Ministry of Higher Education and Scientific Research

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LECTURE (2)

Dilutions : Simple and Serial Dilutions

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In biological systems, many chemical compounds perform their functions at very low concentrations. Too much (or too little) of a good thing can disrupt the homeostatic mechanisms of a living cell. Thus, if an investigator is attempting to simulate or manipulate the conditions in a living cell, it is often necessary to use extremely low concentrations of reagents of interest.

A. Solutions, Suspensions and Colloids

. A chemical mixture is defined as a heterogeneous association of substances that cannot be represented by a single chemical formula. A mixture may be solid, liquid, or gaseous, or a combination of phases. In a mixture

- **the major component represents the greater proportion, and is known as the solvent.**
- **the minor component represents the lesser proportion, and is known as the solute.**

Living organisms are primarily aqueous. A typical mammal body is 50 – 65% water, whereas a common jellyfish may be 98% water. Thus, the major component in biological mixtures is usually water. Various solute molecules perform and participate in a vast array of biological functions. Aqueous mixtures of liquid and solid can be categorized by the size of the particle (Figure 1). Each type of mixture has definitive physical properties.

1. Solution

A solution is a homogenous mixture of solvent and solutes of particle diameter less than 1.0nm. In a true solution, the solute interacts with the solvent at the molecular or ionic level, and the two cannot be optically distinguished from one another.

In biological systems, a gas or solid dissolved in water is invariably the minor component, that gas or solid will always be the solute.

When two **liquids** are mixed, the minor component is designated as the solute, and the major component as the solvent. (If the liquids are present in equal proportion, either one can be designated as the solute/solvent. Just to keep things complicated.)

2. Colloid

A colloid is a heterogenous mixture of solvent and solutes of particle diameter between 1.0 - 1000nm. Solute is distributed evenly throughout the solvent. Although the particles are larger than those in a solution, they do not settle out if the solution is allowed to stand without being mixed. Familiar colloids include milk, fog, aerosol sprays, and toothpaste.

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3. Suspension

A suspension is a heterogenous mixture of solvent and solutes of particle diameter greater than 1000nm. If a suspension is allowed to stand without mixing, gravity will pull solute particles out of the mixture so that they settle to the bottom.

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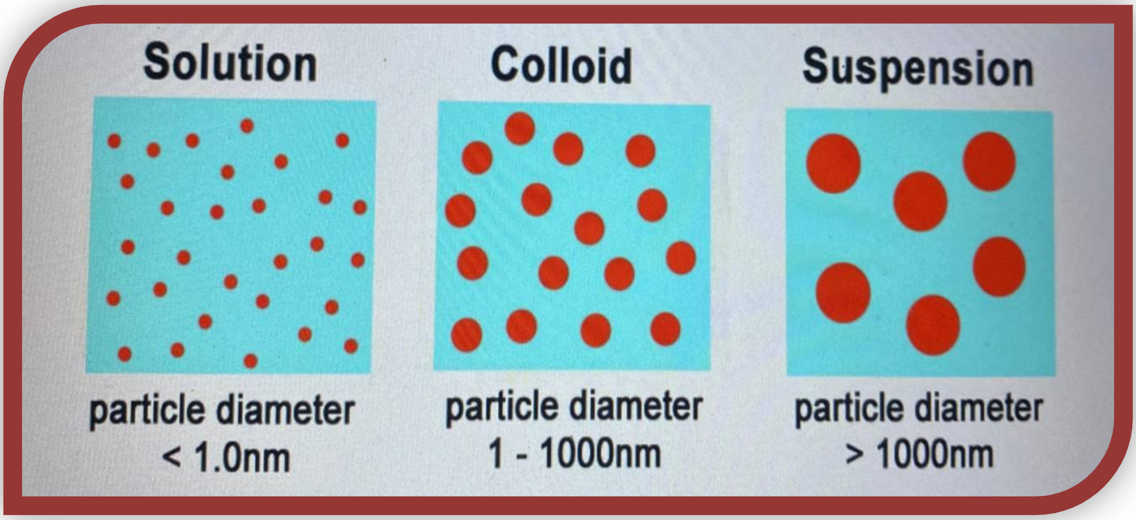

Figure 1. Mixtures can be categorized by particle size. Each type of mixture has specific physical properties that define it as a solution, colloid, or suspension

The number of particles in a mixture defines its concentration. A relatively concentrated mixture contains more particles than a relatively dilute mixture containing fewer particles. An investigator wishing to investigate the function of an aqueous biological system may wish to manipulate its concentration via dilution, decreasing the proportion of solute by increasing the proportion of solvent.

B. Dilution Ratio: Simple Dilutions

A liquid solute can be diluted via the addition of a known quantity of an appropriate solvent, also known as the diluent. The dilution ratio is the ratio of solute to solvent. It is typically expressed as two numbers separated by a colon (e.g., 1:10).

• first number represents the number of volumes of solute liquid.

• second number represents the number of volumes of solvent liquid.

• dilution ratio = volumes of solute : volumes of solvent For example, a dilution ratio of 1:5 means that •

one volume of solute is combined with

• five volumes of solvent, for a total of

• six volumes of finished, diluted product.

C. Dilution Factor: Serial Dilutions

A dilution factor describes the ratio of the volume of solute to the total, final volume of the entire diluted solution. Dilution ratio is sometimes confused with dilution factor, but they are not the same.

Unfortunately, a dilution factor, like a dilution ratio, also is typically expressed as two numbers separated by a colon (e.g., 1:10). However, in this case

• first number represents the number of volumes of solute liquid.

• second number represents the number of volumes of the entire solution.

• dilution factor = volumes of solute : volume of entire solution

For example, a dilution factor of 1:5 means that

• one volume of solute is combined with

• four volumes of solvent, for a total of

• five volumes of finished, diluted product

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D. Serial Dilution

Serial dilutions are widely used in experiments requiring

• a very dilute solution

• a series of sequentially more dilute solutions The dilution factor at each dilution step is usually kept constant, resulting in a logarithmic decrease in solute concentration at each step (Figure 2).

For example a tenfold serial dilution of a 1M solution would result in sequentially more diluted solutions, as follows:

1M à 0.1M à 0.01M à 0.001M à 0.0001M …and so on.

Serial dilutions can be used to prepare solutions of any desired concentration, no matter how dilute.

You will perform a simple exercise to be sure you understand how to do this, as it will be important in your future biological research endeavors. In a pinch, here's a Serial Dilution