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SEAWEEDS CHROMATOGRAPHY- PRINCIPLES AND APPLICATION IN NUTRIENT PROFILING

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Chromatography is the technique used for the separation of the components in a sample mixture. It is the process of analyzing the different components present in a mixture qualitatively and quantitatively by separating them from each other. Chromatography was introduced by Mikhail Tswett in 1906. The name chromatography was derived from the terms "Chroma" which means colour; and graphein means written, as this technique was initially used for the separation of coloured compounds. Later, this technique became applicable even to colourless compounds. Chromatography can separate large as well as small amounts of compounds.

Principles

Chromatography is based on the principle where molecules in a mixture are applied onto the surface or into the solid, and the fluid stationary phase (stable phase) separates from each other while moving with the aid of a mobile phase. Chromatography allows the separation of components of a mixture on the basis of their nature, structure, size, and other properties. The essential components of chromatography are stationary phase and mobile phase.

Stationary phase

The majority of materials used as stationary phases include pores, which enable components to adhere during chromatography. Depending on the type of chromatography being used and the characteristics of the components that need to be separated, a stationary phase must be chosen. The stationary phase can be made of gel beads, thin uniform paper, silica, glass, certain gases, or even liquid components, depending on the type of chromatography being utilised.

Mobile phase

Depending on the nature of the components to be separated and the kind of chromatography, materials employed as mobile phases are chosen for a chromatographic procedure. The mobile phase in various chromatographic procedures is frequently made up of alcohol, water, acetic acid, acetone, or certain gases. If the mobile phase is liquid it is termed liquid chromatography (LC), and if it is gas then it is called gas chromatography (GC). Gas

chromatography is applied for gases, mixtures of volatile liquids, and solid materials. Liquid chromatography is used especially for thermal unstable, and non-volatile samples.

Term	Definition
Mobile Phase or carrier	Solvent moving through the column
Stationary Phase or absorbent	Substance that stays fixed inside the column
Eluent	Fluid entering the column
Eluate	Fluid exiting the column (that is collecting in flasks)
Elution	The process of washing out a compound through a column using a suitable solvent
Analyte	A mixture whose individual components have to be separated and analyzed

Separation mechanism

The separation of compounds is achieved by the differential partition between the stationary phase and the mobile phase. During differential partition, the compounds distribute or partition between the two phases, depending on their relative affinity to the phase. Relative affinity depends on their molecular structure and weak intermolecular forces such as hydrogen bonding or van der waals forces. If the affinity of the compounds is greater for the stationary phase, slower will be movement and vice-versa. This migration results in the separation of the compounds.

The time taken for the distribution of the compounds between the phases much be rapid compared to the velocity of mobile phase. The stationary phase, which is an immobile matrix contains sites, in which, the compounds passed along the mobile phase can bind. If the compounds interact or bind to the solid matrix, their movement through the stationary phase is retarded. This is called “impedance”.

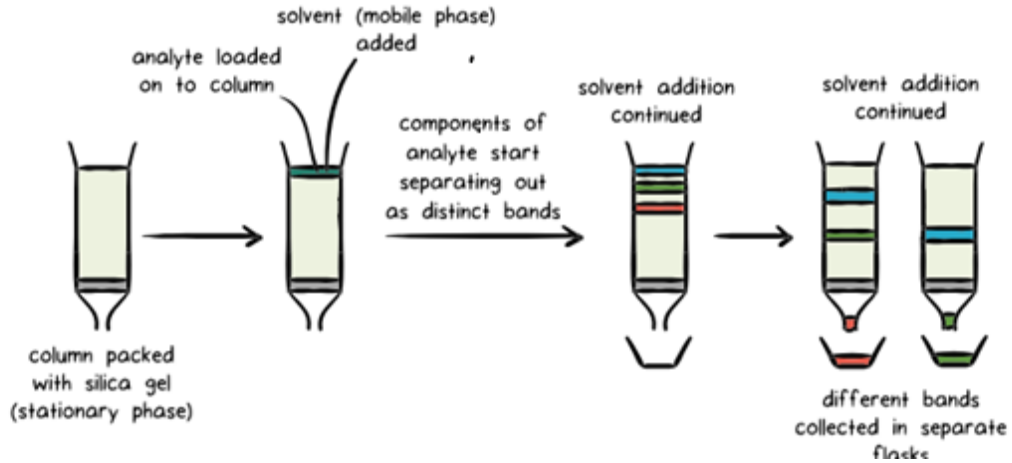
Factors influencing separation

There are two major factors that affect the resolution of the compound ina chromatographic separation. They are

- Distribution coefficient
- Sharpness of compound band

The distribution coefficient or partition coefficient (K_d) is defined as the ratio of the concentration of the compound in the mobile phase (c_m) to the concentration of the compound in the stationary phase (c_s). The K_d of the compound depends on its molecular structure, the nature of two phases, and temperature of the chromatographic column or system.

Separation mechanism of compounds



$K_d = c_m / c_s$

The sharpness of the compound band depends on the number of equilibrations. The number of equilibrations is also termed as “Theoretical plates”. If the number of theoretical plates is more, the compound band will be sharp and the column will be more efficient for the separation.

Types of chromatography

The chromatography techniques are broadly classified into adsorption chromatography and partition chromatography.

Adsorption chromatography

Adsorption chromatography or liquid-solid chromatography, first discovered by Tswett in 1903, is probably the oldest mode of chromatography. The adsorption chromatography is used for solid-gas chromatography and solid-liquid chromatography. Adsorption Chromatography involves the separation of a chemical mixture based on the interaction of the adsorbate with the adsorbent. In this process, the mixture of gas or liquid gets separated on the adsorbent bed which adsorbs different compounds at different rates. Column chromatography is an example of adsorption chromatography. Methods for vitamin K analysis in seafood use a combination of adsorption chromatography for sample clean-up, and reversed-phase LC on C-18 supports for the quantification.

Factors affecting the adsorption chromatography

- Choice of the adsorbent
- Selection of the solvent for the mixture

- The rate of flow of the solvent
- The temperature of the system
- The column height for the procedure.

Types of Adsorption Chromatography

There are four types of adsorption chromatography.

1. Thin Layer Chromatography
2. Solid Liquid Chromatography
3. Gas-Liquid Chromatography
4. Column Chromatography

Partition chromatography

Partition chromatography was introduced by Martin and Synge in 1941 for the separation of acetylated amino acids and was first applied to the separation of alkaloids by Evans and Partridge in 1948. The stationary phase in partition chromatography is a liquid or semi-liquid immobilized on the surface of a solid support with the help of a substance (polymer) to form a thin film. A filter paper or a column can act as a solid support. The mobile phase is usually "liquid". The separation is achieved based on the partition of the compound between two solvents i.e. stationary and mobile phases. E.g. Paper chromatography, high-pressure liquid chromatography, and gas chromatography.

Types of chromatographs used in nutrient profiling

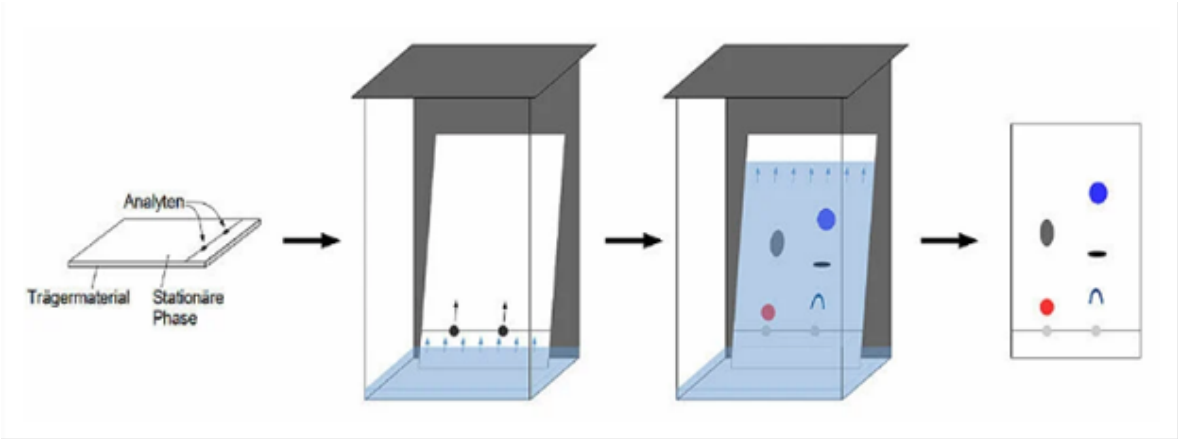
Chromatography can be classified based on mobile phase, stationary phase, forces of separation, or method of separation. There are several types of chromatography techniques, each with its own principles and applications. Chromatography techniques are widely used in seafood analysis to separate, identify, and quantify various compounds such as contaminants, flavor compounds, and nutritional components. These techniques are crucial for ensuring the nutrients, safety, quality, and authenticity of seafood products. Here are some of the most common types of chromatography used in nutrient profiling.

Preliminary screening and qualitative analysis of seafood extracts

Thin-layer chromatography (TLC)

Thin-layer chromatography is a separation technique where the stationary phase is applied as a thin layer on a solid support plate with a liquid mobile phase. This chromatography technique is based on the principle that components of a mixture are separated when the component having an affinity towards the stationary phase binds to the stationary phase. In contrast, other components are eluted with the mobile phase. The substrate/ ligand is bound to the stationary phase so that the reactive sites for the binding of components are exposed. Now, the mixture is passed through the mobile phase where the components with binding sites for the substrate bind to the substrate on the stationary phase while the rest of the components are eluted out with the mobile phase. After separation, the molecules are seen as spots at different location

throughout the stationary phase. The detection of molecules is performed by various techniques. TLC is a simple and cost-effective technique for separating and identifying compounds in seafood extracts. It is often used in preliminary screening and qualitative analysis.



Thin layer chromatography

Amino acid and vitamin analysis

High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography is a modified form of column chromatography where the components of a mixture are separated on the basis of their affinity with the stationary phase. This technique is based on the principle of differential adsorption where different molecules in a mixture have a varying degree of interactions with the absorbent present on the stationary phase. The molecules having higher affinity remain adsorbed for a longer time decreasing their speed of movement through the column. However, the molecules with lower affinity move with a faster movement, thus allowing the molecules to be separated in different fractions. This process is slightly different from the column chromatography as in this case; the solvent is forced under high pressures of up to 400 atmospheres instead of allowing it to drip down under gravity. HPLC is one of the most commonly used chromatography techniques used in the determination of amino acid and fat-soluble vitamins. Using UV detection for amino acids in most cases requires using the absorption of the carboxyl group (-COOH) in the 200 to 210 nm range. Some amino acids with benzene rings can also be detected in the 250 to 280 nm range, but in general, they are difficult to analyze as it is sufficient sensitivity and selectivity. The most commonly used detectors in liquid chromatography are Diode-Array Detector (DAD), Fluorescence Detector (FLD), Mass spectrophotometry (MS), and evaporative light scattering detector (ELSD).

LC-MS combines liquid chromatography with mass spectrometry detection and is used for the analysis of complex mixtures in seafood, including the detection of various contaminants,

pesticides, and toxins. LC-MS is highly sensitive and can provide structural information about the compounds.

Volatile fatty acids and Volatile organic compound analysis

Gas Chromatography (GC)

Gas chromatography is a separation technique in which the molecules are separated on the basis of their retention time depending on the affinity of the molecules to the stationary phase. The sample is either liquid or gas that is vaporized in the injection point. Gas chromatography is based on the principle that components having a higher affinity to the stationary phase have a higher retention time as they take a longer time to come out of the column. However, the components having a higher affinity to the stationary phase have less retention time as they move along with the mobile phase. The mobile phase is a gas, mostly helium that carries the sample through the column. The sample once injected in converted into the vapor stage is then passed through a detector to determine the retention time. The components are collected separately as they come out of the stationary phase at different times.

Gas Chromatography-Mass Spectrometry (GC-MS): GC-MS is employed for the analysis of volatile and semi-volatile compounds in seafood, including the detection of flavor compounds, lipid oxidation products, and contaminants like PCBs, dioxins, and pesticides.

Polysaccharide and protein analysis

Size-Exclusion Chromatography (SEC)

Size-exclusion chromatography (SEC) is the conventional name for a separation method used most frequently for the fractionation and analytical characterization of macromolecules of biological or synthetic origin and less frequently for the separation of colloidal particles. Size exclusion chromatography (SEC) separates molecules based on their size by filtration through a gel. The gel consists of spherical beads containing pores of a specific size distribution. Separation occurs when molecules of different sizes are included or excluded from the pores within the matrix. The invention of small porous particles with a typical diameter between 1 and 10 μm brought about an important technological improvement in SEC. The consequent miniaturization of the columns allowed the reduction of the analysis time to minutes or even to tens of seconds. The development of this separation method is reflected by the numerous names that have been given to this process; for example, gel filtration, gel chromatography, gel filtration chromatography, gel exclusion chromatography, gel permeation chromatography, restricted diffusion chromatography, size separation chromatography, and molecular sieve chromatography. SEC is employed for the separation and quantification of macromolecules like proteins and polysaccharides in seafood products. It is useful for assessing protein quality and freshness.

High-Resolution Mass Spectrometry (HRMS)

High-resolution mass spectrometry (HRMS) is an analytical technique that is used to determine the exact molecular masses of compounds present in a sample. The highly accurate nature of HRMS makes it ideal for the identification of molecular structures, ranging from small organic molecules to large biological macromolecules. HRMS analysis begins by passing a sample into the spectrometer, where it is ionized. The formed ions will travel along the spectrometer's length, separated by their relative charges and masses. Once the ions reach the end of the spectrometer, they are picked up by a detector, and the information is logged on a computer.

Conclusion

Chromatography techniques have diverse applications in various fields, including chemistry, biochemistry, pharmaceuticals, environmental science, and food analysis, among others. These chromatography techniques, when appropriately selected and applied, enable seafood analysts to assess the safety, quality, and nutritional value of seafood products, as well as to detect and quantify contaminants and flavor compounds that are critical for consumer satisfaction and regulatory compliance.

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