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**The Association for  
Clinical Biochemistry**

# CALCULATIONS IN LABORATORY SCIENCE

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**Worked Answers to Further Questions**

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## Chapter 1

(Atomic weights: C = 12; H = 1; O = 16; Ca = 40; N = 14)

### Q 1 (1)

- a) 125 mg% is the same as 125 mg/100 mL

Multiply by 10 to convert volume from 100 mL to 1000 mL (i.e 1 L)

Divide by 1000 to convert from mg to g (there are 1000 mg in one g)

$$125 \text{ mg\%} = \frac{125 \times 10}{1000} = \mathbf{1.25 \text{ g/L}}$$

- b) There are 1000 mmol in one mol. Therefore multiply by 1000.

$$0.25 \text{ mol/L} = 0.25 \times 1000 = \mathbf{250 \text{ mmol/L}}$$

- c) One nmol =  $1.0 \times 10^{-9}$  mol, one  $\mu\text{mol}$  =  $1.0 \times 10^{-6}$  mol.

Therefore one  $\mu\text{mol}$  =  $1.0 \times 10^3$  nmol = 1000 nmol

Division of 1 nmol/L by 1000 converts to  $\mu\text{mol/L}$

$$0.236 \text{ nmol/L} = \frac{0.236}{1000} = \mathbf{0.000236 \mu\text{mol/L}}$$

- d) There are 1000000 (or  $1.0 \times 10^6$ ) ng in 1 mg

There are 1000 mL in one L

Therefore multiplication by 1000000 and division by 1000 converts from mg/L to ng/mL:

$$1.6 \text{ mg/L} = \frac{1.6 \times 1000000}{1000} = \mathbf{1600 \text{ ng/mL}}$$

### Q 1 (2)

- a) SI units for glucose are mmol/L

120 mg% can also be written 120 mg/100 mL

$$\text{Concentration (mmol/L)} = \frac{\text{Concentration (mg/L)}}{\text{Molecular weight}}$$

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Multiplication of concentration in mg/100 mL by 10 converts to mg/L

Formula of glucose =  $C_6H_{12}O_6$

Atomic wt carbon = 12 therefore  $C_6 = 6 \times 12 = 72$

Atomic wt hydrogen = 1 therefore  $H_{12} = 12 \times 1 = 12$

Atomic wt oxygen = 16 therefore  $O_6 = 6 \times 16 = \underline{96}$

Molecular weight of glucose = 180

Therefore 120 mg% glucose =  $\frac{120 \times 10}{180} = \mathbf{6.7 \text{ mmol/L}}$  (2 sig figs)

b) The SI units for calcium are mmol/L

Concentration (mEq/L) = Concentration (mmol/L) x valency

Calcium ions are divalent (i.e.  $Ca^{++}$ ) so that the valency is 2

Therefore, serum calcium (mmol/L) =  $\frac{4.0}{2} = \mathbf{2.0 \text{ mmol/L}}$

c) SI units for serum urea = mmol/L

mg% can also be written mg/100 mL

Multiplication of BUN mg% by 10 converts to mg/L (since 1 L = 1000 mL)

Division of blood urea nitrogen (BUN) in mg% by the molecular weight of nitrogen gives the blood urea nitrogen in mmol/L.

The formula of molecular nitrogen is  $N_2$ . The atomic weight of nitrogen is 14.

Molecular weight of nitrogen ( $N_2$ ) =  $2 \times 14 = 28$

The formula for urea is  $CO(NH_2)_2$ . Therefore each mol of urea contains one mol of nitrogen ( $N_2$ ).

Therefore, serum urea (mmol/L) =  $\frac{\text{BUN (mg\%)} \times 10}{28}$

Serum urea (mmol/L) =  $\frac{21 \times 10}{28} = \mathbf{7.5 \text{ mmol/L}}$

d) The SI units for creatinine are  $\mu\text{mol/L}$

There are 1000  $\mu\text{g}$  in one mg so that multiplication by 1000 converts from

mg% to µg%

µg% can also be written µg/100 mL

Multiplication by 10 converts from µg/100 mL to µg/L (1 L = 1000 mL)

Division by the molecular weight of creatinine converts from µg/L to µmol/L. Formula of creatinine is: C<sub>4</sub>H<sub>7</sub>ON<sub>3</sub>

Carbon atomic wt	= 12	C <sub>4</sub>	= 4 x 12	= 48
Hydrogen atomic wt	= 1	H <sub>7</sub>	= 7 x 1	= 7
Oxygen atomic weight	= 16	O	= 1 x 16	= 16
Nitrogen atomic wt	= 14	N <sub>3</sub>	= 3 x 14	= <u>42</u>

Creatinine molecular weight = 113

$$\text{Creatinine } (\mu\text{mol/L}) = \frac{\text{Creatinine (mg\%)} \times 10 \times 1000}{\text{Molecular weight}}$$

$$\text{Creatinine } (\mu\text{mol/L}) = \frac{0.66 \times 10 \times 1000}{113} = \mathbf{58 \mu\text{mol/L}} \text{ (2 sig figs)}$$

**Q 1 (3)**

- a) Division by 10 converts from mmol/L to mmol/100 mL (since 1 L = 1000 mL)

Multiplication by the molecular weight of glucose converts from mmol/100 mL to mg/100 mL. Formula of glucose is C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>.

Atomic wt carbon	= 12	therefore C <sub>6</sub>	= 6 x 12	= 72
Atomic wt hydrogen	= 1	therefore H <sub>12</sub>	= 12 x 1	= 12
Atomic wt oxygen	= 16	therefore O <sub>6</sub>	= 6 x 16	= <u>96</u>

Molecular weight of glucose = 180

$$\text{Therefore, glucose (mg/100 mL)} = \frac{\text{Glucose (mmol/L)} \times 180}{10}$$

$$\text{Glucose (mg/100 mL)} = \frac{20 \times 180}{10} = \mathbf{360 \text{ mg/100 mL}}$$

- b) Concentration (mEq/L) = Concentration (mmol/L) x valency

Calcium ions are divalent (i.e. Ca<sup>++</sup>) so that the valency is 2

$$\text{Therefore, calcium (mEq/L)} = \text{calcium (mmol/L)} \times 2$$

## WORKED ANSWERS TO FURTHER QUESTIONS

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$$\text{Calcium (mEq/L)} = 3.2 \times 2 = \mathbf{6.4 \text{ mEq/L}}$$

c) Division by 10 converts from mmol/L to mmol/100 mL (since 1L = 1000 mL), which can also be written mmol%.

Since the formula of urea is  $\text{CO}(\text{NH}_2)_2$ , each mol contains 1 mol of molecular nitrogen ( $\text{N}_2$ ). The atomic weight of nitrogen is 14, so its molecular weight ( $\text{N}_2$ ) is  $2 \times 14 = 28$ . Therefore multiplication of urea in mmol% by 28 gives the BUN in mg%.

$$\text{Therefore, BUN (mg\%)} = \frac{\text{Urea (mmol/L)} \times 28}{10}$$

$$\text{BUN (mg\%)} = \frac{30.6 \times 28}{10} = \mathbf{86 \text{ mg\%}} \text{ (2 sig figs)}$$

d) Division by 10 converts from  $\mu\text{mol/L}$  to  $\mu\text{mol}/100 \text{ mL}$  (since 1 L = 1000 mL), which can also be written as  $\mu\text{mol}\%$ .

Division by 1000 converts from  $\mu\text{mol}\%$  to mmol% (since 1000  $\mu\text{mol} = 1 \text{ mmol}$ ).

Multiplication by the molecular weight of creatinine converts from mmol% to mg%. Creatinine has the formula  $\text{C}_4\text{H}_7\text{ON}_3$ .

Carbon atomic wt	= 12	$\text{C}_4$	= 4 x 12	= 48
Hydrogen atomic wt	= 1	$\text{H}_7$	= 7 x 1	= 7
Oxygen atomic weight	= 16	$\text{O}$	= 1 x 16	= 16
Nitrogen atomic wt	= 14	$\text{N}_3$	= 3 x 14	= <u>42</u>

$$\text{Creatinine molecular weight} = 113$$

$$\text{Therefore, creatinine (mg\%)} = \frac{\text{creatinine } (\mu\text{mol/L}) \times 113}{10 \times 1000}$$

$$\text{Creatinine (mg\%)} = \frac{250 \times 113}{10 \times 1000} = \mathbf{2.8 \text{ mg\%}} \text{ (2 sig figs)}$$

### Q 1 (4)

a) 'M' is an abbreviation for mol/L. There are 1000 mmol in one mol.

Therefore, multiplication of a concentration in mol/L by 1000 converts it to mmol/L.

$$1.5 \times 10^{-3} \text{ is the same as } \frac{1.5}{10^3}$$

$10^3$  means 10 multiplied by itself 3 times i.e.  $10 \times 10 \times 10 = 1000$

$$\text{Therefore, } 1.5 \times 10^{-3} = \frac{1.5}{1000} = 0.0015$$

Another way of looking at it is that the power minus 3 means that we must move the decimal point 3 places to the left (as opposed to a positive power which would have meant moving it 3 places to the right). Moving the decimal point one place to the left gives 0.15, 2 places gives 0.015 and 3 places gives 0.0015.

Combining these moves:

$$1.5 \times 10^{-3} \text{ M} = 0.0015 \times 1000 = \mathbf{1.5 \text{ mmol/L}}$$

In other words whenever we see a molar concentration with the term ‘ $\times 10^{-3}$ ’, it is really the same as the concentration in mmol/L.

Another way to approach this problem is that multiplying by 1000 to convert from mol to mmol is the same as multiplying by  $10^3$  in which case the calculation becomes:

$$1.0 \times 10^{-3} \times 10^3 = 1.0$$

since the  $10^{-3}$  and  $10^3$  cancel (i.e. we move the decimal point 3 places to the left then back 3 places to the right to the original position).

b) The symbol ‘M’ stands for mol/L.

Multiplication by 1000000 converts from mol to  $\mu\text{mol}$  (since there are 1000000  $\mu\text{mol}$  in one mol). 1000000 can also be written as  $10^6$

$$\text{Therefore } 1.25 \times 10^{-5} \text{ M} = 1.25 \times 10^{-5} \times 10^6 \mu\text{mol/L}$$

Since  $10^{-5} \times 10^6 = 10^1$  (which is simply 10) the calculation becomes:

$$1.25 \times 10^{-5} \text{ M} = 1.25 \times 10 = \mathbf{12.5 \mu\text{mol/L}}$$

This is the same as moving the decimal point 5 places to the left, then 6 places to the right i.e. a net movement of one place to the right.

c) Multiplication by 10 converts from mg/100 mL to mg/L (since 1L = 1000 mL). Division by 1000 converts from mg/L to g/L (since there are 1000 mg in one g)

$2.5 \times 10^2$  mg/100 mL means 2.5 multiplied by 10 squared (i.e. 100) = 250 mg/100 mL

$$\text{Therefore, } 2.5 \times 10^2 \text{ mg/100 mL} = \frac{250 \times 10}{1000} = \mathbf{2.5 \text{ g/L}}$$

Another way of doing this is to first move the decimal point 2 places to the right (the same as multiplying by  $10^2$ ), then a further one place to the right to convert from 100 mL to 1000 mL (making 3 moves to the right altogether). A further 3 moves to the left (the same as dividing by 1000 to convert from mg to g) takes us back to the starting position and an answer of 2.5 g/L.

- d) Multiplication of mmol/L by 1000 converts to  $\mu\text{mol/L}$  (since 1 mmol = 1000  $\mu\text{mol}$ ). Multiplication by 1000 is the same as multiplication by  $10^3$

$$\text{Therefore, } 3.25 \times 10^{-6} \text{ mmol/L} = 3.25 \times 10^{-6} \times 10^3 = \mathbf{3.25 \times 10^{-3} \mu\text{mol/L}}$$

$3.25 \times 10^{-3} \mu\text{mol/L}$  can also be written 0.00325  $\mu\text{mol/L}$ .

(The same result is obtained by moving the decimal point 6 places to the left then back 3 places to the right).

### Q 1 (5)

- a) If the concentration in the reaction mixture is  $3.00 \times 10^{-3}$  M then each litre contains  $3.0 \times 10^{-3}$  mol of product.

Division by 10 gives the number of mol in 100 mL (since 1L = 1000 mL)

Multiplication by 1000 converts from mol to mmol (since 1 mol = 1000 mmol)

$$3.00 \times 10^{-3} \text{ can also be written as } \frac{3.00}{10^3} \text{ or } \frac{3.00}{1000}$$

$$\text{Therefore concentration (mmol/100 mL)} = \frac{\text{concentration (mol/L)} \times 1000}{10}$$

$$\text{Amount of product in 100 mL} = \frac{3.00 \times 1000}{10 \times 1000} = \mathbf{0.30 \text{ mmol}}$$

- b)  $3.00 \times 10^{-3}$  M means  $3.00 \times 10^{-3}$  mol/L

Multiplication by 1000000 converts from mol/L to  $\mu\text{mol/L}$  (since 1 mol =

1000000  $\mu\text{mol}$ ). 1000000 can also be written as  $1.0 \times 10^6$ .  
 Division by 4 gives the amount of product present in 250 mL (since  $1000/4 = 250$ ).

Since this amount of product was formed over 30 min, division by 30 gives the amount which would be formed in one minute.

Therefore number of  $\mu\text{mol}$  formed in 250 mL in one minute

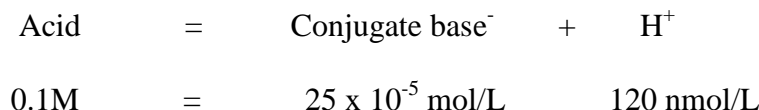
$$= \frac{\text{Molar concentration} \times 10^6}{4 \times 30}$$

$$= \frac{3.00 \times 10^{-3} \times 10^6}{4 \times 30} = \mathbf{25 \mu\text{mol}/\text{min}/250 \text{ mL}}$$

NB.  $10^{-3} \times 10^6 = 10^3$  i.e. move decimal point 3 places to the left, then 6 places to the right making 3 places to the right overall, which is the same as multiplying by 1000.

### Q 1 (6)

The dissociation of the acid can be written:



Before calculating the dissociation constant (K) convert all concentrations to the same units. It doesn't really matter which units but conventionally molar concentrations (mol/L) are used.

0.1M undissociated acid is the same as 0.1 mol/L of acid, which can also be written as  $1.0 \times 10^{-1}$  mol/L.

There are 1000000000 (i.e.  $10^9$ ) nmol in one mol so that 120 nmol/L hydrogen ions can be written  $120 \times 10^{-9}$  mol/L or more conveniently  $1.20 \times 10^{-7}$  mol/L (moving the decimal point 2 places to the left and decreasing the power of 10 by 2 from -9 to -7).

The conjugate base concentration is already in mol/L, but would be more conventionally written as  $2.5 \times 10^{-4}$  (reduction from 25 to 2.5 involves moving the decimal point one place to the left so that the power of 10 must be increased by one i.e. changed from -5 to -4).

The expression for the dissociation constant, with molar concentrations in square brackets [ ] can be written as:

$$K = \frac{[\text{Conjugate base}] (\text{mol/L}) \times [\text{Hydrogen ions}] (\text{mol/L})}{[\text{Acid}] (\text{mol/L})}$$

An 'expression' for the units of K can be written as:

$$\frac{(\text{mol/L}) \times (\text{mol/L})}{(\text{mol/L})}$$

One set of (mol/L) above the line cancels with (mol/L) below the line leaving **mol/L** as the units.

Calculation of K from these data gives:

$$K = \frac{2.5 \times 10^{-4} \times 1.20 \times 10^{-7}}{1.0 \times 10^{-1}} = 2.5 \times 1.20 \times 10^{-10} = \mathbf{3.0 \times 10^{-10} \text{ mol/L}}$$

## Chapter 2

(Atomic weights: H = 1; C = 12; O = 16; P = 31; Na = 23; K = 39; Ca = 40; S = 32; Cl = 35.5)

### Q 2 (1)

70 g/L is the same as 70 g/1000 mL (since 1L = 1000 mL)

If 1000 mL contain 70 g of albumin then each mL contains  $\frac{70}{1000}$  g

then 100 mL must contain 100 times this amount, so that the weight required to make 100 mL of 70 g/L albumin

$$= \frac{70 \times 100}{1000} = \mathbf{7 \text{ g}}$$

Another way of looking at this is that 100 mL is one tenth of 1 L (i.e. 1000 mL) so that one tenth of the amount present in 1 L is required.

### Q 2 (2)

Concentration of sodium chloride = 85 g/L

Division by the molecular weight of sodium chloride gives the concentration in mol/L:

$$\text{Concentration (mol/L)} = \frac{\text{Concentration (g/L)}}{\text{Molecular weight}}$$

Multiplication by 1000 converts from mol/L to mmol/L (since 1 mol = 1000 mmol)

Formula for sodium chloride = NaCl

Atomic weight of Na = 23; atomic weight of Cl = 35.5

Therefore molecular weight of NaCl = 23 + 35.5 = 58.5

$$\text{Therefore, NaCl (mmol/L)} = \frac{\text{NaCl (g/L)} \times 1000}{58.5}$$

Since each molecule of NaCl dissociates to give one ion of Na<sup>+</sup>, this is also the concentration of sodium ions in mmol/L.

$$\text{Concentration of Na}^+ \text{ (mmol/L)} = \frac{85 \times 1000}{58.5} = \mathbf{1453 \text{ mmol/L}}$$

### Q 2 (3)

Calcium carbonate has the formula CaCO<sub>3</sub> so that each mol contains 1 mol of calcium. Therefore, the standard solution will need to contain 5.0 mmol/L of CaCO<sub>3</sub>.

$$\begin{array}{l} \text{Atomic wt calcium} = 40 \quad \text{therefore Ca} = 1 \times 40 = 40 \\ \text{Atomic wt carbon} = 12 \quad \text{therefore C} = 1 \times 12 = 12 \\ \text{Atomic wt of oxygen} = 16 \quad \text{therefore O}_3 = 3 \times 16 = \underline{48} \end{array}$$

$$\text{Molecular weight of CaCO}_3 = 100$$

1 L of 1 mol/L will contain 100 g of CaCO<sub>3</sub>

1 L of 1 mmol/L will contain  $\frac{100}{1000}$  g of CaCO<sub>3</sub> (since 1 mol/L = 1000 mmol/L)

1 L of 5.0 mmol/L will contain  $\frac{100 \times 5.0}{1000}$  g CaCO<sub>3</sub>

500 mL of 5.0 mmol/L will contain  $\frac{100 \times 5.0}{1000 \times 2}$  g CaCO<sub>3</sub> (1 L = 1000 mL)

$$= \mathbf{0.25\ g} \quad (= 250\ \text{mg})$$

**Q 2 (4)**

The total amount of sucrose (as opposed to concentration) remains the same after dilution. The amount of sucrose in a given volume of solution is equal to the volume multiplied by concentration. Therefore, the following expression can be written:

$$\text{Initial volume} \times \text{Initial concentration} = \text{Final volume} \times \text{Final concentration}$$

The units for volume and concentration must be the same on both sides of the equation.

$$\begin{aligned} \text{Initial volume} &= \text{unknown} \\ \text{Initial concentration} &= 5\ \% \\ \text{Final volume} &= 500\ \text{mL} \\ \text{Final concentration} &= 1\ \% \end{aligned}$$

Substituting these values the initial volume can be calculated:

$$\text{Initial volume (mL)} \times 5 = 500 \times 1$$

$$\text{Initial volume (mL)} = \frac{500 \times 1}{5} = \mathbf{100\ \text{mL}}$$

Another way to do this is that the final concentration (1%) is one fifth of the initial value (5%) so that the 5% sucrose solution has to be diluted 5-fold. The volume required is 500 mL so that one fifth of this, 100 mL, has to be diluted.

**Q 2 (5)**

Both volumes must be expressed in the same units. Multiplication of the volume of water (5 mL) by 1000 gives its volume in  $\mu\text{L}$  (5000  $\mu\text{L}$ ) since 1 mL = 1000  $\mu\text{L}$ .

The total volume of diluted urine is the sum of the volumes of urine and water:

$$\text{Final volume } (\mu\text{L}) = 50 + 5000 = 5050\ \mu\text{L}$$

The dilution is the number of times the urine was diluted which is the final volume divided by the initial volume:

$$\text{Dilution} = \frac{\text{Final volume}}{\text{Initial volume}} = \frac{5050}{50} = \mathbf{101}$$

N.B. Concentration is the reciprocal of dilution. In this case  $1/101 = 0.0099$ .

**Q 2 (6)**

First calculate the molecular weight of sulphuric acid:

$$\begin{array}{l} \text{Atomic wt of hydrogen} = 1 \quad 2\text{H} = 2 \times 1 = 2 \\ \text{Atomic wt of sulphur} = 32 \quad \text{S} = 1 \times 32 = 32 \\ \text{Atomic wt of oxygen} = 16 \quad 4\text{O} = 4 \times 16 = \underline{64} \end{array}$$

$$\text{Molecular weight H}_2\text{SO}_4 = 98$$

Therefore 1 L of 1 mol/L requires 98 g H<sub>2</sub>SO<sub>4</sub>

1 L of 0.1 mol/L requires  $\frac{98}{10}$  g H<sub>2</sub>SO<sub>4</sub> (since it is 1/10<sup>th</sup> the strength of 1 mol/L)

Since the sulphuric acid is only 96% pure this weight must be multiplied by 100/96:

$$\text{Weight H}_2\text{SO}_4 \text{ required} = \frac{98 \times 100}{10 \times 96} = 10.21 \text{ g (4 sig figs)}$$

The volume required can be calculated from the specific gravity:

$$\text{Specific gravity (SG)} = \frac{\text{Weight}}{\text{Volume}}$$

$$\text{Volume} = \frac{\text{Weight}}{\text{Specific gravity}}$$

We are told that the specific gravity of H<sub>2</sub>SO<sub>4</sub> is 1.16 so that 1 mL weighs 1.16 g. The volume which weighs 102.08 g is calculated as follows:

$$\text{Volume (mL)} = \frac{10.21}{1.16} = \mathbf{8.8 \text{ mL}}$$

**Q 2 (7)**

First calculate the molar concentration of each individual solution:

Potassium chloride (KCl). Atomic wt K = 39, atomic wt Cl = 35.5  
Molecular weight of KCl = 39 + 35.5 = 74.5

$$\text{KCl (mol/L)} = \frac{\text{KCl (g/L)}}{\text{Molecular wt}} = \frac{5.0}{74.5} = 0.0671 \text{ mol/L (3 sig figs)}$$

WORKED ANSWERS TO FURTHER QUESTIONS

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Sodium chloride (NaCl). Atomic wt Na = 23, atomic wt Cl = 35.5.  
 Molecular weight NaCl = 23 + 35.5 = 58.5

$$\text{NaCl (mol/L)} = \frac{\text{NaCl (g/L)}}{\text{Molecular wt}} = \frac{50}{58.5} = 0.855 \text{ (3 sig figs)}$$

*For potassium:*

50 mL 0.0671 M KCl + 100 mL 0.855 M NaCl → 150 mL mixture

0.0671 M KCl contains 0.06371 M K<sup>+</sup>. 50 mL is diluted to 150 mL.

$$\text{Final K}^+ \text{ (mol/L)} \times \text{Final vol (mL)} = \text{Initial K}^+ \text{ (mol/L)} \times \text{Initial vol (mL)}$$

$$\begin{aligned} \text{Final K}^+ \text{ (mol/L)} \times 150 &= 0.0671 \times 50 \\ \text{Final K}^+ \text{ (mol/L)} &= \frac{0.0671 \times 50}{150} = \mathbf{0.022 \text{ mol/L}} \text{ (2 sig figs)} \end{aligned}$$

*For sodium:*

100 mL NaCl 0.855 mol/L + 50 mL KCl → 150 mL mixture

$$\text{Final Na}^+ \text{ (mol/L)} \times \text{Final vol (mL)} = \text{Initial Na}^+ \text{ (mol/L)} \times \text{Initial vol (mL)}$$

$$\text{Final Na}^+ \text{ (mol/L)} \times 150 = 0.855 \times 100$$

$$\text{Final Na}^+ \text{ (mol/L)} = \frac{0.855 \times 100}{150} = \mathbf{0.57 \text{ mol/L}}$$

*For chloride:*

100 mL NaCl 0.855 mol/L + 50 mL KCl 0.0671 = 150 mL mixture

Chloride arises from both solutions (NaCl and KCl):

$$\text{Final Cl}^- \text{ (mol/L)} \times \text{Final vol (mL)} = [\text{Initial Cl}^- \text{ from KCl (mol/L)} \times$$

$$\text{vol KCl (mL)}] + [\text{Initial Cl}^- \text{ from NaCl (mol/L)} \times \text{vol NaCl (L)}]$$

$$\text{Final Cl}^- \text{ (mol/L)} \times 150 = [0.0671 \times 50] + [0.855 \times 100]$$

$$\text{Final Cl}^- \text{ (mol/L)} = \frac{[(0.0671 \times 50) + (0.855 \times 100)]}{150}$$

$$\begin{aligned}
 &= \frac{3.355 + 85.5}{150} \\
 &= \frac{88.855}{150} \\
 &= \mathbf{0.59 \text{ mol/L}} \text{ (2 sig figs)}
 \end{aligned}$$

**Q 2 (8)**

Initial vol (mL) x Initial concn (%) = Final vol (mL) x Final concn (%)

$$\begin{aligned}
 650 \quad \times \quad 95 &= \text{Final vol (mL)} \quad \times \quad 65 \\
 \text{Final vol (mL)} &= \frac{650 \times 95}{65} = \mathbf{950 \text{ mL}}
 \end{aligned}$$

N.B. The expected volume of water to be added to the 95% ethanol (950 - 650 = 300 mL) will be insufficient because mixing an alcohol with water always results in some contraction of the total volume. Therefore further water should be added until a final volume of 950 mL is reached.

**Q 2 (9)**

First calculate the weight of sodium dihydrogen orthophosphate dihydrate ( $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ) which should have been used. Adding individual atoms together gives the empirical formula:  $\text{NaH}_6\text{PO}_6$

$$\begin{aligned}
 \text{Atomic wt sodium} &= 23 \text{ therefore Na} = 1 \times 23 = 23 \\
 \text{Atomic wt hydrogen} &= 1 \text{ therefore 6H} = 6 \times 1 = 6 \\
 \text{Atomic wt phosphorus} &= 31 \text{ therefore P} = 1 \times 31 = 31 \\
 \text{Atomic wt oxygen} &= 16 \text{ therefore 6O} = 6 \times 16 = \underline{96}
 \end{aligned}$$

$$\text{Molecular weight NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O} = 156$$

Therefore 1 L 1.0 mol/L contains 156 g  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$

$$\text{so that 1 L 0.2 mol/L contains } \frac{156}{5} \text{ g} = 31.2 \text{ g NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$$

Next calculate the molar concentration if this weight (31.2 g) of anhydrous sodium dihydrogen orthophosphate ( $\text{NaH}_2\text{PO}_4$ ) was dissolved in 1 L of water.

$$\begin{aligned}
 \text{Atomic wt sodium} &= 23 \text{ therefore Na} = 1 \times 23 = 23 \\
 \text{Atomic wt hydrogen} &= 1 \text{ therefore 2H} = 2 \times 1 = 2 \\
 \text{Atomic wt phosphorus} &= 31 \text{ therefore P} = 1 \times 31 = 31 \\
 \text{Atomic wt oxygen} &= 16 \text{ therefore 4O} = 4 \times 16 = \underline{64}
 \end{aligned}$$

WORKED ANSWERS TO FURTHER QUESTIONS

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$$\text{Molecular weight NaH}_2\text{PO}_4 = 120$$

$$\text{Concentration (mol/L)} = \frac{\text{Concentration (g/L)}}{\text{Molecular weight}}$$

$$\text{Concentration (mol/L)} = \frac{31.2}{120} = 0.26 \text{ mol/L}$$

As a short cut the target concentration (0.2 mol/L) could be simply multiplied by the ratio of the molecular weight of  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  to that of  $\text{NaH}_2\text{PO}_4$ :

$$\text{Actual concentration (mol/L)} =$$

$$\frac{\text{Target concentration (0.2 mol/L)} \times \text{MW NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}}{\text{MW NaH}_2\text{PO}_4}$$

$$= \frac{0.2 \times 156}{120} = 0.26 \text{ mol/L}$$

Working standard was prepared by diluting 5 mL of this stock standard to 250 mL.

$$\text{Initial concn (mol/L)} \times \text{Initial vol (mL)} = \text{Final concn (mol/L)} \times \text{Final vol (mL)}$$

$$0.26 \times 5 = \text{Final concn (mol/L)} \times 250$$

$$\text{Final concn (mol/L)} = \frac{0.26 \times 5}{250} = 0.0052 \text{ mol/L}$$

Multiplication by 1000 (since there are 1000 mmol in a mol) converts this concentration to mmol/L:

$$\text{Working phosphate standard concentration} = 0.0052 \times 1000 = \mathbf{5.2 \text{ mmol/L}}$$

**Q 2 (10)**

First calculate the molecular weight of anhydrous sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ ):

$$\begin{array}{l} \text{Atomic wt sodium} = 23 \text{ therefore Na} = 1 \times 23 = 23 \\ \text{Atomic wt hydrogen} = 1 \text{ therefore 2H} = 2 \times 1 = 2 \\ \text{Atomic wt phosphorus} = 31 \text{ therefore P} = 1 \times 31 = 31 \\ \text{Atomic wt oxygen} = 16 \text{ therefore 4O} = 4 \times 16 = \underline{64} \end{array}$$

$$\text{Molecular weight NaH}_2\text{PO}_4 = 120$$

Next calculate the molar concentration of a solution containing 12 g/L:

$$\text{Concentration (mol/L)} = \frac{\text{Concentration (g/L)}}{\text{Molecular weight}}$$

$$\text{Concentration (mol/L)} = \frac{12}{120} = 0.1 \text{ mol/L}$$

Multiplication by 1000 gives the concentration in mmol/L (since 1 mol = 1000 mmol):

$$\text{Phosphate concentration} = 0.1 \times 1000 = \mathbf{100 \text{ mmol/L}}$$

To prepare 1 L of 4 mmol/L phosphate:

$$\text{Initial vol (mL)} \times \text{Initial conc (mmol/L)} = \text{Final vol (mL)} \times \text{Final conc (mmol/L)}$$

$$\text{Initial vol (mL)} \times 100 = 1000 \times 4$$

$$\text{Initial vol (mL)} = \frac{1000 \times 4}{100} = \mathbf{40 \text{ mL}}$$

### Chapter 3

#### Q 3 (1)

First calculate molar concentration of 0.5% HCl:

$$0.5 \% \text{ w/v} = 0.5 \text{ g/100 mL} = 0.5 \times 10 = 5.0 \text{ g/L}$$

$$\text{MW HCl} = 1 + 35.5 = 36.5$$

$$\text{Molar conc} = \frac{\text{g/L}}{\text{MW}} = \frac{5.0}{36.5} = 0.137 \text{ mol/L (3 sig figs)}$$

Next calculate pH assuming complete dissociation of HCl:

$$\text{pH} = -\log_{10} [\text{H}^+]$$

Substitute  $[\text{H}^+] = 0.137 \text{ mol/L}$

$$\text{pH} = -\log_{10} 0.137 = -(-0.86) = \mathbf{0.86} \text{ (2 sig figs)}$$

**Q 3 (2)**

$$\text{pH} = -\log_{10} [\text{H}^+]$$

Rearranging:  $\log_{10} [\text{H}^+] = -\text{pH}$

Therefore  $[\text{H}^+] = \text{antilog}_{10} (-\text{pH})$

Substitute  $\text{pH} = 7.35$ :

$$\begin{aligned} [\text{H}^+] &= \text{antilog}_{10} (-7.35) = 4.47 \times 10^{-8} \text{ mol/L} \\ &= 44.7 \times 10^{-9} \text{ mol/L} = \mathbf{45 \text{ nmol/L}} \text{ (2 sig figs)} \end{aligned}$$

(since there are  $10^9$  or 1000000000 nmol in a mol)

Substitute  $\text{pH} = 7.45$

$$\begin{aligned} [\text{H}^+] &= \text{antilog}_{10} (-7.45) = 3.55 \times 10^{-8} \text{ mol/L} \\ &= 35.5 \times 10^{-9} \text{ mol/L} = \mathbf{36 \text{ nmol/L}} \text{ (2 sig figs)} \end{aligned}$$

**Q 3 (3)**

$$\text{pH} = -\log_{10} [\text{H}^+] \quad \text{therefore} \quad [\text{H}^+] = \text{antilog}_{10} (-\text{pH})$$

For urine substitute  $\text{pH} = 6.0$ :

$$\begin{aligned} [\text{H}^+] &= \text{antilog}_{10} (-6.0) = 1.0 \times 10^{-6} \text{ mol/L} \\ &= 1000 \times 10^{-9} \text{ mol/L} = 1000 \text{ nmol/L} \end{aligned}$$

For blood substitute  $\text{pH} = 7.40$ :

$$\begin{aligned} [\text{H}^+] &= \text{antilog}_{10} (-7.40) = 3.98 \times 10^{-8} \\ &= 39.8 \times 10^{-9} \text{ mol/L} = 40 \text{ nmol/L} \end{aligned}$$

$$\text{Gradient} = \frac{[\text{H}^+] \text{ in urine}}{[\text{H}^+] \text{ in blood}} = \frac{1000}{40} = \mathbf{25:1}$$

Another way of approaching this problem is to use the fact that the ratio of two values is equal to the antilog of the difference between their logarithms.

In other words, substitute  $\text{antilog}_{10} (-\text{pH})$  for  $[\text{H}^+]$ :

$$\begin{aligned}
 \text{Gradient} &= \frac{[\text{H}^+]_{\text{urine}}}{[\text{H}^+]_{\text{blood}}} = \text{antilog}_{10} \left( \frac{-\text{pH}_{\text{urine}}}{(-\text{pH}_{\text{blood}})} \right) \\
 &= \text{antilog}_{10} \{-\text{pH}_{\text{urine}} - (-\text{pH}_{\text{blood}})\} \\
 &= \text{antilog}_{10} (\text{pH}_{\text{blood}} - \text{pH}_{\text{urine}}) \\
 &= \text{antilog}_{10} (7.4 - 6.0) \\
 &= \text{antilog}_{10} 1.4 \\
 &= \mathbf{25} \quad (2 \text{ sig figs})
 \end{aligned}$$

**Q 3 (4)**

The dissociation to be considered is:



$$\text{pH} = \text{pKa} + \log_{10} \frac{[\text{Na}_2\text{HPO}_4]}{[\text{NaH}_2\text{PO}_4]}$$

Rearranging:  $\text{pKa} = \text{pH} - \log_{10} \frac{[\text{Na}_2\text{HPO}_4]}{[\text{NaH}_2\text{PO}_4]}$

Next calculate the molar concentration of each phosphate.

$$[\text{Na}_2\text{HPO}_4] = \frac{12.85 \times 10}{1000 \times \text{MW}}$$

(Multiplication by 10 converts mg/100 mL to mg/L. Division by 1000 converts mg/L to g/L)

$$\begin{aligned}
 \text{MW Na}_2\text{HPO}_4 &= (2 \times 23) + 1 + 31 + (4 \times 16) = 142 \\
 [\text{Na}_2\text{HPO}_4] &= \frac{12.85 \times 10}{1000 \times 142} = 0.000905 \text{ mol/L}
 \end{aligned}$$

$$[\text{NaH}_2\text{PO}_4] = \frac{6.88 \times 10}{1000 \times \text{MW}}$$

$$\text{MW NaH}_2\text{PO}_4 = 23 + (2 \times 1) + 31 + (4 \times 16) = 120$$

$$[\text{NaH}_2\text{PO}_4] = \frac{6.88 \times 10}{1000 \times 120} = 0.000573 \text{ mol/L}$$

Next substitute these molar concentrations into the rearranged Henderson-Hasselbalch equation and solve for pKa:

$$\text{pKa} = 7.0 - \log_{10} \frac{0.000905}{0.000573}$$

$$\text{pKa} = 7.0 - \log_{10} 1.58 = 7.0 - 0.20 = \mathbf{6.80}$$

**Q 3 (5)**

The relevant dissociation is:



$$\text{pH} = \text{pKa} + \log_{10} \frac{[\text{CO}_3^{2-}]}{[\text{HCO}_3^-]}$$

Rearrange, substitute  $\text{pH} = 10.7$  and  $\text{pKa} = 10.3$ , then calculate ratio:

$$\log_{10} \frac{[\text{CO}_3^{2-}]}{[\text{HCO}_3^-]} = \text{pH} - \text{pKa} = 10.7 - 10.3 = 0.4$$

$$\frac{[\text{CO}_3^{2-}]}{[\text{HCO}_3^-]} = \text{antilog } 0.4 = 2.51 \quad \dots\dots\dots \text{(i)}$$

Since the total concentration of both bicarbonate and carbonate in the buffer is 0.2 mol/L:

$$0.2 = [\text{HCO}_3^-] + [\text{CO}_3^{2-}] \quad \dots\dots\dots \text{(ii)}$$

$$[\text{HCO}_3^-] = 0.2 - [\text{CO}_3^{2-}]$$

Substitute for  $[\text{HCO}_3^-]$  in (i) and solve for  $[\text{CO}_3^{2-}]$ :

$$\frac{[\text{CO}_3^{2-}]}{0.2 - [\text{CO}_3^{2-}]} = 2.51$$

$$[\text{CO}_3^{2-}] = (2.51 \times 0.2) - 2.51 [\text{CO}_3^{2-}]$$

$$[\text{CO}_3^{2-}] + 2.51 [\text{CO}_3^{2-}] = 0.502$$

$$3.51 [\text{CO}_3^{2-}] = 0.502$$

$$[\text{CO}_3^{2-}] = \frac{0.502}{3.51} = 0.143 \text{ mol/L}$$

Substitute  $[\text{CO}_3^{2-}] = 0.143$  into (ii) and solve for  $[\text{HCO}_3^-]$ :

$$0.2 = [\text{HCO}_3^-] + 0.143$$

$$[\text{HCO}_3^-] = 0.2 - 0.143 = 0.057 \text{ mol/L}$$

Calculate weights of both sodium carbonate and bicarbonate needed to prepare 500 mL of buffer:

$$\text{Weight required (g)} = \frac{\text{Molar concentration (mol/L)} \times \text{MW}}{2}$$

$$\text{MW Na}_2\text{CO}_3 = (2 \times 23) + 12 + (3 \times 16) = 106$$

$$\text{MW NaHCO}_3 = 23 + 1 + 12 + (3 \times 16) = 84$$

$$\text{Wt Na}_2\text{CO}_3 = \frac{0.143 \times 106}{2} = \mathbf{7.58 \text{ g}} \text{ (3 sig figs)}$$

$$\text{Wt NaHCO}_3 = \frac{0.057 \times 84}{2} = \mathbf{2.39 \text{ g}} \text{ (3 sig figs)}$$

**Q 3 (6)**

The relevant dissociation is:



$$\text{pH} = \text{pKa} + \text{Log}_{10} \frac{[\text{Lact}^-]}{[\text{LactH}]}$$

Substitute pH = 7.4 and pKa = 3.86 then calculate ratio:

$$7.4 = 3.86 + \text{Log}_{10} \frac{[\text{Lact}^-]}{[\text{LactH}]}$$

$$\text{Log}_{10} \frac{[\text{Lact}^-]}{[\text{LactH}]} = 7.4 - 3.86 = 3.54$$

$$\frac{[\text{Lact}^-]}{[\text{LactH}]} = \text{antilog}_{10} 3.54 = 3467 \dots\dots(i)$$

The concentration is not given but we are told that the solution must be isotonic. Assuming physiological osmolarity is 285 mmol/L:

$$285 = [\text{LactH}] + [\text{Lact}^-] + [\text{Na}^+]$$

Where the concentrations of LactH, Lact<sup>-</sup> and Na<sup>+</sup> are mmol/L.  
 Since the concentrations of Lact<sup>-</sup> and Na<sup>+</sup> are equal:

$$285 = [\text{LactH}] + 2 [\text{Lact}^-] \dots\dots\dots (\text{ii})$$

Rearranging:

$$[\text{LactH}] = 285 - 2 [\text{Lact}^-]$$

Substitute for [LactH] in (i) and solve for [Lact<sup>-</sup>]:

$$\frac{[\text{Lact}^-]}{285 - 2 [\text{Lact}^-]} = 3467$$

$$[\text{Lact}^-] = 3467 (285 - 2 [\text{Lact}^-])$$

$$[\text{Lact}^-] = 988095 - 6934 [\text{Lact}^-]$$

$$[\text{Lact}^-] + 6934 [\text{Lact}^-] = 988095$$

$$6934 [\text{Lact}^-] = 988095$$

$$[\text{Lact}^-] = \frac{988095}{6934} = 142 \text{ mmol/L (3 sig figs)}$$

Substitute [Lact<sup>-</sup>] = 142 into (ii) and solve for [LactH]:

$$285 = [\text{LactH}] + (2 \times 142)$$

$$[\text{LactH}] = 285 - 284 = 1 \text{ mol/L}$$

Calculate weight of sodium lactate:

$$\text{Sodium lactate (g/2.5 L)} = \frac{[\text{Lact}^-] \text{ mmol/L} \times \text{MW} \times 2.5}{1000}$$

$$\text{MW CH}_3\text{CH(OH)COONa} = 23 + (3 \times 12) + (5 \times 1) + (3 \times 16) = 112$$

$$\text{Wt sodium lactate} = \frac{142 \times 112 \times 2.5}{1000} = \mathbf{39.8 \text{ g}}$$

Calculate weight of lactic acid:

$$\text{Lactic acid (g/2.5 L)} = \frac{[\text{LactH}] \text{ mmol/L} \times \text{MW} \times 2.5}{1000}$$

$$\text{MW CH}_3\text{CH(OH)COOH} = (3 \times 12) + (6 \times 1) + (3 \times 16) = 90$$

$$\text{Wt lactic acid} = \frac{1 \times 90 \times 2.5}{1000} = 0.225 \text{ g}$$

Convert to mL 85% lactic acid SG = 1.2:

$$\begin{aligned} \text{Vol lactic acid (mL)} &= \frac{\text{Wt (g)} \times 100}{\% \text{ purity} \times \text{SG}} \\ &= \frac{0.225 \times 100}{85 \times 1.2} = \mathbf{0.22 \text{ mL}} \quad (2 \text{ sig figs}) \end{aligned}$$

**Q 3 (7)**

The reaction occurring when secreted hydrogen ions are buffered by phosphate in the glomerular filtrate is:



And the corresponding Henderson-Hasselbalch equation is:

$$\text{pH} = \text{pKa} + \log_{10} \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]}$$

Calculate the ratio of the two phosphate ions in fresh glomerular filtrate (i.e. pH = 7.4):

$$\begin{aligned} 7.4 &= 6.8 + \log_{10} \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} \\ \log_{10} \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} &= 7.4 - 6.8 = 0.6 \\ \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} &= \text{antilog}_{10} 0.6 = 3.98 \dots\dots\dots(i) \end{aligned}$$

For simplicity assume a urine volume of 1 L so that the total phosphate concentration is 65 mmol/L. (We are told the amount not the concentration but, since we are dealing with ratios, any volume could be used).

$$\begin{aligned} [\text{Total phosphate}] &= [\text{HPO}_4^{2-}] + [\text{H}_2\text{PO}_4^-] \\ 65 &= [\text{HPO}_4^{2-}] + [\text{H}_2\text{PO}_4^-] \\ [\text{H}_2\text{PO}_4^-] &= 65 - [\text{HPO}_4^{2-}] \end{aligned}$$

WORKED ANSWERS TO FURTHER QUESTIONS

Substitute for  $[\text{H}_2\text{PO}_4^-]$  in (i) then solve for  $[\text{HPO}_4^{2-}]$ :

$$\frac{[\text{HPO}_4^{2-}]}{65 - [\text{HPO}_4^{2-}]} = 3.98$$

$$[\text{HPO}_4^{2-}] = 3.98 (65 - [\text{HPO}_4^{2-}])$$

$$[\text{HPO}_4^{2-}] = 258.7 - 3.98[\text{HPO}_4^{2-}]$$

$$[\text{HPO}_4^{2-}] + 3.98 [\text{HPO}_4^{2-}] = 258.7$$

$$4.98 [\text{HPO}_4^{2-}] = 258.7$$

$$[\text{HPO}_4^{2-}] = \frac{258.7}{4.98} = 51.9 \text{ mmol/L (3 sig figs)}$$

Repeat this procedure for acidified glomerular filtrate i.e. urine pH = 5.5

$$5.5 = 6.8 + \log_{10} \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]}$$

$$\log_{10} \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} = 5.5 - 6.8 = -1.3$$

$$\frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} = \text{antilog}_{10} -1.3 = 0.050$$

Substitute  $[\text{H}_2\text{PO}_4^-] = 65 - [\text{HPO}_4^{2-}]$  and solve for  $[\text{HPO}_4^{2-}]$ :

$$\frac{[\text{HPO}_4^{2-}]}{65 - [\text{HPO}_4^{2-}]} = 0.050$$

$$[\text{HPO}_4^{2-}] = 0.050 (65 - [\text{HPO}_4^{2-}])$$

$$[\text{HPO}_4^{2-}] + 0.050 [\text{HPO}_4^{2-}] = 0.050 \times 65 = 3.25$$

$$1.05 [\text{HPO}_4^{2-}] = 3.25$$

$$[\text{HPO}_4^{2-}] = \frac{3.25}{1.05} = 3.10 \text{ mmol/L}$$

The titratable acidity is the concentration (or rather amount) of  $\text{HPO}_4^{2-}$  consumed as the pH of the glomerular filtrate is reduced from 7.4 to 5.5 and is the difference between the two phosphate concentrations:

$$\text{Titratable acidity} = 51.9 - 3.1 = \mathbf{49 \text{ mmol}} \text{ (2 sig figs)}$$

**Q 3 (8)**

The dissociation to be considered is:



And the relevant form of the Henderson-Hasselbalch equation is:

$$\text{pH} = \text{pKa} + \log_{10} \frac{[\text{Ac}^-]}{[\text{HAc}]}$$

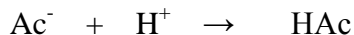
Determine pKa by substituting the pH (4.74) and concentrations of Ac<sup>-</sup> (0.1 mol/L) and HAc (0.1 mol/L):

$$4.74 = \text{pKa} + \log_{10} \frac{0.1}{0.1}$$

Since  $0.1/0.1 = 1$  and  $\log_{10} 1$  is 0, then  $\text{pKa} = 4.74$

Calculate the adjusted concentrations of Ac<sup>-</sup> and HAc, and substitute into the Henderson-Hasselbalch equation (using pKa = 4.74) then solve for pH:

Addition of HCl to this buffer converts some of the acetate ions to acetic acid:



$$\text{Final } [\text{Ac}^-] = \text{Initial } [\text{Ac}^-] - \text{Added } [\text{HCl}]$$

Allowance must be made for the dilution resulting from mixing 10 mL buffer with 4 mL HCl (total volume = 14 mL):

$$\text{Initial } [\text{Ac}^-] = \frac{0.1 \times 10}{14} = 0.071 \text{ mol/L}$$

$$\text{Added } [\text{HCl}] = \frac{\text{Initial } [\text{HCl}] \times 4}{14} = \frac{0.025 \times 4}{14} = 0.0071 \text{ mol/L}$$

$$\text{Final } [\text{Ac}^-] = 0.071 - 0.0071 = 0.0639 \text{ mol/L}$$

Similarly:

$$\text{Final } [\text{HAc}] = \text{Initial } [\text{HAc}] + \text{Added } [\text{HCl}]$$

$$\text{Since Initial } [\text{HAc}] = \text{Initial } [\text{Ac}^-]$$

$$\text{Final } [\text{HAc}] = 0.071 + 0.0071 = 0.0781 \text{ mol/L}$$

$$\begin{aligned}
 \text{Therefore: } \text{pH} &= 4.74 + \log_{10} \frac{0.0639}{0.0781} \\
 &= 4.74 + \log_{10} 0.818 \\
 &= 4.74 + (-0.087) = \mathbf{4.65}
 \end{aligned}$$

## Chapter 4

### Q 4 (1)

Only absorbance is proportional to concentration, so the first step is to calculate the absorbance of the final solution which absorbs 30% of the light entering into it.

$$\text{Absorbance (A)} = \log_{10} \frac{I_0}{I}$$

$$I_0 = \text{intensity of incident light} = 100\%$$

$$I = \text{intensity of transmitted light. Since 30\% was absorbed, } 100 - 30 = 70\% \text{ is transmitted. Therefore, } I = 70\%$$

Substitute these values to obtain A:

$$A = \log_{10} \frac{100}{70} = \log_{10} 1.429 = 0.155 \text{ (3 sig figs)}$$

$$\text{Let } x = \text{vol of solution to be added to 100 mL water} = x \text{ mL}$$

$$\text{Final volume} = (100 + x) \text{ mL}$$

$$\text{Initial volume} \times \text{Initial concn} = \text{Final volume} \times \text{Final concn}$$

Since Beer's Law is obeyed, absorbance can be substituted for concentration:

$$\text{Initial volume} \times \text{Initial absorbance} = \text{Final volume} \times \text{Final absorbance}$$

Substitute values for volumes and absorbances:

$$x \text{ (mL)} \times 0.23 = (100 + x) \times 0.155$$

$$x \text{ (mL)} = \frac{(100 + x) \times 0.155}{0.23}$$

$$x = \frac{15.5 + 0.155x}{0.23}$$

$$0.23x = 15.5 + 0.155x$$

$$0.23x - 0.155x = 15.5$$

$$0.075x = 15.5$$

$$x = \frac{15.5}{0.075} = \mathbf{207 \text{ mL}} \text{ (3 sig figs)}$$

**Q 4 (2)**

If  $I_o$  is the intensity of incident light and  $I$  the intensity of transmitted light, then:

$$\text{transmittance (\%T)} = \frac{I \times 100}{I_o} \quad \text{and} \quad \text{absorbance (A)} = \log_{10} \frac{I_o}{I}$$

The expression for %T can be arranged to:  $\frac{I_o}{I} = \frac{100}{\%T}$

Which can be substituted into the expression for A to give:

$$A = \log_{10} \frac{100}{\%T} = \log_{10} 100 - \log_{10} \%T$$

Substituting 2 for  $\log_{10} 100$  gives the following useful expression:

$$\mathbf{A = 2 - \log_{10} \%T}$$

All that is required is to substitute values for %T into this expression to obtain A:

a)  $\%T = 95$

$$A = 2 - \log_{10} 95 = 2 - 1.978 = \mathbf{0.022} \text{ (3 sig figs)}$$

b)  $\%T = 75$

$$A = 2 - \log_{10} 75 = 2 - 1.875 = \mathbf{0.125} \text{ (3 sig figs)}$$

c)  $\%T = 50$

$$A = 2 - \log_{10} 50 = 2 - 1.699 = \mathbf{0.301} \text{ (3 sig figs)}$$

d)  $\%T = 25$

$$A = 2 - \log_{10} 25 = 2 - 1.398 = \mathbf{0.602} \text{ (3 sig figs)}$$

e)  $\%T = 10$

$$A = 2 - \log_{10} 10 = 2 - 1.000 = \mathbf{1}$$

f)  $\%T = 1$

$$A = 2 - \log_{10} 1 = 2 - 0 = \mathbf{2}$$

**Q 4 (3)**

The expression used to calculate  $A$  from  $\%T$  can be rearranged to enable direct calculation of  $\%T$  from  $A$ :

$$A = 2 - \log_{10} \%T$$

$$A + \log_{10} \%T = 2$$

$$\log_{10} \%T = 2 - A$$

$$\mathbf{\%T = \text{antilog}_{10} (2 - A)}$$

Therefore substitute values for  $A$  into this expression then evaluate  $\%T$ :

a)  $A = 0.1$

$$\%T = \text{antilog}_{10} (2 - 0.1) = \text{antilog}_{10} 1.9 = \mathbf{79\%} \text{ (2 sig figs)}$$

b)  $A = 0.25$

$$\%T = \text{antilog}_{10} (2 - 0.25) = \text{antilog}_{10} 1.75 = \mathbf{56\%} \text{ (2 sig figs)}$$

c)  $A = 0.50$

$$\%T = \text{antilog}_{10} (2 - 0.50) = \text{antilog}_{10} 1.50 = \mathbf{32\%} \text{ (2 sig figs)}$$

d)  $A = 0.75$

$$\%T = \text{antilog}_{10} (2 - 0.75) = \text{antilog}_{10} 1.25 = \mathbf{18\%} \text{ (2 sig figs)}$$

e)  $A = 1.00$

$$\%T = \text{antilog}_{10}(2 - 1.00) = \text{antilog}_{10} 1 = \mathbf{10\%}$$

f)  $A = 2.00$

$$\%T = \text{antilog}_{10}(2 - 2) = \text{antilog}_{10} 0 = \mathbf{1\%}$$

**Q 4 (4)**

First convert percentage transmittance (%*T*) to absorbance (*A*):

$$\begin{aligned} A &= 2 - \log_{10} \%T \\ &= 2 - \log_{10} 18.4 \\ &= 2 - 1.2648 \\ &= \mathbf{0.735} \quad (\text{3 sig figs}) \end{aligned}$$

Use this absorbance to calculate the molar absorptivity using the Beer-Lambert Law:

$$A = abc$$

Where

<i>A</i>	=	absorbance reading	=	0.735
<i>a</i>	=	molar absorptivity	=	unknown
<i>b</i>	=	light path length	=	1 cm
<i>c</i>	=	concentration	=	1.00 mmol/L

Since the question asks for calculation of molar absorptivity, the concentration must be divided by 1000 to convert it to mol/L (1 mol = 1000 mmol):

$$\text{Concentration (mol/L)} = \frac{1 \text{ (mmol/L)}}{1000} = 0.001 \text{ mol/L}$$

Substitute *A*, *b* and *c* into the Beer-Lambert equation and solve for *a*:

$$\begin{aligned} 0.735 &= a \times 1 \times 0.001 \\ a &= \frac{0.735}{1 \times 0.001} = \mathbf{735} \end{aligned}$$

The units can be derived by entering the individual units into the same equation (remembering that absorbance is the logarithm of a ratio so has no units):

$$a = \frac{\text{—}}{\text{cm} \times \text{mol/L}} = \frac{\text{L}}{\text{cm} \times \text{mol}} = \text{L/cm/mol (or L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}\text{)}$$

$$\text{Therefore molar absorptivity} = 735 \text{ L.mol}^{-1}.\text{cm}^{-1}$$

**Q 4 (5)**

Since absorbance, not transmittance, is linearly proportional to concentration, the first step is to convert the transmittance (%*T*) to absorbance (*A*):

$$\begin{aligned} A &= 2 - \log_{10} \%T \\ &= 2 - \log_{10} 45 \\ &= 2 - 1.6532 \\ &= 0.3468 \end{aligned}$$

Assuming NADH obeys Beer's Law, the absorbance of a 1 in 5 dilution of this solution will be a fifth of this value:

$$\text{Absorbance of 1 in 5 dilution} = \frac{0.3468}{5} = \mathbf{0.069} \quad (2 \text{ sig figs})$$

**Q 4 (6)**

First use the Beer-Lambert equation to calculate the porphyrin concentration in the extract:

$$A = abc$$

$$\begin{aligned} \text{Where } A &= \text{absorbance} &= 0.35 \\ a &= \text{molar absorptivity} &= 2.75 \times 10^5 \text{ L.mol}^{-1}.\text{cm}^{-1} \\ b &= \text{path length} &= 1 \text{ cm} \\ c &= \text{concentration} &= \text{mol/L} \end{aligned}$$

$$0.35 = 2.75 \times 10^5 \times 1 \times c$$

$$c = \frac{0.35}{2.75 \times 10^5 \times 1} = 1.273 \times 10^{-6} \text{ mol/L}$$

The answer is required in nmol not mol so this value must be multiplied by  $10^9$  (since  $1 \text{ mol} = 10^9 \text{ nmol}$ ):

$$c \text{ (nmol/L)} = 1.273 \times 10^{-6} \times 10^9 = 1.273 \times 10^3 \text{ nmol/L}$$

Since the faecal sample produced 4.5 mL of extract, the amount of porphyrin in the sample is obtained by dividing by 1000 (to convert from nmol/L to nmol/mL), the multiplying by the volume of extract (4.5 mL):

$$\text{Porphyrin in sample} = \frac{1.273 \times 10^3 \times 4.5}{1000 (= 10^3)} = 5.729 \text{ nmol}$$

The porphyrin content (expressed as nmol/g fresh weight of faeces) is obtained by dividing the porphyrin extracted (5.729 nmol) by the weight of sample used to prepare the extract (75 mg = 0.075 g):

$$\text{Porphyrin (nmol/g fresh stool)} = \frac{5.729}{0.075} = 76.4 \text{ nmol/g fresh wt (3 sig figs)}$$

To express this result on a dry weight basis, multiply by the fresh weight of faeces used for the dry weight determination then divide by its dry weight:

$$\text{Porphyrin content} = \frac{76.4 \times 0.250}{0.125} = \mathbf{153 \text{ nmol/g dry faeces (3 sig figs)}}$$

#### Q 4 (7)

First convert the % transmittance (%*T*) to absorbance (*A*):

$$A = 2 - \log_{10} \%T$$

$$A = 2 - \log_{10} 75$$

$$= 2 - 1.875$$

$$= 0.125$$

Provided the substance obeys Beer's Law over the range of concentrations (i.e. absorbance is directly proportional to concentration), then the absorbance of a different concentration (4 g/L) can be calculated from the relationship:

$$\frac{\text{Absorbance}_2}{\text{Concentration}_2} = \frac{\text{Absorbance}_1}{\text{Concentration}_1}$$

$$\text{Rearranging: Absorbance}_2 = \frac{\text{Absorbance}_1 \times \text{Concentration}_2}{\text{Concentration}_1}$$

$$\text{Substitute: absorbance}_2 = \text{unknown}; \quad \text{absorbance}_1 = 0.125;$$

$$\text{concentration}_2 = 4 \text{ g/L}; \quad \text{concentration}_1 = 3 \text{ g/L}$$

$$\text{Absorbance}_{(4\text{g/L})} = \frac{4 \times 0.125}{3} = 0.1667 \text{ (4 sig figs)}$$

Convert this absorbance to % transmittance:

$$\text{Log}_{10}\%T = 2 - A = 2 - 0.1667 = 1.833$$

$$\%T = \text{antilog}_{10} 1.833 = 68 \% \quad (2 \text{ sig figs})$$

To calculate molar absorption coefficient use either pairs of concentration and absorption:

$$A = abc$$

Where  $A = \text{absorbance} = 0.167$  (for a concentration of 4 g/L)

$a = \text{molar absorption coefficient} = ?$

$b = \text{cell path length} = 1 \text{ cm}$

$c = \text{concentration} = 4\text{g/L.}$

Since  $\text{MW} = 400$

$\text{molar concentration} = 4/400 = 1/100 = 1.0 \times 10^{-2} \text{ mol/L}$

$$0.167 = a \times 1.0 \times 1.0 \times 10^{-2}$$

$$a = \frac{0.167}{1.0 \times 10^{-2}} = 0.167 \times 10^2 = 16.7 \text{ L.mol}^{-1}.\text{cm}^{-1}$$

#### Q 4 (8)

Both NAD and NADH absorb at the two wavelengths used (260 nm and 340 nm). Absorbances are additive, therefore at either wavelength:

$$\text{Total absorbance} = \text{Absorbance of NAD} + \text{Absorbance of NADH}$$

At any wavelength the absorbance of NAD or NADH is given by:

$$\text{Absorbance} = \text{Molar extinction coefficient} \times \text{Molar concentration} \times \text{Cell path}$$

Therefore for each wavelength equations can be set up relating measured total absorbance to the sums of the individual absorbances of NAD and NADH:

$$\text{Measured absorbance} = (\text{NAD}_{\text{Conc}} \times \text{NAD}_{\text{Coeff}}) + (\text{NADH}_{\text{Conc}} \times \text{NADH}_{\text{Coeff}})$$

$$\text{At 340 nm: } 0.337 = 1.0 \times 10^{-3} [\text{NAD}] + 6.3 \times 10^3 [\text{NADH}] \dots\dots\dots(i)$$

$$\text{At 260 nm: } 1.23 = 1.8 \times 10^4 [\text{NAD}] + 1.5 \times 10^4 [\text{NADH}] \dots\dots\dots(ii)$$

(The cell path is 1 cm and can be ignored)

These form a pair of simultaneous equations which can be solved for [NAD] and [NADH] in the usual manner. However, solving a set of simultaneous equations can be a lengthy process. Therefore we should look for approximations and short cuts. In this particular example it is possible to considerably simplify the

calculation. The molar extinction coefficient of NAD at 340 nm is much lower than that of NADH (by a factor of approx.  $10^{-6}$ ) so that the contribution of NAD to the absorbance at this wavelength can be ignored. Equation (i) can then be simplified to:

$$0.337 = 6.3 \times 10^3 [\text{NADH}]$$

$$[\text{NADH}] = \frac{0.337}{6.3 \times 10^3} = 5.35 \times 10^{-5} \text{ M} = \mathbf{53.5 \mu\text{mol/L}}$$

[NAD] can be calculated by substituting  $[\text{NADH}] = 5.35 \times 10^{-5}$  into equation (ii):

$$1.23 = 1.8 \times 10^4 [\text{NAD}] + (1.5 \times 10^4 \times 5.35 \times 10^{-5})$$

$$1.23 = 1.8 \times 10^4 [\text{NAD}] + (8.03 \times 10^{-1})$$

$$1.8 \times 10^4 [\text{NAD}] = 1.23 - (8.03 \times 10^{-1}) = 0.427$$

$$[\text{NAD}] = \frac{0.427}{1.8 \times 10^4} = 2.37 \times 10^{-5} \text{ M} = \mathbf{23.7 \mu\text{mol/L}}$$

#### Q 4 (9)

First use the absorbance reading to calculate the actual concentration of bilirubin in the final solution:

$$A = a \times b \times c$$

Where

$A$	=	absorbance	=	0.502
$a$	=	molar absorptivity	=	$6.07 \times 10^4 \text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$
$b$	=	path length	=	1 cm
$c$	=	concentration in mol/L	=	?

$$0.502 = 6.07 \times 10^4 \times 1 \times c$$

Rearranging and solving for  $c$ :

$$c = \frac{0.502}{6.07 \times 10^4} = 8.27 \times 10^{-6} \text{ mol/L} = 8.27 \times 10^{-3} \text{ mmol/L}$$

Use this concentration of the final solution to calculate the bilirubin content of the weighed bilirubin:

The final solution was prepared by diluting 200  $\mu\text{L}$  (i.e. 0.2 mL) of stock to 250 mL

Therefore actual concentration of stock

$$= \frac{8.27 \times 10^{-3} \times 250}{0.2} = 10.34 \text{ mmol/L}$$

4 mL (the volume of DMSO the bilirubin was dissolved in) contains:

$$\frac{10.34 \times 4}{1000} = 0.04136 \text{ mmol bilirubin}$$

Convert to wt of bilirubin:

$$\text{Wt bilirubin (mg)} = \text{mmol bilirubin} \times \text{MW}$$

$$\text{MW bilirubin} = (33 \times 12) + (36 \times 1) + (6 \times 16) + (4 \times 14) = 584$$

$$\text{Therefore wt bilirubin} = 0.0414 \times 584 = 24.15 \text{ mg}$$

$$\% \text{ purity} = \frac{\text{Amount of bilirubin by assay} \times 100}{\text{Amount of bilirubin weighed}}$$

$$= \frac{24.15 \times 100}{25} = \mathbf{97\%} \text{ (2 sig figs)}$$

#### Q 4 (10)

First subtract the reagent blank (i.e. the reading obtained when using water as sample) from each absorbance reading:

	<b>Absorbance</b>	<b>Corrected Absorbance</b>
Blank (water as sample)	0.050	0.000
Creatinine standard (200 $\mu\text{mol/L}$ )	0.250	0.200
Serum sample	0.125	0.075
Urine sample (prediluted 1 in 50 with water)	0.200	0.150

$$\frac{\text{Corrected absorbance of unknown}}{\text{Concentration of unknown}} = \frac{\text{Corrected absorbance of standard}}{\text{Concentration of standard}}$$

$$\text{Concentration of unknown} =$$

$$\frac{\text{Corrected absorbance of unknown} \times \text{Concentration of standard}}{\text{Corrected absorbance of standard}}$$

For serum:

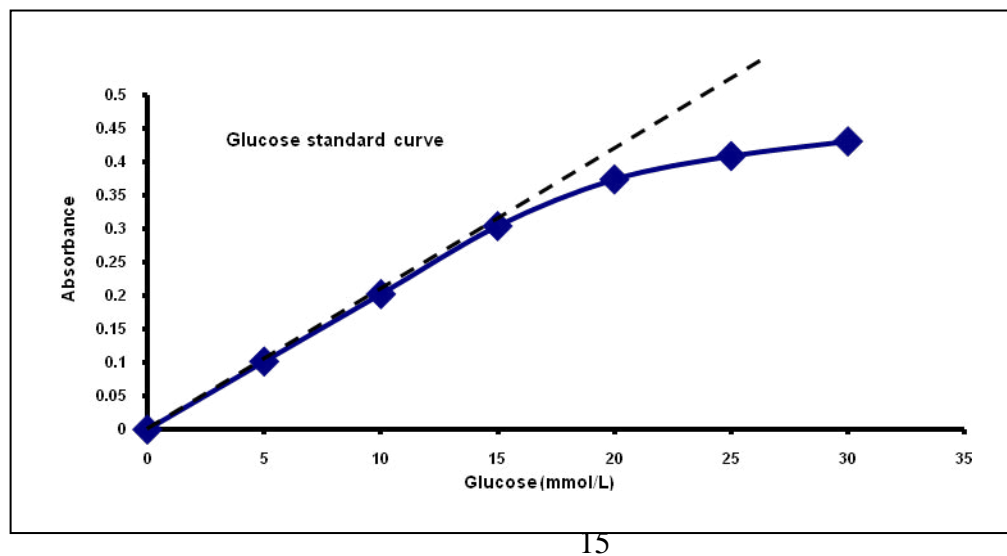
$$\text{Serum creatinine } (\mu\text{mol/L}) = \frac{0.075 \times 200}{0.200} = \mathbf{75 \mu\text{mol/L}}$$

For urine the calculation is the same except that the result must be multiplied by 50 to allow for the predilution of the sample prior to assay, then divided by 1000 to convert from  $\mu\text{mol/L}$  to  $\text{mmol/L}$  ( $1 \text{ mmol} = 1000 \mu\text{mol}$ ):

$$\text{Urine creatinine (mmol/L)} = \frac{0.150 \times 50 \times 200}{0.200 \times 1000} = 7.5 \text{ mmol/L}$$

**Q 4 (11)**

Plot the absorbance (vertical scale) against the standard concentration (horizontal scale) including the zero as a point (since the blank was used to zero the instrument):



Therefore  $1 A = \frac{1}{0.02}$  so that  $0.250 A = \frac{0.250}{0.02} = 12.5 \text{ mmol/L}$

**Chapter 5**

**Q 5 (1)**

$$\text{Creatinine excretion (mmol/24h)} = \text{creatinine concentration (mmol/L)} \times 24\text{h urine volume (L)}$$

Divide the creatinine concentration by 1000 to convert from  $\mu\text{mol/L}$  to  $\text{mmol/L}$  ( $1000 \mu\text{mol} = 1 \text{ mmol}$ ).

Divide the urine volume by 1000 to convert from mL to l L ( $1000 \text{ mL} = 1 \text{ L}$ ).

WORKED ANSWERS TO FURTHER QUESTIONS

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$$\text{Creatinine excretion (mmol/24h)} = \frac{8500 \times 1850}{1000 \times 1000} = \mathbf{15.7 \text{ mmol/24h}}$$

**Q 5 (2)**

First convert the filtration rate from mL/min to L/12 h. Multiply by 60 (to convert from min to h), then by 12 (to convert from h to 12 h) and finally divide by 1000 (to convert from mL to L):

$$\text{Filtration rate (L/12 h)} = \frac{110 \times 60 \times 12}{1000} = 79.2 \text{ L/12h}$$

The answer is required in mmol so divide the plasma concentration by 1000 to convert from  $\mu\text{mol/L}$  to  $\text{mmol/L}$  ( $1000 \mu\text{mol} = 1 \text{ mmol}$ ):

$$\text{Plasma creatinine (mmol/L)} = \frac{180}{1000} = 0.18 \text{ mmol/L}$$

$$\text{Creatinine filtered (mmol/12 h)} =$$

$$\text{Plasma creatinine (mmol/L)} \times \text{Filtration rate (L/12h)}$$

$$\text{Creatinine filtered (mmol/12h)} = 0.18 \times 79.2 = \mathbf{14.3 \text{ mmol/12h}}$$

**Q 5 (3)**

$$\text{Creatinine clearance (mL/min)} =$$

$$\frac{\text{Urine creatinine (mmol/L)} \times \text{Urine flow rate (mL/min)}}{\text{Plasma creatinine (mmol/L)}}$$

$$\text{Urine creatinine concentration} = 7.2 \text{ mmol/L}$$

$$\text{Plasma creatinine} = 94 \mu\text{mol/L} = \frac{94}{1000} \text{ mmol/L}$$

$$\text{Urine flow rate} = 3.2 \text{ L/24h} = \frac{3.2}{24} \text{ L/h} = \frac{3.2}{24 \times 60} \text{ L/min} = \frac{3.2 \times 1000}{24 \times 60} \text{ mL/min}$$

$$\text{Creatinine clearance} = \frac{7.2 \times 3.2 \times 1000}{24 \times 60 \times 94} \times 1000 = \mathbf{170 \text{ mL/min}}$$

This creatinine clearance is a little high. The most likely cause is that the 24 collection was made over a longer period than 24h – perhaps the bladder was not

emptied at the start of the collection period (or if emptied it was added to the collection instead of being discarded).

**Q 5 (4)**

First calculate the total amount of the compound filtered over a 24h period (based on the assumption that the compound is freely filtered at the glomerulus):

$$\text{Amount filtered (mg)} = \text{GFR (L/24h)} \times \text{Plasma concentration (mg/L)}$$

$$\text{GFR} = 110 \text{ mL/min} = \frac{110}{1000} \text{ L/min} = \frac{110 \times 60}{1000} \text{ L/h} = \frac{110 \times 60 \times 24}{1000} \text{ L/24h}$$

$$\text{Amount filtered (mg/24h)} = \frac{110 \times 60 \times 24 \times 10}{1000} = \mathbf{1584 \text{ mg/24h}}$$

The rate of excretion (316.8 mg/24h) is much less than this suggesting that the compound is either not freely filtered at the glomerulus or considerable amounts are reabsorbed from the filtrate.

N.B. The urine volume was not used in this calculation. Another approach (which would utilize urine volume) would be to calculate the clearance of the compound (which comes out at 22 mL/min) then compare it with the GFR.

**Q 5 (5)**

When a steady state is reached the rate of excretion is equal to the rate of infusion and the plasma concentration reaches a constant value.

$$\text{Clearance (mL/min)} = \frac{\text{Excretion rate } (\mu\text{mol/min})}{\text{Plasma concentration } (\mu\text{mol/mL})}$$

$$\text{Excretion rate} = \text{infusion rate} = 100 \mu\text{mol/min}$$

$$\text{Plasma concentration} = 200 \mu\text{mol/L} = \frac{200}{1000} \mu\text{mol/mL}$$

$$\text{Clearance (mL/min)} = \frac{100 \times 1000}{200} = \mathbf{500 \text{ mL/min}}$$

The clearance of the drug far exceeds the GFR suggesting that the mode of excretion is predominantly tubular secretion.

**Q 5 (6)**

This question involves calculating the urinary excretion when the plasma concentration and clearance is known. The expression for clearance is:

$$\text{Clearance (mL/min)} = \frac{\text{Urine creatinine (mmol/L)} \times \text{Urine flow rate (mL/min)}}{\text{Plasma creatinine (mmol/L)}}$$

Rearranging this expression:

$$\text{Urine creatinine (mmol/L)} = \frac{\text{Clearance (mL/min)} \times \text{Plasma creatinine (mmol/L)}}{\text{Urine flow rate (mL/min)}}$$

$$\text{Creatinine clearance} = 100 \text{ mL/min}$$

$$\text{Plasma creatinine} = 100 \mu\text{mol/L} = \frac{100}{1000} \text{ mmol/L}$$

$$\text{Urine flow rate} = 100 \text{ mL/6 h} = \frac{100}{6} \text{ mL/h} = \frac{100}{6 \times 60} \text{ mL/min}$$

$$\text{Urine creatinine (mmol/L)} = \frac{100 \times 100 \times 6 \times 60}{1000 \times 100} = \mathbf{36 \text{ mmol/L}}$$

**Q 5 (7)**

First calculate the amount of sodium filtered at the glomerulus in mmol/min:

$$\text{Na filtered (mmol/min)} = \text{GFR (mL/min)} \times \text{Plasma Na (mmol/mL)}$$

$$\text{Plasma Na} = 140 \text{ mmol/L} = \frac{140}{1000} \text{ mmol/mL}$$

$$\text{Na filtered (mmol/min)} = \frac{95 \times 140}{1000} = 13.3 \text{ mmol/min}$$

If the amount reabsorbed decreases by 1% then the amount excreted in the urine will increase by 1% of that filtered:

$$\text{Increase in Na excretion} = \frac{13.3 \times 1}{100} = 0.133 \text{ mmol/min}$$

Therefore the increase in urine Na over a 24h period =

$$0.133 \times 60 \times 24 = \mathbf{192 \text{ mmol/24h}}$$

**Q 5 (8)**

$$\text{Fractional excretion}_{\text{Na}} = \frac{\text{Na excreted in urine}}{\text{Na filtered}}$$

If 90 % of filtered Na is reabsorbed then  $100 - 90 = 10 \%$  must be excreted i.e. fractional excretion ( $FE_{\text{Na}}$ ) = 10 %

$FE_{\text{Na}}$  is calculated from the expression:

$$FE_{\text{Na}} (\%) = \frac{(\text{Urine}_{\text{Na}} \times \text{Plasma}_{\text{Creatinine}})}{\text{Urine}_{\text{Creatinine}} \times \text{Plasma}_{\text{Na}}} \times 100$$

Which can be rearranged to give an expression for urine sodium:

$$\text{Urine}_{\text{Na}} = \frac{FE_{\text{Na}} \times \text{Urine}_{\text{Creatinine}} \times \text{Plasma}_{\text{Na}}}{\text{Plasma}_{\text{Creatinine}} \times 100} \text{ mmol/L}$$

Substitute these values to obtain the urine sodium concentration. N.B units must be the same so divide plasma creatinine ( $\mu\text{mol/L}$ ) by 1000 to convert it to  $\text{mmol/L}$ .

$$\text{Urine}_{\text{Na}} = \frac{10 \times 12.5 \times 155 \times 1000}{200 \times 100} = 969 \text{ mmol/L}$$

Since the 24h urine volume is 1250 mL (=1.25 L) the amount excreted in 24h is:

$$969 \times 1.25 = 1211 \text{ mmol/24h}$$

Convert to g/24h:

$$\text{Na (g/24h)} = \text{Na (mol/24h)} \times \text{MW}$$

Divide the sodium output by 1000 to convert from  $\text{mmol/24h}$  to  $\text{mol/24h}$ . MW Na = 23.

$$\text{Na (g/24h)} = \frac{1211 \times 23}{1000} = \mathbf{28 \text{ g}} \text{ (2 sig figs)}$$

Or expressed as NaCl (MW = 58.5):

$$\text{NaCl (g/24h)} = \frac{1211 \times 58.5}{1000} = \mathbf{71 \text{ g}} \text{ (2 sig figs)}$$

Another approach to this problem would be to first calculate the GFR from the creatinine results, then use this to calculate the Na filtered etc.

**Q 5 (9)**

$$FE_{Na} = \frac{U_{Na} \times Plasma_{Creatinine}}{U_{Creatinine} \times Plasma_{Na}}$$

All units need to be the same, if mmol/L used then:

$$Plasma \text{ creatinine} = 250 \mu\text{mol/L} = \frac{250}{1000} \text{ mmol/L}$$

$$FE_{Na} = \frac{90 \times 250}{1000 \times 2.4 \times 135} = \mathbf{0.069} \text{ (or 6.9 \%)}$$

**Q 5 (10)**

Since body weight is given, the Cockcroft-Gault formula for females can be used:

$$GFR \text{ (mL/min)} = \frac{(140 - \text{age in yrs}) \times \text{Body wt (kg)} \times 1.2 \times 0.85}{\text{Plasma creatinine } (\mu\text{mol/L})}$$

$$GFR \text{ (mL/min)} = \frac{(140 - 45) \times 56 \times 1.2 \times 0.85}{150}$$

$$= 36 \text{ mL/min}$$

Next calculate the patient's body surface area (*A*) using the body weight in kg (*W*) and height in cm (*H*):

$$A = \text{antilog}_{10} [(0.425 \times \log_{10} W) + (0.725 \times \log_{10} H) - 2.144] \text{ m}^2$$

$$A = \text{antilog}_{10} [(0.425 \times \log_{10} 56) + (0.725 \times \log_{10} 155) - 2.144]$$

$$= \text{antilog}_{10} [(0.425 \times 1.75) + (0.725 \times 2.19) - 2.144]$$

$$= \text{antilog}_{10} [0.744 + 1.588 - 2.144]$$

$$= \text{antilog}_{10} 0.188$$

$$= 1.54 \text{ m}^2$$

$$\text{Corrected } GFR \text{ (mL/min/1.73m}^2) = \frac{\text{Measured } GFR \text{ (mL/min)} \times 1.73}{A \text{ (m}^2)}$$

$$= \frac{36 \times 1.73}{1.54}$$

$$= \mathbf{40 \text{ mL/min/1.73 m}^2}$$

Alternatively the abbreviated MDRD formula can be used (height not required).

**Q 5 (11)**

First calculate the fractional excretion of glucose ( $FE_{\text{Glucose}}$ ):

$$FE_{\text{Glucose}} = \frac{\text{Urine}_{\text{Glucose}} \times \text{Plasma}_{\text{Creatinine}}}{\text{Urine}_{\text{Creatinine}} \times \text{Plasma}_{\text{Glucose}}}$$

All units must be the same so first correct plasma creatinine to mmol/L:

$$\text{Plasma creatinine} = 120 \mu\text{mol/L} = \frac{120}{1000} \text{ mmol/L}$$

$$FE_{\text{Glucose}} = \frac{50 \times 120}{1000 \times 6.0 \times 10} = 0.1$$

This is the fraction of filtered glucose which is NOT reabsorbed by the tubules. The fraction reabsorbed ( $TR$ ) is next calculated:

$$TR = 1 - FE = 1 - 0.1 = 0.9$$

To convert this reabsorption fraction to the absolute amount reabsorbed (i.e. the  $Tm/GFR$ ), multiply by the plasma concentration:

$$\begin{aligned} Tm/GFR &= TR \times \text{Plasma concentration} \\ &= 0.9 \times 10 \\ &= \mathbf{9 \text{ mmol/L glomerular filtrate}} \end{aligned}$$

**Q 5 (12)**

First calculate the osmolar clearance ( $C_{\text{osm}}$ ):

$$C_{\text{osm}} = \frac{U_{\text{osm}} \times V}{P_{\text{osm}}}$$

$$P_{\text{osm}} = 260 \text{ mmol/kg}$$

$$U_{\text{osm}} = 200 \text{ mmol/kg}$$

$$V = \text{urine flow rate} = 800 \text{ mL/6h}$$

$$= \frac{800 \text{ mL/h}}{6} = \frac{800}{6 \times 60} \text{ mL/min} = 2.22 \text{ mL/min}$$

WORKED ANSWERS TO FURTHER QUESTIONS

$$C_{\text{osm}} = \frac{200 \times 2.22}{260} = 1.71 \text{ mL/min}$$

The free water clearance ( $C_{\text{water}}$ ) is the difference between the urine flow rate and the osmolar clearance:

$$C_{\text{water}} = V - C_{\text{osm}}$$

$$C_{\text{water}} = 2.22 - 1.71 = \mathbf{0.51 \text{ mL/min}}$$

**Q 5 (13)**

First calculate the GFR using the abbreviated MDRD formula by substituting values for serum creatinine (130  $\mu\text{mol/L}$ ) and age (57 y) – remembering to multiply by 0.742 since the patient is female:

$$\begin{aligned} \text{GFR (mL/min/1.73m}^2) &= 186 \times [130 \times 0.011312]^{-1.154} \times [57]^{-0.203} \times 0.742 \\ &= 186 \times 1.471^{-1.154} \times 57^{-0.203} \times 0.742 \\ &= 186 \times \text{antilog}_{10} [-1.154 \times \log_{10} 1.471] \times \text{antilog}_{10} [-0.203 \times \log_{10} 57] \times 0.742 \\ &= 186 \times \text{antilog}_{10} [-1.154 \times 0.1676] \times \text{antilog}_{10} [-0.203 \times 1.7559] \times 0.742 \\ &= 186 \times \text{antilog}_{10} (-0.1934) \times \text{antilog}_{10} (-0.3565) \times 0.742 \\ &= 186 \times 0.6406 \times 0.4400 \times 0.742 = \mathbf{39 \text{ mL/min/1.73m}^2} \text{ (2 sig figs)} \end{aligned}$$

Next calculate the creatinine clearance:

$$\text{Creatinine clearance (mL/min)} = \frac{\text{Urine creatinine (mmol/L)} \times \text{Urine flow rate (mL/min)}}{\text{Serum creatinine (mmol/L)}}$$

$$\text{Urine creatinine} = 4.7 \text{ mmol/L}$$

$$\text{Serum creatinine} = 130 \mu\text{mol/L} = \frac{130}{1000} \text{ mmol/L}$$

$$\text{Urine flow rate} = 1.1 \text{ L/24h} = 1.1 \times 1000 \text{ mL/24h}$$

$$= \frac{1.1 \times 1000}{24} \text{ mL/h} = \frac{1.1 \times 1000}{24 \times 60} \text{ mL/min}$$

$$\begin{aligned} \text{Creatinine clearance (mL/min)} &= \frac{4.7 \times 1.1 \times 1000 \times 1000}{24 \times 60 \times 130} \\ &= \mathbf{28 \text{ mL/min}} \end{aligned}$$

There are several possible reasons for the discrepancy between the derived GFR and the calculated clearance:

- Inaccuracy in the timed urine collection. **This is potentially the greatest source of error.** Although the 24h volume of 1.1 L seems reasonable the calculated creatinine excretion seems low ( $1.1 \times 4.7 = 5.2 \text{ mmol/24h}$ ) – unless the lady has a very low muscle mass – suggesting that the collection is incomplete.
- Failure to correct the creatinine clearance for body surface area (this would require knowledge of weight and height). However, the MDRD formula does not take into account individual variation in body surface area either, but just assumes an average value based on the patient’s age and sex.
- Creatinine is secreted by tubules into the urine so that creatinine clearance measurements always overestimate GFR.

## Chapter 6

### Q 6 (1)

First calculate the osmolalities due to glucose and sodium chloride individually.

Formula for glucose =  $\text{C}_6\text{H}_{12}\text{O}_6$

$$\begin{aligned} \text{AW C} = 12 \text{ therefore } \text{C}_6 &= 6 \times 12 = 72 \\ \text{AW H} = 1 \text{ therefore } \text{H}_{12} &= 12 \times 1 = 12 \\ \text{AW O} = 16 \text{ therefore } \text{O}_6 &= 6 \times 16 = \underline{96} \end{aligned}$$

$$\text{MW} = 180$$

$$\text{Osmolality}_{\text{Glucose}} = \frac{\text{Glucose concentration (g/L)}}{\text{MW}}$$

Glucose concentration = initially 5% = finally 2.5% (since mixed with an equal volume of saline).

$$\begin{aligned} 2.5 \% \text{ glucose} &= 2.5 \text{ g/100 mL} = 25 \text{ g/L} \\ \text{Osmolality}_{\text{Glucose}} &= \frac{25}{180} = 0.139 \text{ mol/kg} = 139 \text{ mmol/kg} \end{aligned}$$

First calculate mmolar sodium chloride concentration:

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$$\text{NaCl} = 0.9\% = 0.9 \text{ g}/100 \text{ mL} = 9 \text{ g/L}$$

Final concentration (after mixing with an equal volume of 5% glucose) is one half of this i.e. 4.5 g/L. MW of NaCl = 23 + 35.5 = 58.5.

$$\text{NaCl (mol/L)} = \frac{\text{NaCl (g/L)}}{\text{MW}} = \frac{4.5}{58.5} = 0.077 \text{ mol/L} = 77 \text{ mmol/L}$$

Sodium chloride dissociates to give two osmotically active species – Na<sup>+</sup> and Cl<sup>-</sup>

$$\text{Therefore, Osmolality}_{\text{NaCl}} = 2 \times 77 = 154 \text{ mmol/kg}$$

$$\begin{aligned} \text{Osmolality}_{\text{Total}} &= \text{Osmolality}_{\text{Glucose}} + \text{Osmolality}_{\text{NaCl}} \\ &= 139 + 154 \\ &= \mathbf{293 \text{ mmol/kg}} \quad (\text{i.e. essentially isosmolar}) \end{aligned}$$

### Q 6 (2)

Calculate individual osmolalities separately.

Mannitol is undissociated so that  $\text{Osmolality}_{\text{Mannitol}} = \text{Molar concentration}$

$$\text{Concentration of mannitol} = 10\% = 10 \text{ g}/100 \text{ mL} = 100 \text{ g/L}$$

$$\begin{aligned} \text{Osmolality}_{\text{Mannitol}} &= \frac{\text{Mannitol (g/l)}}{\text{MW}} = \frac{100}{182} \text{ mol/kg} \\ &= \frac{100 \times 1000}{182} \text{ mmol/kg} = 549 \text{ mmol/kg} \end{aligned}$$

$$\text{Concentration of NaCl} = 0.9\% = 0.9 \text{ g}/100 \text{ mL} = 9 \text{ g/L}$$

$$\text{MW NaCl} = 23 + 35.5 = 58.5$$

$$\text{NaCl (mmol/L)} = \frac{\text{NaCl (g/L)} \times 1000}{\text{MW}} = \frac{9 \times 1000}{58.5} = 154 \text{ mmol/L}$$

Each molecule of NaCl dissociates into 2 ions (Na<sup>+</sup> and Cl<sup>-</sup>).

$$\text{Osmolality}_{\text{NaCl}} = 2 \times \text{NaCl (mmol/L)} = 2 \times 154 = 308 \text{ mmol/kg}$$

Adding these together gives the total osmolality:

$$\begin{aligned} \text{Osmolality}_{\text{Total}} &= \text{Osmolality}_{\text{Mannitol}} + \text{Osmolality}_{\text{NaCl}} \\ &= 549 + 308 \end{aligned}$$

$$= \quad \mathbf{857 \text{ mmol/kg}}$$

**Q 6 (3)**

First calculate the osmotic load of 500 mL of each solution.

For 20 % mannitol:

$$\text{Concentration} = 20 \% = 20 \text{ g/100 mL} = 200 \text{ g/L}$$

$$\text{AW C} = 12 \text{ therefore } C_6 = 6 \times 12 = 72$$

$$\text{AW H} = 1 \text{ therefore } H_{14} = 14 \times 1 = 14$$

$$\text{AW O} = 16 \text{ therefore } O_6 = 6 \times 16 = \underline{96}$$

$$\text{MW} = 182$$

$$\text{Osmolality}_{\text{Mannitol}} = \frac{\text{Mannitol (g/L)}}{\text{MW}} = \frac{200}{182} \text{ mol/kg}$$

$$= \frac{200 \times 1000}{182} \text{ mmol/kg} = 1099 \text{ mmol/kg}$$

$$\text{Therefore osmotic load of 500 mL} = \frac{1099}{2} = 550 \text{ mmol}$$

For 0.9 % saline:

$$\text{Concentration} = 0.9 \% = 0.9 \text{ g/100 mL} = 9 \text{ g/L}$$

$$\text{MW NaCl} = 23 + 35.5 = 58.5$$

$$\text{Osmolality}_{\text{NaCl}} = \frac{\text{NaCl (g/L)} \times 2}{\text{MW}} \text{ mol/kg} = \frac{9 \times 2}{58.5} \text{ mol/kg}$$

$$= \frac{9 \times 2 \times 1000}{58.5} \text{ mmol/kg} = 308 \text{ mmol/kg}$$

(factor of 2 introduced since NaCl dissociates into 2 ions (Na<sup>+</sup> and Cl<sup>-</sup>)).

$$\text{Osmolar load due to 500 mL NaCl} = \frac{308}{2} = 154 \text{ mmol}$$

$$\text{Extra osmolal load} = \text{Osmolal load}_{\text{Mannitol}} - \text{Osmol load}_{\text{NaCl}}$$

$$= 550 - 154$$

$$= \quad \mathbf{396 \text{ mmol}}$$

**Q 6 (4)**

First convert ethanol concentration to mmol/L:

$$\text{Ethanol concentration} = 92 \text{ mg/dL} = 920 \text{ mg/L}$$

Formula for ethanol = C<sub>2</sub>H<sub>5</sub>OH

$$\begin{aligned} \text{AW C} &= 12 \text{ therefore } \text{C}_2 = 2 \times 12 = 24 \\ \text{AW H} &= 1 \text{ therefore } \text{H}_6 = 6 \times 1 = 6 \\ \text{AW O} &= 16 \text{ therefore } \text{O} = 1 \times 16 = \underline{16} \\ \text{MW} &= 46 \end{aligned}$$

$$\text{Osmolality}_{\text{Ethanol}} = \frac{\text{Ethanol (mg/L)}}{\text{MW}} = \frac{920}{46} = \mathbf{20 \text{ mmol/kg}}$$

**Q 6 (5)**

a) First calculate osmolality due to Na<sup>+</sup>, glucose and urea:

$$\begin{aligned} \text{Osmolality} &= 1.86 [\text{Na}^+] + [\text{glucose}] + [\text{urea}] + 9 \\ \text{mmol/kg} &\quad \text{mmol/L} \quad \text{mmol/L} \quad \text{mmol/L} \end{aligned}$$

$$\text{Osmolality} = (1.86 \times 141) + 3.2 + 3.5 + 9 = 278 \text{ mmol/kg}$$

Next calculate the osmolal gap:

$$\begin{aligned} \text{Osmolal gap} &= \text{Osmolality}_{\text{Measured}} - \text{Osmolality}_{\text{Calculated}} \\ &= 330 - 278 \\ &= \mathbf{52 \text{ mmol/kg}} \end{aligned}$$

b) Calculate the expected contribution from ethanol:

$$\text{Ethanol concentration} = 270 \text{ mg/dL} = 2700 \text{ mg/L}$$

Formula of ethanol = C<sub>2</sub>H<sub>5</sub>OH

$$\begin{aligned} \text{AW C} &= 12 \text{ therefore } \text{C}_2 = 2 \times 12 = 24 \\ \text{AW H} &= 1 \text{ therefore } \text{H}_6 = 6 \times 1 = 6 \\ \text{AW O} &= 16 \text{ therefore } \text{O} = 1 \times 16 = \underline{16} \\ \text{MW} &= 46 \end{aligned}$$

$$\text{Osmolality}_{\text{Ethanol}} = \frac{\text{Ethanol (mg/L)}}{\text{MW}} = \frac{2700}{46} = 59 \text{ mmol/kg}$$

The osmolal gap is in reasonable agreement with the expected osmolal contribution from ethanol. Therefore the ethanol concentration explains the observed osmolal gap.

## Chapter 7

### Q 7 (1)

The first order elimination rate equation is:

$$\ln C_{p_t} = \ln C_{p_0} - k_d \cdot t$$

Where  $C_{p_t}$  = drug concentration at time  $t$  = 20 mg/L  
 $C_{p_0}$  = initial drug concentration = 50 mg/L  
 $k_d$  = elimination rate constant

$k_d$  can be calculated from the half-life ( $t_{1/2} = 30\text{h}$ ):

$$k_d = \frac{0.693}{t_{1/2}} = \frac{0.693}{30} = 0.023 \text{ h}^{-1}$$

Substitute these values into the rate equation and solve for  $t$ :

$$\begin{aligned} \ln 20 &= \ln 50 - 0.023 \cdot t \\ 3.00 &= 3.91 - 0.023 \cdot t \\ 0.023 \cdot t &= 3.91 - 3.00 = 0.91 \\ t &= \frac{0.91}{0.023} = \mathbf{40 \text{ h}} \text{ (2 sig figs)} \end{aligned}$$

### Q 7 (2)

- a) The volume of distribution of a drug is usually calculated by dividing the total dose administered by the plasma concentration. In this question we do not have a reliable estimate of the amount ingested. Since lithium is readily water soluble its volume of distribution approximates to total body water volume.

$$\text{Total body water (L)} = \text{Body wt (kg)} \times \frac{\% \text{ Body water}}{100}$$

Assuming an average body water content of 60%:

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$$\text{Volume of distribution } (V_d) = \frac{65 \times 60}{100} = \mathbf{39 \text{ L}}$$

- b) Lithium is excreted from the body by glomerular filtration (with some reabsorption by the proximal tubule which we shall ignore) and so its elimination follows first order kinetics:

$$\ln C_{p_t} = \ln C_{p_0} - k_d \cdot t$$

$$C_{p_0} = \text{initial concentration (before dialysis)} = 4.1 \text{ mmol/L}$$

$$C_{p_t} = \text{concentration at time } t = 1.5 \text{ mmol/L}$$

$$t = \text{time taken (in hours) to reach the 'safe' level of 1.5 mmol/L}$$

$$k_d = \text{elimination rate constant}$$

The clearance of the drug is given as 0.03 L/h/kg. Multiply by the patient's weight to give the total clearance:

$$\text{Clearance} = 0.03 \text{ (L/h/kg)} \times 65 \text{ (kg)} = 1.95 \text{ L/h}$$

The elimination rate constant ( $k_d$ ) can be calculated from the clearance ( $Cl$ ) and the volume of distribution ( $V_d$ ):

$$k_d = \frac{Cl}{V_d} = \frac{1.95}{39} = 0.050 \text{ h}^{-1}$$

Substitute for  $C_{p_t}$ ,  $C_{p_0}$  and  $k_d$  then solve for  $t$ :

$$\ln 1.5 = \ln 4.1 - 0.050 \cdot t$$

$$0.405 = 1.411 - 0.050 \cdot t$$

$$0.050 \cdot t = 1.411 - 0.405 = 1.006$$

$$t = \frac{1.006}{0.050} = \mathbf{20 \text{ h}} \text{ (2 sig figs)}$$

**Q 7 (3)**

Assuming distribution throughout total body water, then  $V_d =$  total body water vol:

Assume body water is 60 % of body weight.

$$\text{Total body water (L)} = \text{Body Wt (kg)} \times 60\%$$

$$= \frac{80 \times 60}{100} = 48 \text{ L}$$

$$V_d = \frac{\text{Amount of drug in body (dose)}}{\text{Plasma drug concentration}}$$

$$\text{Plasma drug level (mg/L)} = \frac{\text{Dose (mg)}}{V_d \text{ (L)}} = \frac{60}{48} = \mathbf{1.25 \text{ mg/L}}$$

If the drug is only distributed throughout the ECF, the  $V_d$  must be adjusted. ECF is normally 20 % of body wt.

$$V_d \text{ (L)} = \text{Body wt (Kg)} \times 20 \%$$

$$= \frac{80 \times 20}{100} = 16 \text{ L}$$

$$\text{Plasma drug level (mg/L)} = \frac{\text{Dose (mg)}}{V_d \text{ (L)}} = \frac{60}{16} = \mathbf{3.75 \text{ mg/L}}$$

Alternatively, since a third of body water is in the ECF, the drug level will be 3 times higher than if it were distributed throughout total body water.

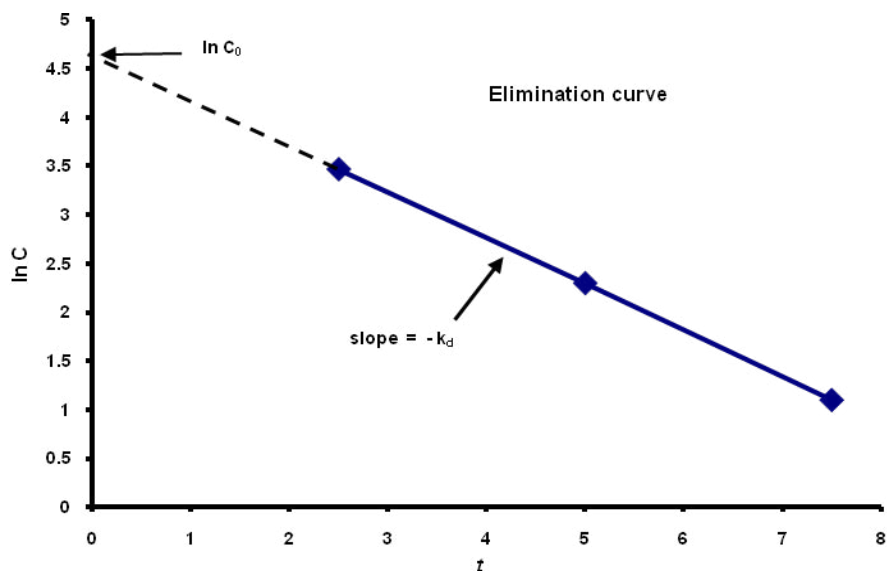
**Q 7 (4)**

- a) Assuming the clearance of the drug follows first-order elimination kinetics then the data should be described by the expression:

$$\ln C_p_t = \ln C_p_0 - k_d \cdot t$$

Therefore a plot of  $\ln C$  versus  $t$  should be linear with an intercept on the  $\ln C$  axis of  $C_p_0$  and slope  $-k_d$ :

Time (h)	Conc (mg/L)	$\ln C$
2.5	32	3.47
5	10	2.30
7.5	3	1.10



This plot clearly demonstrates that elimination of the drug follows first order kinetics so that  $Cp_0$  and  $k_d$  could be determined directly from the graph. Alternatively any 2 values can be substituted into the rate equation and solved for  $k_d$ :

Let  $Cp_0$  be the concentration at time 2.5 h = 32 mg/L  
 Let  $Cp_t$  be the concentration at time 5 h = 10 mg/L  
 $t = 2.5$  h (the time difference between  $Cp_0$  and  $Cp_t$ ).

Therefore:  $\ln_{10} = \ln_{32} - k_d \cdot 2.5$

$$2.303 = 3.466 - 2.5 k_d$$

$$2.5 k_d = 3.466 - 2.303 = 1.163$$

$$k_d = \frac{1.163}{2.5} = 0.465 \text{ h}^{-1}$$

a) Half-life ( $t_{1/2}$ ) can be calculated from  $k_d$ :

$$t_{1/2} = \frac{0.693}{k_d} = \frac{0.693}{0.465} = \mathbf{1.5 \text{ h}} \text{ (2 sig figs)}$$

b) First calculate the initial concentration ( $Cp_0$ ) using one other value (e.g. 2.5 h = 32 mg/L as  $Cp_t$  and  $t = 2.5$  h) and the value for  $k_d$ :

$$\ln 32 = \ln Cp_0 - (0.465 \times 2.5)$$

$$3.466 = \ln C_{p0} - 1.163$$

$$\ln C_{p0} = 3.466 + 1.163 = 4.629$$

$$C_{p0} = \text{antilog}_e 4.629 = 102 \text{ mg/L}$$

The  $V_d$  is then calculated from dose and  $C_{p0}$ :

$$V_d (\text{L}) = \frac{\text{Dose (mg)}}{C_{p0} (\text{mg/L})} = \frac{6000}{102} = \mathbf{59 \text{ L}} \quad (2 \text{ sig figs})$$

**Q 7 (5)**

The first order rate equation is:

$$\ln C_{p_t} = \ln C_{p_0} - k_d \cdot t$$

where  $C_{p_0} = 200 \text{ nmol/L}$ ;  $k_d = 0.34 \text{ h}^{-1}$

Calculation of  $t$  when  $C_{p_t} = 100 \text{ nmol/L}$ :

$$\ln 100 = \ln 200 - 0.34 \cdot t$$

$$4.605 = 5.298 - 0.34 \cdot t$$

$$0.34 \cdot t = 5.298 - 4.605 = 0.693$$

$$t = \frac{0.693}{0.34} = \mathbf{2.0 \text{ h}} \quad (2 \text{ sig figs}) \text{ i.e. 10 am}$$

Calculation of  $t$  when  $C_{p_t} = 75 \text{ nmol/L}$

$$\ln 75 = \ln 200 - 0.34 \cdot t$$

$$4.317 = 5.298 - 0.34 \cdot t$$

$$0.34 \cdot t = 5.298 - 4.317 = 0.981$$

$$t = \frac{0.981}{0.34} = \mathbf{2.9 \text{ h}} \quad (2 \text{ sig figs}) \text{ i.e. 11 am}$$

**Q 7 (6)**

Assuming elimination follows first order kinetics:

$$\ln C_{p_t} = \ln C_{p_0} - k_d \cdot t$$

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Where  $Cp_t$  = concentration at time  $t$  = 10 %  
 $Cp_0$  = initial concentration = 100 %  
 $t$  = time when  $Cp_t$  reaches 10 %

Calculate  $k_d$  from  $t_{1/2}$ :

$$k_d = \frac{0.693}{t_{1/2}} = \frac{0.693}{2} = 0.347 \text{ h}^{-1}$$

Substitute these values into the rate equation and solve for  $t$ :

$$\begin{aligned} \ln 10 &= \ln 100 - 0.347.t \\ 2.303 &= 4.605 - 0.347.t \\ 0.347.t &= 4.605 - 2.303 = 2.302 \\ t &= \frac{2.302}{0.347} = \mathbf{6.6 \text{ h}} \text{ (2 sig figs)} \end{aligned}$$

**Q 7 (7)**

$$\text{Loading dose (LD)} = \frac{V_d \times (Cp_{\text{target}} - Cp_{\text{initial}})}{S \times F}$$

Where  $V_d$  = volume of distribution = 0.5 L/kg  
 $Cp_{\text{target}}$  = desired drug concentration = 12 mg/L  
 $Cp_{\text{initial}}$  = starting drug concentration = 4 mg/L  
 $S$  = salt factor = 0.8  
 $F$  = bioavailability (not given so assume a value of 1)

$$\begin{aligned} \text{Patients } V_d &= \text{Body weight (kg)} \times 0.5 \\ &= 80 \times 0.5 = 40 \text{ L} \end{aligned}$$

Substitute these values in order to obtain  $LD$ :

$$\begin{aligned} LD &= \frac{40 \times (12 - 4)}{0.8} \\ &= \frac{40 \times 8}{0.8} \\ &= \mathbf{400 \text{ mg}} \end{aligned}$$

**Q 7 (8)**

The following expression allows calculation of the maintenance dose:

$$\text{Maintenance dose} = \frac{Cp_{ss} \times Cl \times \tau}{S \times F}$$

Where:

$$\begin{aligned} Cp_{ss} &= \text{steady state plasma concentration} = 75 \text{ mg/L} \\ Cl &= \text{clearance} = 10 \text{ mL/h/kg} \\ \tau &= \text{dosing interval} = 12 \text{ h (i.e. twice daily)} \\ S &= \text{salt factor} = 0.85 \\ F &= \text{bioavailability} = 0.7 \end{aligned}$$

First correct the clearance for the body weight and express it in litres (to be compatible with the drug concentration which is given in mg/L):

$$\begin{aligned} Cl \text{ (L/h)} &= \frac{Cl \text{ (mL/h/kg)} \times \text{Body wt (kg)}}{1000} \\ &= \frac{10 \times 55}{1000} = 0.55 \text{ L/h} \end{aligned}$$

Substitute these values into the expression for maintenance dose:

$$\begin{aligned} \text{Maintenance dose (mg)} &= \frac{75 \times 0.55 \times 12}{0.85 \times 0.7} \\ &= \mathbf{832 \text{ mg}} \end{aligned}$$

**Chapter 8**

**Q 8 (1)**

Draw up a table of fluid gains and losses then calculate the total of each. Assume a value of 400 mL per day for *net insensible losses*.

<b>Fluid gains</b>		<b>Fluid losses</b>	
Oral	750 mL	Urine output	1250 mL
IV	2000 mL	Loss via fistula	600 mL
		Net insensible loss	400 mL
<b>Total</b>	<b>2750 mL</b>		<b>2250 mL</b>

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$$\begin{aligned}\text{Fluid balance (mL)} &= \text{Net fluid intake (mL)} - \text{Net fluid loss (mL)} \\ &= 2750 - 2250 \\ &= \mathbf{500 \text{ mL}}\end{aligned}$$

i.e. there is a net fluid gain of 500 mL.

**Q 8 (2)**

The average adult male has a body water content of approximately 60%. If the body water deficit is  $x$  L, then the initial body water content can be calculated:

$$\begin{aligned}\text{Initial body water (L)} &= \frac{(85 + x) \times 60}{100} \\ &= \frac{5100 + 60x}{100} \\ &= 51 + 0.6x\end{aligned}$$

Assuming a normal initial osmolality (say 285 mmol/kg) the total amount (in mmol) of osmotically active species present in the body can be calculated:

$$\begin{aligned}\text{Osmolality (mmol/kg)} &= \frac{\text{Total solutes (mmol)}}{\text{Initial body water (kg)}} \\ 285 &= \frac{\text{Total solutes (mmol)}}{51 + 0.6x} \\ \text{Total solutes (mmol)} &= 285 (51 + 0.6x) \\ &= 14535 + 171x\end{aligned}$$

On presentation his body weight is 85 kg. Assuming the total amount of solutes in the body is unchanged, then the body water volume can be calculated from the current osmolality:

$$\begin{aligned}\text{Final osmolality (mmol/kg)} &= \frac{\text{Total solutes (mmol)}}{\text{Final body water (kg)}} \\ 324 &= \frac{14535 + 171x}{(51 + 0.6x) - x}\end{aligned}$$

$$\begin{aligned}
 324 &= \frac{14535 + 171x}{51 - 0.4x} \\
 324(51 - 0.4x) &= 14535 + 171x \\
 16524 - 130x &= 14535 + 171x \\
 171x + 130x &= 16524 - 14535 \\
 301x &= 1989 \\
 x &= \frac{1989}{301} \\
 &= \mathbf{6.6\ L}
 \end{aligned}$$

If it is assumed that the change in body wt is negligible (or that the initial body water was the same as for an average 70 kg adult) then a simpler calculation (using Eq 8.3) can be used and gives a slightly different result which may be adequate as a rough guide in clinical practice:

$$\begin{aligned}
 \text{Fluid loss (L)} &= 42 - \left[ \frac{12000}{\text{Osmolality (mmol/kg)}} \right] \\
 &= 42 - \frac{[12000]}{324} \\
 &= 42 - 37 \\
 &= \mathbf{5L}
 \end{aligned}$$

### Q 8 (3)

Making a number of assumptions:

- That it is plasma glucose which is measured rather than whole blood glucose.
- That as a result of insulin deficiency there is no increase in glucose concentration in the intracellular fluid (ICF)
- That the plasma glucose has equilibrated with interstitial fluid so that its concentration in the extracellular fluid (ECF) is the same as in plasma.
- That there is negligible change in the concentrations of solutes other than glucose, sodium and chloride.

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- That the ratio of ICF:ECF volumes is 2 (i.e. ECF = 14 L, ICF = 28 L for average adult male) and that the total body water is that of an average male i.e. 42 L

The effect of an increase in plasma (and hence ECF) glucose is to raise plasma (and ECF) osmolarity. The body will retain water (stimulation of thirst increases intake and stimulation of ADH reduces renal loss) until osmotic equilibrium is restored. If there is a plentiful supply of water then the plasma osmolarity is returned to normal and since the plasma glucose has risen by 10 mmol/L the plasma sodium must have fallen by  $10/2 = 5$  mmol/L. However, this question states that **there is no net loss or gain of body water**. Therefore, water will move, by osmosis, from the ICF compartment (isoosmolar) to the ECF (now hyperosmolar) until osmotic equilibrium is established. Since movement of water from the ICF leads to an increase in ICF osmolarity, the movement of water is restricted and at equilibrium the ECF will reach a value somewhere in-between normality and the original value i.e. the osmotic load is shared between the ECF and ICF compartments, both of which become hyperosmolar.

The plasma glucose has risen by  $15 - 5 = 10$  mmol/L

Rise in **amount** of glucose in ECF =

$$\begin{aligned} \text{Rise in plasma glucose concentration (mmol/L)} \times \text{ECF vol (L)} \\ = 10 \times 14 = 140 \text{ mmol} \end{aligned}$$

(a slight underestimate since there has been a small expansion in ECF vol)

At equilibrium, the rise in osmolarity (which is the same in the ECF and ICF) is given by:

$$\begin{aligned} \frac{\text{Increase in amount of glucose in body (mmol)}}{\text{Total body fluid (ECF + ICF) volume (L)}} \\ = \frac{140}{42} = 3.33 \text{ mmol/L} \end{aligned}$$

Since the plasma osmolarity has risen by 3.33 mmol/L and the plasma glucose by 10 mmol/L then the concentration of NaCl which has been displaced by glucose is

$$10 - 3.33 = 6.67 \text{ mmol/L}$$

and so the sodium has fallen by  $\frac{6.67}{2} = 3.34$  mmol/L

i.e. the plasma sodium concentration has decreased by approximately **3 mmol/L**.

**Q 8 (4)**

Flame photometry measures sodium as concentration in plasma i.e. 140 mmol/L of **plasma**.

A direct-reading ion-selective electrode measures sodium as activity i.e. 140 mmol/L of plasma **water**. Large molecules such as proteins occupy significant space in solution i.e. displace plasma water. If plasma contains 70 g/L protein then this is equivalent to 0.070 kg/L. Assuming that 1 kg of protein occupies a volume of 1 L then the volume of plasma water in which the sodium is dissolved is  $(1.0 - 0.07) = 0.93$  L. Assuming that the activity is the same as concentration for sodium in plasma water (i.e. the activity coefficient is one), for a plasma sodium of 140 mmol/L of plasma, the true concentration of sodium in plasma water is:

$$\text{Plasma sodium} = \frac{140}{0.93} = 150.5 \text{ mmol/L water}$$

There are two ways in which the ISE reading can be converted to the same as that obtained by flame photometry (140 mmol/L):

- Subtraction of 10.5 mmol/L from the result
- Multiplication of the result by the factor  $140/150.5$  i.e. 0.930

At a protein concentration of 90 g/L (occupying 0.090 L plasma), the concentration of sodium in plasma water will be:

$$\frac{140}{(1.00 - 0.09)} = \frac{140}{0.91} = 153.8 \text{ mmol/L plasma water}$$

Carrying out the two adjustments by the instrument:

- Subtraction of 10.5 gives  $153.8 - 10.5 = 143.3$  mmol/L.
- Multiplication by 0.930 gives  $153.8 \times 0.930 = 143.0$  mmol/L

Therefore expected ISE reading = **143 mmol/L**

**Chapter 9**

**Q 9 (1)**

One international unit of activity is the amount of enzyme present in 1 L of serum which catalyses the conversion of 1  $\mu\text{mol}$  substrate per min under the conditions of the assay.

First calculate the absorbance change per min:

$$\Delta A/\text{min} = \frac{\Delta A/5\text{min}}{5} = \frac{0.150}{5} \Delta A/\text{min}$$

Convert the absorbance change to concentration change per min:

$$\Delta A = a.b. \Delta c$$

Where  $\Delta A/\text{min} = \frac{0.150}{5}$

$$a = \text{molar absorptivity} = 6.30 \times 10^3 \text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$$

$$b = \text{light path length} = 0.5 \text{ cm}$$

$$\Delta c = \text{change in concentration (mol/min)}$$

Substitute these values then rearrange to give an expression for  $\Delta c/\text{min}$ :

$$\frac{0.150}{5} = 6.30 \times 10^3 \times 0.5 \times \Delta c/\text{min}$$

$$\Delta c/\text{min} = \frac{0.150}{5 \times 6.30 \times 10^3 \times 0.5} \text{ mol/min/L reaction mixture}$$

Multiply by 1000000 to convert the concentration units from mol to  $\mu\text{mol}$  (1 mol = 1000000  $\mu\text{mol}$ ):

$$\Delta c/\text{min} = \frac{0.150 \times 1000000}{5 \times 6.30 \times 10^3 \times 0.5} \mu\text{mol/min/L reaction mixture}$$

The final step is to convert the activity to  $\mu\text{mol}/\text{min}/\text{L}$  serum. In the assay 100  $\mu\text{L}$  of serum was mixed with 2.7 mL buffer and 100  $\mu\text{L}$  of substrate.

$$\text{Total assay volume} = 2.7 + 0.1 + 0.1 = 2.9 \text{ mL}$$

$$\begin{aligned} \Delta c/\text{min/L serum} &= \frac{\Delta c/\text{min/L assay mixture} \times \text{Total assay vol (mL)}}{\text{Serum vol (mL)}} \\ &= \frac{0.150 \times 1000000 \times 2.9}{5 \times 6.30 \times 10^3 \times 0.5 \times 0.1} \\ &= \mathbf{276 \text{ IU/L serum}} \end{aligned}$$

**Q 9 (2)**

- a) One international unit is the amount of enzyme which liberates one  $\mu\text{mol}$  of product per minute. Therefore to calculate the alk phos activity in IU/L serum the following steps are involved:

Determine the rate of absorbance change in  $\Delta A/\text{min}$ .

$$\Delta A/\text{min} = \frac{\Delta A/5\text{min}}{5} = \frac{0.180}{5}$$

Convert to the rate of change in concentration using the molar absorptivity and pathlength:

$$\Delta A = a.b. \Delta c$$

$$\Delta A = \text{rate of absorbance change} = \frac{0.180}{5} \text{ A/min}$$

$$a = \text{molar absorptivity of 4-nitrophenol} = 1.88 \times 10^4 \text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$$

$$b = \text{pathlength of cuvette} = 1 \text{ cm}$$

$$\Delta c = \text{rate of change of concentration in mol/L/min} = ?$$

Substitute these values and re-arrange to give an expression for  $\Delta c/\text{min}$ :

$$\frac{0.180}{5} = 1.88 \times 10^4 \times 1 \times \Delta c/\text{min}$$

$$\Delta c/\text{min} = \frac{0.180}{5 \times 1.88 \times 10^4 \times 1} \text{ mol/L/min}$$

Multiply by 1000000 to convert units from mol/L/min to  $\mu\text{mol/L/min}$  (1 mol = 1000000  $\mu\text{mol}$ ):

$$\Delta c/\text{min} = \frac{0.180 \times 1000000}{5 \times 1.88 \times 10^4 \times 1} \mu\text{mol/min/L reaction mixture}$$

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To convert to activity per L serum multiply by the total volume of reaction mixture and divide by the sample volume – using the same units:

Serum	=	0.05 mL
Buffer	=	2.70 mL
<u>Substrate</u>	=	<u>0.20 mL</u>
Total	=	2.95 mL

$$\begin{aligned} \text{Alk phos activity} &= \frac{0.180 \times 1000000 \times 2.95}{5 \times 1.88 \times 10^4 \times 1 \times 0.05} \quad \mu\text{mol/min/L serum} \\ &= \mathbf{113 \text{ IU/L}} \end{aligned}$$

b) One Katal is the amount of enzyme that catalyses the reaction of 1 mol substrate per second

$$\text{Alk phos activity} = 113 \text{ IU/L } (\mu\text{mol/min/L})$$

Divide by 1000000 to convert from  $\mu\text{mol}$  to mol, then by 60 to convert from min to seconds.

$$\begin{aligned} 113 \text{ IU/L} &= \frac{113}{1000000 \times 60} \quad \text{Katal/L} \\ &= \mathbf{1.88 \times 10^{-6} \text{ Kat/L}} \end{aligned}$$

### Q 9 (3)

Somogyi units = mg glucose/30 min/100 mL serum

International units =  $\mu\text{mol/min/L}$  serum

Consider a sample with activity of  $x$  Somogyi units

First convert from mg glucose to  $\mu\text{mol}$  glucose:

Glucose formula =  $\text{C}_6\text{H}_{12}\text{O}_6$

$$\begin{array}{l} \text{AW C} = 12 \quad \text{therefore } \text{C}_6 = 6 \times 12 = 72 \\ \text{AW H} = 1 \quad \text{therefore } \text{H}_{12} = 12 \times 1 = 12 \\ \text{AW O} = 16 \quad \text{therefore } \text{O}_6 = 6 \times 16 = \underline{96} \\ \text{MW} = 180 \end{array}$$

Activity (Somogyi units) =  $x$  mg/30 min/100 mL

$$\text{Activity (mmol/30 min/100 mL)} = \frac{x}{180}$$

Multiply by 1000 to convert from mmol to  $\mu\text{mol}$ :

$$\text{Activity } (\mu\text{mol/30 min/100 mL}) = \frac{x \times 1000}{180}$$

Divide by 30 to obtain the rate per minute:

$$\text{Activity } (\mu\text{mol/min/100 mL}) = \frac{x \times 1000}{180 \times 30}$$

Multiply by 10 to obtain the activity per litre:

$$\begin{aligned} \text{Activity } (\mu\text{mol/min/L}) &= \frac{x \times 1000 \times 10}{180 \times 30} \\ &= x \times 1.85 \end{aligned}$$

$$\text{IU/L} = 1.85 \times \text{Somogyi Units}$$

#### Q 9 (4)

International units =  $\mu\text{mol/min/L}$  serum

Wroblewski-LaDue (W-L-D units) = 0.001  $\Delta A/\text{min/mL}$  serum (total volume 3 mL)

Multiply by 3 to obtain absorbance change obtained with 1 mL of undiluted serum, then by 1000 to obtain the absorbance change due to 1 L serum:

$$1 \text{ W-L-D unit} = 0.001 \times 3 \times 1000 \Delta A/\text{min/L serum}$$

Next convert  $\Delta A/\text{min}$  to  $\Delta c$  (i.e.  $\text{mol/L/min}$ ):

$$\Delta A = a.b. \Delta c$$

$$\Delta A = \text{absorbance change} = 0.001 \times 3 \times 1000 A/\text{min}$$

$$a = \text{molar absorptivity of NADH} = 6.3 \times 10^3 \text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$$

$$b = \text{cuvette pathlength} = \text{not given so assume } 1 \text{ cm}$$

$$\Delta c = \text{rate of change of concentration in mol/L/min}$$

Substitute these values and rearrange to give an expression for  $\Delta c$ :

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$$0.001 \times 3 \times 1000 = 6.3 \times 10^3 \times 1 \times \Delta c$$

$$\Delta c = \frac{0.001 \times 3 \times 1000}{6.3 \times 10^3 \times 1} \text{ mol/min/L serum}$$

Multiply by 1000000 to convert from mol to  $\mu\text{mol}$ :

$$1 \text{ W-L-D unit} = \frac{0.001 \times 1000 \times 3 \times 1000000}{6.3 \times 10^3 \times 1} \mu\text{mol/min/L reaction mixture}$$

$$1 \text{ W-L-D unit} = 476 \mu\text{mol/min/L serum}$$

$$\text{i.e. } 1 \text{ W-L-D unit} = 476 \text{ IU/L}$$

$$\text{Therefore activity (IU/L)} = \mathbf{476} \times \text{Wroblewski-LaDue Units}$$

**Q 9 (5)**

The Michaelis-Menten equation is:

$$v = \frac{V_{max} [S]}{K_m + [S]}$$

Where

$v$	=	initial velocity	
$V_{max}$	=	maximal velocity	
$[S]$	=	substrate concentration	= 10 mmol/L
$K_m$	=	Michaelis constant	= 2.5 mmol/L

Substitute and solve for  $v$ :

$$v = \frac{10 V_{max}}{2.5 + 10} = \frac{10 V_{max}}{12.5} = \mathbf{0.8 V_{max}}$$

**Q 9 (6)**

a) When  $1/v = 0$ , the value for  $1/[S]$  is  $-1/K_m$

$$\text{Therefore } -\frac{1}{K_m} = -12.5 \times 10^6 \text{ L/mol}$$

Which can be rearranged to give the value of  $K_m$ :

$$K_m = \frac{-1}{-12.5 \times 10^6} = 0.08 \times 101^{-6} = \mathbf{8.0 \times 10^{-8} \text{ mol/L}}$$

b) When  $1/[S] = 0$ ,  $1/v = 1/V_{max}$

$$\text{Therefore } \frac{1}{V_{max}} = 5.2 \times 10^6 \text{ min/mol}$$

Rearrange and solve for  $V_{max}$ :

$$V_{max} = \frac{1}{5.2 \times 10^6} = 0.19 \times 10^{-6} = \mathbf{1.9 \times 10^{-7} \text{ mol/min}}$$

c) When  $1/[S] = 0$ ,  $1/v = 1/V_{max}$

$$\text{Therefore } \frac{1}{V_{max}} = 6.5 \times 10^6 \text{ min/mol}$$

Rearrange and solve for  $V_{max}$ :

$$V_{max} = \frac{1}{6.5 \times 10^6} = 0.15 \times 10^{-6} = \mathbf{1.5 \times 10^{-7} \text{ mol/min}}$$

The slope of the line gives  $K_m/V_{max}$

$$\text{Therefore } \frac{K_m}{V_{max}} = 100 \text{ min/L}$$

Substitute  $V_{max} = 1.5 \times 10^{-7} \text{ mol/min}$  and solve for  $K_m$ :

$$\frac{K_m}{1.5 \times 10^{-7}} = 100$$

$$K_m = 100 \times 1.5 \times 10^{-7} = \mathbf{1.5 \times 10^{-5} \text{ mol/L}}$$

### Q 9 (7)

a) If  $[S] = \text{mmol/L} = 10^{-3} \text{ mol/L}$

$$\frac{1}{[S]} = \frac{1}{10^{-3} \text{ mol/L}} = \mathbf{10^3 \text{ L/mol}}$$

If  $v = \mu\text{mol/min} = 10^{-6} \text{ mol/min}$

$$\frac{1}{v} = \frac{1}{10^{-6} \text{ mol/min}} = \mathbf{10^6 \text{ mol/min}}$$

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$$b) \quad \frac{[S]}{v} = \frac{\text{mmol/L}}{\mu\text{mol/min}} = \frac{10^{-3} \text{ mol/L}}{10^{-6} \text{ mol/min}} = \mathbf{10^3 \text{ min/L}}$$

$$[S] = \text{mmol/L} = \mathbf{10^{-3} \text{ mol/L}}$$

$$c) \quad v = \mu\text{mol/min} = \mathbf{10^{-6} \text{ mol/min}}$$

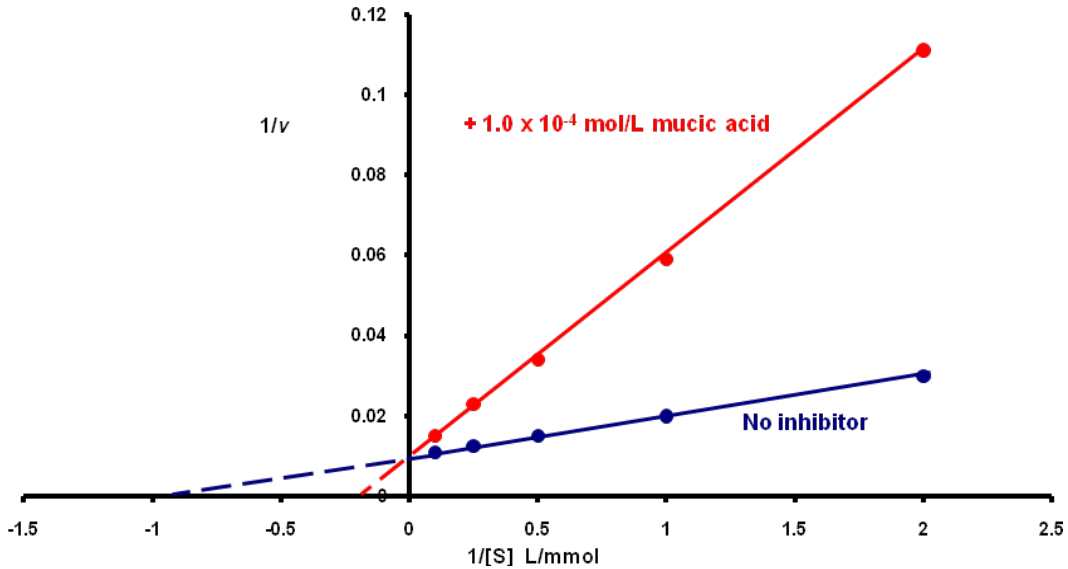
$$\frac{v}{[S]} = \frac{\mu\text{mol/min}}{\text{mmol/L}} = \frac{10^{-6} \text{ mol/min}}{10^{-3} \text{ mol/L}} = \mathbf{10^{-3} \text{ L/min}}$$

**Q 9 (8)**

Substrate Concentration (mmol/L)	Reaction velocity	
	No inhibitor	Mucic acid
0.5	33	9
1.0	50	17
2.0	67	29
4.0	80	44
10	91	67

The first step is to plot the data. Any linear transformation of the Michaelis-Menten equation can be used but the double-reciprocal plot is probably the simplest. Calculated reciprocals are:

1/[S] L/mmol	1/v	
	No inhibitor	Mucic acid
2.0	0.030	0.111
1.0	0.020	0.059
0.5	0.015	0.034
0.25	0.0125	0.023
0.10	0.011	0.015



Without inhibitor, when  $1/v = 0$ ,  $1/[S] = -1/K_m$

Intercept on  $1/[S]$  without inhibitor =  $-0.947$  L/mmol

$$\text{Therefore } K_m = \frac{-1}{-0.947} = 1.06 \text{ mmol/L} = 1.06 \times 10^{-3} \text{ mol/L}$$

Since the lines cross on the  $1/v$  axis the type of inhibition is **competitive**.

With inhibitor, when  $1/v = 0$ ,  $1/[S] = -1/K_m^{app}$

Intercept on  $1/[S]$  with inhibitor =  $-0.193$  L/mmol

$$\text{Therefore } K_m^{app} = \frac{-1}{-0.193} = 5.18 \text{ mmol/L} = \mathbf{5.18 \times 10^{-3} \text{ mol/L}}$$

For competitive inhibition:  $K_m^{app} = K_m (1 + [I]/K_i)$

Substitute  $K_m^{app} = 5.18 \times 10^{-3} \text{ mol/L}$ ,  $K_m = 1.06 \times 10^{-3} \text{ mol/L}$ ,  $[I] = 1.0 \times 10^{-4} \text{ mol/L}$  then solve for  $K_i$ :

$$5.18 \times 10^{-3} = 1.06 \times 10^{-3} \{1 + (1.0 \times 10^{-4}/K_i)\}$$

$$\frac{5.18 \times 10^{-3}}{1.06 \times 10^{-3}} = 1 + \frac{1.0 \times 10^{-4}}{K_i}$$

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$$4.89 - 1 = \frac{1.0 \times 10^{-4}}{K_i}$$

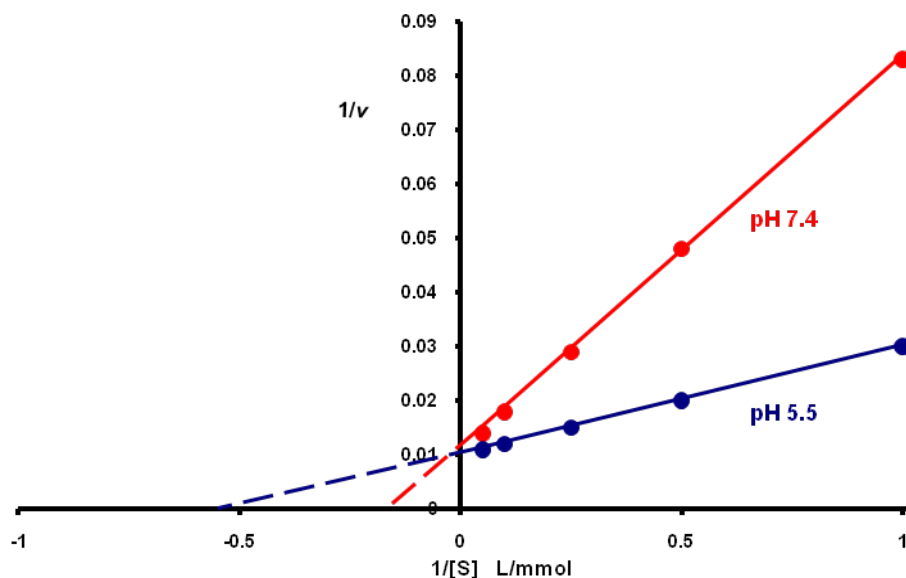
$$K_i = \frac{1.0 \times 10^{-4}}{3.89} = 2.6 \times 10^{-5} \text{ mol/L}$$

Q 9 (9)

Lactate concentration mmol/L	Reaction velocity	
	pH 7.4	pH 5.5
1	12	33
2	21	50
4	35	67
10	57	83
20	73	91

Calculate reciprocals then plot  $1/v$  versus  $1/[S]$  at each pH:

$1/[S]$ L/mmol	$1/v$	
	pH 7.4	pH 5.5
1	0.083	0.030
0.5	0.048	0.020
0.25	0.029	0.015
0.1	0.018	0.012
0.05	0.014	0.011



At pH 7.4, when  $1/v = 0$ ,  $1/[S] = -0.162$  L/mmol

$$\text{Therefore } K_m = \frac{-1}{-0.162} = 6.2 \text{ mmol/L} = \mathbf{6.2 \times 10^{-3} \text{ mol/L}}$$

At pH 5.5, when  $1/v = 0$ ,  $1/[S] = -0.545 \text{ L/mmol}$

$$\text{Therefore } K_m = \frac{-1}{-0.545} = 1.84 \text{ mmol/L} = \mathbf{1.84 \times 10^{-3} \text{ mol/L}}$$

Assuming that equilibrium conditions apply (i.e. that  $k_{+1} \gg K_{+2}$ ) then the  $K_m$  is the dissociation constant of the enzyme-substrate complex and is inversely proportional to the affinity of the enzyme for the substrate. The  $K_m$  is lower at pH 5.5 than at pH 7.4. **Therefore the enzyme has greatest affinity for its substrate at pH 5.5. The same conclusion could also be reached simply by inspecting the double-reciprocal plots. The intercept on the  $1/[s]$  axis is greatest (and therefore  $K_m$  lower) at pH 5.5.**

**Q 9 (10)**

Inhibitor concentration (mmol/L)	Apparent value	
	$K_m$ (mmol/L)	$V_{max}$ ( $\mu\text{mol/min}$ )
5	10	7.5
10	7	5
15	5	4
20	4	3

A competitive inhibitor causes an increase in the apparent  $K_m$ . As the  $K_m$  is actually decreasing as inhibitor concentration increases this mode of inhibition can be ruled out. The apparent  $V_{max}$  is decreasing as inhibitor concentration is increased; this behaviour is seen both with non-competitive and uncompetitive inhibition. However, in non competitive inhibition the  $K_m$  is unaffected by the inhibitor whereas in uncompetitive inhibition the apparent  $K_m$  decreases with increasing inhibitor concentration. Therefore these data are consistent with **uncompetitive inhibition**.

The value for  $K_i$  can be obtained from secondary plots of either  $1/K_m$  or  $1/V_{max}$  versus  $[I]$ .

The relationship between  $K_m^{app}$  and  $[I]$  for an uncompetitive inhibitor is:

$$K_m^{app} = \frac{K_m}{(1 + [I]/K_i)}$$

Inversion of this expression gives:

$$\frac{1}{K_m^{app}} = \frac{(1 + [I]/K_i)}{K_m}$$

Which can also be written:

$$\frac{1}{K_m^{app}} = \left( \frac{1}{K_i K_m} \times [I] \right) + \frac{1}{K_m}$$

Therefore a plot of  $1/K_m^{app}$  versus  $[I]$  is linear.

When  $1/K_m^{app} = 0$ :

$$0 = \left( \frac{1}{K_i K_m} \times [I] \right) + \frac{1}{K_m}$$

Which can be rearranged to give:

$$-\frac{1}{K_m} = \frac{[I]}{K_i K_m}$$

Multiplying both sides by  $K_m K_i$  and changing the signs gives:

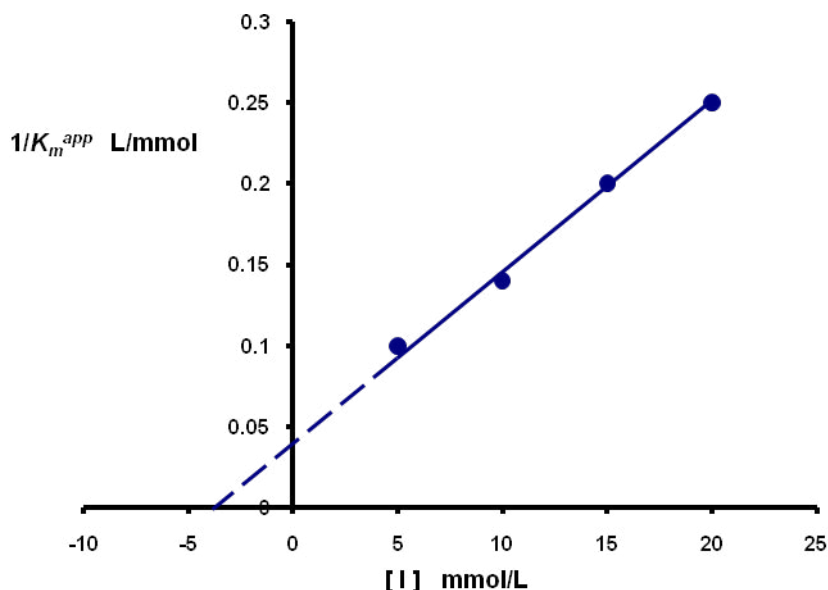
$$\frac{K_m K_i}{K_m} = - [I]$$

Cancelling  $K_m$ :

$$K_i = - [I]$$

Therefore the intercept on the  $[I]$  axis is  $-K_i$

$[I]$ (mmol/L)	5	10	15	20
$1/K_m^{app}$ (L/mmol)	0.1	0.14	0.2	0.25



When  $1/K_m^{app} = 0$ ,  $[I] = -K_i$

From graph, when  $K_m^{app} = 0$ ,  $[I] = -3.67$  mmol/L

Therefore  $K_i = -(-3.67) = 3.67$  mmol/L =  **$3.7 \times 10^{-3}$  mol/L** (2 sig figs)

The relationship between  $V_{max}^{app}$  and  $K_i$  for an uncompetitive inhibitor is:

$$V_{max}^{app} = \frac{V_{max}}{(1 + [I]/K_i)}$$

Inversion gives:

$$\frac{1}{V_{max}^{app}} = \frac{(1 + [I]/K_i)}{V_{max}}$$

Which can also be written:

$$\frac{1}{V_{max}^{app}} = \left( \frac{1}{K_i V_{max}} \times [I] \right) + \frac{1}{V_{max}}$$

When  $1/V_{max}^{app} = 0$ :

$$0 = \left( \frac{1}{K_i V_{max}} \times [I] \right) + \frac{1}{V_{max}}$$

Which can be rearranged to:

$$- \frac{I}{V_{max}} = \frac{[I]}{K_i V_{max}}$$

$$- \frac{K_i V_{max}}{V_{max}} = [I]$$

Cancelling  $V_{max}$  and changing the sign on both sides gives:

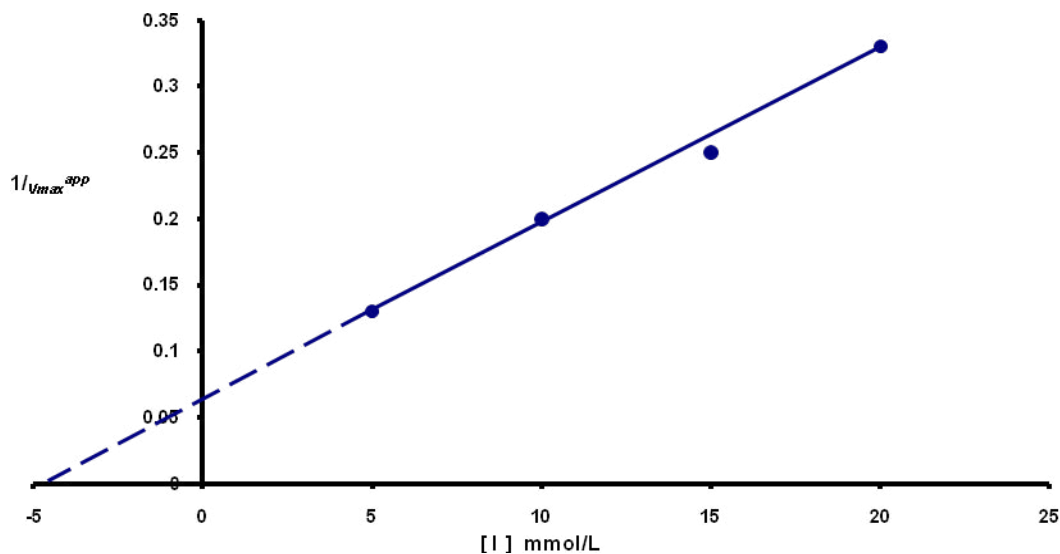
$$K_i = - [I]$$

Therefore the intercept on the  $[I]$  axis is  $- K_i$ .

Calculating  $1/V_{max}$ :

$[I]$ mmol/L:	5	10	15	20
$1/V_{max}^{app}$	0.13	0.20	0.25	0.33

Then plotting  $1/V_{max}$  versus  $[I]$ :



When  $1/V_{max}^{app} = 0$ ,  $[I] = - K_i$ .

From graph, when  $1/V_{max}^{app} = 0$ ,  $[I] = - 4.59$  mmol/L

Therefore  $K_i = 4.59$  mmol/L =  **$4.6 \times 10^{-3}$  mol/L** (2 sig figs)

The  $K_i$ s from the two plots do not agree exactly but the value is approximately  $4 \times 10^{-3}$  mol/L. This is due to errors inherent in manually constructing the plots and reading off the values of the intercepts.

**Chapter10**

**Q 10 (1)**

Construct a table with columns for protein result ( $x$ ) and  $x^2$ , then obtain the sum of the results in each column:

Result ( $x$ )	$x^2$
70	4900
68	4624
71	5041
65	4225
68	4624
70	4900
73	5329
69	4761
75	5625
74	5476
69	4761
<u>71</u>	<u>5041</u>

**Total:**  $\Sigma x = 843$        $\Sigma x^2 = 59307$

Number of values of  $x$  ( $n$ ) = 12

$$\text{Mean } (m) = \frac{\Sigma x}{n} = \frac{843}{12} = \mathbf{70.25 \text{ g/L}}$$

$$\text{Variance } (s^2) = \frac{\Sigma (x - m)^2}{n - 1}$$

As a short cut use the identity:

$$\begin{aligned} \Sigma (x - m)^2 &= \Sigma x^2 - \frac{(\Sigma x)^2}{n} \\ &= 59307 - \frac{843^2}{12} \\ &= 59307 - 59221 \end{aligned}$$

$$= 86 \text{ g/L}$$

$$\text{Therefore, variance } (s^2) = \frac{86}{(12 - 1)} = \frac{86}{11} = \mathbf{7.82 \text{ g/L}}$$

$$\text{Standard deviation } (s) = \sqrt{s^2} = \sqrt{7.82} = \mathbf{2.80 \text{ g/L}}$$

$$\text{Coefficient of variation } (CV) = \frac{s \times 100}{m} = \frac{2.80 \times 100}{70.25} = \mathbf{4.0 \%}$$

The confidence limits of the mean are:

$$\text{Mean} - (z \times s) \text{ to } \text{mean} + (z \times s)$$

For 95 % confidence limits  $z = 1.96$  so that this expression becomes:

$$\text{Mean} - (1.96 \times s) \text{ to } \text{mean} + (1.96 \times s)$$

Substituting mean = 70.25 g/L and  $s = 2.80$  g/L gives the 95 % confidence limits:

$$= \begin{array}{l} 70.25 - (1.96 \times 2.80) \text{ to } 70.25 + (1.96 \times 2.80) \\ 70.25 - 5.49 \text{ to } 70.25 + 5.49 \end{array}$$

$$= \mathbf{64.8 \text{ to } 75.7 \text{ g/L}} \quad (3 \text{ sig figs})$$

### Q 10 (2)

Assume that the normal range is the mean  $\pm 2$  standard deviations.

The mean will be the mean of the upper and lower limits:

$$m = \frac{(50 + 150)}{2} = \frac{200}{2} = 100 \text{ nmol/L}$$

The upper and lower reference limits will span 4 standard deviations:

$$s = \frac{(150 - 50)}{4} = \frac{100}{4} = 25 \text{ nmol/L}$$

Next calculate the  $z$  value for a result of 165 nmol/L:

$$z = \frac{x - m}{s} = \frac{(165 - 100)}{25} = \frac{65}{25} = 2.6$$

From tables of  $z$ , the value of  $P$  when  $z$  is equal to 2.6 is 0.002. Therefore, 0.002 of results fall outside the range: mean  $\pm$  65 nmol/L and a half of these (0.001) will be greater than 165 nmol/L.

$$\begin{aligned} \text{Number of results} > 165 \text{ nmol/L} &= 0.001 \times 10000 \\ &= \mathbf{10 \text{ results}} \end{aligned}$$

**Q 10 (3)**

First calculate the total variation in terms of  $CV\%$ :

$$\begin{aligned} CV_{\text{Total}} &= \sqrt{(CV_{\text{Analytical}})^2 + (CV_{\text{Intra-individual}})^2} \\ &= \sqrt{(2.4)^2 + (4.7)^2} \\ &= \sqrt{(5.76 + 22.09)} \\ &= \sqrt{27.85} \\ &= 5.28 \% \end{aligned}$$

For a change to be significant the overall difference must be at least 2.8 CVs:

$$\text{Least significant change} = 2.8 \times 5.28 = 14.8 \text{ mmol/L}$$

Calculate the actual percentage change in the patient's result:

$$\begin{aligned} \text{Actual \% change} &= \frac{(6.9 - 5.9) \times 100}{6.9} \\ &= \frac{1.0 \times 100}{6.9} \\ &= \mathbf{14.5 \%} \end{aligned}$$

Since this percentage change is not greater than 14.8 %, the change is **not quite statistically significant** at the 5 % level of probability.

**Q 10 (4)**

The probability of a channel failing QC is 1% = 0.01

There are only two possible outcomes - pass or fail.

Therefore the probability of a channel passing QC is 1 - 0.01 = 0.99

This problem is analogous to flipping a coin. The joint probability of two *independent* events is the product of their individual probabilities.

Thus if a coin is tossed once the probability of 'heads' is 0.5. If the coin is tossed again then the probability of it landing 'heads' on *both* occasions is  $0.5 \times 0.5 = 0.25$ . Similarly if the probability of one channel passing QC is 0.99, then the probability of two channels passing is  $0.99 \times 0.99 = 0.98$ . The chance of three different channels passing is given by  $0.99 \times 0.99 \times 0.99 = 0.97$  i.e.  $(0.99)^3$ .

The general rule is:

$$\begin{aligned} \text{Probability of event occurring on } n \text{ occasions} &= \\ &(\text{probability of event occurring on a single occasion})^n \end{aligned}$$

$$\text{Therefore the probability of 30 channels passing QC} = (0.99)^{30} = 0.74$$

If your calculator does not have the facility to calculate  $x^y$  then the result can be easily calculated using logs:

$$\begin{aligned} \text{Log}_{10} (\text{probability of 30 channels passing}) &= 30 \times \text{Log}_{10} 0.99 \\ &= 30 \times (-0.00436) \\ &= -0.131 \end{aligned}$$

$$\text{Probability of 30 channels passing} = \text{antilog} (-0.131) = \mathbf{0.74}$$

### Q 10 (5)

There are two problems with this set of data:

1. The individual results are not given, only the number of results falling into each class interval. The easiest way to deal with this is to assume that the results fall in the middle of the range i.e. there are 5 results within the range 0.5 to 1.49 so assume there are 5 results of the mid-point value (1.0 mU/L), similarly there are 3 samples with a value of 2 mU/L. Using this approach 10 individual results are produced which can be processed in the usual way.
2. The data are obviously skewed and do not form a Gaussian distribution. This can be overcome to some extent by taking logarithms (to the base 10) of the results then calculating the mean, SD and 95% confidence limits in the usual way. Taking antilogarithms of the confidence limits then gives the reference range.

A table can be completed in the following way:

TSH result	$x = \log_{10}$ TSH result	$x^2$
1.0	0	0
1.0	0	0
1.0	0	0
1.0	0	0
1.0	0	0
2.0	0.301	0.0906
2.0	0.301	0.0906
2.0	0.301	0.0906
4.0	0.602	0.3624
5.0	0.699	0.4886
$n = 10$	$\Sigma x = 2.204$	$\Sigma x^2 = 1.123$
Mean	$= \frac{\Sigma x}{n} = \frac{2.204}{10} = 0.220$	
$s^2$	$= \frac{\Sigma x^2 - (\Sigma x)^2 / n}{n - 1} = \frac{1.123 - 2.204^2 / 10}{10 - 1} = 0.0708$	
$s$	$= \sqrt{0.0708} = 0.266$	

Alternatively the mean and  $s$  can be calculated directly on most modern pocket calculators. The 95% confidence are given by mean - 1.96  $s$  to mean + 1.96  $s$

$$= 0.220 - (1.96 \times 0.266) \text{ to } 0.220 + (1.96 \times 0.266)$$

$$= -0.301 \text{ to } 0.741 \quad (\text{these values are logs and so do NOT have units})$$

Taking antilogs (to the base 10) gives the 95% confidence limits in mU TSH/L:

**0.50 to 5.51 mU/L**

Although the original data may have been expressed to one or two decimal places, this information has been lost by grouping the data into class intervals. Therefore it would be more correct to quote a reference range of **less than 6 mU/L**.

**Q 10 (6)**

Assume that the error is required as 95 % confidence limits i.e.  $\pm 2s$ .

a) Using the graduated pipette:

Calculate  $s$  when mean = 9 mL and  $CV = 2\%$ :

$$CV (\%) = \frac{s \times 100}{m} =$$

$$\text{Therefore: } s = \frac{CV (\%) \times m}{100}$$

$$s = \frac{2 \times 9}{100} = \frac{18}{100} = 0.18 \text{ mL}$$

$$\text{Therefore 95 \% limits} = \pm 2s = \pm 2 \times 0.18 = \pm 0.36 \text{ mL}$$

**Error = plus/minus 0.36 mL**

b) Similarly calculate the error for each of the bulb pipettes:

For 5 mL bulb with  $CV = 1\%$

$$s = \frac{1 \times 5}{100} = \frac{5}{100} = 0.05 \text{ mL}$$

For 2 mL bulb with  $CV = 1\%$ :

$$s = \frac{1 \times 2}{100} = \frac{2}{100} = 0.02 \text{ mL}$$

To pipette 9 mL the 5ml bulb is used once and the 2 mL bulb twice. Calculate the overall  $s$ :

$$\begin{aligned} s_{\text{Total}} &= \sqrt{(s_{5\text{mL}}^2 + s_{2\text{mL}}^2 + s_{2\text{mL}}^2)} \\ &= \sqrt{(0.05^2 + 0.02^2 + 0.02^2)} \\ &= \sqrt{(0.0025 + 0.0004 + 0.0004)} \\ &= \sqrt{0.0033} \\ &= 0.0574 \text{ mL} \end{aligned}$$

$$\text{Therefore total error } (2s) = 2 \times 0.0574 = 0.11 \text{ mL (2 sig figs)}$$

**Error = plus/minus 0.11 mL**

**Q 10 (7)**

The relationship between the overall variation, analytical variation and biological variation is:

$$CV_{\text{Total}}^2 = CV_{\text{Analytical}}^2 + CV_{\text{Biological}}^2$$

Both the analytical and biological CV's share the same mean.

When the analytical variation is one half of the biological variation:

$$CV_{\text{Analytical}} = 0.5 CV_{\text{Biological}}$$

Substitute this value for the analytical CV so as to obtain the total CV expressed in terms of the biological CV:

$$\begin{aligned} CV_{\text{Total}} &= \sqrt{[(0.5 CV_{\text{Biological}})^2 + CV_{\text{Biological}}^2]} \\ &= \sqrt{[0.25 \times CV_{\text{Biological}}^2 + CV_{\text{Biological}}^2]} \\ &= \sqrt{1.25 \times CV_{\text{Biological}}^2} \\ &= 1.118 CV_{\text{Biological}} \end{aligned}$$

The reference range encompasses a span of 4 CV's

Therefore biological reference range spans 4 CV's and the total reference range spans  $4 \times 1.118 CV_{\text{Biological}} = 4.47 CV_{\text{Biological}}$  's

Therefore the percentage expansion is:

$$\begin{aligned} &\frac{(4.47 CV_{\text{Biological}} - 4CV_{\text{Biological}}) \times 100}{4CV_{\text{Biological}}} \\ &\frac{CV_{\text{Biological}}(4.47 - 4) \times 100}{4CV_{\text{Biological}}} \\ \frac{(4.47 - 4) \times 100}{4} &= \frac{0.47 \times 100}{4} = \mathbf{11.8 \%} \quad (3 \text{ sig figs}) \end{aligned}$$

**Chapter 11**

**Q 11 (1)**

Construct a table with columns for result ( $x$ ) and  $x^2$ , then obtain the sum of the results in each column:

$x$	$x^2$
109	11881
91	8281
105	11025
112	12544
90	8100
115	13225
89	7921
113	12769
93	8649
94	8836

**Total:**  $\sum x = 1011$     $\sum x^2 = 103231$

$$n = 10$$

$$\text{Mean } (m) = \frac{\sum x}{n} = \frac{1011}{10} = \mathbf{101 \text{ (3 sig figs)}}$$

$$\text{Variance } (s^2) = \frac{\sum (x - m)^2}{n - 1}$$

$$\begin{aligned} \sum (x - m)^2 &= \sum x^2 - \frac{(\sum x)^2}{n} \\ &= 103231 - \frac{1011^2}{10} \\ &= 103231 - 102212 \\ &= 1019 \end{aligned}$$

$$s^2 = \frac{\sum (x - m)^2}{n - 1} = \frac{1019}{(10 - 1)} = \frac{1019}{9} = 113.2$$

$$\text{Standard deviation } (s) = \sqrt{s^2} = \sqrt{113.2} = \mathbf{10.64}$$

$$\text{Standard error of the mean } (SE_m) = \frac{s}{\sqrt{n}} = \frac{10.64}{\sqrt{10}} = \frac{10.64}{3.16} = \mathbf{3.4}$$

**Q 11 (2)**

First lab:  $m_1 = 145 \text{ mmol/L}; s_1 = 3 \text{ mmol/L}$

2<sup>nd</sup> lab:  $m_2 = 147 \text{ mmol/L}; s_2 = 2 \text{ mmol/L}$

$n = 10$  for each lab

To check for bias carry out a *t*-test:

$$\begin{aligned} t &= \frac{m_1 - m_2}{\sqrt{(s_1^2/n + s_2^2/n)}} \\ &= \frac{145 - 147}{\sqrt{(3^2/10 + 2^2/10)}} \\ &= \frac{-2}{\sqrt{(0.9 + 0.4)}} \\ &= \frac{-2}{\sqrt{1.3}} = \frac{-2}{1.14} = -1.75 \end{aligned}$$

Next calculate degrees of freedom (*DF*):

$$\begin{aligned} DF &= \frac{(s_1^2/n_1 + s_2^2/n_2)^2}{[(s_1^2/n_1)^2/(n_1 - 1)] + [(s_2^2/n_2)^2/(n_2 - 1)]} \\ &= \frac{(0.9 + 0.4)^2}{0.9^2/9 + 0.4^2/9} \\ &= \frac{1.3^2}{0.09 + 0.018} \\ &= \frac{1.69}{0.108} \\ &= 15.6 \end{aligned}$$

From tables when  $t = 1.75$  with 16 degrees of freedom,  $P = 0.10$ . Therefore there is no significant difference between the means of the two set of results i.e. **no evidence of bias**.

To compare imprecision perform an  $F$  ratio test:

$$F = \frac{s_1^2}{s_2^2} = \frac{3^2}{2^2} = \frac{9}{4} = 2.25$$

From tables when  $F = 3.18$  (with 9 degrees of freedom for both variances),  $P = 0.05$ . Therefore there is no significant difference between the two variances i.e. **no evidence of difference in imprecision**.

### Q 11 (3)

Assume that the normal range is the mean  $\pm 2s$ .

The mean is the average of the upper and lower reference limit:

$$\text{Mean } (m) = \frac{(50 + 150)}{2} = \frac{200}{2} = 100 \text{ nmol/L}$$

The reference limits span  $4s$  units so that  $s$  is a quarter of the range:

$$\text{Standard deviation } (s) = \frac{(150 - 50)}{4} = \frac{100}{4} = 25 \text{ nmol/L}$$

Standard error of the mean ( $SE_m$ ) for 9 results

$$= \frac{s}{\sqrt{n}} = \frac{25}{\sqrt{9}} = \frac{25}{3} = 8.33 \text{ nmol/L}$$

Calculate  $t$  for 9 results with  $m = 125$  nmol/L, population mean ( $\mu$ ) = 100 nmol/L and  $SE_m = 8.33$  nmol/L:

$$t = \frac{m - \mu}{SE_m} = \frac{(125 - 100)}{8.33} = \frac{25}{8.33} = 3.00 \quad (DF = n - 1 = 8)$$

From tables, for  $t = 3.00$  with 8 degrees of freedom  $P =$  approx 0.02. Therefore 0.02 of results fall outside of the range mean  $\pm 25$  nmol/L and a half of these results, 0.01, will be greater than 125 nmol/L.

Probability of mean of 9 results being greater than 125 nmol/L = **0.01**.

**Q 11 (4)**

Since these are paired samples the results should be compared using the paired *t*-test. Construct a table with the individual differences between each pair of results ( $d = A - B$ ),  $d^2$ , the difference between each  $d$  and the overall mean ( $m_d$ ) for all the values of  $d$  (i.e.  $d - m_d$ ) and their squares i.e.  $(d - m_d)^2$ .

A	B	$d$	$d^2$	$d - m_d$	$(d - m_d)^2$
6.8	7.2	-0.4	0.16	-0.24	0.058
4.2	4.5	-0.3	0.09	-0.14	0.020
5.0	4.8	0.2	0.04	0.36	0.130
5.6	5.9	-0.3	0.09	-0.14	0.120
8.5	8.7	-0.2	0.04	-0.04	0.002
2.9	2.8	0.1	0.01	0.26	0.070
4.8	4.9	-0.1	0.01	0.06	0.004
7.6	8.1	-0.5	0.25	-0.34	0.116
6.5	6.4	0.1	0.01	0.26	0.070
5.0	5.2	-0.2	0.04	-0.04	0.002

$$n = 10 \quad \sum d = -1.6 \quad \sum d^2 = 0.74 \quad \sum (d - m_d)^2 = 0.592$$

$$\text{Mean difference } (m_d) = \frac{\sum d}{n} = \frac{-1.6}{10} = -0.16$$

$$\text{Paired } t = \frac{m_d}{s_d/\sqrt{n}}$$

$$\begin{aligned} s_d &= \sqrt{[\sum (d - m_d)^2 / (n - 1)]} \\ &= \sqrt{[0.592 / 9]} \\ &= \sqrt{0.0658} \\ &= 0.256 \end{aligned}$$

Use this  $s_d$  to calculate the paired *t*:

$$\text{Paired } t = \frac{m_d}{s_d/\sqrt{n}} = \frac{-0.16}{0.256/\sqrt{10}}$$

WORKED ANSWERS TO FURTHER QUESTIONS

$$s_d/\sqrt{n} \qquad 0.256/\sqrt{10}$$

$$= \frac{-0.16}{0.256/3.16} \qquad = \frac{-0.16}{0.081} \qquad = -1.98$$

From tables, for  $t = 1.98$  (degrees of freedom =  $n - 1 = 9$ )  $P$  is greater than  $0.05$ . Therefore there is no significant bias between the two methods.

**Q 11 (5)**

Calculate  $n$ ,  $\sum x$ ,  $m$ ,  $\sum x^2$ ,  $(\sum x)^2/n$  and  $\sum x^2 - (\sum x)^2/n$  for each lab:

	Lab				
	A	B	C	D	
	7.6	7.5	7.0	7.7	
	7.3	7.6	7.4	7.8	
	7.5	7.2	7.7	7.4	
	7.7	7.5	7.5	7.5	
	7.5	7.7	7.4	7.6	
	7.6	7.4	7.2	7.5	
	7.4	7.8	7.5	7.3	
	7.8	7.5	7.2	7.8	
	7.2	7.3	7.5	7.6	
	7.5	7.4	7.3	7.6	
					<b>Totals</b>
$\sum x$	75.1	74.9	73.7	75.8	299.5
$n$	10	10	10	10	40
$m$	7.51	7.49	7.37	7.58	
$\sum x^2$	564.29	561.29	543.53	574.8	2243.91
$(\sum x)^2/n$	564.001	561.001	543.69	574.564	2242.735
$\sum x^2 - (\sum x)^2/n$	0.289	0.289	-0.16	0.236	

Number of groups ( $u$ ) = 4, number in each group ( $v$ ) = 10,  $uv = 40$

$$\begin{aligned} \text{Between groups sum of squares} &= \sum (\sum x)^2/n - (\sum \sum x)^2/uv \\ &= 2242.735 - 299.5^2/40 \\ &= 2242.735 - 2242.5063 \\ &= 0.2287 \end{aligned}$$

$$\text{Within groups sum of squares} = \sum \sum x^2 - \sum (\sum x)^2/n$$

$$\begin{aligned}
 &= 2243.91 - 2242.735 \\
 &= 1.175 \\
 \text{Total sum of squares} &= \sum \cdot \sum x^2 - (\sum \cdot \sum x)^2 / uv \\
 &= 2243.91 - 299.5^2 / 40 \\
 &= 2243.91 - 2242.5063 \\
 &= 1.4037
 \end{aligned}$$

Divide each sum of squares by the degrees of freedom (*DF*) to give the corresponding variance. The ratio of the between groups to the within groups variance gives the *F* value.

Source	Sum of squares	<i>DF</i>	<i>s</i> <sup>2</sup>	<i>F</i>
Between groups	0.2287	3	0.0762	2.34
Within groups	1.175	36	0.0326	
Total	1.4037	39	0.0360	

From tables the probability of obtaining an *F* value greater than 2.84 (for 3 and 40 degrees of freedom) is 0.05. Therefore the data is homogeneous and there is **no evidence for bias between the four laboratories.**

## Chapter 12

### Q 12 (1)

- a) Regression equation for new standards (*y*) upon old standards (*x*):

$$y = 1.10x + 1.0$$

Substitute old standard containing 15 mmol/L for *x* then solve for *y*:

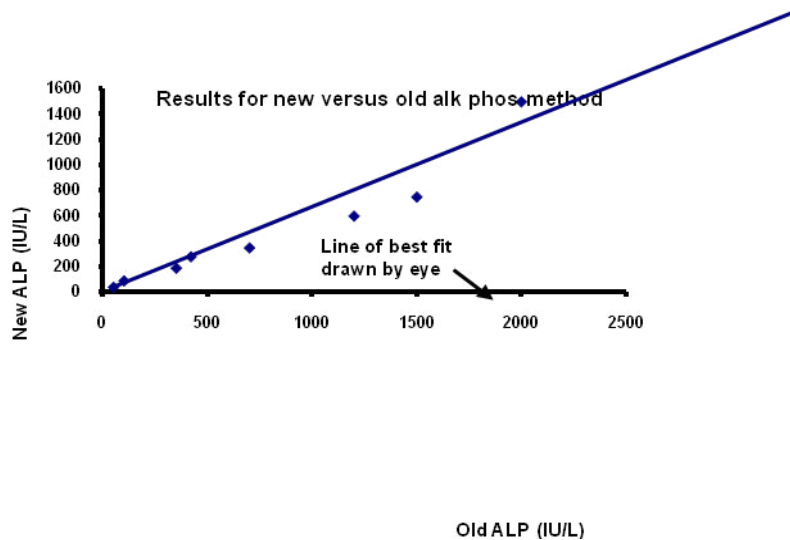
$$\begin{aligned}
 y &= (1.10 \times 15) + 1.0 \\
 &= 16.5 + 1.0 \\
 &= \mathbf{17.5 \text{ mmol/L}}
 \end{aligned}$$

b) Substitute old standard containing 150 mmol/L for  $x$  then solve for  $y$ :

$$\begin{aligned}
 y &= (1.10 \times 150) + 1.0 \\
 &= 165 + 1.0 \\
 &= \mathbf{166 \text{ mmol/L}}
 \end{aligned}$$

**Q 12 (2)**

The first step is to check that there is a linear relationship between the two methods. This is best done by plotting the results using the new method ( $y$ -axis) against those obtained using the old method ( $x$ -axis):



50	40	2500	1600	2000
350	190	122500	36100	66500
700	350	490000	122500	245000
100	90	10000	8100	9000
1500	750	2250000	562500	1125000
2000	1500	4000000	2250000	3000000
420	280	176400	78400	117600
1200	600	1440000	360000	720000
$\Sigma x = 6320$	$\Sigma y = 3800$	$\Sigma x^2 = 8491400$	$\Sigma y^2 = 3419200$	$\Sigma xy = 5285100$

$$\text{Slope of regression line } (b) = \frac{\Sigma (x - m_x)(y - m_y)}{\Sigma (x - m_x)^2}$$

$$= \frac{\Sigma xy - (\Sigma x \Sigma y / n)}{\Sigma x^2 - (\Sigma x)^2 / n}$$

$$\begin{aligned}
 & \Sigma x^2 - (\Sigma x)^2/n \\
 b &= \frac{5285100 - (6320 \times 3800/8)}{8491400 - (6320^2/8)} \\
 &= \frac{5285100 - 3002000}{8491400 - 4992800} \\
 &= \frac{2283100}{3498600} \\
 &= 0.653 \quad (3 \text{ sig figs})
 \end{aligned}$$

The value for the intercept ( $a$ ) can be obtained by substituting the slope ( $b$ ), the mean of  $x$  for  $x$  and the mean of  $y$  for  $y$  into the linear expression  $y = bx + a$ , then solving for  $a$ :

$$\begin{aligned}
 m_x &= \frac{\Sigma x}{n} = \frac{6320}{8} = 790 \text{ IU/L} \\
 m_y &= \frac{\Sigma y}{n} = \frac{3800}{8} = 475 \text{ IU/L} \\
 475 &= (0.653 \times 790) + a \\
 a &= 475 - (0.653 \times 790) \\
 &= 475 - 516 \\
 &= -41
 \end{aligned}$$

Therefore regression equation of  $y$  (new results) upon  $x$  (old results):

$$\text{New method} = (\text{Old method} \times 0.65) - 41$$

Rearranging to enable easy conversion of new to old results:

$$\text{Old method} \times 0.65 = \text{New method} + 41$$

$$\text{Old method} = \frac{\text{New method} + 41}{0.65}$$

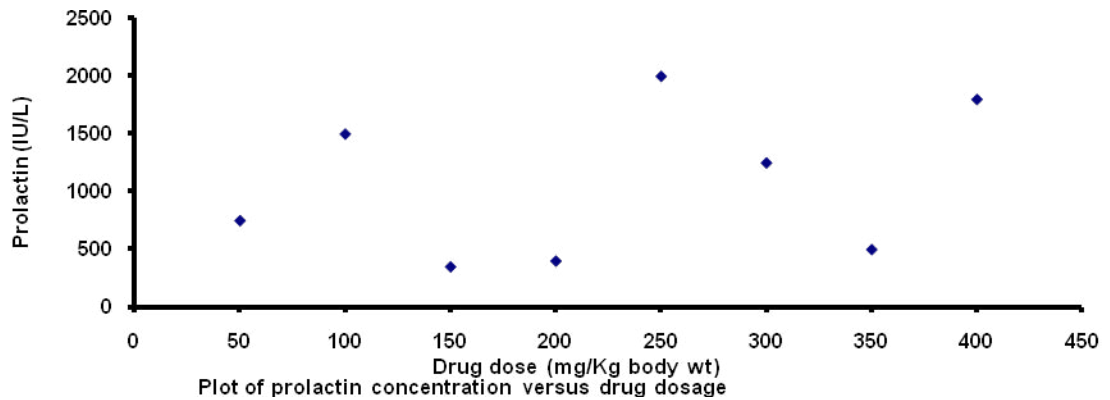
Inspection of the plot suggests that the highest pair of values ( $x = 2000$ ,  $y = 1500$ ) may be an outlier. If this point is omitted and the calculations repeated then the

slope becomes 0.508 and the intercept 13, giving an alternative conversion formula:

$$\text{Old method} = \frac{\text{New method} - 13}{0.508}$$

**Q 12 (3)**

The first step is to plot the data with prolactin as the y-axis and drug dosage as the x-axis:



Visual inspection suggests that there is no significant relationship between serum prolactin concentration and drug dosage. Further evidence could be obtained by calculating the correlation coefficient:

$x$	$x^2$	$y$	$y^2$	$xy$
50	2500	750	562500	37500
100	10000	1500	2250000	150000
150	22500	350	122500	52500
200	40000	400	160000	80000
250	62500	2000	4000000	500000
300	90000	1250	1562500	375000
350	122500	500	250000	175000
400	160000	1800	3240000	720000

$$\Sigma x = 1800 \quad \Sigma x^2 = 510000 \quad \Sigma y = 8550 \quad \Sigma y^2 = 12147500 \quad \Sigma xy = 2090000$$

$$n = 8$$

$$\begin{aligned}
 r &= \frac{\sum xy - (\sum x \sum y / n)}{\sqrt{\{ [\sum x^2 - (\sum x)^2 / n] [\sum y^2 - (\sum y)^2 / n] \}}} \\
 &= \frac{2090000 - (1800 \times 8550 / 8)}{\sqrt{\{ [510000 - 1800^2 / 8] [12147500 - 8550^2 / 8] \}}} \\
 &= \frac{2090000 - 1923750}{\sqrt{\{ [510000 - 405000] [12147500 - 9137813] \}}} \\
 &= \frac{166250}{\sqrt{\{ 105000 \times 3009687 \}}} \\
 &= \frac{166250}{562154} \\
 &= 0.30 \text{ (2 sig figs)}
 \end{aligned}$$

From tables, for  $r = 0.30$  with 7 degrees of freedom,  $P > 0.1$ . Therefore there is **no significant correlation between drug dosage and serum prolactin**.

**Q 12 (4)**

No evidence is presented that the relationship between the two variables is linear.

Correlation analysis is not the best approach to comparing two analytical methods – as they both measure the same analyte it would be surprising if there were no correlation. Analysis of difference plots would be more appropriate.

The standard error of the slope (1.05) is not given.

The standard deviation of the residual ( $s_{res}$  or  $sy_x$ ) is not given – this is the best indicator of the goodness of fit of the data to the regression line.

**Chapter 13**

**Q 13 (1)**

a) It is easiest to work with proportions rather than percentages or absolute numbers of results. The contingency table to use is:

	Positive result	Negative result	Total
Patients <i>with</i> disease	TP	FN	Prevalence

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**Patients without disease**      FP                      TN                      1 - prevalence

If the prevalence of disease is 1 in 2 i.e. 0.5, then 1 - prevalence is also 0.5 so this table becomes:

	<b>Positive result</b>	<b>Negative result</b>	<b>Total</b>
<b>Patients with disease</b>	TP	FN	0.5
<b>Patients without disease</b>	FP	TN	0.5

The next task is determine values for TP, FN, FP and TN using the stated sensitivity and specificity:

$$\text{Sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}} = 0.95$$

Substitute (TP + FN) = 0.5, then solve for TP:

$$\frac{\text{TP}}{0.5} = 0.95 \text{ so that } \text{TP} = 0.5 \times 0.95 = 0.475$$

$$\text{and } \text{FN} = 0.5 - \text{TP} = 0.5 - 0.475 = 0.025$$

Similarly using specificity:

$$\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}} = 0.95$$

$$\frac{\text{TN}}{0.5} = 0.95 \text{ so that } \text{TN} = 0.5 \times 0.95 = 0.475$$

$$\text{and } \text{FP} = 0.5 - \text{TN} = 0.5 - 0.475 = 0.025$$

Inserting these values into the contingency table gives:

	<b>Positive result</b>	<b>Negative result</b>	<b>Total</b>
<b>Patients with disease</b>	0.475	0.025	0.5
<b>Patients without disease</b>	0.025	0.475	0.5

These values are then used to calculate positive and negative predictive values:

$$PV(+)=\frac{TP}{TP+FP}=\frac{0.475}{0.475+0.025}=0.95\text{ (95\%)}$$

$$PV(-)=\frac{TN}{TN+FN}=\frac{0.475}{0.475+0.025}=0.95\text{ (95\%)}$$

b) With a prevalence of 1 in 5000 (= 0.0002) the contingency table becomes:

	Positive result	Negative result	Total
<b>Patients <i>with</i> disease</b>	TP	FN	0.0002
<b>Patients <i>without</i> disease</b>	FP	TN	0.9998

$$TP=0.0002\times 0.95=0.00019$$

$$FN=0.0002-0.00019=0.00001$$

$$TN=0.9998\times 0.95=0.94981$$

$$FP=0.9998-0.94981=0.04999$$

So the contingency table becomes:

	Positive result	Negative result	Total
<b>Patients <i>with</i> disease</b>	0.00019	0.00001	0.0002
<b>Patients <i>without</i> disease</b>	0.04999	0.94981	0.9998

Use these values to calculate positive and negative predictive values:

$$PV(+)=\frac{TP}{TP+FP}=\frac{0.00019}{0.00019+0.04999}=0.004\text{ (0.4\%)}$$

$$PV(-)=\frac{TN}{TN+FN}=\frac{0.94981}{0.94981+0.00001}=1.00\text{ (100\%)}$$

### Q 13 (2)

- a) The number of patients with pheochromocytoma missed by the VMA test is the number of *false negatives* using this test.

## WORKED ANSWERS TO FURTHER QUESTIONS

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First calculate the proportion of false negatives i.e use the sensitivity expressed as a proportion (0.967) rather than percentage (96.7%) and the prevalence calculated as follows:

$$\text{Prevalence} = \frac{0.5}{100} = 0.005$$

$$\text{Sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}}$$

Substitute  $(\text{TP} + \text{FN}) = \text{prevalence} = 0.005$ , and  $\text{sensitivity} = 0.967$  and solve for TP:

$$\text{Sensitivity} = \frac{\text{TP}}{0.005} = 0.967$$

$$\text{TP} = 0.967 \times 0.005 = 0.004835$$

Since  $\text{TP} + \text{FN} = 0.005$

$$\text{FN} = 0.005 - 0.004835 = 0.000165$$

Multiply this *proportion* by the total number screened to obtain the *number* of false negatives (i.e. cases of phaeochromocytoma missed):

$$\begin{aligned} \text{Patients missed} &= 0.000165 \times 100000 \\ &= \mathbf{16.5} \text{ (2 sig figs)} \end{aligned}$$

b) The proportion of patients incorrectly diagnosed with phaeochromocytoma using the metanephrine test is the proportion of false positives which can be calculated from the specificity and prevalence:

$$\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}} = 0.98$$

$$\text{TN} + \text{FP} = 1 - \text{prevalence} = 1 - 0.005 = 0.995$$

$$\text{Specificity} = \frac{\text{TN}}{0.995} = 0.98$$

$$\text{TN} = 0.98 \times 0.995 = 0.9751$$

$$\text{FP} = (1 - \text{prevalence}) - \text{TN}$$

$$\text{FP} = 0.995 - 0.9751 = 0.0199$$

Multiply the proportion of false positives by the total number tested to give the absolute number of false positives i.e. the number of patients incorrectly diagnosed with pheochromocytoma by the metanephrine test:

$$\begin{aligned} \text{Number incorrectly diagnosed} &= 0.0199 \times 100,000 \\ &= \mathbf{1990} \end{aligned}$$

- c) Probably the best way to decide which is the best test is to calculate the positive and negative predictive values for each test:

**For VMA:**

	<b>Positive result</b>	<b>Negative result</b>	<b>Total</b>
<b>Patients <i>with</i> disease</b>	0.004835	0.00165	0.005
<b>Patients <i>without</i> disease</b>	0.008955	0.986045	0.995
 PV(+) = $\frac{TP}{TP + FP}$	 = $\frac{0.004835}{0.004835 + 0.008955}$	 = $\frac{0.004835}{0.01379}$	 = <b>0.35</b>
 PV(-) = $\frac{TN}{TN + FN}$	 = $\frac{0.986045}{0.986045 + 0.00165}$	 = $\frac{0.986045}{0.987695}$	 = <b>0.998</b>

**For metanephrines:**

	<b>Positive result</b>	<b>Negative result</b>	<b>Total</b>
<b>Patients <i>with</i> disease</b>	0.005	0.000	0.005
<b>Patients <i>without</i> disease</b>	0.0199	0.9751	0.995
 PV(+) = $\frac{TP}{TP + FP}$	 = $\frac{0.005}{0.005 + 0.0199}$	 = $\frac{0.005}{0.0249}$	 = <b>0.20</b>
 PV(-) = $\frac{TN}{TN + FN}$	 = $\frac{0.9751}{0.9751 + 0.000}$	 = $\frac{0.9751}{0.9751}$	 = <b>1.00</b>

To summarize:

<b>Test</b>	<b>PV(+)</b>	<b>PV(-)</b>
<b>VMA</b>	0.35	0.998
<b>Metanephrines</b>	0.20	1.00

Although the VMA test produces less false positives (i.e. higher PV+) this is achieved at the expense of missing approximately 1 in 3 (FN/prevalence = 0.33) patients with phaeochromocytoma. Although the metanephrine test produces more false positives (i.e. lower PV+) this is achieved without missing any cases of phaeochromocytoma (i.e. no false negatives). **On balance total metanephrines is the better test.**

**Q 13 (3)**

Start by drawing up a contingency table:

	<b>Positive result</b>	<b>Negative result</b>	<b>Total</b>
<b>Patients <i>with</i> disease</b>	TP (Sens x prev)	FN (Prev - TP)	Prev (TP + FN)
<b>Patients <i>without</i> disease</b>	FP [(1 - prev) - TN]	TN [Spec x (1 - prev)]	1 - prev (FP + TN)
<b>Total</b>	TP + FP	TN + FN	1

Using sensitivity and specificity expressed as proportions instead of percentages i.e. sensitivity = 0.85 and specificity = 0.90 fill in the above table:

	<b>Positive result</b>	<b>Negative result</b>	<b>Total</b>
<b>Patients <i>with</i> disease</b>	0.085 (Sens x prev)	0.015 (Prev - TP)	0.10 (TP + FN)
<b>Patients <i>without</i> disease</b>	0.09 [(1 - prev) - TN]	0.81 [Spec x (1 - prev)]	0.90 (FP + TN)
<b>Total</b>	0.175 TP + FP	0.825 TN + FN	1

a) Predictive value of a positive result:

$$PV(+) = \frac{TP}{TP + FP} = \frac{0.085}{0.175} = \mathbf{0.49} \text{ (2 sig figs)}$$

b) Predictive value of a negative result:

$$PV(-) = \frac{TN}{TN + FN} = \frac{0.81}{0.825} = \mathbf{0.98} \text{ (2 sig figs)}$$

**Q 13 (4)**

The prevalence of disease amongst the population of 200 is 76, so that (1 - prevalence) = 200 - 76 = 124

64 of these 76 gave a positive test result = true positives

10 were positive amongst those without celiac disease = false positives

Set up a 2 x 2 contingency table for these results:

	<b>Positive result</b>	<b>Negative result</b>	<b>Total</b>
<b>Patients <i>with</i> disease</b>	TP (Sens x prev)	FN (Prev - TP)	Prev (TP + FN)
<b>Patients <i>without</i> disease</b>	FP [(1 - prev) - TN]	TN [Spec x (1 - prev)]	1 - prev (FP + TN)
<b>Total</b>	TP + FP	TN + FN	1

Fill in this table working with the data given in the question:

	<b>Positive result</b>	<b>Negative result</b>	<b>Total</b>
<b>Patients <i>with</i> disease</b>	64	12	76
<b>Patients <i>without</i> disease</b>	10	114	124
<b>Total</b>	74	126	200

N.B. In a question like this when the sensitivity and specificity is not given and actual numbers of results are supplied it is probably easiest to work with absolute numbers rather than proportions.

$$\text{Sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}} = \frac{64}{76} = \mathbf{0.84} \text{ (or 84\%)} \text{ 2 sig figs}$$

$$\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}} = \frac{114}{124} = \mathbf{0.92} \text{ (or 92\%)} \text{ 2 sig figs}$$

$$\text{PV(+)} = \frac{\text{TP}}{\text{TP} + \text{FP}} = \frac{64}{74} = \mathbf{0.86} \text{ (or 86\%)} \text{ 2 sig figs}$$

WORKED ANSWERS TO FURTHER QUESTIONS

**Q 13 (5)**

Set up a 2 x 2 contingency table then fill in the gaps using a prevalence of 0.4, sensitivity of 0.92 and specificity of 0.88:

	<b>Positive result</b>	<b>Negative result</b>	<b>Total</b>
<b>Patients <i>with</i> disease</b>	TP (Sens x prev)	FN (Prev - TP)	Prev (TP + FN)
<b>Patients <i>without</i> disease</b>	FP [(1 - prev) - TN]	TN [Spec x (1 - prev)]	1 - prev (FP + TN)
<b>Total</b>	TP + FP	TN + FN	1

	<b>Positive result</b>	<b>Negative result</b>	<b>Total</b>
<b>Patients <i>with</i> disease</b>	0.368 (Sens x prev)	0.032 (Prev - TP)	0.4 (TP + FN)
<b>Patients <i>without</i> disease</b>	0.072 [(1 - prev) - TN]	0.528 [Spec x (1 - prev)]	0.6 (FP + TN)
<b>Total</b>	0.44	0.56	1

$$PV(+)=\frac{TP}{TP+FP}=\frac{0.368}{0.44}=\mathbf{0.84}\text{ or }84\% \text{ (2 sig figs)}$$

Recalculate the above table using a prevalence of 0.4 % (i.e. 0.004):

	<b>Positive result</b>	<b>Negative result</b>	<b>Total</b>
<b>Patients <i>with</i> disease</b>	0.00368 (Sens x prev)	0.00032 (Prev - TP)	0.004 (TP + FN)
<b>Patients <i>without</i> disease</b>	0.12 [(1 - prev) - TN]	0.876 [Spec x (1 - prev)]	0.996 (FP + TN)
<b>Total</b>	0.12368	0.87632	1

$$PV(+)=\frac{TP}{TP+FP}=\frac{0.00368}{0.12368}=\mathbf{0.030}\text{ or }3.0\% \text{ (2 sig figs)}$$

**Q 13 (6)**

The 2 x 2 contingency table can be set up as follows:

	Positive result	Negative result	Total
<b>Patients <i>with</i> disease</b>	TP (Sens x prev)	FN (Prev - TP)	Prev (TP + FN)
<b>Patients <i>without</i> disease</b>	FP [(1 - prev) - TN]	TN [Spec x (1 - prev)]	1 - prev (FP + TN)
<b>Total</b>	TP + FP	TN + FN	1

Complete the table using a prevalence of 5% = 0.05 which with a total of 400 individuals gives a prevalence in absolute numbers of 0.05 x 400 = 20.

	Positive result	Negative result	Total
<b>Patients <i>with</i> disease</b>	15	5 (Prev - TP)	20 (TP + FN)
<b>Patients <i>without</i> disease</b>	30	350 (1 - prev) - FP	380 (FP + TN)
<b>Total</b>	45	355	400

a)  $PV(+)$  =  $\frac{TP}{TP + FP}$  =  $\frac{15}{45}$  = **0.33** or 33% (2 sig figs)

b) Pre-test odds =  $\frac{\text{Prevalence}}{1 - \text{prevalence}}$   
 =  $\frac{20}{380}$   
 = 0.053 (2 sig figs)

c)  $LR+$  =  $\frac{\text{probability of +ve test with disease}}{\text{probability of a +ve test without disease}}$  =  $\frac{\text{sensitivity}}{(1 - \text{specificity})}$

Sensitivity =  $\frac{TP}{TP + FN}$  =  $\frac{15}{20}$  = 0.75

Specificity =  $\frac{TN}{TN + FP}$  =  $\frac{350}{380}$  = 0.92 (2 sig figs)

$LR+$  =  $\frac{0.75}{(1 - 0.92)}$  =  $\frac{0.75}{0.08}$  = **9.4** (2 sig figs)

d) Post-test odds = Pre-test odds x  $LR+$

WORKED ANSWERS TO FURTHER QUESTIONS

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$$= 0.053 \quad \times \quad 9.4$$

$$= \mathbf{0.50} \quad (2 \text{ sig figs})$$

e) Post-test probability =  $\frac{\text{Post-test odds}}{(1 + \text{post-test odds})}$

$$= \frac{0.50}{(1 + 0.50)}$$

$$= \frac{0.50}{1.50}$$

$$= \mathbf{0.33}$$

**Q 13 (7)**

If the prevalence of disease is 0.0005 then the pre-test odds can be calculated:

$$\text{Pre-test odds} = \frac{\text{Prevalence}}{(1 - \text{prevalence})} = \frac{0.0005}{(1 - 0.0005)} = \frac{0.0005}{0.9995} = 0.000500$$

$$\text{LR+} = \frac{\text{probability of +ve test with disease}}{\text{probability of a +ve test without disease}} = \frac{\text{sensitivity}}{(1 - \text{specificity})}$$

For the 1<sup>st</sup> test:  $\text{LR+} = \frac{0.98}{(1 - 0.95)} = \frac{0.98}{0.05} = 19.6$

For the 2<sup>nd</sup> test:  $\text{LR+} = \frac{0.95}{(1 - 0.99)} = \frac{0.95}{0.01} = 95$

Post test odds =

$$\text{Post test odds} = \text{Pre-test odds} \times \text{likelihood ratio (1<sup>st</sup> test)} \times \text{likelihood ratio (2<sup>nd</sup> test)} = 0.000500 \times 19.6 \times 95 = 0.931$$

Post-test probability =  $\frac{\text{Post-test odds}}{(1 + \text{post test odds})}$

$$= \frac{0.931}{1.931}$$

$$= \mathbf{0.48} \text{ or } 48\% \quad (2 \text{ sig figs})$$

**Chapter 14**

**Q 14 (1)**

The power can be calculated from the following expression:

$$z_{\alpha} + z_{\beta} = \frac{\Delta \sqrt{n}}{s}$$

$\Delta$  = difference between the means of the two groups = 30 - 25 = 5 g/L

$n$  = number of subjects in the study = 40

$s$  = standard deviation = 10 g/L

Substitute these values to obtain  $z_{\alpha} + z_{\beta}$

$$z_{\alpha} + z_{\beta} = \frac{5 \sqrt{40}}{10} = \frac{5 \times 6.32}{10} = 3.16$$

Since the probability ( $P$ ) used as a decision level is 0.05, the corresponding  $z$  value (obtainable from tables) is 1.96 (the question only requires detection of a change – which could be either positive or negative – so both sides of the distribution are being used). Therefore,  $\alpha = 0.05$  and  $z_{\alpha}$  is 1.96. Substitute this value for  $z_{\alpha}$  and solve for  $z_{\beta}$ :

$$z_{\beta} = 3.16 - z_{\alpha} = 3.16 - 1.96 = 1.20$$

From tables of  $z$ , the value for  $\beta$  (i.e. proportion of area under the curve) corresponding to a  $z$  of 1.20 is 0.1151 (single sided probability).

Therefore power =  $(1 - \beta) = (1 - 0.1151) = \mathbf{0.88}$  or 88 % (2 sig figs)

**Q 14 (2)**

The expression for calculating sample size is:

$$n = [s (z_{\alpha} + z_{\beta}) / \Delta]^2$$

$s$  = standard deviation = 2.5 mmol/L

$\Delta$  = difference between the means

= Final cholesterol - Initial cholesterol

= (90 % x 7.5) - 7.5 (since cholesterol is required to be lowered by 10%)

$$= 6.75 - 7.5$$

$$= -0.75 \text{ mmol/L}$$

The required power is 90 %

$$\text{Therefore } (1 - \beta) = 0.9 \text{ and } \beta = 1 - 0.9 = 0.1$$

From tables the corresponding  $z$  value (i.e.  $z_{\beta}$ ) is 1.28 (one sided value).

The decision level used is a probability of 0.05 ( $\alpha$ ) with a corresponding  $z$  value for one side of the distribution (since we are required to detect a decrease in cholesterol) ( $z_{\alpha}$ ) of 1.64.

Substitute these values and solve for  $n$ :

$$\begin{aligned} n &= [2.5 (1.64 + 1.28) / -0.75]^2 \\ &= [2.5 \times 2.92 / -0.75]^2 \\ &= 9.73^2 \\ &= \mathbf{95} \text{ (2 sig figs)} \end{aligned}$$

## Chapter 15

### Q 15 (1)

$$\text{Recovery \%} = \frac{\text{Increase in concentration upon adding standard} \times 100}{\text{Concentration of standard added}}$$

Allowance must be made for dilution of both the sample and standard when they are mixed – since only 0.5 mL of the mixture is used for the assay.

Concentration of Y from urine in the mixture

$$= \frac{\text{Initial concentration} \times \text{Volume of urine (mL)}}{\text{Volume of mixture (mL)}}$$

Since initial concentration = 320 nmol/L

Mixture = 0.5 mL urine + 0.1 mL standard = 0.6 mL

$$\text{Concentration of Y from urine} = \frac{320 \times 0.5}{0.6} = 266.7 \text{ nmol/L}$$

Similarly concentration of standard in mixture =  $\frac{880 \times 0.1}{0.6} = 146.7 \text{ nmol/L}$

0.6

$$\begin{aligned}
 \text{Recovery (\%)} &= \frac{(\text{Measured concn} - \text{concn from urine}) \times 100}{\text{Standard added}} \\
 &= \frac{(405 - 266.7) \times 100}{146.7} \\
 &= \frac{138.3 \times 100}{146.7} \\
 &= \mathbf{94 \%} \quad (2 \text{ sig figs})
 \end{aligned}$$

**Q 15 (2)**

Calculate the expected concentrations in the mixture from the urine and the standard separately:

$$\text{Urine HCG in mixture} = \frac{8240 \times 450}{500} = 7416 \text{ IU/L}$$

$$\text{Standard HCG in mixture} = \frac{50000 \times 50}{500} = 5000 \text{ IU/L}$$

$$\begin{aligned}
 \% \text{ recovery} &= \frac{\text{HCG recovered} \times 100}{\text{HCG added}} \\
 &= \frac{(\text{Measured HCG in mixture} - \text{Expected HCG from urine}) \times 100}{\text{HCG added}} \\
 &= \frac{(12100 - 7416) \times 100}{5000} \\
 &= \frac{4684 \times 100}{5000} \\
 &= \mathbf{94 \%} \quad (2 \text{ sig figs})
 \end{aligned}$$

**Q 15 (3)**

Assuming the clearance of AFP follows first-order kinetics the rate equation is:

$$\ln C_{p_t} = \ln C_{p_0} - k_d \cdot t$$

$C_{p_t}$  = concentration of AFP after 21 days =  $C_{p_{21}}$   
 $C_{p_0}$  = initial concentration of AFP = 10200 U/L  
 $t$  = time period = 21 days  
 $k_d$  = elimination rate constant which can be calculated from the half-life ( $t_{1/2}$ ):

$$k_d = \frac{0.693}{t_{1/2}} = \frac{0.693}{5.5} = 0.126 \text{ days}^{-1}$$

Substitute these values and solve for  $C_{p_{21}}$ :

$$\ln C_{p_{21}} = \ln 10200 - 0.126 \times 21$$

$$\ln C_{p_{21}} = 9.230 - 2.646 = 6.584$$

$$C_{p_{21}} = \text{antilog}_e 6.584 = \mathbf{723 \text{ U/L}}$$

#### Q 15 (4)

The decay of a radioisotope follows first-order kinetics:

$$\ln A_t = \ln A_0 - k_d \cdot t$$

$A_t$  = activity at time  $t$  = 0.1 (10% of the initial value)

$A_0$  = initial activity = 1

$t$  = time for activity to fall to 10 % of initial value = ?

$k_d$  = decay constant which can be calculated from the given half-life ( $t_{1/2}$ ):

$$k_d = \frac{0.693}{t_{1/2}} = \frac{0.693}{21} = 0.033 \text{ days}^{-1}$$

Substitute these values and solve for  $t$ :

$$\ln 0.1 = \ln 1 - 0.033 \cdot t$$

$$-2.303 = 0 - 0.033 \cdot t$$

$$0.033 \cdot t = 2.303$$

$$t = \frac{2.303}{0.033}$$

$$= \mathbf{70 \text{ days}} \quad (2 \text{ sig figs})$$

An alternative approach is to use the expression:

$$\log_{10} AR = -0.30.N$$

$AR$  = ratio of final to initial activity = 0.1

$N$  = number of half-lives for this change to occur

Therefore  $\log_{10} 0.1 = -0.30.N$

$$\begin{aligned} -1 &= -0.30.N \\ N &= \frac{1}{0.30} = 3.333 \text{ half-lives} \end{aligned}$$

As  $t_{1/2} = 21$  days,  $t = 3.333 \times 21 = 70$  days

**Q 15 (5)**

Exponential growth obeys the first-order rate equation:

$$\ln Cp_t = \ln Cp_0 + k_d.t$$

If we take 1 as the initial concentration then a 10-fold increase will result in a concentration of 10.

$Cp_t$  = concentration after time  $t = 10$

$Cp_0$  = initial concentration = 1

$k_d$  = specific growth rate, which can be calculated from the doubling time ( $t_d$ ):

$$k_d = \frac{0.693}{t_d} = \frac{0.693}{2} = 0.3465 \text{ day}^{-1}$$

$t$  = time taken for concentration to increase 10-fold = ?

Substitute these values and solve for  $t$ :

$$\ln 10 = \ln 1 + 0.3465.t$$

$$2.303 = 0 + 0.3465.t$$

$$0.3465.t = 2.303$$

$$t = \frac{2.303}{0.3465} = \mathbf{6.6 \text{ days}} \text{ (2 sig figs)}$$

Alternatively the following expression can be used:

$$\log_{10} CR = 0.30 N$$

where  $CR$  is the concentration ratio = 10:1

$N$  = number of doubling times required to achieve this ratio

Substitute  $CR = 10$  and solve for  $N$ :

$$\begin{aligned} \log_{10} 10 &= 0.30 N \\ 1 &= 0.30 N \\ N &= \frac{1}{0.30} = 3.333 \end{aligned}$$

$$\begin{aligned} \text{Therefore time taken} &= N \times t_d \\ &= 3.333 \times 2 \\ &= 6.7 \text{ days} \end{aligned}$$

**Q 15 (6)**

$$\begin{aligned} \text{Nitrogen excretion (g/24h)} &= \frac{\text{Urea excretion (mmol/24h)} \times 28}{1000} \\ &= \frac{580 \times 28}{1000} \\ &= 16.24 \text{ g/24g} \end{aligned}$$

$$\begin{aligned} \text{Nitrogen balance (g/24h)} &= \text{Nitrogen intake (g/24h)} - \text{Nitrogen excretion (g/24h)} \\ &= 11.8 - 16.24 \\ &= -4.44 \text{ g/24h} \end{aligned}$$

If 20% is added to the urinary excretion to allow for other urinary losses and a further 2 g/day added to allow for losses by other routes then the nitrogen excretion becomes:

$$\begin{aligned} \text{Corrected nitrogen excretion} &= [\text{Urea nitrogen excretion (g/24h)} \times 1.2] + 2.0 \\ &= (16.24 \times 1.2) + 2.0 \\ &= 19.49 + 2.0 \\ &= 21.49 \text{ g/24h} \end{aligned}$$

and the corrected nitrogen balance becomes:

$$\begin{aligned} \text{Corrected nitrogen balance (g/24h)} &= 11.8 - 21.49 \\ &= \mathbf{-9.7 \text{ g (Negative balance)}} \end{aligned}$$

**Q 15 (7)**

$$M_1 \times V_1 = M_2 \times V_2$$

$$\begin{aligned} M_1 &= \text{molar concentration of HCl in gastric fluid} = ? \\ V_1 &= \text{volume of gastric fluid used in titration} = 5 \text{ mL} \\ M_2 &= \text{molar concentration of NaOH} = 0.1 \text{ M} \\ V_2 &= \text{titre of NaOH} = 2.5 \text{ mL} \end{aligned}$$

$$M_1 \times 5 = 0.1 \times 2.5$$

$$M_1 = \frac{0.1 \times 2.5}{5}$$

$$= 0.05 \text{ M}$$

Since the answer is required in mmol multiply by 1000:

$$\text{HCl concentration} = 0.05 \times 1000 = 50 \text{ mmol HCl/L gastric fluid}$$

Divide by 1000 to give the acid output per mL of gastric fluid then multiply by the total volume of gastric fluid collected (27 mL) to obtain the total output of acid:

$$\text{Total HCl output} = \frac{50 \times 27}{1000} = 1.35 \text{ mol HCl / 27 mL gastric fluid}$$

Since the gastric fluid was collected over 30 min, multiply this result by 2 to obtain the amount of HCl secreted in 1 h:

$$\begin{aligned} \text{Rate of HCl excretion} &= 1.35 \times 2 \\ &= \mathbf{2.7 \text{ mmol/h}} \end{aligned}$$

**Q 15 (8)**

First calculate the fatty acid concentration in the homogenate:

$$M_1 \times V_1 = M_2 \times V_2$$

## WORKED ANSWERS TO FURTHER QUESTIONS

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$M_1$  = molar concentration of fatty acids in homogenate = ?  
 $V_1$  = volume of homogenate titrated = 10 mL  
 $M_2$  = molar concentration of NaOH used in titration = 0.05 M  
 $V_2$  = titre of 0.05 M NaOH = 48 mL

$$M_1 \times 10 = 0.05 \times 48$$

$$M_1 = \frac{0.05 \times 48}{10} = 0.24 \text{ mol/L}$$

Multiply by 1000 to convert this concentration to mmol/L:

$$\begin{aligned} \text{Fatty acid concentration} &= 0.24 \times 1000 \\ &= 240 \text{ mmol fatty acid/L homogenate} \end{aligned}$$

Multiply by the total volume (in litres) of the homogenate to obtain the total fatty acid output over the 5-day collection period:

$$\text{Fatty acid output} = 240 \times 1.50 = 360 \text{ mmol fatty acid/5 days}$$

Division by 5 gives the daily fatty acid output:

$$\text{Daily fatty acid output} = \frac{360}{5} = 72 \text{ mmol fatty acid/24h}$$

Assuming that all the fatty acids were liberated from triglyceride then division by 3 gives the total fat output:

$$\text{Fat output} = \frac{72}{3} = 24 \text{ mmol fat/24h (as triglyceride)}$$

### Q 15 (9)

Divide the drug peak area by the internal standard peak area to give the peak height ratio (PHR) for both standard and patient:

Sample	Peak area		PHR
	Internal standard	Drug	
Standard (200 nmol/L)	50000	200000	4.00
Patient	40000	150000	3.75

Assuming that the PHR is directly proportional to concentration then concentration of the drug in the patient sample can be calculated from the relationship:

$$\frac{\text{PHR}_{\text{Patient}}}{\text{Concentration}_{\text{Patient}}} = \frac{\text{PHR}_{\text{Standard}}}{\text{Concentration}_{\text{Standard}}}$$

Substitute the PHR values and standard concentration to obtain the drug concentration in the patient sample:

$$\begin{aligned} \frac{3.75}{\text{Concentration}_{\text{Patient}}} &= \frac{4.00}{200} \\ \text{Concentration}_{\text{Patient}} &= \frac{3.75 \times 200}{4.00} \\ &= \mathbf{188 \text{ nmol/L}} \quad (3 \text{ sig figs}) \end{aligned}$$

**Q 15 (10)**

$$\text{Frequency of allele A} = p = 0.65$$

$$\text{Frequency of allele B} = q = 0.35$$

The possible combinations are AA, AB and BB.

If the conditions for the Hardy-Weinberg equilibrium are met then the frequencies of the three genotypes are:

$$AA = p^2 = 0.65^2 = 0.4225$$

$$AB = 2pq = 2 \times 0.65 \times 0.35 = 0.455$$

$$BB = q^2 = 0.35^2 = 0.1225$$

$$\text{Therefore \% heterozygotes (AB)} = 0.455 \times 100 = \mathbf{45.5 \%}$$

$$\text{and \% homozygotes (AA and BB)} = (0.4225 + 0.1225) \times 100 = \mathbf{54.5 \%}$$

(or simply subtract 45.5 from 100)

**Q 15 (11)**

Let the dominant gene be *A* and the recessive gene *a*. As the inheritance of the disease is autosomal recessive only the homozygous recessive genotype (*aa*) expresses the disease.

WORKED ANSWERS TO FURTHER QUESTIONS

$$\text{The incidence of the recessive disorder } (aa) = 1 \text{ in } 2500 = \frac{1}{2500} = 0.00040$$

$$\text{Incidence of carriers } (Aa) = 1 \text{ in } 50 = \frac{1}{50} = 0.020$$

Since the total of all frequencies must equal 1, the frequency of the remaining homozygous dominant genotype, AA (which does not express disease nor have carrier status) can be calculated by difference:

$$\begin{aligned} \text{Incidence of AA} &= 1 - (0.00040 + 0.020) \\ &= 1 - 0.0204 \\ &= 0.9796 \end{aligned}$$

To summarize the *observed* frequencies of the three genotypes are:

Genotype	AA	Aa	aa
Observed frequency	0.9796	0.020	0.00040

Next calculate the expected frequencies if the Hardy-Weinberg equilibrium is operating starting with the frequency of *aa* which is the frequency of the disorder i.e. 1 in 2500.

$$\text{Frequency of affected individuals } (aa) = 0.00040 = q^2$$

$$\begin{aligned} \text{Therefore } q &= \sqrt{q^2} = \sqrt{0.00040} = 0.020 \\ \text{Since } p + q &= 1 \end{aligned}$$

$$p = 1 - q = 1 - 0.020 = 0.98$$

Using these values for *p* and *q* the frequencies of the other two genotypes can be calculated:

$$\text{Frequency of } Aa = 2pq = 2 \times 0.98 \times 0.020 = 0.0392$$

$$\text{Frequency of } AA = p^2 = 0.98^2 = 0.9604$$

Tabulate this data then calculate  $X^2$ :

$$X^2 = \sum (O - E)^2/E$$

Genotype	Frequency		(O - E)	(O - E) <sup>2</sup>	(O - E) <sup>2</sup> /E
	Observed	Expected			
AA	0.9796	0.9604	0.0192	0.00036864	0.000383840

<i>Aa</i>	0.02	0.0392	-0.0192	0.00036864	0.009404082
<i>aa</i>	0.0004	0.0004	0	0	0
Total:	1.0000	1.0000	0	0.0007372	0.0097878

$X^2$  is the sum of all the values in the final column = **0.010** (2 sig figs)  
 Normally the degrees of freedom would be  $3 - 1 = 2$ . However, since one of the observations (frequency of disease) was used to estimate the expected values, a further degree of freedom is lost leaving only one.

From tables, the value for  $P$  when  $X^2 = 0.010$  is somewhere between 0.95 and 0.90. Therefore there is no significant difference between the observed and expected frequencies so that **the data fit the Hardy-Weinberg equilibrium.**

**Q 15 (12)**

Sample	Duplicate cpm	
	1	2
TC	15100	15900
NSB	320	380
TB	11350	11650
0.2 nmol/L standard	10320	10980
0.4 “ “	9250	8340
0.8 “ “	6782	6630
1.2 “ “	5104	5890
2.4 “ “	3700	3430
4.8 “ “	1350	1650
Patient serum	4350	5000

$$\text{Mean NSB} = (320 + 380)/2 = 350 \text{ cpm}$$

$$\text{Mean TB} = (11350 + 11650)/2 = 11500 \text{ cpm}$$

$$B_0 = \text{Mean TB} - \text{Mean NSB} = 11500 - 350 = 11150 \text{ cpm}$$

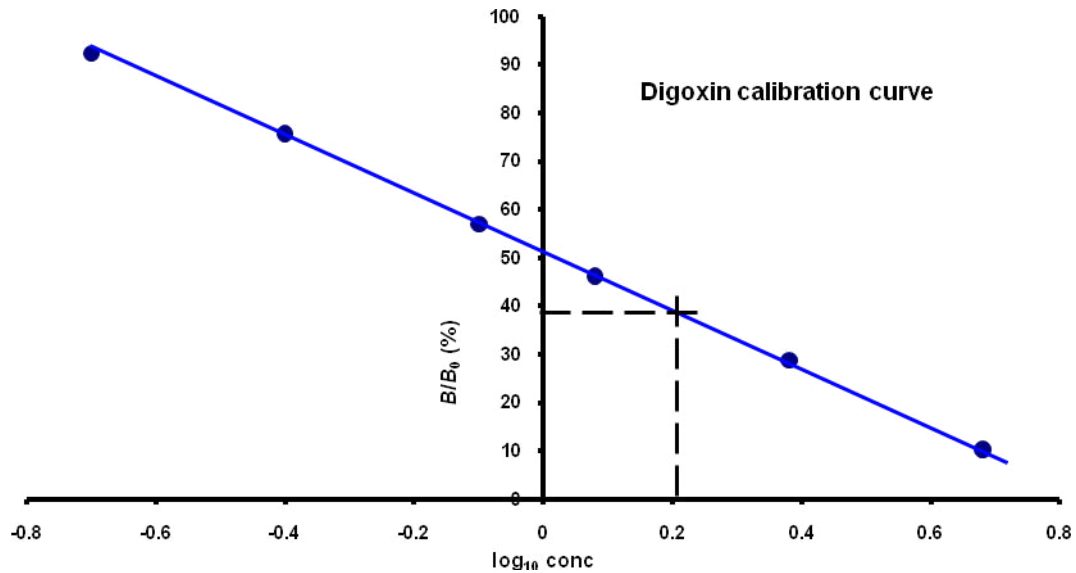
Calculate the mean for each pair of duplicates then  $B/B_0$  (%) using the formula:

$$B/B_0 (\%) = \frac{(\text{Mean cpm standard/sample} - \text{Mean NSB}) \times 100}{B_0}$$

These calculations are performed in the following table:

WORKED ANSWERS TO FURTHER QUESTIONS

Sample (%)	Conc	$\log_{10}$ conc	Duplicate cpm	Mean cpm	Mean - NSB	B/B <sub>0</sub>
NSB	-	-	320 380	350		
TB	0	-	11350 11650	11500	1150	100
Standard	0.2	-0.70	10320 10980	10650	10300	92.3
"	0.4	-0.40	9250 8340	8795	8445	75.7
"	0.8	-0.10	6782 6630	6706	6356	57.0
"	1.2	0.08	5104 5890	5497	5147	46.2
"	2.4	0.38	3700 3430	3565	3215	28.8
"	4.8	0.68	1350 1650	1500	1150	10.3
Serum	?	?	4350 5000	4675	4325	38.8



From calibration curve  $\log_{10}$  conc when serum  $B/B_0$  (%) = 0.21

Serum digoxin (nmol/L) =  $\text{antilog}_{10} 0.21 = 1.6 \text{ nmol/L}$