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A Short Note on Liquid chromatography and its Types

Lin Fuxian*

Department of chemistry, Bahir Dar University, Bahir Dar City, Ethiopia

Perspective

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***For Correspondence:**
Lin Fuxian, Department of chemistry, Bahir Dar University, Bahir Dar City, Ethiopia
E-mail: linfuxian01@gmail.com

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ABSTRACT

Liquid chromatography is a physical separation technology that distributes the components of a liquid mixture across two immiscible phases, stationary and mobile. Adsorption chromatography, partition chromatography, ion-exchange chromatography, size-exclusion chromatography, and affinity chromatography are the five categories of LC practice. The Reverse-Phase (RP) mode of the partition chromatography technique, which uses a nonpolar (hydrophobic) stationary phase and a polar mobile phase, is the most extensively utilised of these. The stationary matrix is made by attaching long-chain alkyl groups (e.g., n-octadecyl or C_{18}) to the external and internal surfaces of irregularly or spherically shaped 5 m diameter porous silica particles in common applications.

INTRODUCTION

In HPLC, 20 ml of the sample of interest is typically injected into the mobile phase stream, which is provided by a high-pressure pump. The analytes-containing mobile phase permeates the stationary phase bed in a certain direction. The components of the mixture are separated into mobile and stationary phases based on their chemical affinity. When the liquid interacts with the stationary bed, it goes through a series of sorption and desorption

stages. The liquid solvent (mobile phase) is injected into a packed column containing the stationary phase at high pressure (up to 400 bar or 300.000 torr). For reproducible chromatography tests, high pressure is required to maintain a steady flow rate. The components of the sample will flow out of the column at different times depending on how the mobile and stationary phases are partitioned. The column is the most important part of the LC system because it is designed to withstand the liquid's high pressure. Conventional LC columns have an exterior diameter of 6.4 mm (1/4 inch) and an internal diameter of 3.0–4.6 mm and are 100–300 mm long. Chromatography columns with 3–5 m diameter packing particles might be shorter (30–50 mm) for applications using LC-MS. Other LC columns include the narrow bore, microbore, microcapillary, and nano-LC versions, in addition to the regular variant. These columns feature smaller internal diameters, which allows for better separation and can handle liquid flows of less than 1 ml/min (the conventional flow rate). Ultra-High-Performance Liquid Chromatography (UHPLC) can be used instead of HPLC to improve separation efficiency and peak resolution. This LC type requires higher working pressures in the range of 310000 to 775000 torr and uses columns packed with smaller *silica* particles (1.7 m diameter) (6000 to 15000 psi, 400 to 1034 bar).

Types

Normal phase chromatography: This method uses a continuous polar stationary phase and a non-polar liquid solvent to measure non-polar materials. Because of the polarity difference, the least polar molecules separate first, followed by the most polar compounds. The compounds are separated using gravity to drive them apart.

Reverse phase chromatography: Reverse phase polarity is the polar opposite of normal phase polarity, and it uses a polar liquid mobile phase and a non-polar stationary phase to separate the most polar chemicals from the less polar molecules. HPLC also use reverse phase chromatography.

Partition chromatography: Partition chromatography is a liquid-liquid method that requires the stationary and mobile phases to be immiscible, or split into components when mixed together like gasoline and water.

Chiral chromatography: This approach separates chiral molecules, which are non-superimposable mirror images. While chiral molecules are symmetrical, they are not interchangeable and can be selectively isolated from a racemic solution, or one that contains the opposing chiral molecules, based on one of the chiral bonds present in the stationary or mobile phase. This is primarily used in biochemistry.