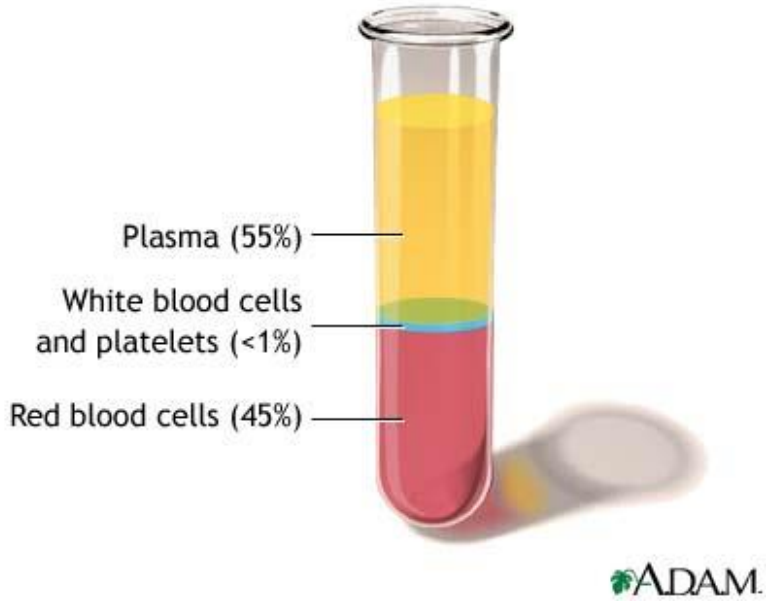
LECTURES IN PRACTICAL BIOCHEMISTRY

TYPES OF SPCEMIN: Lab (1)

There are three types of blood specimens- serum, plasma and whole blood. Each different specimen is collected for various reasons. When blood is removed from the body, typically, it will coagulate or clot within 30 to 60 minutes. Serum can be separated from blood by centrifugation. Centrifugation is a process that spins the blood at high speeds in a machine called a centrifuge. This spinning separates the serum from the blood cells enmeshed in blood clot. Blood serum looks pale-yellow and has a similar composition to plasma. However, serum does not contain fibrinogen. Laboratory tests, like chemistry and immunology test are commonly performed on serum.

Coagulation tests cannot be performed on serum because the coagulation factors are separated out of the serum during the centrifuge process.

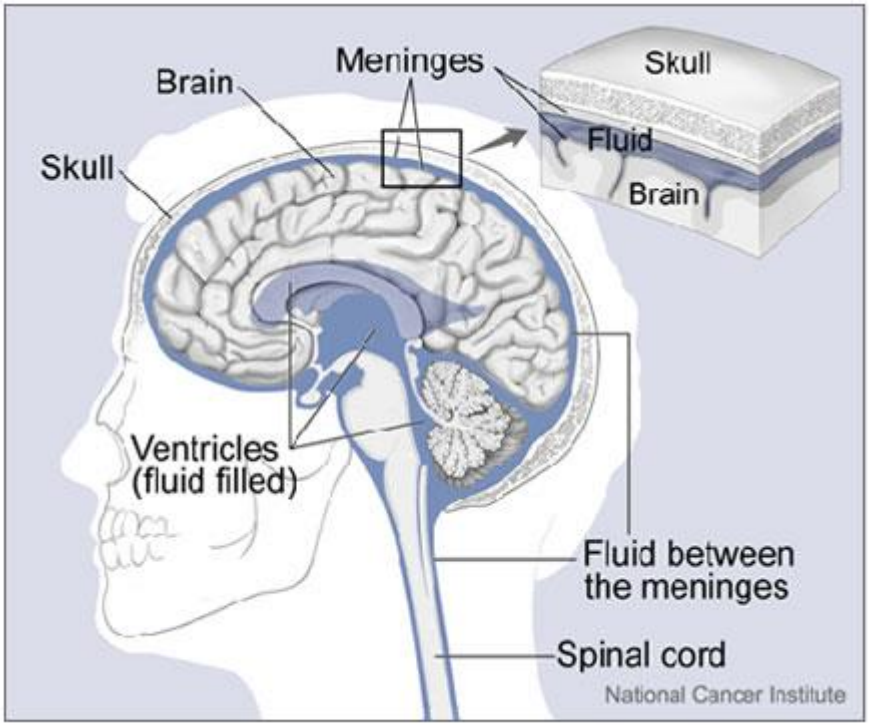
1- Whole blood specimens are usually required for hematology tests. These types of tests require the blood to remain in the same form as it is in the bloodstream. It is important that the blood specimen does not clot or separate. An anticoagulant must be added and the specimen should be mixed for at least 2 minutes immediately before performing the test.



2- Urine has a long, rich history as a source for measuring health and well-being and remains an important tool for clinical diagnosis. The clinical information obtained from a urine specimen is influenced by the collection method, timing and handling.

3- Saliva testing is a diagnostic technique that involves laboratory analysis of saliva to identify markers of endocrine, immunologic, inflammatory, infectious, and other types of conditions. Saliva is a useful biological fluid for assaying steroid hormones such as cortisol, genetic material like RNA, proteins such as enzymes and antibodies, and a variety of other substances. Saliva testing is used to screen for or diagnose numerous conditions and disease states, including Cushing's disease, anovulation, HIV, cancer, parasites, hypogonadism, and allergies.

4- Cerebrospinal fluid (CSF) is a clear watery liquid that is formed and secreted by the choroid plexus, a special tissue that has many blood vessels and that lines the small cavities or chambers (ventricles) in the brain. A bout 17 ounces (500 mL) are produced each day. This rate of production means that all of the CSF is replaced every few hours. A CSF analysis is a group of tests that evaluate substances present in CSF in order to diagnose conditions affecting the central nervous system.



ANTICOAGULANT

An **anticoagulant** is a substance that prevents coagulation (clotting) of blood. A group of pharmaceuticals called anticoagulants can be used *in vivo* as a medication for thrombotic disorders. Some anticoagulants are used in medical equipment, such as test tubes, blood transfusion bags, and renal dialysis equipment.

Heparin is a biological substance, usually made from pig intestines. It works by activating antithrombin III, which blocks thrombin from clotting blood. Heparin can be used *in vivo* (by injection), and also *in vitro* to prevent blood or plasma clotting in or on medical devices.

Anticoagulants outside the body

Laboratory instruments, blood transfusion bags, and medical and surgical equipment will get clogged up and become nonoperational if blood is allowed to clot. In addition, test tubes used for laboratory blood tests will have chemicals added to stop blood clotting. Apart from heparin, most of these chemicals work by binding calcium ions, preventing the coagulation proteins from using them.

- EDTA is denoted by mauve or purple caps on Vacutainer(A **vacutainer** blood collection tube is a sterile glass or plastic tube with a closure that is evacuated to create a vacuum inside the tube facilitating the draw of a predetermined volume of liquid. Most commonly used to draw a blood sample directly from the vein, these also are used to collect urine samples) brand test tubes. This chemical strongly and irreversibly binds calcium. It is in a powdered form.
- Citrate is usually in blue Vacutainer tube. It is in liquid form in the tube and is used for coagulation tests, as well as in blood transfusion bags. It binds the calcium, but not as strongly as EDTA. Correct proportion of this anticoagulant to blood is crucial because of the dilution. It can be in the form of sodium citrate or ACD.
- Oxalate has a mechanism similar to that of citrate. It is the anticoagulant used in fluoride (grey top) tubes.

Spectrophotometry Lab(2)

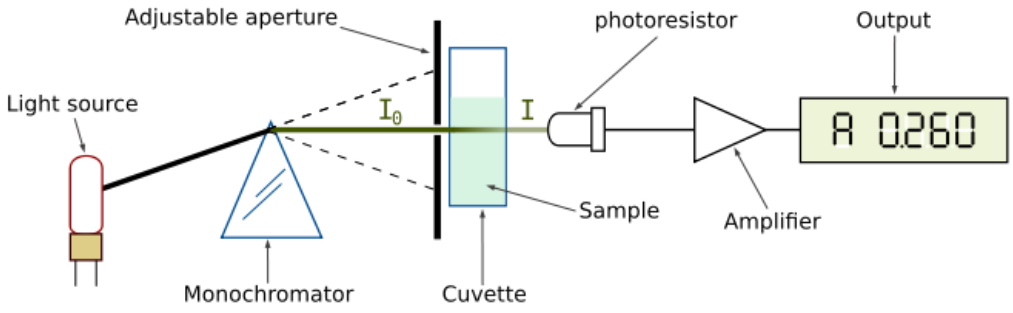
In chemistry, **spectrophotometry** is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared.

Spectrophotometry involves the use of a spectrophotometer. A spectrophotometer is a photometer that can measure intensity as a function of the light source wavelength. Important features of spectrophotometers are spectral bandwidth and linear rangeabsorption or reflectance measurement

In short, the sequence of events in a modern spectrophotometer is as follows:

1. The light source is imaged upon the sample
2. A fraction of the light is transmitted or reflected from the sample
3. The light from the sample is imaged upon the entrance slit of the monochromator

The monochromator separates the wavelengths of light and focuses each of them onto the photodetector sequentially



Beer-Lambert Law

Introduction

The Beer-Lambert law (or Beer's law) is the linear relationship between absorbance and concentration of an absorbing species. The general Beer-Lambert law is usually written as:

$A = a(\lambda) * b * c$

where A is the measured absorbance, $a(\lambda)$ is a wavelength-dependent absorptivity coefficient, b is the path length, and c is the analyte concentration. When working in concentration units of molarity, the Beer-Lambert law is written as:

$A = \varepsilon * b * c$

where ε is the wavelength-dependent molar absorptivity coefficient with units of M^{-1} cm^{-1}.

Instrumentation

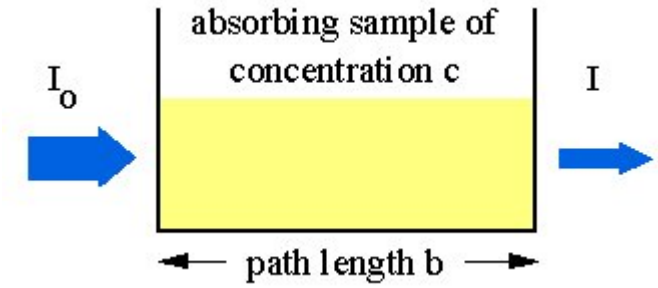
Experimental measurements are usually made in terms of transmittance (T), which is defined as:

$T = I / I_o$

where I is the light intensity after it passes through the sample and I_o is the initial light intensity. The relation between A and T is:

$A = -\log T = -\log (I / I_o)$.

Absorption of light by a sample



Modern absorption instruments can usually display the data as either transmittance, %-transmittance, or absorbance. An unknown concentration of an analyte can be determined by measuring the amount of light that a sample absorbs and applying Beer's law. If the absorptivity coefficient is not known, the unknown concentration can be determined using a working curve of absorbance versus concentration derived from standards.