concentration of an

IgG solution with a concentration of 1 μg/mL. The molecular weight of an IgG is approximately 150 kDa, therefore

$$1\ \frac{\mu g}{mL}=1\ \frac{mg}{L}=10^{-3}\ \frac{g}{L}=\frac{10^{-3}\ \frac{g}{L}}{1.5\cdot 10^{5}\ \frac{g}{mol}}=6.67\cdot 10^{-9}\ \text{M} = 6.67\ \text{nM} \tag{66}$$

Using this dissociation constant, we are ready to substitute into equation (65):

$$c_{RL}=R\frac{c_{L,tot}}{c_{L,tot}+K_D}=5\cdot 10^{5}\frac{6.67\ \text{nM}}{6.67\ \text{nM}+8\ \text{nM}}=5\cdot 10^{5}\cdot \underbrace{0.455}_{\text{fraction of occupied receptors}}=227{,}335 \tag{67}$$

i.e., 45.5% of the receptors are bound to the antibody, and the number of bound antibodies/cell is 227,335.

3.2. Calculate ligand depletion

A 50 μL cell suspension contains 2·10⁵ cells expressing 10⁶ EGF receptors in the plasma membrane. Cells in this 1 mL suspension are labeled with an anti-EGF receptor antibody at a concentration of 10 nM. The K_D of this antibody is 8 nM. How many antibodies are bound to cells? How large fraction of the antibody is bound to cells?

Let us substitute into equation (65) to calculate the fraction of antibody-bound receptors:

$$\textit{occupied fraction}=\frac{c_{L,tot}}{c_{L,tot}+K_D}=\frac{10\ \text{nM}}{10\ \text{nM} + 8\ \text{nM}}=0.556 \tag{68}$$

There are $2\cdot 10^5$ cells each expressing 10^6 receptors. Consequently, there are

$$2\cdot 10^{5}\cdot 10^{6}=2\cdot 10^{11} \tag{69}$$

receptors in the 50 μL suspension. According to equation (68), 55.6% of these receptors are antibody-bound, so the number of antibody-receptor complexes is

$$2\cdot 10^{11}\cdot 0.556=1.11\cdot 10^{11} \tag{70}$$

The total number of antibodies present in the 50 μL cell suspension can be determined from its total concentration (10 nM). The molar quantity of the antibody in the 50 μL cell suspension is

$$10\ \text{nM}\ 50\ \mu\text{L} = 10^{-8}\ \frac{mol}{L}\ 5\cdot 10^{-5}\ \text{L} = 5\cdot 10^{-13}\ \text{mol} \tag{71}$$

This amount corresponds to

$$5\cdot 10^{-13}\ \text{mol}\cdot 6\cdot 10^{23}\frac{1}{mol}=3\cdot 10^{11} \tag{72}$$

antibody molecules in the cell suspension. According to equation (70) $1.11\cdot10^{11}$ of them are receptor bound, so the fraction of cell-bound antibodies is

$$\frac{1.11\cdot10^{11}}{3\cdot10^{11}}=0.37 \qquad (73)$$

According to this result, 37% of the antibodies are bound to receptors. This calculation contains obvious simplifications since

- we conclude that ligand depletion is not negligible (37% of the antibodies are bound)
- but we still used equation (65) for solving the problem.

Therefore, the actual numerical values calculated in the solution are not accurate, but the calculation shows that ligand depletion can take place in non-extreme situations commonly encountered in cell biology. Strictly speaking, the simple Michaelis-Menten type equation, equation (65), is not valid in such cases. A more complicated solution to the equilibrium binding problem leading to a quadratic equation can be derived, but this equation is beyond the scope of this booklet.

4. Exercises

4.1. The molecular weight of a substance is 2300 g/mol, the concentration of its solution 6 μg/mL. What is the molar concentration of the solution?

4.2. The concentration of a stock solution of a substance is 2.5 mM. How do you prepare 500 μL solution with a concentration of 100 μM?

4.3. Convert 12 μg/mL to ng/mL.

4.4. The molecular weight of a substance is 12 kDa, how to prepare a 10 mL solution with a concentration of 100 μM?

4.5. Convert 16 μM to nM and mM.

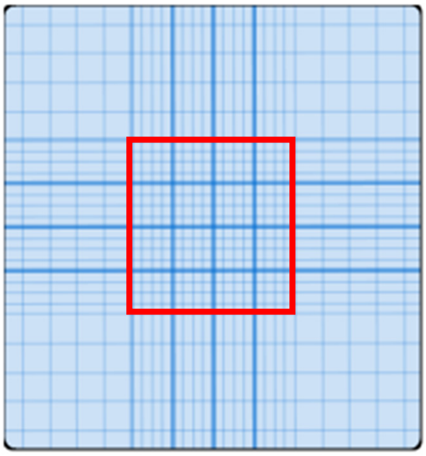
4.6. A tube contains 500 μL cell suspension. The concentration of the stock solution of a peptide is 2 μM. How much stock to add to the cell suspension so that the final peptide concentration is

a. 50 nM?

b. 500 nM?

4.7. The molecular weight of a molecule is 890 Da. In how much volume do you have to dissolve 1.2 mg to obtain a stock solution with a concentration of 1 mM?

4.8. You have 2 mL cell suspension from which you take 50 μL, dilute it to 500 μL. You pipette a small aliquot of this 10-fold diluted cell suspension into a Bürker chamber, and the number of cells counted in the highlighted area is 185.



a. How many cells do you have in your original cell suspension?

b. In how many mL do you have to resuspend them after centrifugation to obtain a cell suspension with a density of $2 \cdot 10^6$ cells/mL?

5. Solution to exercises

4.1. $2.6 \cdot 10^{-6}$ M

4.2. 20 μL stock + 480 μL solvent

4.3. $1.2 \cdot 10^7$ ng/L

4.4. 12 mg substance in 10 mL solution

4.5. 16,000 nM, 0.016 mM

4.6. a. 12.5 μL, b. 166.67 μL

4.7. 1.35 mL

4.8. a. 37 million, b. 18.5 mL