Quantitative Methods: Solutions and Dilutions

A biologist must be able to work with a variety of mixtures. He or she must be able to plan the preparation of mixtures, read formulas for mixtures, describe them, store them properly, dilute them, analyze them for content and/or concentration, pipette them, and handle them safely. This talk will present basic concepts and definitions, and the rationale behind descriptions of types of mixtures. It is part of a presentation on the methodology related to understanding and preparing solutions.

Many, but not all, mixtures used in a biology laboratory are made by mixing a solid with water. Many, but not all, of these mixtures are true solutions. Here are some examples of mixtures that a biologist might encounter in a laboratory. Some are true solutions and some are not.

physiological saline solutions	buffers	cell suspensions
soil suspensions		staining solutions
microbiological media		
chromatography slurries	dishwater	milk
protein solutions		DNA solutions
	density gradients	

The word "mixture" can be defined as a heterogeneous association of substances that cannot be represented by a single chemical formula. This definition does not limit mixtures to solids mixed with liquids. Two or more gases, solids, or liquids can be mixed, and two or more different phases of matter can be combined in a mixture. Because of the importance of liquid solutions and similar mixtures to biology, this series of talks will focus primarily on mixtures in which the major component is a liquid.

References:

Lewis, R.J., Sr. (1997). *Hawley's Condensed Chemical Dictionary (*13th ed.). Van Nostrand Reinhold.

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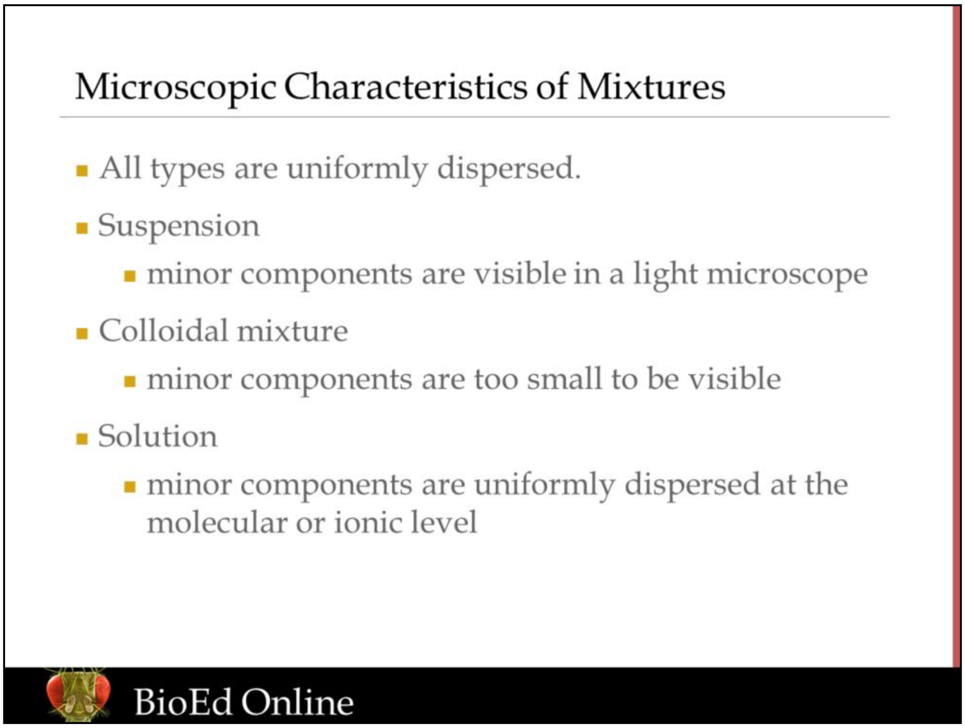
[This handbook serves as a good general reference for laboratory techniques]



Microscopic Characteristics of Mixtures

Wilhelm Ostwald, Nobel Laureate in Chemistry in 1909, was one of the founders of modern physical chemistry. He is reported to have said, "There are no sharp differences between mechanical suspensions, colloidal solutions, and molecular [true] solutions. There is a gradual and continuous transition from the first through the second to the third."

A mixture, regardless of type, is described as "uniformly dispersed." This means that one or more minor components are evenly distributed throughout a major component. The major component is the substance that is present in the greatest proportion. In the biology laboratory, the major component often is a liquid, and minor components can be solids, other liquids, or even gases.

The "mechanical suspension" to which Ostwald referred is the easiest to describe. The minor component in a suspension is typically visible in an optical microscope and often is visible to the naked eye.

A colloidal mixture is sometimes called a colloidal system, a colloidal suspension, or simply a "colloid." The smallest dimension of the minor component of a colloidal mixture can range from approximately one nanometer (1 billionth of a meter) to one micrometer (1 millionth of a meter). Examples of liquid colloidal mixtures are milk, paints, and muddy water. The medium can be a gas, in the cases of smog, smoke, or aerosol sprays. Some solids are considered to be colloidal mixtures, as in steel or foam rubber.

In a true solution, one or minor components interact at the molecular level or ionic level with the major component. The minor components are atoms or molecules, and are not distinguishable in any optical microscope.

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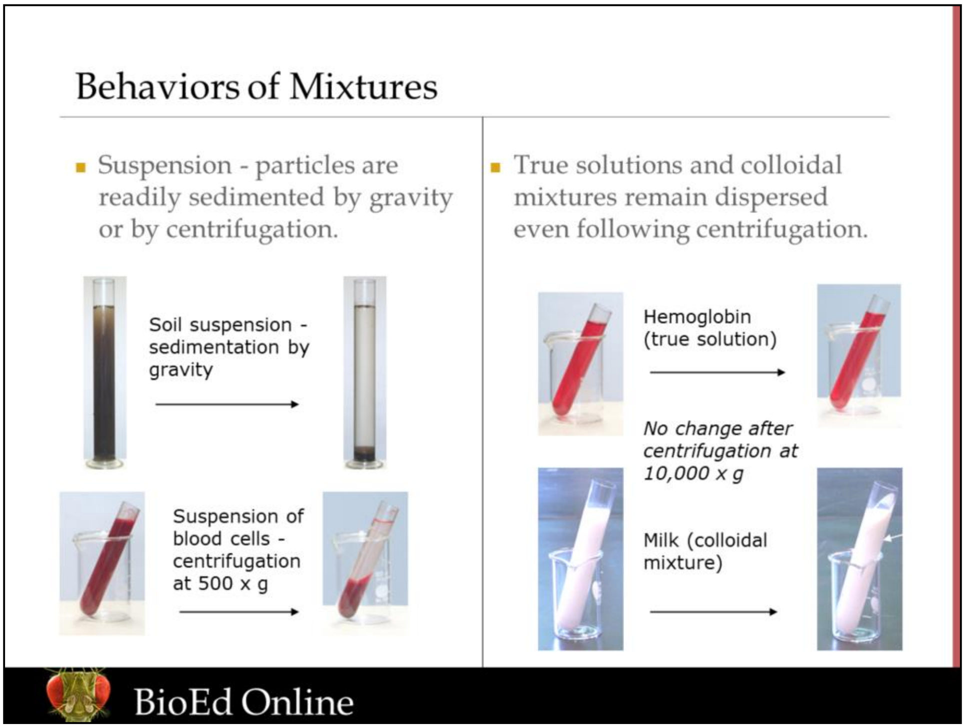

Behaviors of Mixtures

If a simple suspension is left undisturbed, gravity causes the minor components to separate from the major component. Gravity can bring down the minor or major component, depending on their relative densities. For example, a handful of soil from a forest floor forms a suspension when mixed with water. Sand and clay particles settle to the bottom when the suspension is left alone, while some plant materials float to the surface.

Smaller particles separate more slowly. For example, whole blood treated with anticoagulant may take hours to separate completely, with hemoglobin-rich red blood cells on the bottom, the lighter white cells in a layer above the red cells, and plasma on top. Centrifugation speeds up the process.

The properties of a mixture must be observed in order to confirm that it is colloidal. Even prolonged centrifugation cannot separate the minor components from the major component of a colloidal mixture. For very small particles the resistance to movement effectively opposes the force causing such movement. Neither gravity nor centrifugal force can separate components if they are of equal densities. The nature of colloidal systems prevents their separation even by extremely high centrifugal forces.

True solutions, like colloidal systems, remain dispersed even with conventional centrifugation, but different principles are involved. Molecular interactions between minor components and the major component of a true solution make it energetically unfavorable for separation to occur. However, separation can be accomplished by prolonged centrifugation in an ultracentrifuge. One of the earliest methods for determination of the molecular weights of proteins involved ultracentrifugation of protein solutions.

References:

Ford, T.C. & Graham, J. M. (1991). *An Introduction to Centrifugation*. Bios.

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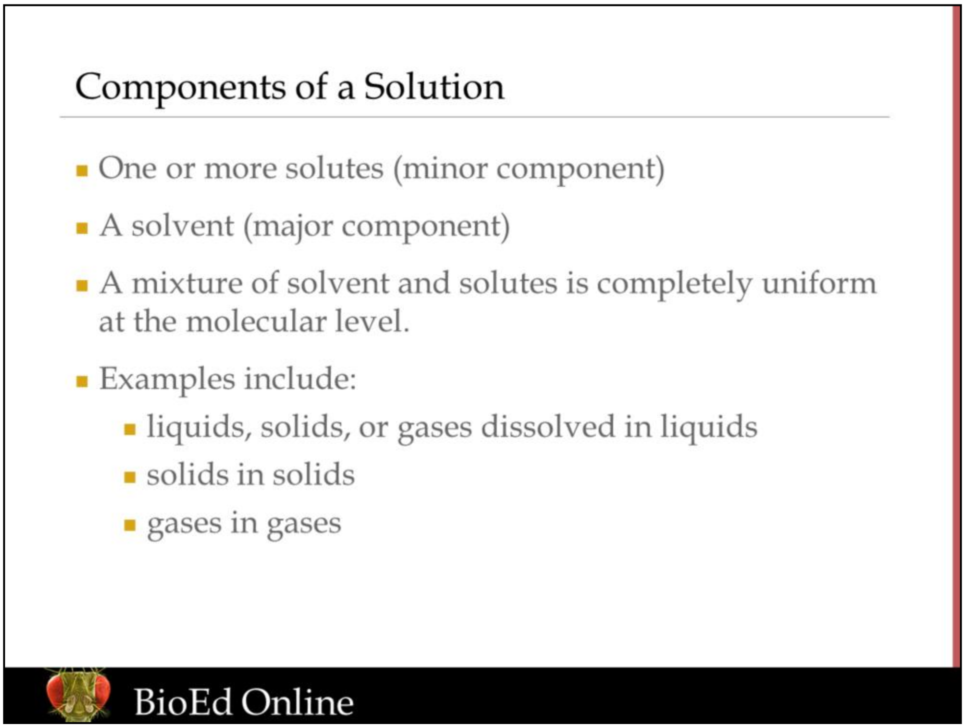

Components of a Solution

A true solution consists of a minimum of two components, namely a solvent and a solute. The solvent is the major component of a solution, and frequently is a liquid matrix in which one or more solutes (minor components) are dissolved. Solutions are completely homogeneous mixtures, a property that often is attributed to suspensions and colloids as well. The minor components of a true solution, however, remain dispersed, due to interactions at the molecular level. A substance is considered to be soluble in a particular solvent if it is capable of interaction with the solvent so as to form a solution.

By definition, a solution can consist of a liquid, solid, or gas, or combination of these types of substances, dissolved in a liquid. A solid can also be dissolved in another solid such as is the case for some frozen drinks or metal alloys. Gases, unless compressed, typically are mutually soluble.

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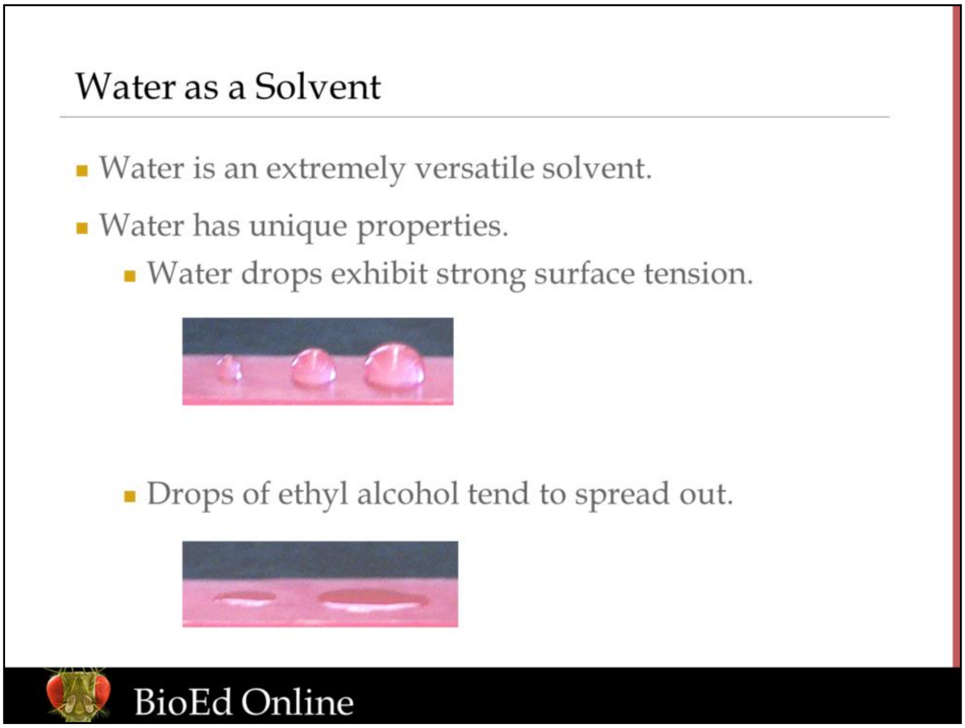

Water as a Solvent

Water may be the most versatile solvent known. It is capable of dissolving both acids and bases, nearly all biologically important molecules, and a wide range of organic (carbon-based) and inorganic substances. More substances will dissolve in water than in any other known solvent, and it is the most abundant liquid solvent on Earth. The behavior of drops of water provides a clue as to why it is such a versatile solvent.

Water exhibits a property known as surface tension. It will form round beads on a surface, rather than spread out into a film. On the other hand, a similar quantity of oil or ethyl alcohol spreads out on a surface. It may seem strange to think of a liquid as having structure. Nevertheless there must be some structure, that is, some special interaction among water molecules. A liquid consisting of molecules that do not interact at all would be expected to spread out on a surface in a layer one molecule thick.

References:

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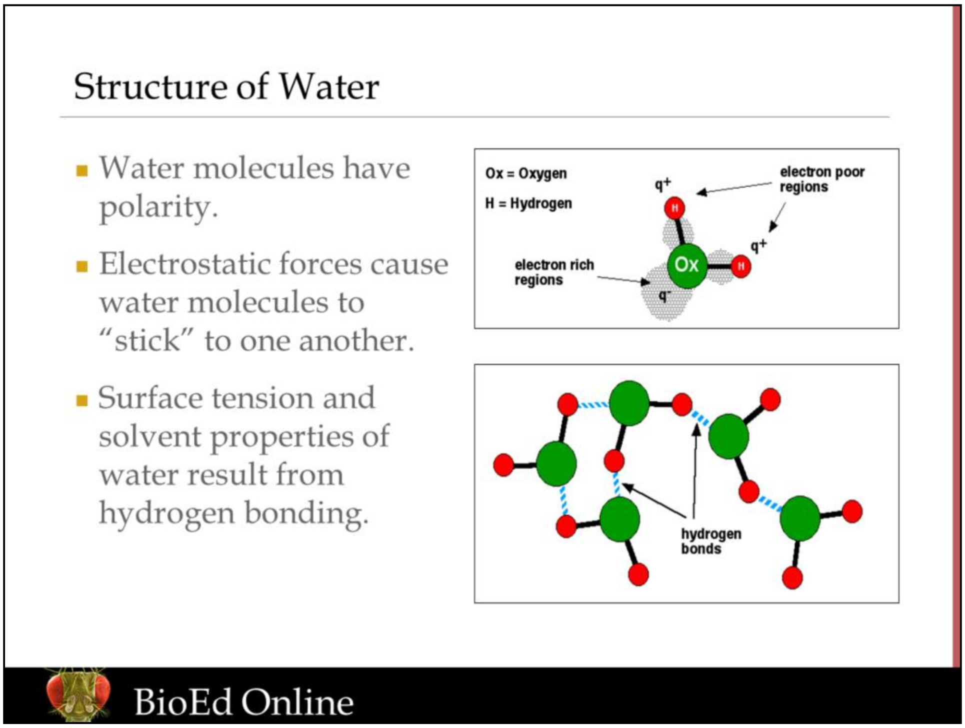

Structure of Water

A molecule carries no net electric charge when there are as many negatively charged orbiting electrons as there are positively charged protons in its atomic nuclei. Different parts (poles) of an electrically neutral molecule can nevertheless carry a partial positive or negative charge that can attract or repel other charged structures. Such molecules are called polar molecules.

A water molecule consists of two hydrogen atoms and one oxygen atom and is electrically neutral. Water molecules are highly polar, however. Oxygen has a greater affinity for electrons than does hydrogen, so on average, the outer electrons are closer to the oxygen atom than to either of the two hydrogen atoms. The result is that the oxygen atom carries a partial negative charge and the hydrogen atoms each carry a partial positive charge.

Because opposite charges attract each other, the hydrogen atoms of a water molecule tend to "stick" to oxygen atoms on nearby water molecules. This property is called hydrogen bonding. Hydrogen bonding keeps water from spreading out when it is placed on a surface, that is, it gives water the property of surface tension. This structure of water is so stable that when something else is tossed into water it tends to separate out (that is, the water molecules separate themselves from the intruding molecules) unless the new substance contributes something "special" to the structure of water. In scientific terms, it takes free energy to disrupt the molecular structure of water or of any solvent. Therefore, if a substance is to go into solution spontaneously, it must make the structure of water even more stable.

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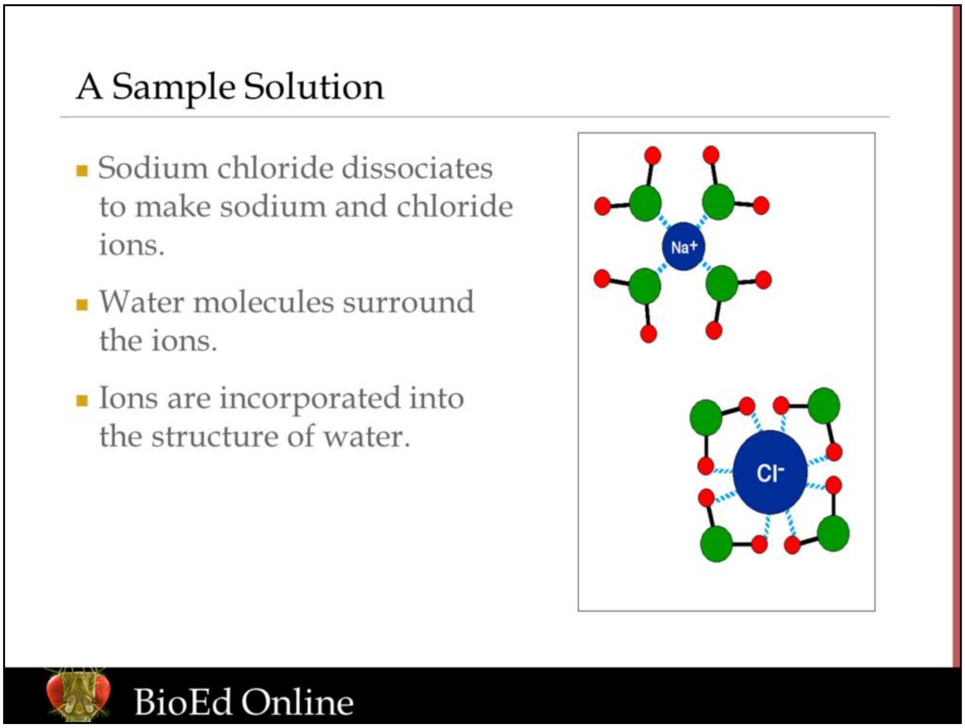

A Sample Solution A Sample Solution

For a substance to go into and remain in solution, it must be more energetically favorable for the substance to remain mixed with water than to separate out. Solutions are stable. Gravity will take particles out of suspension, but gravity will not take a solute out of solution.

For example, sodium chloride dissociates into ions when added to water, forming a stable solution. Why?

A sodium atom readily gives up an electron to become positively charged. Because they are positively charged, sodium ions are attracted to the partial negative charge on the oxygen atoms of water molecules. Similarly, a chlorine atom gains an electron to become a negatively charged chloride ion. Chloride ions are attracted to the partial positive charges on the hydrogen atoms of water molecules. In either case, the result is a stable structure, called a hydration shell, that surrounds the ion. It isn't energetically favorable for the ion to separate from the surrounding solvent molecules.

Even in biology, not all solutions require water to be used as a solvent. For example, some solutes must be dissolved in ethanol or another organic solvent. Non-polar solutes will not dissolve in water at all. The molecular properties of solute and solvent, and how they interact, determine what combinations are most appropriate. When a potential solute is readily dissolved in a given solvent, we refer to the solute and solvent as being compatible. Sodium chloride is compatible with water. Oil is not. Which is another way of saying, "oil and water don't mix."

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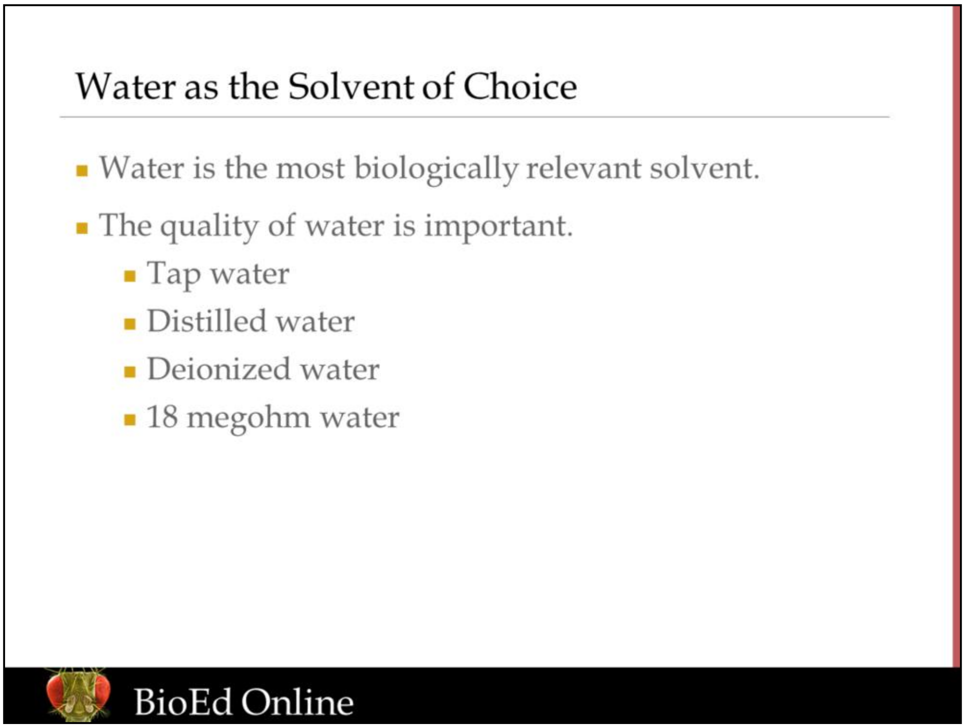

Water as the Solvent of Choice

Water is by far the most commonly used solvent in biology because it is the major component of all living organisms. Most known biochemical reactions take place in an aqueous environment, and water is frequently a reactant in, or a product of, biochemical reactions. Biologically important macromolecules, organelles, cells, and organs all are designed to function in an aqueous environment.

Water quality is highly variable, and for any task an appropriate grade of water must be chosen. For example, tap water is fine for washing dishes, but it is not recommended for making solutions because the quality of such water is unknown. Tap water typically contains sediments (suspended particles), metal and other ions, deliberately added chemicals such as chlorine or fluoride, and/or traces of organic solvents. Although tap water is generally safe for drinking and other personal uses, materials in tap water can be toxic to some cells or may interfere with assays or biochemical reactions. Therefore, tap water is inappropriate for making solutions. Also, it is recommended that glassware that has been washed and rinsed in tap water be thoroughly rinsed with a higher quality water before use in the laboratory.

Distilled water, obtained from the condensation of steam, is of better quality because distillation eliminates all the sediment and most of the inorganic solutes. Organic contaminants and some of the inorganic contaminants remain.

Deionized water is produced by running tap water through a resin cartridge, or series of cartridges. A home deionizing system might simply replace divalent cations with sodium ions, producing what is commonly known as “soft” water. Laboratory deionized water is usually treated to remove both cations and anions, which are exchanged for hydrogen and hydroxyl ions, respectively. Deionized water often is of better quality than distilled water. However, on the downside, the resins used in the cartridges may release organic contaminants into the water.

The highest grade of water is called 18 megohm water. Eighteen megohms is 18 million ohms, which are units representing resistance to the flow of electricity. Eighteen meghoms is more than a million times the electrical resistance of a typical household electric circuit. Very pure water does not conduct electricity as well as contaminated water because it contains no inorganic ions with which to carry electric current. Eighteen megohm water is usually produced in multiple steps, including reverse osmosis and the passage of product through ion exchange resins, activated carbon beds and filters.

Pure water is somewhat acidic, with pH close to 5. It is also what we call an aggressive reagent, meaning that it will leech ions from plastic or glass containers. It does so because of the polar nature of water molecules. Ions dissolve most readily in 18 megohm water because the system (water plus dissolved ions) is more stable than when pure water is separated from soluble materials. Because very pure water accumulates contaminants during storage, it should be freshly prepared. Avoid use of plastic tubing, funnels, and especially metal containers.

References:

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