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## Assignment Advanced in medical lab technology

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**Definition:**

* A spectrophotometer is an analytical instrument used to quantitatively measure the transmission or reflection of visible light, UV light or infrared light. Spectrophotometers measure intensity as a function of light source wavelength.
* A method in which the absorption or transmission properties of a material is quantitatively measured as a function of wavelength. The basic principle behind this method is that: Each compound absorbs or transmits light over a certain range of wavelength.
* Spectrophotometer is one of the most useful methods of quantitative analysis in various fields such as chemistry, physics, biochemistry, material and chemical engineering and clinical applications.

**Introduction:**

* Every chemical compound absorbs, transmits, or reflects light (electromagnetic radiation) over a certain range of wavelength. Spectrophotometry is a measurement of how much a chemical substance absorbs or transmits.
* Spectrophotometry is widely used for quantitative analysis in various areas (e.g., chemistry, physics, biology, biochemistry, material and chemical engineering, clinical applications, industrial applications, etc.). Any application that deals with chemical substances or materials can use this technique.
* In biochemistry, for example, it is used to determine enzyme-catalyzed reactions
* A spectrophotometer is an instrument that measures the amount of photons (the intensity of light) absorbed after it passes through sample solution. With the spectrophotometer, the amount of a known chemical substance (concentrations) can also be determined by measuring the intensity of light detected. Depending on the range of wavelength of light source, it can be classified into two different types:

1. **UV-visible spectrophotometer:** Uses light over the ultraviolet range (185 - 400 nm) and visible range (400 - 700 nm) of electromagnetic radiation spectrum.
2. **IR spectrophotometer:** Uses light over the infrared range (700 - 15000 nm) of electromagnetic radiation spectrum.

**Device in spectrophotometry:**

* It consists of a light source, a collimator, a monochromatic, a wavelength selector, a curette for sample solution, a photoelectric detector, and a digital display or a meter.
* A spectrophotometer, in general, consists of two devices; a spectrometer and a photometer.

1**: spectrometer**:

* A spectrometer is a device that produces, typically disperses and measures light. It produces a desired range of wavelength of light. First a collimator (lens) transmits a straight beam of light (photons) that passes through a monochromatic (prism) to split it into several component wavelengths (spectrum).

2**. Photometer:**

* A photometer indicates the photoelectric detector that measures the intensity of light. After the desired range of wavelength of light passes through the solution of a sample in cuvette, the photometer detects the amount of photons that is absorbed and then sends a signal to a galvanometer or a digital display.

**TYPE OF SPECTROPHOTMETRY:**

* There are two major classes of devices: single beam and double beam. A double beam spectrophotometer compares the light intensity between two light paths, one path containing a reference sample and the other the test sample.
* A single-beam spectrophotometer measures the relative light intensity of the beam before and after a test sample is inserted. Although comparison measurements from double-beam instruments are easier and more stable, single-beam instruments can have a larger dynamic range and are optically simpler and more compact

**Application:**

* Detection of concentration of substances
* Detection of impurities
* Structure elucidation of organic compounds
* Monitoring dissolved oxygen content in freshwater and marine ecosystems
* Characterization of proteins
* Detection of functional groups
* Respiratory gas analysis in hospitals
* Molecular weight determination of compounds
* The visible and UV spectrophotometer may be used to identify classes of compounds in both the pure state and in biological preparations.

**History:**

* The spectrophotometer was invented in 1940, by Arnold J. Beckman and his colleagues at National Technologies Laboratories, the company Beckman had started in 1935. They were led by project leader Howard H. Cary. The spectrophotometer was the company's greatest discovery.

**Accuracy:**

* Before 1940, the chemical analysis process was a long venture taking weeks to complete with only 25 percent accuracy according to the MIT Inventor of the Week archive.
* In 1940 when the Beckman DU Spectrophotometer was introduced, it simplified the process greatly, requiring only a few minutes for analysis. According to the same source, this test offered 99.99 percent accuracy on the analysis.

**Design:**

* The model B spectrophotometer used a quartz prism instead of a glass prism this improved the UV capabilities of the device.
* Model C soon followed with changes that raised wavelength resolution in the UV and three subsequent Model C spectrophotometers were made. In 1941 the Model D, also known as the Model DU was produced with a hydrogen lamp and other improvements.

This design remained essentially unchanged from 1941 to 1976 w

**Answer no question 2**

1. **IMMUNOELECTRON MICROSCOPY:**

* It is used to detect or identified the viruses
* It is the most fastest and sensitive method for detection and diagnosis of viruses.
* Immune electron microscopy applied for diagnosis of many viral infection .A difficult procedures immunoelectron microscopy was developed as a diagnostic aid for detecting transmissible gastroenteritis virus and rotavirus in fecal and intestinal contest from case of the gastroenteritis in young pigs.
* The virus is reacted with the immune serum, resulting in clumping that can be seen when viewed under the electron microscope

1. **PCR:** ( polymerase chain reaction)

* It is used to detect or to identified the specific virus or specific genes sequence
* It is a method widely uses to rapidly make million to billion of copies of a specific DNA sample allowing scientist to take small sample of DNA and amplifying it to large amount to study in details.
* It is used in analyzing clinical specimen for the presence infection agent includes HIV, human pipillomaviruse.also used for detection of malaria.
* PCR in vitro method of DNA replication is capable of amplifying DNA segments by more than one million fold

1. **ELISA:**

* It is abbreviated as enzyme linked immunosorbent assay, it is a type of serological test and immunoassay technique
* In ELISA a specific type of enzymes is linked to an antibody to detect the presence of protein like the antigen
* ELISA method was elevated from RIA technique; therefore ELISA technique is less similar to RIA where the antigen is radio labeled
* ELISA technique is widely used as compared to RIA technique due to environmental pollution and to detect the viral infection or diagnosis

1. **ELECTRON MICRSCOPY (EM):**

* it is used to demonstrate viruses in clinical samples

In this technique of negative contrast EM distilled water lysates of clinical specimen are stained with a solution of heavy atoms

* The technique is primarily used for the examination of those clinical specimen expected to contain a large number of viral particles such as feces (Corona virus, pro viruses) and vascular and pox like lesion (Herpes and pox viruses)
* Specimen’s preparation and EM examination usually can be completely within 30 mints.

1. **VIRUS NEUTRALIZATION(VN):**

* . It is used to detect and measure antibody Virus neutralization is the most widely used method to detect and measure antibodies to viruses of veterinary.
* When vertebrate is infected with viruses antibodies are produced against many epitopes on multiple virus protein.
* The subset of these antibodies can block virus infection by process called neutralization