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A REVIEW ON FLASH CHROMATOGRAPHY AND ITS PHARMACEUTICAL APPLICATIONS

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Abstract

Earlier column chromatography was used for preparative purposes as well as for reaction control in organic synthesis. Column chromatography is an extremely tedious stage in any laboratory and can quickly become a point of congestion for any process lab. Flash chromatography is a technique developed as a modification of preparative column chromatography. This is an air pressure driven technique comprising of medium and short column chromatography, optimised for rapid separation of organic compounds. Modern flash chromatographic system consists of pre- packed plastic cartridges where in the solvent is pumped through the cartridge. By employing high pressure gas, the efficiency and speed of classical column chromatography can be increased. Flash chromatography is an easy & quick approach that is economical to preparative liquid chromatography. The content mentioned in this article mainly focuses on the various components, general principles, procedures and applications of flash chromatography.

KEYWORDS:

Flash chromatography, column chromatography, liquid chromatography, air pressure.

Introduction

Excluding TLC, all the Chromatographic techniques use columns for separation. It is also known as medium pressure chromatography. In flash Chromatography, generally the solvent is forcefully driven down the column by positive air pressure. Because of restricted flow of solvent, pressurized gas is employed to drive the solvent through the column of stationary phase. It is an advantageous technique as it allows faster rate of solvent flow. When compared to simple gravity flow, flash chromatography offers rapid separation of molecules from a mixture and is used in drug discovery process. Flash chromatography differs from other techniques in different aspects as follows:

a) It uses silica gel particles which pass through 250-400mesh.
b) Pressurized gas (ca.10-15psi) is used to drive the solvent through column of stationary phase.

The main applications of flash chromatography are Isolation of intended compounds from products natural origin; refining synthetic products; isolation of purified compounds for further analysis; segregation of complex mixtures into simple fractions. The most used stationary phase is silica and $SiO_2$and Al_2O_3 are the two adsorbents. The size of adsorbent particle affects the flow of solvent. There is no requirement of additional supports like cotton glass wool / sand when there is a porous plate to the column. Manufacturers are developing automated flash chromatographic systems. Two main types of automated flash chromatographic systems are LPLC (low pressure liquid chromatography) &MPLC (medium pressure liquid chromatography).

Principle:

The eluent (developing solvent) under pressurized gas is rapidly pushed through a short glass column that is packed with an adsorbent of a well-defined particle size with a large inner diameter. Most commonly used stationary phase is silica gel (40-63 micrometers) and other agents with varying particle sizes can also be used. Low viscosity mobile phases should be employed in case of particles with size smaller than 25micrometres, to avoid very low flow rate.

Usually, gel beds are about 15cm high with 1.5-2.0 bar pressure. Mostly unmodified silica was used as stationary phase, so that the normal phase chromatography was possible.
However, parallel to HPLC reversed phase material can be used in flash Chromatography.

Theory:
Chromatography exploits the differences in partitioning behavior between a mobile phase and a stationary phase to separate the components in a mixture.[1] Compounds of the mixture interact with either the stationary phase or relative solubility. In a continuous development system like HPLC or GC, the retention is measured as retention time (rt) i.e., the time between injection and detection. In un-interrupted development system, the retention is measured as retention factor (Rf)i.e., the run length of the compound divided by the run length of eluent front.

Rf = distance travelled by the solvent front.[4]

Chromatography exploits variance in partitioning behavior between a stationary phase and mobile phase in order to isolate the components of a mixture.

1. Various Components of Flash Chromatography

Sorbent selection:
Proper selection of adsorbent is the basic pre-requisite for successful separation of components.$SiO_2$and Al_2O_3 are the commonly used adsorbents.

These adsorbents are available in different mesh sizes as indicated by a no of bottle label i.e."silica gel 60 "or "silica gel 230-400".The density of powdered silica gel is 0.75g/ml .Silica, florisil, alumina, reverse phase silica are mainly used in flash chromatography. If your Rf is a≈0.2, you will require a volume of dissolvable ≈5X the volume of the dry silica gel to run your column.[4]

Solvent Systems:
A mixture of 2 solvents i.e., a polar & a non-polar component is used to carry out a flash column chromatography.[6] Single solvent can be used in rare cases. One component solvent system are mentioned here from least polar to the most polar systems. Streak fragment Chromatography is by and large finished with a mix of two solvents with a polar and a non-polar portion.
These are a couple of adsorbents which are basically used as a piece of blast Chromatography.[7] As acidic corrosive bubbles at a lower BP than toluene, this will eliminate the corrosive without uncovering the flawless compound to it (1) (5), as shown in table (1).

a) Hydrocarbons: pentane, petroleum, ether, hexanes.
b) Ether and dichloromethane.
c) Ethyl acetate

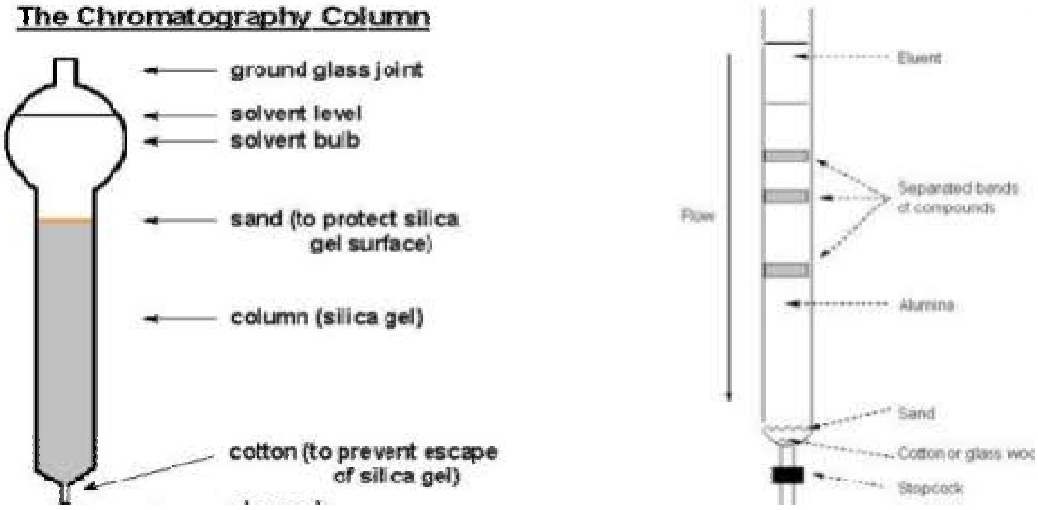
Two- component solvent system includes:

a) Ether / petroleum ether, Ether/hexane and Ether/pentane.
b) Ethyl acetate / Hexane.

Table 1:The properties of commonly used flash solvents[2]

Solvent	Density (g/ml)	Elution Strength	Solvent Group	Boiling Point (°C)	UV Cut-off (nm)	TLV (ppm)
n-Hexane	0.66	0.01	1	69	195	100
2 2 4-Trimethylpentane	0.69	0.02	1	99	210	300
Cyclohexane	0.77	0.03	1	81	200	100
1 1 2-Trichloromethane	1.48	0.31	8	61	245	50
Toluene	0.87	0.22	7	110	285	100
Dichloromethane	1.33	0.30	5	40	232	100
Ethyl Acetate	0.90	0.45	6	77	256	400
Methyl-t-butyl ether	0.74	0.48	2	55	210	40
Acetone	0.79	0.53	6	56	330	750
Tetrahydrofuran	0.89	0.35	4	66	212	200
Acetonitrile	0.78	0.50	6	82	190	40
Isopropanol	0.79	0.60	3	82	205	400
Ethanol	0.79	0.88	3	78	210	1000
Methanol	0.79	0.70	3	65	205	200
Water	1.00	0.073	8	100	180	-

Figure 1: Flash chromatography column.



Packing the column

Take a glass column and make sure that it has a plug of cotton wool directly above the stopcock in order to prevent the escape of silica gel from the column.

The dissolvable evading from the stopcock ought not be warm or hot. [8] Also stuffed segment ought not have any breaks or fixes.[9]

Put a ½ inch of layer of sand above the plug of cotton wool. Use in a quantity such that a flat surface is obtained with a same diameter as that of body of the column. Add dry silica gel absorbent usually the jar is labelled as "for flash Chromatography".

Figure 2: Method of packing the column

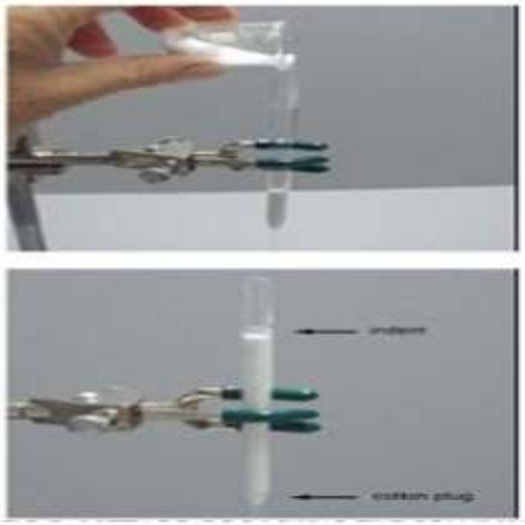


Figure 3: Method of packing the column

Advanced Detection Techniques of Flash Chromatography:

UV detection is one of the traditional methods in flash chromatography to monitor and fractionate peaks while the process of purification is on-going. Few detecting options are available in flash chromatography for the compounds lacking chromophores and thus can't be detected by UV. If UV chromophores are absent, the invisible compounds may not be detected with UV. UV absorption of mobile phase may interfere with absorption maximum of compound. In other cases, the absorption spectrum of compounds may be unknown, therefore not detected. These advanced

detection techniques allow users to fractionate the compounds very easily.

Figure 5: Instrumentation of Flash Chromatography

Modern Flash Chromatography:

In modern flash chromatographic system, pre-packed plastic cartridges are placed in place of glass column which are safer and reproducible. The solvent is pumped through the cartridge. For the system automation, detectors and fraction collectors are linked. Introduction of gradient pumps indicates quicker isolations, less solvent usage and greater flexibility.

Column characteristics:

a. Disposable plastic cartridges
b. Cartridges of different size
c. Solid sample module and injection value
d. Pressure up to 100psi
e. Narrow particle distribution

2. Green Flash Chromatography:

Green flash chromatography is the ultimate flash chromatographic technology which attains the most efficient sample purification. Minimum elution solvent is sufficient for sample run. It reduces run time and use of solvent during separation.

Features of green flash chromatography:

Optimal parameters for flow rate, run time, fraction volume etc. will be calculated and set automatically upon selecting a column on" GREEN FLASH" software. Software provides the maximum sample load information for the selected column.

State- of-The-Art software based on true theory of chromatography. Sample Eluting position and Resolution can be fully controlled for systems.

Automatic method setup for Reverse phase chromatography. Parallel detection of UV detector and RI detector or ELSD.

1. Flash Chromatography With TLC Image Reader:

The system consists of a built-in UV light source and a camera. By shooting the TLC plate and clicking the target compound on the TLC plate, the Rf value of the target compound will be calculated. The TLC plate is displayed on the screen during sample run. Compound spots on the TLC plate and the compound peaks are displayed on the screen. Both the photographic image and the purification are saved as a data file. Click the target compound and the nearest impurity on the TLC plate. The maximum sample load for each column will be automatically calculated which enable the chemists to choose the best suited column for their sample.

Applications of Flash Chromatography:

Natural products/ Nutraceuticals Application:

1. Separation and Isolation of α &β -Santalol from sandalwood extraction.
2. Isolation and purification of chromophoric and Nonchromophoric compounds.
3. Isolation and purification of flavonoids.
4. Isolation and purification of ginsenosides from Red Panax ginseng extract.
5. Isolation and purification of catechins.
6. Purification of ferulic acid in rhizoma and chuanxiong extract.

Carbohydrate Applications:

1. Purification of conjugated Quercetin and Rutinose
2. Impurity isolation of valproic acid from cyclodextrin during Encapsulation.

Isolation of amino glycoside antibiotics.

Lipid Application:

1. Purification of fatty acid methyl esters (FAMEs)
2. Purification of sterols.

3. Pharmaceutical /Small Molecules Application:

1. Bile acid purification during lead generation.
2. In impurity isolation during drug purification.
3. Mestranol purification during chemical synthesis.
4. In anti-malarial drug purification.
5. It is used for purification of protected peptide.
6. Used for purification of trace compounds from organic mixtures.
7. Used as a tool to monitor the reaction progress
8. It is used for high-speed flash fractionation of natural products.

4. Advantages:

- Fast and economic methods.
- Ideal method for separation of compounds up to gram quantities.
- Equipment required is cheap /affordable.
- Automated changes between normal phase and reversed phase chromatography.

5. Conclusion:

Flash Chromatography is a simple, fast, cost effective approach to preparative liquid chromatography. In flash chromatography, separations are based up on traditionally obtained TLC results. It is very useful technique for quick separation of increasing quantities of samples. The results obtained are predictable and easy to scale up & down as required. Flash Chromatography is an alternative technique to preparative HPLC as it saves time and solvent. Modern flash chromatography with pre-packed plastic cartridges and advanced techniques are applicable to wide range of compounds.

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