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Research Paper Chemistry

History and Introduction to Chromatographic Techniques

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History of chromatography

In 1903 a Russian botanist Mikhail Tweet produced a colorful separation of plant pigments through calcium carbonate column. Chromatography word came from Greek language chrome color and graphing to write i.e. color writing or chromatography.

Introduction of Chromatography

Modern pharmaceutical formulations are complex mixtures containing one or more therapeutically active ingredients, to a number of inert materials like excipients, disintegrates, colors and flavors. In order to ensure quality and stability of the final product, the pharmaceutical analyst must be able to separate the mixtures into individual components prior to quantitative analysis. Chromatography is the powerful techniques in which differential migration of components take place between two phases, one is stable which is known as stationary phase and another is movable which is known as a mobile phase. Species in the sample undergo repeated interactions (partitions) between the mobile phase and stationary phase. The stationary phase may be solid or a liquid (supported on a solid or a gel), and packed in a column, spread as a layer or film. The mobile phase may be gaseous or liquid.

Those solutes, distributed preferentially in the mobile phase, will move rapidly through the system than those distributed preferentially in the stationary phase. This forms the basis of separation of component present in a sample. The distribution of a solute between two phases results from the balance of forces between solute molecules and the molecule of each phase.

Chromatographic method must having essentially,

- Stationary phase,
- Mobile phase,
- Sample injection system,
- Solvent delivery system,
- Column (support for stationary phase),
- Detection by detecting agent

All chromatographic methods involve modifications in these basic components.

HISTORY OF HPLC

Prior to the 1970's, few reliable chromatographic methods were commercially available to the laboratory scientist. During 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography, and thin-layer chromatography. However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds. During this time, pressure liquid chromatography began to be used to decrease flow through time, thus reducing purification times of compounds being isolated by column chromatography. However, flow rates were inconsistent, and the question of whether it was better to have constant flow rate or constant pressure was debated.

High pressure liquid chromatography was developed in the mid-1970's and quickly improved with the development of column packing materials and the additional convenience of on-line detectors. In the late 1970's, new methods including reverse phase liquid chromatography allowed for improved separation between very similar compounds.

By the 1980's HPLC was commonly used for the separation of chemical compounds. New techniques improved separation, identification, purification and quantification far above the previous techniques. Computers and automation added to the convenience of HPLC. Improvements in type of columns and thus reproducibility were made as such terms as micro-column, affinity columns, and Fast HPLC began to immerge.

Modern High Performance Liquid Chromatography (HPLC)

The highly sophisticated reliable and fast liquid chromatographic (LC) separation techniques are become a requirement in many industries like pharmaceuticals, agrochemicals, dyes, petrochemicals, natural products and others.

Early LC used gravity fed open tubular columns with particles 100s of microns in size; the human eye was used for a detector and separations often took hours to develop. Today's HPLC requires very special apparatus which includes the following.

i. Extremely precise gradient mixers.
ii. HPLC high pressure pumps with very constant flow.
iii. Unique high accuracy, low dispersion, HPLC sample valves.
iv. Very high efficiency HPLC columns with inert packing materials.
v. High sensitivity low dispersion HPLC detectors.
vi. High speed data acquisition systems.
vii. Low dispersion connecting tubes for valve to column and column to detector.

HPLC Gradient mixtures

HPLC gradient mixers must provide a very precise control of solvent composition to maintain a reproducible gradient profile. This can be complicated in HPLC by the small elution volumes required by many systems. It is much more difficult to produce a constant gradient when mixing small volumes then when mixing large volumes.

HPLC Pumps

Because of the small particles used in modern HPLC, modern LC pumps need to operate reliably and precisely at pressures of 10,000 p.s.i. or at least 6,000 p.s.i. To operate at these pressures and remain sensibly inert to the wide variety of solvents used HPLC pumps usually have sapphire pistons, stainless steel cylinders and return valves fitted with sapphire balls and stainless steel seats.

HPLC Sample Valves

Since sample valves come between the pump and the column it follows that HPLC sample valves must also tolerate pressures up to 10,000 p.s.i., For analytical HPLC, the sample volume should be selectable from sub micro liter to a few micro liters, whereas in preparative HPLC the sample volume may be even greater than 10 ml.

HPLC Columns

HPLC columns are packed with very fine particles (usually a few microns in diameter).

The very fine particles are required to attain the low dispersion that give the high plate counts expected of modern HPLC. Plate counts in excess of 25,000 plates per column are possible with modern columns, however, these very high efficiencies are very rarely found with real samples because of the dispersion associated with injection valves, detectors, data acquisition systems and the dispersion due to the higher molecular weight of real samples as opposed to the common test samples. Packing these small particles into the column is a difficult technical problem but even with good packing a great amount of care must be given to the column end fittings and the inlet and outlet connection to keep dispersion to a minimum.

HPLC Detectors

In order to give an accurate chromatographic profile the detector sampling (cell) volume must be a small fraction of the solute elution vol-

ume. If the detector volume were larger than the elution volume then you would have peaks that appeared with flat tops as the whole peak would be resident in the detector at the same time. This means that as column volumes decrease and system efficiencies increase the volume of the detector cell volume must also decrease.

HPLC Data acquisition

In HPLC data acquisition system the higher sampling rate needed for the rapidly eluting narrow peaks of the HPLC chromatogram. Although the theoretical number of samples needed for good quantization are actually quite small, for real systems a hundred samples or more per peak is recommended; thus, for a 4 sec wide peak, a rate of 25 samples per second may be required. The same data analysis and reporting software can be used as in ordinary LC.

Conclusion

HPLC is probably the most universal type of analytical procedure; its application areas include quality control, process control, forensic analysis, environmental monitoring and clinical testing. In addition HPLC also ranks as one of the most sensitive analytical procedures and is unique in that it easily copes with multi-component mixtures.

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