CMS COLLEGE OF SCIENCE AND COMMERCE (AUTONOMOUS),

Department of Biotechnology

Ist Year M. Sc., Biotechnology

Biochemistry practical notes – 1

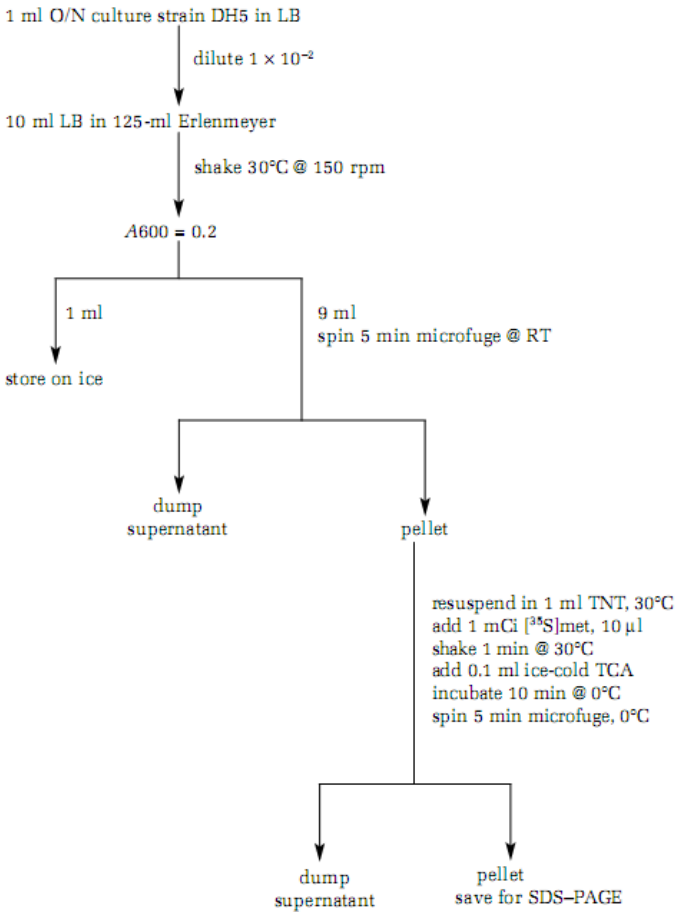
Preparation of Flow charts:

Prepare a flow chart in ink (not pencil) prior to each experiment and include it in your lab report. You may not participate in the laboratory exercise without a flow chart.

A flow chart outlines each procedure step by step and guides you through the experiment. If you modify a procedure during the course of an experiment, note these changes on the flow chart. Record observations on a separate page as you work.

Flow charts contain words, symbols, diagrams and arrows. Begin your flow chart by listing the first step of the procedure. Use an arrow to connect the first step to the second, and so forth. The arrows indicate major procedural steps and direct your attention to the next task. The steps taken to proceed from one intermediate to the next are listed beside each arrow. A sample flow chart appears on the next page.

A. Sample Flow Chart



Cleaning Laboratory Glassware:

The results of your experiments will depend, to a great extent, on the cleanliness of your equipment. There are at least two reasons for this.

a) Many of the chemicals and biochemicals will be used in milligram or microgram amounts. Any contamination, whether on the inner walls of a beaker, in a pipette, or in a glass cuvette, could be a significant percentage of the total experimental sample.

b) Many biochemicals and biochemical processes are sensitive to one or more of the following common contaminants: metal ions, detergents, and organic residues. In fact, the objective of many experiments is to investigate the effect of a metal ion, organic molecule, or other chemical agent on a biochemical process. Contaminated glassware will virtually ensure failure.

Glassware

Many contaminants, including organics and metal ions, adhere to the inner walls of glass containers. Washing glassware, including pipettes, with dilute detergent (0.5% in water) followed by five to ten water rinses is probably sufficient for most purposes. The final rinse should be with distilled or deionized water. Metal ion contamination can be greatly reduced from glassware by rinsing with concentrated nitric acid followed by extensive rinsing with purified water.

Dry equipment is required for most processes carried out in biochemistry laboratory. When you needed dry glassware in organic laboratory, you probably rinsed the piece of equipment with acetone, which rapidly evaporated, leaving a dry surface. Unfortunately, that surface is coated with an organic residue consisting of nonvolatile contaminants in the acetone. Since this residue could interfere with your experiment, it is best to refrain from acetone washing. Glassware and plastic ware should be rinsed well with purified water and dried in an oven designated for glassware, not one used for drying chemicals.

Quartz and Glass Cuvettes

Never clean cuvettes or any optically polished glassware with ethanolic KOH or other strong base, as this will cause etching. All cuvettes should be cleaned carefully with 0.5% detergent solution, in a sonicator bath, or in a cuvette washer.

Exercise 1: Biochemical calculations for Biotechnology and Molecular biology:

Metric prefixes in Laboratory:

Name	Abbreviation	Power of 10
Giga	G	10^9
Mega	M	10^6
Kilo	k	10^3
Milli	m	10^{-3}
Micro	µ	10^{-6}
Nano	n	10^{-9}
Pico	p	10^{-12}
Femto	f	10^{-15}
Atto	a	10^{-18}

Exponents and Scientific Notation:

An exponent is a number written above and to the right of (and smaller than) another number (called the base) to indicate the power to which the base is to be raised. Exponents of base 10 are used in scientific notation to express very large or very small numbers in a shorthand form. For example, for the value 10^3, 10 is the base and ^{3} is the exponent. This means that 10 is multiplied by itself three times (10^3 = 10 x 10 x 10 = 1000). For numbers less than 1.0, a negative exponent is used to express values as a reciprocal of base 10. For example,

$$10^{-3} = \frac{1}{10^3} = \frac{1}{10 \times 10 \times 10} = \frac{1}{1000} = 0.001$$

Adding and subtracting numbers written in scientific notation:

When adding or subtracting numbers expressed in scientific notation, it is simplest first to convert the numbers in the equation to the same power of 10 as that of the highest exponent. The exponent value then does not change when the computation is finally performed.

Conversion calculations in Biotechnology Laboratory:

1. Converting percentage solutions to Molarity (% to M):

eg., What is the molar concentration of a 10% NaCl solution?
10g/100ml = xg/1000ml
10 X 1000 = 100 X x
10000/100 = x
100 = x

100g/1000 ml
Molecular weight od Nacl = 58.44
xM/100g = 1M/58.44g
x = 100/58.44 = 1.71 M

2. Dilusion of Molar solutions:

eg., From 1M Tris solution, how is a 400ml of 0.2 M Tris prepared?
1M X (xml/400ml) = 0.2 M
X = 0.2 X 400
X = 80

3. Converting molarity to percentage solutions:

eg., Convert 2.5M NaCl as % solution?
Molarity = Grams/Litre
Molecular weight of NaCl = 58.44
Calculate how many grams of NaCl in 2.5 ml solution
58.44/1M = xg/2.5M
(58.44) x (2.5) = 1 x xg
146.1 g = x

% is concentration per 100 ml.

146.1 g/1000 ml = x g/100ml

146.1 g x 100 = x x 1000

(146.1 x 100) /1000 = x

14.6 g = x

14.6%

DILUTION:

C1V1 = C2V2

C1 – Initial concentration of stock solution

V1 – The amount of stock solution taken to perform dilution

C2 – The concentration of the diluted sample

V2 – The final, total volume of the sample

Dimentional analysis:

Starting concentration X Conversion factor X (Unknown volume/Final volume) = Desired concentration

20% X (1ml/1000ml) X (xml/2ml) = 5%

(20%) X x/ (2000) = 5%

X = (5%) (2000)/ (20%) = 500

Concentrations by a factor of X:

Buffers of agarose and polyacrylamide gel electrophoresis are prepared as solutions of 10 fold (10X) more concentrated than their standard running concentration (1X).

eg., To prepare 1000 ml of 1X Trisborate EDTA buffer (TBE) solution to 900 ml of distilled water.

10X buffer X (nml/1000ml) = 1X Buffer

10xn/1000 = 1X

Multiply by 1000,

10xn=1x1000

10xn = 1x1000

n=100

Problem no1:

How to prepare 640 ml of 0.5X buffer prepared from an 8X stock?

By using the above mentioned formula:

8X buffer x (nml/ 640 ml) = 0.5X buffer

(8xn)/ 640 = 0.5

Multiply by 640,

(8X)xn = (0.5X) x 640 = 320X

n = 320/8 = 40

Percentage solution preparations:

How can the following solutions be prepared?

a. 100 ml of 40% (w/v) polyethylene glycol (PEG) 8000?

Weigh out 40 grams of PEG,

Add 60 ml (approx) to dissolve it completely.

Made up to 100 ml using a measuring cylinder.

b. 47 ml of a 7% (w/v) solution of NaCl.

7% of 47 ml must be calculated

47 x (7/100) = 47 x 0.07 = 3.29

To prepare 47 ml of 7% NaCl, weigh out 3.29 g of NaCl and dissolve the crystals in some amount of distilled water and made up to 47 ml using measuring cylinder.

c. 200 ml of a 95% (v/v) solution of ethanol.

Calculated by multiplying 0.95

95/100 x (200) = 0.95 x 200 = 190 ml alcohol made up to 200 ml with water.

Dilution of Percentage solutions:

30 ml of 70% ethanol to be prepared from 95% ethanol stock solution.

(95/100) X (xml/30ml) = 70/100

95x/3000 = 70/100

Multiply by 3000,

95x = (70/100) X (3000)/100

95x = 2100

Multiply by 1/95,

(1/95) x 95x = 100/95

x = 2100/95 = 22

22ml of 95% ethanol stock made up to 30 ml using a measuring cylinder.

Calibration of Micropipettes in Laboratory:

Principle:

Specific gravity of water.

Requirements:

1. Micropipettes with clean tips (to be calibrated)
2. Analytical electronic balance (0.0000g model)
3. Pure, sterile deionized or double distilled water
4. Clean petriplate.

Steps:

Minimal volume:

1. Adjust the P200 pipette at lowest setting (20µL).
2. Carefully pipette deionized water in to the petriplate in digital analytical balance (0.0000g digit model).
3. Record the balance reading in a table.
4. Zero (Tare) the balance reading.
5. Repeat the above steps (1-4) three times.

Middle volume:

1. Adjust the P200 pipette at middle setting (100µL).
2. Carefully pipette deionized water in to the petriplate in digital analytical balance (0.0000g digit model).
3. Record the balance reading in a table.
4. Zero (Tare) the balance reading.
5. Repeat the above steps (1-4) three times.

Maximum volume:

1. Adjust the P200 pipette at highest setting (200µL).
2. Carefully pipette deionized water in to the petriplate in digital analytical balance (0.0000g digit model).
3. Record the balance reading in a table.
4. Zero (Tare) the balance reading.
5. Repeat the above steps (1-4) three times.

Accepted accuracy % for each volumes of micropipettes:

Details of Volume	**P10**	**P20**	**P200**	**P1000**
Lower Volume accuracy % = 1.25%	0.5µL	2µL	20µL	200µL
Middle Volume accuracy % = 1.10%	5µL	10µL	100µL	500µL
Higher Volume accuracy % = 0.40%	10µL	20µL	200µL	1000µL

Calculation of Accuracy %:

Calculation of accuracy % for micropipettes								
Minimal Volume			**Middle volume**			**Maximum volume**		
No	**Grams**	**Corresponding volume in µL**	**No**	**Grams**	**Corresponding volume in µL**	**No**	**Grams**	**Corresponding volume in µL**
1			1			1		
2			2			2		
3			3			3		
4			4			4		
Average Volume			Average Volume			Average Volume		
Accuracy %		(Volume-Average volume)/Volume = 1.25 %	Accuracy %		(Volume-Average volume)/Volume = 1.10 %	Accuracy %		(Volume-Average volume)/Volume = 0.40 %

Formula:

Accuracy % = [(Pipette volume (in µL) – Average volume value)/Pipette volume in µL] × 100
