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Organic Analysis Tools in Dentistry

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Abstract: Organic analytic tools let us to recognize the structural composition and features of unknown constituents. It is particularly valuable in drug production, and therapeutics. Here you will describe how to analyze a completely unknown substance.

Keywords: Ceramics; glass-ionomer; endodontics.

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Organic Analysis Tools:

A majority of methods has been developed for organic analysis; chromatography is very important in trace organic analysis.

1. Chromatography:

Chromatography is a separation method used for chemical analysis. It was initially introduced as an analytical technique in the early twentieth century and was first used as a method of separating colored compounds. Chromatography (chroma) i.e. color where (graphy) means writing. Samples can be gases, liquids or solids, in simple mixtures or in complex blends of widely differing chemicals. The solvent can also be a gas or liquid, depending on the type of chromatography ⁽²⁾.

1.1. **Gas Chromatography - Mass Spectroscopy (GC-MS):**

Gas chromatography-mass spectroscopy (GC-MS) is one of the combined analytical techniques. Actually, it is a combination of two techniques to form a single method of analyzing mixtures of chemicals. Gas chromatography separates the components of a mixture and mass spectroscopy characterizes each of the components individually. They are considered as the gold standard for analyzing lipids, drug metabolites and environmental analysis.

Gas chromatography is used to separate mixtures of compounds that can be vaporized without decomposition into individual components. Once isolated, the components can be evaluated individually. Separation occurs when the sample mixture is injected into a **mobile phase**. The mobile phase is an inert gas such as helium. The mobile phase carries the sample mixture through what is referred to as a **stationary phase**. The stationary phase is a usually chemical (liquid or solid) that can selectively attract components in a sample mixture. The stationary phase is usually contained in a capillary tube which is referred as a **column**. The mixture of compounds in the mobile phase interacts with the stationary phase. Each compound in the mixture interacts at a different rate. Those interact fastest will exit first, while those interact slowest will exit the last. By changing characteristics of the mobile phase and the stationary phase, different mixtures of chemicals can be separated. As the compounds are separated, they enter a **detector**. The detector is capable of creating an electronic signal whenever the presence of a compound is detected.

The signal is then processed by a **computer**. A schematic representation of the device basic component is provided in figure (1).

As the individual compounds enter the mass spectroscopy, they are bombarded with a stream of electrons causing them to break apart into fragments. They determine the mass of a molecule by measuring the mass-to-charge ratio (m/z) of its ion. The computer records a graph for each scan where the x-axis represents the M/Z ratios while, the y-axis represents the signal intensity (abundance) for each of the fragments detected during the scan. The mass spectrum produced by a given chemical compound is essentially a fingerprint for the molecule. This fingerprint can be used to identify the compound.

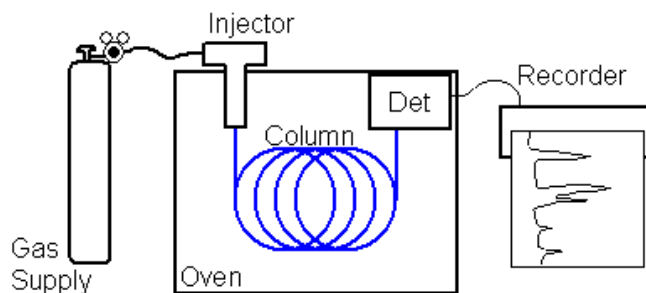


Figure (1): Schematic representation of GC (3).

When GC is combined with MS, a powerful analytical tool is created. A researcher can take an organic solution, inject it into the instrument, separate the individual components, and identify each of them. Furthermore, the researcher can determine the quantities (concentrations) of each of the components (4).

The main *limitation* is that the sample must be easily converted into a gaseous form and withstand heat at high temperatures without breaking down or degrading into another molecule (4).

The main *benefit* is that it is a powerful instrument capable of analyzing, separating and matching all the ingredients of an unknown mixture (4).

1.2. High-Performance Liquid Chromatography (HPLC):

High performance liquid chromatography (HPLC) is now one of the most powerful tools in analytical chemistry. It has the ability to separate a mixture into its individual components. They are coupled with a detection system that can characterize each type of analyte properly. It is used for separation of organic, inorganic, biological compounds, polymers, and thermally labile compounds. They are commonly used as quantitative and qualitative analyses of amino acids, nucleic acids, proteins in physiological samples. Also, for measuring levels of active drugs, synthetic byproducts, degradation products in pharmaceuticals. In addition to, purifying compounds from mixtures.

The fundamental basis for HPLC consists of passing a sample (analyte mixture) in a high-pressure solvent (called the mobile phase) through a steel tube (called a column) packed with sorbents (called the stationary phase). As the analytes pass through the column, it is separated by the attraction of the compound against the material of the column. This polar and non-polar attraction to the column material is the active force that causes the compounds to separate over time. A schematic representation of the device basic component is provided in figure (2).

HPLC has the following advantages over GC that it is quick, accurate, need a smaller sample for analysis, give a higher resolution record that is easily to read and can analyze less stable compounds as the sample is not subjected to heat as compared to GC (5).

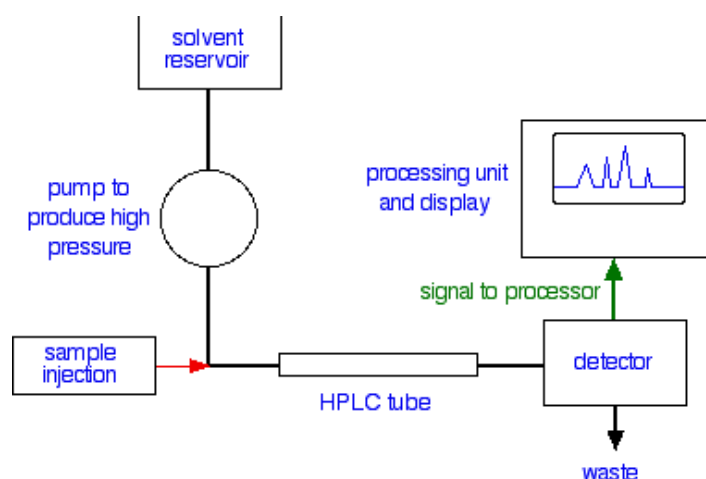


Figure (2): Schematic representation of HPLC ⁽⁶⁾.

1.3. Size Exclusion Chromatography (SEC):

Size exclusion chromatography (SEC) is also called molecular sieve. This analytical method separates molecules based on their molecular sizes and shapes by using the molecular sieve properties of a variety of porous materials. The SEC column is packed with a resin consisting of beads with pores of defined size. This resin is the SEC stationary phase. The mobile phase is a buffer that usually contains a salt, which is necessary to reduce the interaction between the proteins and the beads. The size of the pores or "holes" in these beads is controlled during the manufacturing process. The separation of chemicals by SEC is based on the amount of time molecules spend included in the pores of these beads. SEC is used to separate and analyze a variety of chemicals, including both synthetic polymers and biopolymers. It is a powerful and popular method to purify and determine the molecular weight of proteins. It is very effective method for the separation of the large molecules from the small ⁽⁷⁾.

1.4. Gel permeation Chromatography (GPC):

Gel Permeation Chromatography (GPC), also known as gel filtration Chromatography (GFC), is a chromatographic technique that separates dissolved molecules on the basis of their size by pumping them through specialized columns containing a porous packing material. It is used mainly for analysis of synthetic and biologic polymers, purification of polymers, polymer characterization and for study properties like molecular weight, size and viscosity.

In GPC, a solution of the dissolved sample is passed through a column packed with a controlled-porosity packing, typically a polymeric porous gel typically referred to as a stationary phase. The size and shape of the molecule dictate its ability to interact with the pores on the stationary phase. Small molecules typically enter the pores therefore take the longest to elute from the column. Molecules with a very large molecule weight cannot move into the pore spaces available in the stationary phase and elute relatively quickly. In this manner, a whole range of molecular weights can be characterized when compared to the retention time of standard molecular weights ⁽⁸⁾.

I. Particle Size / Sorting:

Measurement of particle size distributions is carried out across a wide range of analytical tools such as:

1. Flow Cytometer (FC):

Cell sorting is the separation and isolation of various cell populations. Flow cytometers (FC) are automated instruments they can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins.

Flow cytometer use fluorescent probes to identify and characterize cells or particles. Cells or particles tagged with fluorescent molecules enter the cytometer via a fluid stream. The cells then pass through a **laser**, which emits a specific wavelength of light. The fluorescent probes are excited by the laser and then emit light. The fluorescent signal is detected and amplified, then translated into an electronic signal, which is sent to the computer. Information about the size and granularity of a cell is recorded. The result is a visual presentation describing an individual or group of cellular events. This technique is costly and complicated ⁽⁹⁾.

2. Air Separation:

Particle separation is an important process in the fields of industrial production, environmental assessment and chemical or biological research. It is a novel approach for particle separation by sizes, using air–liquid interface in a microfluidic channel. The particles with different sizes can be separated by the action of different speed of the air–liquid interface. This separation technique has several advantages: It is totally independent on the properties of the particle and working fluid (such as the optical, magnetic and surface charge). It does not require any pre-treatment of particles such as optical or magnetic labelling. The process is fast and easy to handle ⁽¹⁰⁾.

3. Dynamic Light Scattering (DLS):

Dynamic light scattering (DLS), also known as photon correlation spectroscopy is a non-invasive, well-established technique used for measuring the size of molecules and particles which have been dispersed or dissolved in a liquid, typically in the submicron and less than 1nm. The Brownian motion of particles or molecules in suspension causes laser light to be scattered at different intensities. The particle size can be determined by analysis of fluctuation intensity versus time using the Stokes-Einstein relationship. Figure (3) represent DLS diagram. Dynamic light scattering technology offers the following advantages: Accurate, reliable, fast, simple, no need for sample preparation and directly measure high concentration and turbid samples, in addition to their ability to measure sizes less than 1nm ⁽¹¹⁾.

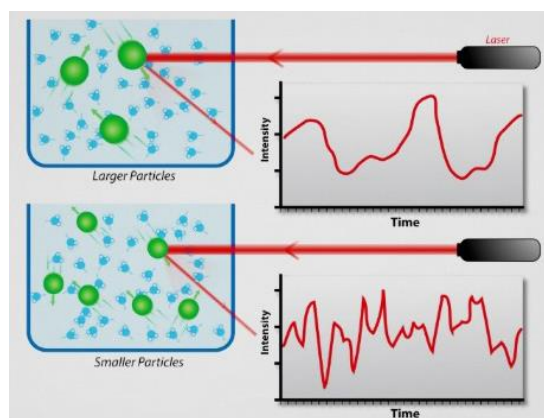


Figure (3): Schematic representation of DLS ⁽¹²⁾.

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