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REVIEW ON: FLASH COLUMN CHROMATOGRAPHY

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ABSTRACT

In previous days, Column chromatography was used for qualitative as well as quantitative analysis but Column chromatography is an extremely time consuming process. This leads to the development of novel preparative liquid chromatography in which mobile phase flows down by positive air pressure called as Flash chromatography. Flash Chromatography is a rapid form of preparative column chromatography. Flash chromatography, also known as medium pressure chromatography, was popularized several years ago by Clark Still of Columbia University, as an alternative to slow and often inefficient gravity-fed chromatography. It is a simple, fast and economical approach to preparative Liquid chromatography.

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INTRODUCTION

All chromatographic methods use columns for the separation process, except TLC. Column chromatography has found its place in many laboratories for preparative purposes as well as for reaction control in organic synthesis. The importance of column chromatography is mainly due to following factors: Simple packing procedure.Low operating pressure. Low expense for instrumentation.

Column chromatography is classified into two categories, depending on how the solvent flows down the column. If the solvent is allowed to flow down the column by gravity, or percolation, it is called Gravity column chromatography. If the solvent is forced down the column by positive air pressure, it is called Flash chromatography. In traditional column chromatography a sample to be separated is placed on the top of a column containing some solid support, often silica gel. The rest of the column is then filled with a solvent (or mixture of solvents) which then runs through the solid support under the force of gravity. The various components to be separated travel through the column at different rates and then can be collected separately as they emerge from the bottom of the column. Unfortunately, the rate at which the solvent percolates through the column is slow. In flash chromatography however air pressure is used to speed up the flow of solvent, dramatically decreasing the time needed to purify the sample, therefore making the column and running the separation could take less than 10-15 minutes.

Preparative HPLC:

Important chromatographic parameters to achieve reliable and accurate results are resolution, peak width and peak symmetry. If more and more sample amount is applied to the column, the peak height and peak area increases but the peak symmetry and the capacity factor remain unchanged. In analytical HPLC the optimal peak shape resembles a Gaussian curve. If more than a certain amount of sample is injected onto the column the adsorption isotherm becomes non-linear. This means the peak becomes unsymmetrical, shows strong tailing and the capacity factor decreases. In preparative HPLC this effect is called concentration overloading. In some cases, depending on the compound, the capacity factor increases with increasing overloading, which leads to a strongly fronting peak. Since the adsorption isotherm is dependent on the compounds the chromatographic system column load ability has to be determined for each preparative HPLC experiment.

Flash chromatography (medium pressure chromatography):

PRINCIPLE OF FLASH CHROMATOGRAPHY:

The principle is that the eluent which is a liquid, under gas pressure (normally nitrogen or compressed air) rapidly pushed through a short glass column. The glass column is packed with an adsorbent of defined particle size with large inner diameter. The most used stationary phase is silica gel 40 – 63 µm, but obviously packing with other particle sizes can be used as well. Particles smaller than 25 µm should only be used with very low viscosity mobile phases, because otherwise the flow rate would be very low. Normally gel beds are about 15 cm high with working pressures of 1.5 – 2.0 bars.

Flash chromatography is a fast and inexpensive separation technique for the purification of organic syntheses products e.g. in drug discovery or from natural extracts. It is a popular alternative when other separation techniques cannot be used or are too difficult.

In flash chromatography Columns are disposable plastic cartridges, advantage of cartridges are time save and reproducibility. Based on sample volume we may select different size of cartridges. Now a days readily prepared cartridges are available based on particle size and stationary phase volume.

Selecting a Solvent System

The compound of interest should have a TLC R f of ≈0.15 to 0.20 in the solvent system you choose. 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. Common binary solvent systems in order of increasing polarity are dichloromethane/hexane, ether/hexane, hexane/ethyl acetate, and dichloromethane/methanol. Hexane/ethyl acetate can be used on the bench, all other solvents should be used in the hood. If your R f 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.

Determining the Quantity of Silica Gel Required

The amount of silica gel depends on the R f difference of the compounds to be separated, and on the amount of sample. For n grams of sample, you should use 30 to 100 n grams of silica gel. For easier separations, ratios closer to 30 : 1 are effective, for difficult separations, more silica gel is often required. However, by using more silica gel, the length of time required for the chromatography is extended. The density of powdered silica gel is about 0.75 g per mL.

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, you will have to put in a somewhat loosely stuffed plug of cotton wool; if you stuff it too much, solvent flow becomes painfully slow even with air pressure above the column). Next, put a ~1/2 in. layer of clean sand above the plug of glass wool. Use only as much as is necessary to obtain a flat surface, with the same diameter as that of the body of the column. Make sure the surface is flat. Then pour in the silica gel using a funnel. DO THIS STEP IN THE HOOD! Silica gel is chemically similar to asbestos, and is a known carcinogen. Manipulations with dry silica should be done only in the hood.

Solvating the Silica Gel Column

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 your elution solvent onto the silica gel. Use pressurized gas to force the solvent through the silica. As you force through a few hundred millilitres, you should see the top part of the silica become more homogeneous. This is because you 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. You 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 you will force air back into the top of the silica, and you will be back where you started.

Applying the Sample

Allow the solvent which remains above the silica to drain down until it is flush with the surface of the silica. If the top surface of the silica gel is not flat, gently tap the side of the column until it is. Dissolve your sample into the minimum volume of the elution solvent. Apply this to the top of the column, being careful not to disturb the top of the silica. Allow the sample to soak into the silica. Next, rinse the sides of the column with as few as possible millilitres of the elution solvent. Let this soak into the silica. After the rinsing's have soaked into the silica gel, add a small amount of sand to protect the top surface of the silica when you add more solvent.

Eluting the Sample

Add a good part of your elution solvent to the column. Apply pressure to force the solvent through the column. The pressure should be the minimum necessary to keep a steady stream coming out of the column. Be aware that if you have chosen your solvent properly, it will take a little while before your compound of interest begins to elute. This means that the solvent, at first, contains none of your compound and can be discarded. If the R f of your compound is 0.33 or less, you should be safe discarding an amount of solvent equal to the volume of the dry silica you used for the column. When you have collected this much solvent, begin collecting the eluted solvent into separate test tubes (fractions). When you have used all of your solvent, your sample should have finished eluting into the test tubes you collected. To maximize the efficiency of your chromatography, the fractions you collect should be no more than about one tenth of the column volume. For example, if you use 25 g of silica gel you should collect fractions of about 3 mL.

Locating the Sample

Use TLC to determine which fractions contain your compound. As the fractions fill, you should analyse each using TLC. It is best to spot 10 fractions on one TLC plate and elute that 10 lane plate once, rather than conducting individual analyses for each fraction. Combine the fractions that contain your sample together in a flask, then concentrate the sample on the rotavap (rotary evaporator).Cleaning the Column Flush all the remaining solvent out of the column using pressurized gas. When all liquid solvent has bee removed from the reservoir, remove the last remnants of solvent by applying a vacuum (from aspirator) to the bottom of the column.

INSTRUMENTATION

Flash chromatography General consist of following parts (Fig. No. 1)

- 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

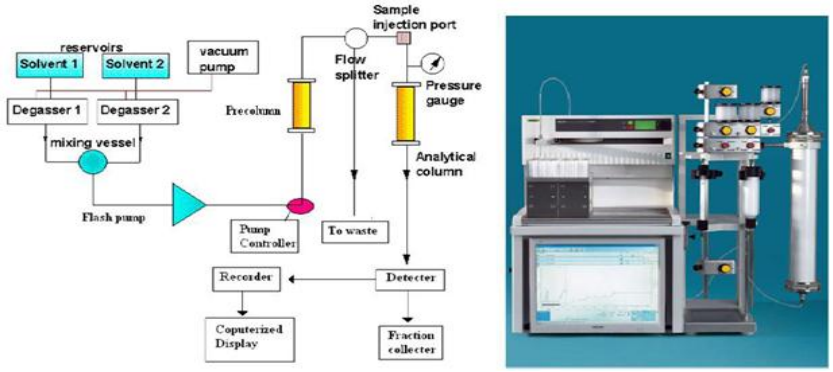

Fig. No. 1: Instrumentation of Flash Chromatography.

Pump Systems

Pump Controller

A pressure range 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.

Pump Controller C-610

The Pump Controller C-610 for one Pump Module C-601 is designed for isocratic separations. The flow rate can be easily adjusted by turning a knob and is indicated by a large illuminated LCD-display. Delivered with a over pressure sensor for maximum safety.

Pump Manager C-615

The Pump Manager C-615 is designed for both isocratic and gradient separations. Fast operation, easy programming and a large graphical display allows a quick and easy set up. Running time, solvent consumption and actual pressure are shown during a separation for maximum optimization. The unit has Input/Outputs for 2 solvent valves and level sensors and includes a pressure sensor and mixing chamber.

Control Unit C-620

The Control Unit C-620 in combination with Sepacore 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 or C-625 for automatic sequential chromatography on up to 5 columns or cartridges The Control Unit C-620 is included in the Sepacore Control
package.

Type of pump

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.

Columns

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.

Pre-columns

Pre-column are minimizing dead volumes and enhance the life time of the main column by trapping contaminants. The small Pre-column, fits to Glass Columns of inner diameter of ID 15, 26, 36 and 49 mm. The large Pre-column, 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 – 200 µm 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 25 µm.

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 Sync ore Racks Optional: Waste Diverter valve and Level sensor.

DETECTOR

3 detectors delivering a very precise analysis of the separation results. For most applications one of the robust UV/Vis detectors would be sufficient 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 Refractometer (RI Detector) in combination with a UV/Vis detector is the preferred setup.

UV Monitor:

Filter Photometer with four standards built in filters at 200 nm, 220 nm, 254 nm and 280 nm. Delivered with built in Deuterium Lamp and a preparative flow cell.

UV Photometer:

Spectral Photometer with a wavelength range between 190 nm and 740 nm. Delivered with built in Deuterium Lamp and a preparative flow cell.

Differential Refractometer:

Refractive Index detector mostly used in combination with a UV/Vis detector for the analysis of low UV/Vis absorbing substances. Delivered with a preparative cell. For a maximal flow rate of 100 ml/min.

Table 1: Difference between FLASH and Preparative chromatography.

Preparative Chromatography	Flash Chromatography
Maximum Quantities of the sample can be separated (0.1-2.0g)	Maximum Quantities of the sample can be separated (0.5-2.0 g)
Separation time is Based on number of compounds present. (10 min to 1 Hour)	Separation time is 10-15 min
Elaborate equipment and the purchase of expensive equipment is necessary	Elaborate equipment and the purchase of expensive equipment is not necessary
Column is highly Expensive	Cartridges are reuse full, Nearly 8 times Cheaper than Preparative columns.
Sample should soluble in mobile phase	No need to solubility of sample in Mobile phase
More useful in Agro chemistry, Synthetic chemistry, Natural products separation.	More useful in separation of various antibiotics, Impurities, Peptides.
This technique saves time.	This technique saves time and solvents
Reliable	Reliable and cost effective

CONCLUSION

As per Table No.1 Preparative HPLC and Flash chromatography both are efficient and advance techniques for separation of various chemical compounds. Flash chromatography is cost effective and low maintenance. In the case of the target molecule or compound is in high concentration, flash Chromatography is preferable. Then we may isolate the compound with high purity. In the case of sample have more chemical constituents, without information of concentrations of that chemical constituents, preparative chromatography is preferable. Flash chromatography also recommend for future research.

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