The Association for Clinical Biochemistry and Laboratory Medicine

SWW Regional Training Day 18th July 2014, Southmead Hospital, Bristol

Organisers: Dr Moya O'Doherty & Dr Tim McDonald

Training Day

0900-0930	Group Calculations
0930-1030	Case presentations: Clinical cases from the Bristol Trainees 1. ALP isoenzymes by Rebecca Rolfe 2. Phaeochromocytoma investigations by Fran Mills 3. An interesting case of renal stone disease by Chris Stockdale
1030-1115	Journal Interpretation
1115-1130	Coffee
1130-1230	Wet practical: Recent experiences of the wet practical & how to prepare. Evaluation and introduction of a new POCT device Nicola Pullan (Clinical scientist , RUH)
1230-1330	LUNCH
1330-1430	How to write a business case. Mr David Gibbs IM&T Project Manager Severn Pathology
1430-1530	Surviving the FRCPATH part 2 viva Vicki Powers (Clinical Scientist, BRI)
1530-1545	Tea
1545- 1645	HPLC Method, optimization and tricks of the trade Steve Jones (BMS, NBT) HPLC applications Ann Bowron (Clinical scientist , BRI)
1645	Close and feedback

Regional training sessions aims

- Trainee lead
- Twice a year
- Network
- Peer support
- Ideas for how to get the best out of training
- Tips for exams
- Share each others strengths and experiences
- To train in some of the softer skills such as communication or leadership skills

Calculations !! With no coffee!!

- **<u>Categories to cover over the next 2-3 years:</u>**

- 1.Dilutions, manipulation, spectrophotometry, recovery, pH, chromatograms
- 2. Pharmacokinetics, elimination of tumour markers, radioactive decay
- 3. Enzymology and competitive binding assays
- 4. Fluids , electrolytes, osmolality and renal
- 5. Statistics

Dilutions and manipulations

A.

$$\text{Moles} = \frac{\text{Mass}}{\text{MW}}$$

B. Preparation of solutions :

1. Density (g/ml) = $\frac{\text{weight (g)}}{\text{volume (ml)}}$

2. Conc impure material = $\frac{\text{Required Conc x \% purity}}{100}$

3. OR, To prepare a solution containing a required concentration then weight of solid required in g/l = $\frac{\text{concentration of given (impure) material x 100}}{\text{\%impurity}}$

4. In preparing solutions you may also require :

Initial volume x initial concentration = Final volume x Final concentration

C. Calculate Molar concentrations of ions in solution:

1. Calculate molarity
2. Calculate how the molecule dissociates
3. Calculate dilution factor of mixing solution
4. Apply dilution factor to molarity

Preparation of a solution

Concentrated sulphuric acid (SG 1.84) is 96% by weight H_2SO_4. Calculate the volume of concentrated acid required to prepare 1L of 0.1M H_2SO_4.

Preparation of a solution

1. To calculate volume required uses formula B from above.

Density = weight/ volume

- Therefore volume = weight/ density.

Specific gravity (SG) is the same as density so this is given (NB its units are g/ml)

- And we can calculate weight from concentration given in moles/L…

Mass = MW x Moles

MW = (2x1)+ 32 + (4 x 16) = 98

Mass = 98 x 0.1 = 9.8g/l

2. However we also need to adapt to impurity (96%)

Therefore we use the second part of B….

$$\frac{\text{Weight} \times 100}{\%\text{impurity}} = \frac{\text{Weight 9.8 g/l (calculated above)} \times 100}{96\%} = 10.2 \text{ g/L}$$

3. Therefore lastly calculate the volume required

Density = 10.2(weight) / 1.84(SG) = 5.5 mls

Dilutions & manipulations

How many grams of anhydrous disodium hydrogen phosphate will be needed to prepare 2 litres with a concentration of 50mmol/l?

Instructions for preparing 1L of a phosphate buffer state that 12.00g of anhydrous sodium dihydrogen phosphate are required. If this material is unavailable how many grams of sodium dihydrogen phosphate dihydrate would be required?

How much to weigh out?

A) How much to weigh out?

Anhydrous disodium hydrogen phosphate = Na_2HPO_4

- MW = (2 x 23) + 1 + 31 + (16 x 4) = 142
- 50mmol/l = 0.05moles/L and if 2 Litres required multiply by 2 = 0.1moles

- Mass required = MW x Moles = 142 x 0.1 = 14.2 g

B) How much to weigh out of an alternative?

Note change in MW between formulae –

- anhydrous dihydrogen phosphate = NaH_2PO_4 (MW = 23 + 2 + 31 + (4x16) = 120)
- sodium dihydrogen phosphate dehydrate = $NaH_2PO_4.2H_2O$ (MW = 23 + 2 + 31 + 4 + 32 = 156)

- The concentration that 12g NaH_2PO_4 provided is 0.1 mole/l
- So for $NaH_2PO_4.2H_2O$ to provide the same concentration , work backwards :
- Mass = MW x mole = 156 x 0.1 = 15.6 g/l

Spectrophotometry

A standard curve for a plasma glucose method was set up by preparing a series of dilutions of a stock glucose standard (containing 50mmol glucose /L) and measuring the absorbance at 500nm in a cuvette using a blank with a zero glucose concentration to zero the instrument. The following readings were obtained:

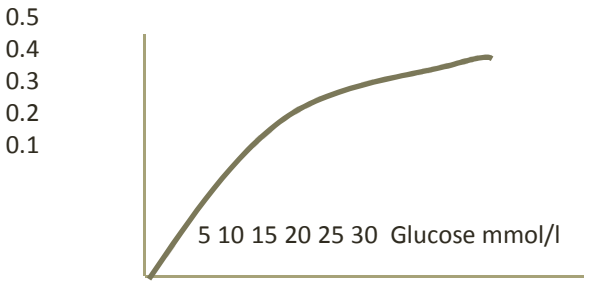
Glucose (mmol/l)	5	10	15	20	25	30
Abs	0.102	0.203	0.305	0.375	0.410	0.432

Does the method obey Beers Law?

What glucose conc corresponds to an absorbance of 0.250?

2 Options

1. plot a calibration curve



- So yes up to 15mmol/l it obeys Beers Law
- The glucose concentration can be read off the graph from the absorbance given = 10mmol/l
- The book actually suggested using A = 0.250 which gives a conc 12.5 mmol/l

2. Calculate the equation which describes the curve

- A = Intercept + (c x slope)
- Where intercept is either the blank or the Absorbance at 0 concentration i.e. here is 0 mmol/l
- And slope is given by the change in Absorbance over 1mmol/l change in concentration
- i.e. Absorbance at 10mmol/l – 5mmol/l ie 0.203 – 0.102 = 0.102 divided by 5 = 0.02

- Using this given the absorbance 0.205
- 0.205 = 0 + (c x 0.02) = 10.25mmol/l
- Again using the books conc 0.250 = 12.5mls

Spectrophotometry

Lactate can be measured enzymatically by oxidation to pyruvate

$$\text{L-lactate} + \text{NAD} \xrightarrow{\text{LDH}} \text{Pyruvate} + \text{NADH} + \text{H+}$$

(NADH has a molar absorption coefficient of 6.22 x 103 L.mol-1.cm-1)

Method

To 2.0 mL buffer, add 0.1 mL sample, 0.2 mL NAD+ (27 mmol/L) and 30 µL LDH solution.

Result

The absorbance change (relative to a reagent blank, using a standard 1 cm cuvette) in the assay was 0.82.

Calculate the lactate concentration in this sample.

How much lactate?

- Each mol of lactate produces 1 mol of NADH
- Therefore NADH = LACTATE

Using A= abc

- $0.82 = 6.22 \times 103 \times \boldsymbol{c} \times 1 = 1.32 \times 10^{-4}$
- As lactate is usually reported in mmol/L, not mol/L, multiply by 1,000
- Lactate concentration = 1.32 x 10-4 x 103 = 0.132 mmol/L

- This is the lactate concentration in the total reaction mixture. To calculate the concentration in the sample ie before dilution multiply by the reaction volume and divide by the sample volume:

$$\textbf{Sample lactate (mmol/L)} = \frac{\textbf{Reaction mixture lactate (mmol/L) x Total vol}}{\textbf{Sample vol}}$$

Reaction mixture consists of:
Buffer = 2.0 mL
Sample = 0.10 mL
NAD+ = 0.2 mL
LDH = 0.030 mL (= 30µL)
Reaction volume = 2.33 mL

$$\textbf{Sample lactate} = \frac{\textbf{0.132 x 2.33}}{\textbf{0.10}} = \textbf{3.1 mmol/L (2 sig figs)}$$

pH

You need to make up a phosphate buffer with a pH of 7.4 and a total phosphate concentration of 50 mmol/L.

Calculate the amounts of sodium dihydrogen phosphate and disodium monohydrogen phosphate that need to be weighed into 1 litre of water, given that the pKa is 6.82.

(atomic weights: Na 23, P 31).

- First calculate concentration of the two phosphate molecules required by using Henderson Hasselbach equation
- Then calculate weights using MW

1. $H_2PO_4 \longrightarrow HPO_4^{2-} + H^+$

- Fit into HH equation:

pH (7.4) = pKa (6.82) + log (Base – HPO_4/ acid – H_2PO_4)

- so rearranging 7.4-6.82 = log(HPO_4/ H_2PO_4)
- Take antilogs =

antilog0.58= 3.8

3.8 = (HPO4/H2PO4)

2. We have been given a concentration that we need , a total phosphate conc = 50mmol/l = 0.05mol/l

- So total PO4 = 0.05mol/l= HPO4 + H2PO4
- Rearranging the equation (0.05-H2PO4)= HPO4

If the two previous equations are combined …..

3.8 = (0.05-H2PO4)/(H2PO4)

3.8(H2PO4) = 0.05-H2PO4

3.8(H2PO4) + H2PO4 = 0.05

4.8(H2PO4) = 0.05

H2PO4 = 0.01 mol/l

3. Then substitute to find HPO4:

0.05 = 0.01 + HPO4

HPO4 = 0.04mol/l

- So then calculate MW

A)NaH_2PO_4 = 120

B)Na_2HPO_4 = 142

Mass = MW x Mole/l

A) = 1.2g

B)= 5.6g