Culture medium and Making Solutions

Lab 4

Ms. Amal Al-ayedh

Culture medium

- The culture medium must provide all the essential nutrients, vitamins, cofactors, metabolic substrates, amino acids, inorganic ions and trace elements needed to support cellular functions and the synthesis of new cells.

- Choice of media depends on the type of cell being cultured.

Types of culture media

1- Basal medium: There are four main categories of basal medium for mammalian cells:

(a) Eagle's minimal essential medium (EMEM) and its derivatives, for example these include alpha minimal essential medium (*αMEM), Dulbecco's modified Eagle's medium (DMEM).*

(b) Media designed at the Roswell Park Memorial Institute (RPMI).

(c) Basal media designed for use with sera, for example Fischer's.

(d) Basal media designed for use in serum-free formulation, for example Iscove's modified Dulbecco's medium (IMDM)

Dulbecco's Modified Eagle : is a widely used basal medium for supporting the growth of many different mammalian cells

Roswell Park Memorial Institute (RPMI) 1640 medium was originally developed to culture human leukemic cells in suspension and as a monolayer

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Basal media are composed of the following sets of components:

1- *A balanced salt solution*

Balanced salt solutions comprise a mixture of inorganic salts designed to:

- maintain osmotic pressure
- maintain physiological pH
- maintain membrane potential
- act as cofactors for enzyme reactions.

The main inorganic ions used are Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-}, PO_4^{3-} and HCO_3^- .

In addition, glucose is often added as an energy source for the cells.

2- A buffering system

buffering is required in order to maintain the cells within their normal pH range and limit the effects of acidic waste products generated by the cell, most notably (e.g.) bicarbonate/CO2 and lactic acid.

3- An energy source

Carbohydrates, glucose and sometimes other sugars such as maltose, sucrose, fructose, galactose and mannose may also be included.

4- Amino acids

- Amino acid glutamine , act as an energy source and as an amine donor in biosynthesis.
- Also require a supply of the essential amino acids (those not synthesized in the body), such as Cysteine and tyrosine.

5- Vitamins

- Several vitamins of the B group are essential for cell growth and multiplication, such as *para-amino benzoic acid, folic* acid, riboflavin and thiamine.

6- Other components

For examples, trace elements such as selenium, iron, zinc and copper.

7- Antibiotics

Types of culture media

ii. Serum This is the liquid excluded from the clot when blood is allowed to coagulate. Because it is rich in growth factors, and has a low antibody concentration, the most widely used serum in mammalian cell culture is FBS, followed by newborn calf serum, calf serum and adult bovine serum. Horse, human and other sera are also used for some purposes.

It consists of proteins, growth factors and hormones.

iii. Complete medium

Complete medium will be a mixture of a basal medium and serum, for example DMEM + 10% FBS.

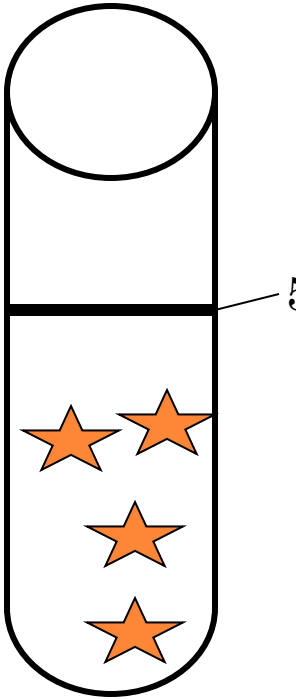
Making Solutions

Key terms

- **Solute**: substances that are dissolved
- **Solvent**: substances in which solutes are dissolved (often times this is water or a buffer)
- **Concentration**: amount per volume
- **Solution**: a homogeneous mixture in which one or more substances are dissolved in another.

Concentration

Cnocentration of a solution is the ratio between solute amount and the solvent (or solution) amount.



If each star represents 1 mg of NaCl,
what is the total amt. of NaCl in the tube?

4 mg

What is the concentration of NaCl in the tube in mg/mL?

4 mg / 5mL = 0.8 mg/mL

Weight per Volume

- Simplest way of expressing a concentration

- Example: 2mg / mL
  - 2mg is the weight of something in 1 mL of solvent

Percents

- May be expressed as weight per volume which is grams per 100 mL (w/v%)
  - Example: 20 grams of KCL in 100 mL of solvent is a 20% solution (w/v)

1- Percentage (%)

Example (1)

To prepare 10% alcohol

10 ml of absolute alcohol

complete volume with distilled water to 100 ml (V/V)

Example (2)

To prepare 2% NaCl

2 gm of NaCl

dissolve in 10 ml water

complete volume with distilled water
to 100 ml (W/V)

Practice Problem

- How would you prepare 500 mL of a 5% (w/v) solution of NaCl?
  - 5% = 5g/100mL
  - $\frac{5}{100} = \frac{x}{500mL}$

100x = 2500 x=25grams of NaCl

  - Weigh out 25 grams of NaCl and add it to a 500 mL volumetric flask. Add approximately 400 mL and mix. Once dissolved, QS to 500 mL. Cap and store.

Percents continued

- May be expressed in volume percent which is (v/v):

$$\frac{\text{mL of solute}}{100\ \text{mL of solvent}}$$

- May be expressed in weight per weight which is (w/w):

$$\frac{\text{grams solute}}{100\ \text{grams solvent}}$$

Practice Problem

- How would you make 500 grams of a 5% solution of NaCl by weight (w/w)?

% strength is 5% w/w, total weight is 500 grams

$$\frac{5 \text{ grams}}{100 \text{ grams}} \times 500 \text{ g} = 25 \text{ grams of NaCl}$$

500 grams total weight – 25 grams NaCl = 475 grams of solvent needed

Weigh out 25 grams of NaCl and add it to 475 grams of water.

The density of water is exactly 1 gram per mL.

1 liter **of pure water** weigh 1000 grams or 1 kilogram.

1 mililiter **of pure water** weigh 1 gram.

2- Molecular (Molar) Solution (M)

To prepare a molar solution, **molecular weight (in gm)** is dissolved in **1 Liter (L) of solution**.

1 L = 1000 ml.

Example (1)

How to prepare molar solution of NaCl ?

- Calculate molecular weight (Mw) of NaCl as follow:

Mw = 23 + 35.5 = 58.5 g/mole

Dissolve 58.5 gm of NaCl in a little distilled water then complete volume to 1 L.

Example (2)

How to prepare half molar (0.5 M) solution of $CuSO_4.7H_2O$?

- **Calculate molecular weight (Mw) of $CuSO_4.7H_2O$**

Mw = 63.5 + 32 + 4(16) + 7(2+16)

63.5 + 32 + 64 + 126 = 285.5

285.5 X 0.5 = 142.75 g/mole

Dissolve 142.75 gm of $CuSO_4.7H_2O$ in a little distilled water, then complete volume to 1 L.

Practice Calculations

- What is the MW for Sulfuric Acid?
  - H_2SO_4
    - 2 x 1.00 = 2.00
    - 1 x 32.06= 32.06
    - 4 x 16 = 64.00

98.06 g/mole

- A 1M solution of sulfuric acid contains 98.06 g of sulfuric acid in 1 L of total solution
  - Mole is an expression of amount
  - Molarity is an expression of concentration

Key unit conversions

- Millimolar (mM)
  - A millimole is 1/1000 of a mole
- Micromolar (μM)
  - A μmole is 1/1,000,000 of a mole

Practice Problem

- Prepare 100 mL of a 0.1M Tris buffer
  - Step 1: what is the MW of Tris
  - MW = 121.1 g/mole
  - What formula are you going to use
  - Molarity
    - moles per liter
    - 121.1 grams per 1L or 1000 mL = moles
  - How much Tris are you going to weight out?

- Start with what you know
  - 1M = 121.1 grams/1L
  - To make 100 mL of 0.1M solution you would add how much Tris buffer?
  - Formula= MW x molarity x volume (L) = g needed

121.1 x 0.1 x 0.1L = 1.211 grams Tris

Weight out 1.211 grams of Tris and add it to 80 mL of water, mix and QS to 100 mL of 0.1M Tris soln.

Practice Problem

- How much solute is required to make 400 mL of 0.8 M $CaCl_2$
  - First what is the MW of $CaCl_2$?
  - 40.08 + (35.45x2) = 110.98
  - 110.98 x 0.8 x 0.4L = 35.51 grams $CaCl_2$ required
  - Weigh out 35.51 grams $CaCl_2$ and add it to approximately 300 mL of water. Mix and then QS to 400 mL using a graduated cylinder.

Preparing dilute solutions from concentrated solutions

- Formula: C1xV1 = C2xV2
  - C1: concentration of stock solution
  - V1: volume of stock solution required
  - C2: concentration you want your dilute solution to be
  - V2: how much of the dilute solution is required

Practice Problem

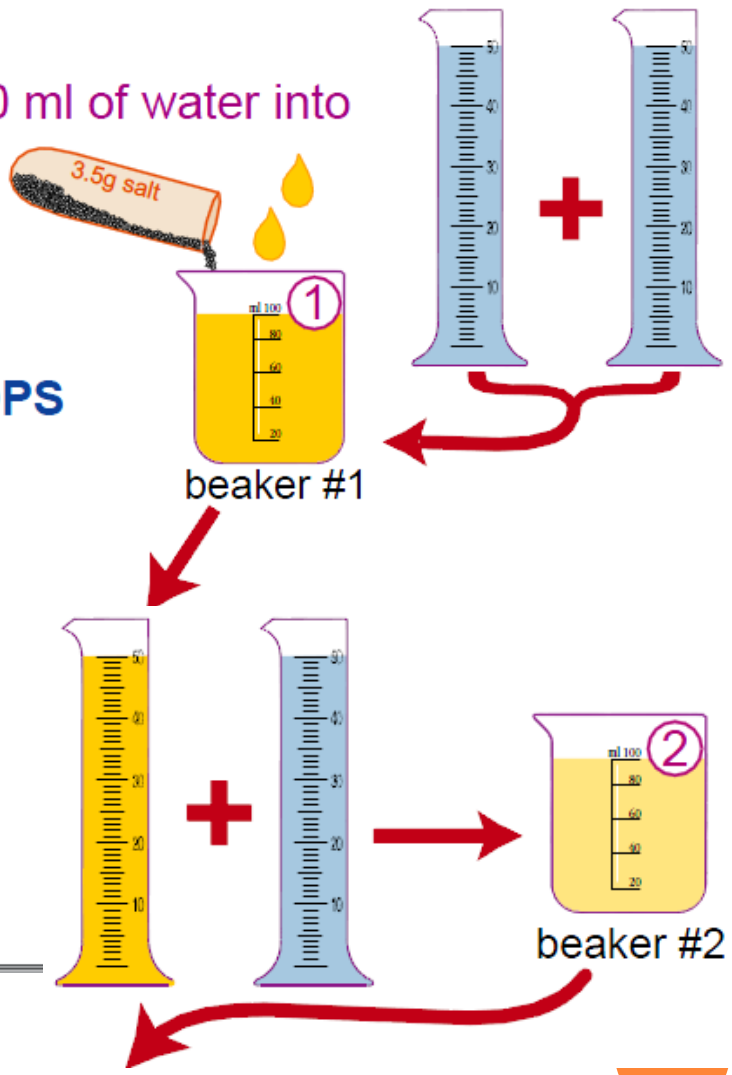
- How would you prepare 500 mL of a 1M Tris buffer from a 3M stock solution?
  - What is C1? 3M
  - What is V1? Unknown
  - What is C2? 1M
  - What is V2? 500 mL
    - 3x = 1(500)
    - X=166.67 mL
    - (500-166.67= 333.33)
    - Measure 166.67 mL of 3M Tris and add it to 333.33 mL of water to make 500 mL of 1M Tris

How to Make Serial Dilutions

1. Using your graduated cylinder, measure 100 ml of water into beaker #1.
   [hint: To get 100 ml, you will need to fill the graduated cylinder to the 50ml line twice]

2. Add 3.5 grams of salt (provided) and **2 DROPS** of yellow food coloring to beaker #1.
   Stir with the stir rod provided.

3. Measure 50 ml of solution #1 with your graduated cylinder.
   Pour it into beaker #2.
   Add 50 ml of water to beaker #2.
   Stir.



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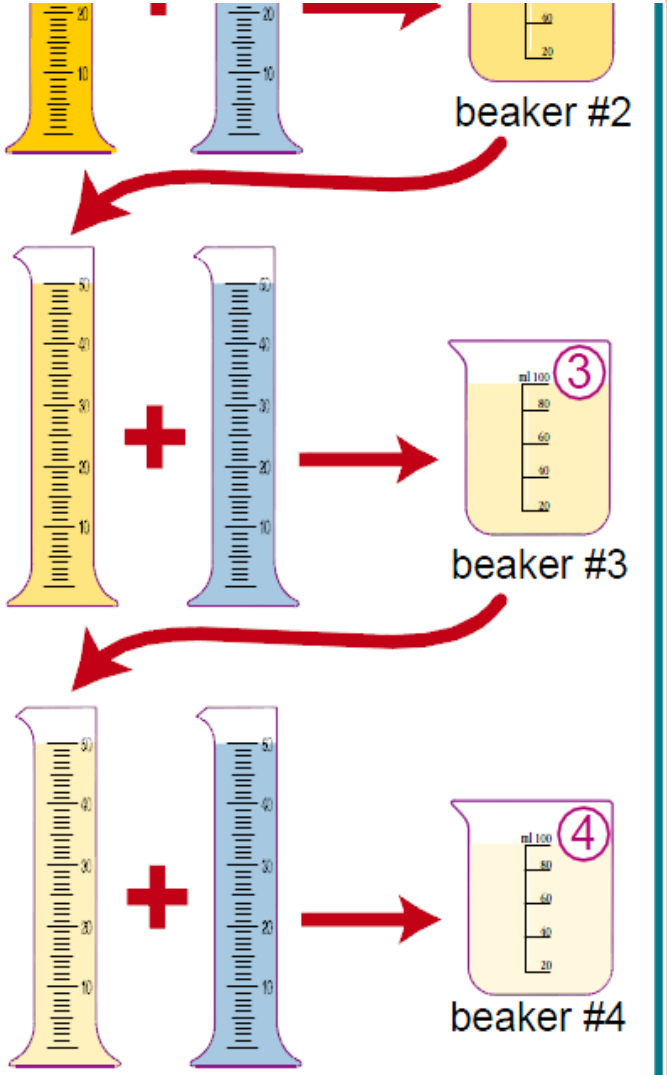
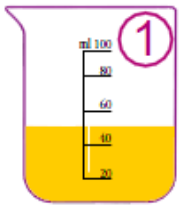
4 Measure 50 ml of solution #2 with your graduated cylinder.
Pour it into beaker #3.
Add 50 ml of water to beaker #3.
Stir.

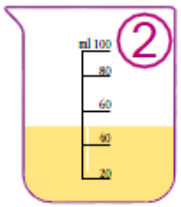
5 Measure 50 ml of solution #3 with your graduated cylinder.
Pour it into beaker #4.
Add 50 ml of water to beaker #4.
Stir.



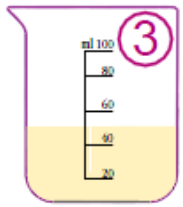
Congratulations!
You have just made serial dilutions!



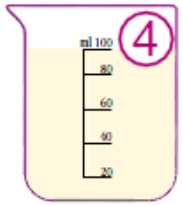
3.5% NaCl



1.75% NaCl
1/2 as much salt as beaker #1



0.875% NaCl
1/2 as much salt as beaker #2



0.4375% NaCl
1/2 as much salt as beaker #3

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