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Column Chromatography and its Methods in Separation for Constituents

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Commentary

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DESCRIPTION

Chromatography is a laboratory technique used in chemical analysis to separate a mixture into its constituents. The mixture is dissolved in a fluid solvent (gas or liquid) known as the mobile phase, which transports it through a system (a column, a capillary tube, a plate or a sheet) that is fixed with a material known as the stationary phase. Because the constituents of the mixture have varying affinities for the stationary phase and are retained for varying lengths of time depending on their interactions with its surface sites, they travel at different apparent velocities in the mobile fluid causing them to separate. The separation is accomplished through differential partitioning of the mobile and stationary phases.

The concept of partition coefficient validates chromatography. Any solute partitioning occurs between two immiscible solvents. When we make one solvent immobile and another mobile, we get the most common chromatographic applications. If the matrix support or stationary phase is polar (e.g., paper, silica etc.) the chromatography is forward phase if it is non-polar (C-18) the chromatography is reverse phase.

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Column chromatography is a method of separation in which the stationary bed is contained within a tube. The particles of the solid stationary phase or the support coated with a liquid stationary phase may fill the entire inside volume of the tube (packed column) or be concentrated on or along the inside tube wall, leaving an open, unrestricted path for the mobile phase in the tube's middle (open tubular column). Different retention times of the sample are calculated based on differences in rates of movement through the medium. The method is very similar to traditional column chromatography with the exception that the solvent is forced through the column by applying positive pressure. Most separations could now be completed in less than 20 minutes, with better separations than the previous method. Modern flash chromatography systems are sold in the form of pre-packaged plastic cartridges with the solvent pumped through them. Systems can also be linked to detectors and fraction collectors allowing for automation. Gradient pumps accelerated separations and reduced solvent consumption. A fluidized bed, rather than a solid phase formed by a packed bed, is used in expanded bed adsorption. For culture broths or slurries of broken cells this allows for the omission of initial clearing steps like centrifugation and filtration. A column is made by packing a solid absorbent into a cylindrical glass or plastic tube. The size is determined by the amount of compound isolated. To keep the solid phase in place, the tube's base contains a filter either a cotton or glass wool plug or glass frit. A solvent reservoir can be attached to the top of the column. The dry method and the wet method are the two most common methods for preparing a column. For the dry method, the column is first filled with dry stationary phase powder, followed by the addition of mobile phase, which is flushed through the column until it is completely wet and is never allowed to run dry from this point on. For the wet method, slurry of the eluent and the stationary phase powder is prepared and carefully poured into the column. The top of the silica should be flat and it can be protected with a layer of sand. To advance the organic material, eluent is slowly passed through the column. The stationary phase retains the individual components differently and separates them while they run at different speeds through the column with the eluent. They elute one at a time at the column's end. The eluent is collected in a series of fractions throughout the chromatography process. Fraction collectors are devices that collect fractions automatically. By running multiple columns at once, chromatography productivity can be increased. Multi-stream collectors are used in this case. The eluent flow's composition can be monitored, and each fraction is analyzed for dissolved compounds using analytical chromatography, UV absorption spectra or fluorescence, for example.

Column chromatography is using a solid as the stationary phase or adsorbent. Silica gel is the most frequently used stationary phase in column chromatography with alumina coming in second. Previously, cellulose powder was widely used. To perform ion exchange chromatography, Reversed-Phase Chromatography (RP), affinity chromatography or Expanded Bed Adsorption (EBA), a wide variety of stationary phases are available. The stationary phases are typically finely ground powders or gels and/or micro porous for increased surface area, though Expanded Bed Adsorption (EBA) employs a fluidized bed. The mobile phase, also known as eluent is a solvent or a mixture of solvents that is used to move compounds through the column. It is chosen so that the retention factor value of the compound of interest is roughly between 0.2 and 0.3 in order to reduce the time and

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amount of eluent required to run the chromatography. The eluent has also been chosen so that the various compounds can be effectively separated.