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R E V I E W A R T I C L E

A Review on Flash Chromatography

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A B S T R A C T

Chromatography was most widely used analytical technique of the separation of mixture of the components with the help of stationary phase and mobile phase. Flash chromatography was one of the advance techniques of normal phase chromatography. In this technique separation of mixture of the components from few milligrams to up to industrial kg scale. Flash chromatography was parallel to the HPLC. Stationary phase purpose glass columns are used and packed with silicagel. The present review explains the various components in flash chromatography and applications. Flash Chromatography is a simple, fast, cost effective Preparative Liquid Chromatography approach. Separations are based upon traditionally obtained TLC results which are simply extrapolated to preparative scale.

Keywords: Flash chromatography, Sillica gel, glass column, Normal phase

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Flash chromatography, also called as medium pressure chromatography it was popularized several years ago by Clark Still of Columbia University; it is an alternative to slow and often inefficient gravity-fed chromatography. It is used in organic research laboratories. Flash chromatography controls the synthesized organic compounds. Flash chromatography differs from the conventional Technique in two ways:

- In the first step slightly smaller silica gel particles (250-400 mesh) are used, and
- Second due to restricted flow of solvent caused by the small gel particles, pressurized gas (*ca.* 10-15 psi) is used to drive the solvent through the column of stationary phase. The net result is a rapid ("over in a flash") and high resolution chromatography.

2. Classification

Flash chromatography classified into two types

1. LPLC - Low pressure liquid chromatography (LPLC) system which operate around at 50 -75 psi.

2. MPLC - Medium pressure liquid chromatography (MPLC) systems which operate above 150 psi.

Principle

The principle is that the eluent is, under gas pressure (normally nitrogen or compressed air) rapidly pushed through a short glass column with large inner diameter. The glass column is packed with an adsorbent. The most used stationary phase is silica gel 40 – 63 μm, but obviously packing with other particle sizes can be used as well. Particles size less than 25 μm should be used for very low viscosity mobile phases, because the flow rate would be very low. In the meantime, however, and parallel to HPLC, in flash chromatography reverse phase materials are most used frequently.

Theory

Chromatography is differences in partitioning behavior between a mobile phase and a stationary phase to separate the mixture into a component. Compounds of the mixture interact with the stationary phase based on charge, relative solubility or adsorption. The retention is a measure of the speed at which a substance moves in a chromatographic system.

Stationary phase of flash Chromatographic System

The basic prerequisite for successful separations is the choice of the proper adsorbent. The most important stationary phase in column chromatography is silica. Silica gel (SiO_2) and alumina (Al_2O_3) are two adsorbents commonly used by the organic chemist for column chromatography. These adsorbents are sold in different mesh sizes, as indicated by a number on the bottle label: "silica gel 60" or "silica gel 230-400" is a couple examples.

These are some stationary phases which are mainly used in flash chromatography.

1. Silica:

Slightly acidic medium. Best for ordinary compounds, good separation is achieved.

2. Florisil:

Mild, neutral medium. 200 mesh can be effective for easy separations. Less than 200 mesh best for purification by filtration. Some compounds stick on florisil, test first.

3. Alumina:

Basic or neutral medium. Can be effective for easy separations, and purification of amines.

4. Reverse phase silica: The most polar compounds elute fastest, the most nonpolar slowest.

Solvent Systems

Flash column chromatography is usually carried out with a mixture of two solvents, with a polar and a non-polar component. In flash chromatography most commonly used solvent systems are,

- One –component solvent system
- Two –component solvent system

The properties of commonly used flash solvents:

Here we can select the compound have a TLC Rf of ≈0.15 to 0.20 in the solvent system. Binary (two component) solvent systems with one solvent having a higher polarity than the other are usually best since they allow for easy adjustment of the average polarity of the eluent. The ratio of solvents determines the polarity of the solvent system, and hence the rates of elution of the compounds to be separated. Higher polarity of solvent increases rate of elution for all compounds. If your Rf is a ≈0.2, you will need a volume of solvent ≈5X the volume of the dry silica gel in order to run your column (table 1).

Column Selection

- Select a column that is 10, 20, 40 mm ID based upon preparative requirements. Indeed, Professor Still et al offered this selection. Single Step Flash Columns (patented) represent an innovative step forward in chromatography.
- Flash Chromatography is a quick and inexpensive technique for the purification of organic compounds.
- Thomson flash columns come in a wide variety of sizes ranging from 4g to 300g silica-based for easy scalability of synthetic reactions.
- Thomson also offers other packing material like Amine and C18 flash columns which enable the end-user to utilize these flash columns for a broad range of reactions.

Typical Data of Silica gel Column Grade Adsorbents [4]:

- Iron Content : <0.02%
- Chloride Content : <0.10%
- Loss on Drying : <3%
- PH (10% suspension) : 7±0.5
- Surface Area: 400–600 m2/gm

Packing the Column:

Obtain a glass column and make sure that it has either a glass frit or a plug of cotton wool directly above the stopcock to prevent the silica gel from escaping from the column through the stopcock. (IF it doesn't have either, we must put in a somewhat loosely stuffed plug of cotton wool; if we stuff it too much; solvent flow becomes painfully slow even with air pressure above the column).Higher polarity of solvent increases rate of elution for all compounds. If your Rf is a ≈0.2, you will need a volume of solvent ≈5X the volume of the dry silica gel in order to run your column.

Solvating the Silica Gel Column 1:

- Next, tap gently and evenly the sides of the column with a piece of rubber tubing to settle the silica gel. Pour a good amount of elution solvent onto the silica gel. Use pressurized gas to force the solvent through the silica.

- As we force through a few hundred milliliters, we should see the top part of the silica become more homogeneous. This is because we are forcing out air that was entrapped in the silica gel. Continue to flush solvent through the silica gel until the entire silica plug becomes homogeneous in appearance.
- We may have to recycle the solvent coming through the column onto the top of the column several times before all the silica gel is solvated. Do not let the top of the column run dry otherwise we will force air back into the top of the silica, and we will be back where you started.

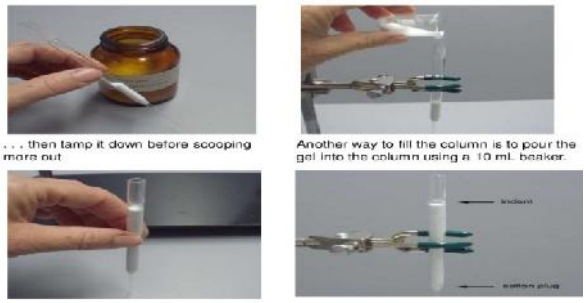

Fig.1: Steps involved in solvating the silica gel column

Load the sample onto the silica gel column:

Two different methods are used to load the column.

- ❖ Wet loading method
- ❖ Dry loading method

Wet Loading Method:

- In the wet method, the sample to be purified (or separated into components) is dissolved in a small amount of solvent, such as hexanes, acetone, or other solvent.
- This solution is loaded onto e column. Sometimes the solvent of choice to load the sample onto the column is more polar than the eluting solvents.
- In this case, if we use the wet method of column loading, it is critical that we only use a few drops of solvent to load the sample.
- If we use too much solvent, the loading solvent will interfere with the elution and hence the purification or separation of the mixture.
- In such cases, the dry method of column loading is recommended.

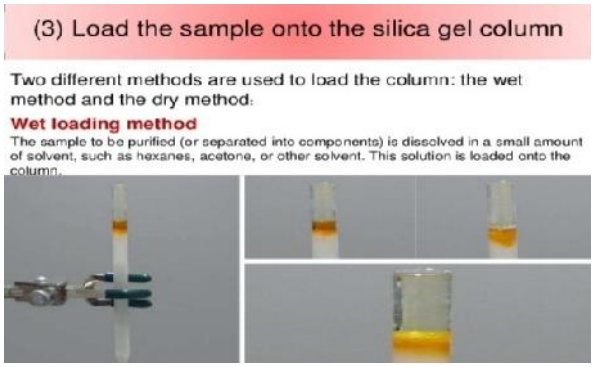

Fig.2: Wet loading method

Dry Loading Method:

- First dissolve the sample to be analyzed in the minimum amount of solvent and add about 100 mg of silica gel. Swirl the mixture until the solvent evaporates and only a dry powder remains.

Place the dry powder on a folded piece of weighing paper and transfer it to the top of the prepared column. Add fresh eluting solvent to the top now we are ready to begin the elution process.

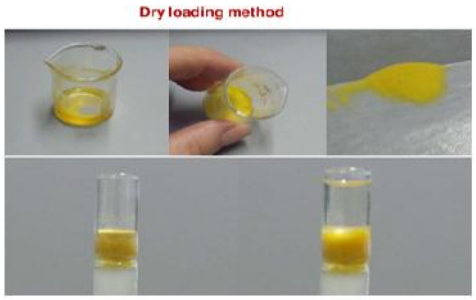

Fig. 3: Dry loading method

Instrumentation

Flash chromatography General consist of following parts

- Pump Systems
- Pump Controller
- Type of pump
- Vacuum Pump/peristaltic Pump
- Sample Injection Systems
- Glass Columns, Filling Sets & Column Valves
- Pre-columns
- Fraction Collector
- Detectors and Chart Recorders
- Computerize LCD Display

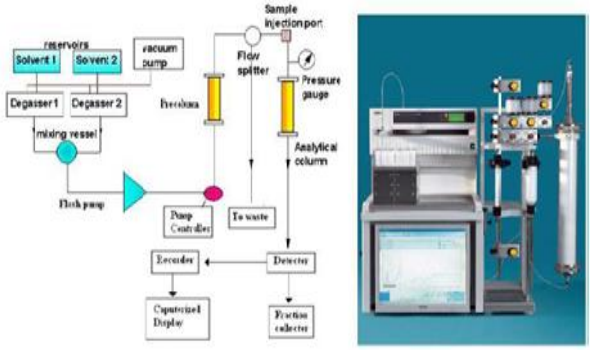

Fig. 4: Instrumentation of Flash chromatography

Pump Controller

A pressure ranges up to either 10 bar or 50 bar gives optimum separation results for a broad range of applications. The pump modules can be controlled by three different units. The Pump Controller C610 (for isocratic separation up to 10 bar), the Pump Manager C615 (for isocratic and gradient separation up to 50 bar) and the Control Unit C620.

3. Type of pumps

Pump Module C-601, 10 bar: The Pump Controller C-610 with a Pump Module C-601 is used for fast isocratic Flash

separations. No programming is needed. The system can be run by two buttons and one knob. Pump Module C-601, 10 bar Silent operating 3-piston Pump Module C-601 for flash chromatography. The pump module provides a constant, pulse-free flow from 2.5 to 250 ml/min and ensures reproducible, fast separation at a maximum working pressure of 10 bar/145 psi.For sample sizes of up to 5 g, pre-packed PP cartridges can be used for the quick, safe implementation of normal phase and reversed phase applications.

Pump Module C-605, 50 bar: The Pump Manager C-615 with a Pump Module C-601/C-605 is used for isocratic Flash separations. This combination allows exploration into the features of the Pump Manager C-615 for solvent selection, timed runs and solvent level control. Pump Module C-605, 50 bar Similar to the Pump Module C-601 but with a maximum working pressure of 50 bar/725 psi. Using the Pump Module C-605, fast separation with reversed phase and separations can be performed with sample sizes up to 100 g. Ideal for use with glass and plunger columns and silica gel particle sizes < 40 µm.

Pump Manager C-615: The Pump Manager C-615 with two Pump Modules C-601/C-605 for binary solvent gradients. The efficient solvent mixing under pressure and the pulsation free solvent flow eliminate vapour bubbles and result in maximum separation performance.

Control Unit C-620:

The Control Unit C-620 in combination with Separate control provides precise control of the chromatography system. The following components can be connected to the Control Unit C-620: 2 to 4 Pump Modules C-601 or C-605 Up to 2 Fraction Collectors Up to 8 Detectors. e. g. UV, RI Sequential Modules C-623 orC-625 for automatic sequential chromatography on up to 5 columns or cartridges The Control Unit C-620 is included in the Sepacore Control package.

Vacuum Pump/peristaltic Pump:

Transfer Solvent from Mobile phase Reservoir to Flash Pump.

Sample Injection Systems

Injection systems are designed to facilitate column loading with liquids and low solubility oils and solids. Regardless of the nature or quantity of the material.

Injection Valve: For the sample injection of 0–5 ml.

Elute Glass Column:

Prep Elute Glass Column for use in combination with the Injection Unit for loading dry or barely soluble samples up to either 18 ml or 53 ml. Pressure Range of up to 50 bar or 40 bar.

Sample Chamber 100 ml:

Sample Chamber 100 ml for use in combination with the Injection Unit for loading sample volumes of 10–v100 ml including N2 gas valve (on/off). Glass parts with larger volumes 250 ml, 500 ml and 1000 ml on demand.

Columns used Flash chromatography

Glass Columns

A wide range of columns offer maximum flexibility for every situation. Depending on the nature and the quantity of the sample offers a series of column types which vary in form, size and performance.

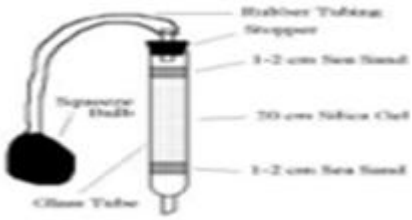

Fig. 5: Glass column

Plastic+Glass Column

Plastic+Glas-coated Glass Columns are available for larger sample amounts and higher-pressure applications on a high safety level. The columns are designed for sample amounts from1 – 100 g and pressures up to 50 bar during preparative separations. Easy fixation on a support rod by using the corresponding pivoting clamp.

Plunger Column C-695

Robust, chemically resistant and biocompatible plunger columns are designed for optimum operational performance and safety. Volume changes in soft gel can be equalized and dead volume will be avoided. 1 – 100 g and pressures up to 50bar during preparative separations. Easy fixation on a support rod by using the corresponding pivoting clamp. An integrated cooling jacket allows separations under constant conditions at a high quality level. Column Length 460 mm.

Precolumns

Precolumn are minimizing dead volumes and enhance the life time of the main column by trapping contaminants. The small Precolumn, fits to Glass Columns of inner diameter of ID 15, 26, 36 and 49 mm. The large Precolumn, fits to Glass Columns of ID 70 and 100 mm inner diameter.

Filling Sets for Glass Columns

Dry Filling Set:

The Dry Filling Set is employed for filling glass columns with silica gel using compressed gas. Silica gel in the size range of 25 – 200µm can be packed with this method.

Slurry Filling Set:

The Slurry Filling Set is used for wet filling and conditioning of glass columns with silica gel particles smaller than 25µm.

Fraction Collector

For simple separations a column, pump and pump controller may be enough. For a greater level of automation with precision, performance and ease of use the Fraction Collector can be incorporated into most setups.

Fraction Collector C-660:

The intelligent, height-adjustable Fraction Collector with dialogue language options for preparative chromatography. The C-660 collects the separated substances according to time, volume or peak. During each run, up to 12 liters can be collected in a maximum of 240 glass tubes With the Teach-In function customer designed racks can be programmed and checked by using the Show mode. Sample collection according to time, volume or peak Total capacity of 12 liters in max. 240 glass tubes. Integrated peak collection for 2 detector signals Teach-In function for customer specific programming RS-232 interface for transferring data to a PC 2 Detector inlets, 2 Recorder

outlets Compatibility with Syncore Racks Optional: Waste Diverter valve and Level sensor.

Detectors and Recorders/Software

For most applications one of the robust UV/Vis detectors would be enough for the systems detection needs. Both detectors are delivered in combination with a preparative flow cell. In the absence of adequate UV/Vis absorption, likely for sugars or polymers, a Differential Refract meter (RI Detector) in combination with a UV/Vis detector is the preferred setup.

4. Applications

- It is used for Purification of Protected Peptide
- It is used for Separation of Closely Related Organic Compounds (Isomer)
- It is used for High Speed Flash Fractionation of Natural Products - Tocopherols Using reversed phase flash chromatography as the preliminary isolation step allows the tocopherols to be concentrated and have fewer oil contaminants thereby increasing the lifetime of the HPLC columns.
- It is used to purify, collect and identify the various aromatic components in a semi-synthetic extract.
- Amino modified silica is used with normal-phase solvents and is better suited for nitrogen heterocyclic purification because the surface chemistry is slightly alkaline.
- Flash systems are powerful tools for purification of trace compounds from organic mixtures.
- By reversing the cartridge and then using ~100mL of methylene chloride as mobile phase. It is used as a tool to monitor the reaction progress and to isolate and identify a mixture's compounds.
- Improving Natural Product Purity by Orthogonal FLASH Purification In this application, several solvent systems were evaluated by TLC. No solvent system was capable of resolving capsaicin, dihydrocapsaicin and lutein from each other. The best solvent mixture for this TLC separation was 90:10 methylene chloride (DCM)/ acetonitrile (ACN).
- It is used to purify, collect and identify the various avermectin components in a semi-synthetic extract.
- The Flex system has been used by many pharmaceutical companies to purify compounds in the process of drug discovery.
- It is used to purify, identify and collect the isomers of an aqueous modified antibiotic precursor. The goal for this work was to isolate each isomer with > 98% purity.

Table 1: Solvents used in flash chromatography

	Properties					
Commonly Used Flash Solvents	**Density (g/ml)**	**Elution strength**	**Solvent Group**	**Boiling Point(0c)**	**UV Cut-off (nm)**	**TLV (PPM)**
n-Hexane	0.66	0.01	1	69	195	100
224-Trimethyl pentane	0.69	0.02	1	99	210	300
Cyclohexane	0.77	0.03	1	81	200	100
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	6	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	-

5. Conclusion

Flash Chromatography is a simple, fast, cost effective Preparative Liquid Chromatography approach. Separations are based upon traditionally obtained TLC results which are simply extrapolated to preparative scale. Flash chromatography is very useful technique for quickly separating increasing quantities of samples. It is predictable and easy to scale up and down as required. Modern instrumentation is making it easier still to take full control over the separation and the technique continues to develop quickly.

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