Vol 3|Issue 1| 2013 |45-49.

International Journal
of
Pharmacy Review & Research
www.ijprr.com

e-ISSN: 2248 – 9207
Print ISSN: 2248 – 9193

REVIEW ON FLASH CHROMATOGRAPHY

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ABSTRACT

In previous days, Column chromatography was used in many laboratories for preparative purposes as well as for reaction control in organic synthesis. Column chromatography is an extremely time consuming stage in any lab and can quickly become the bottleneck for any process lab. This leads to the development of novel preparative liquid chromatography in which mobile phase flows down by positive air pressure called as Flash chromatography. It is a simple, fast and economical approach to preparative Liquid chromatography .This review try to focus on principle, various components, general procedure, advantages and application of Flash chromatography.

Key words: Flash chromatography, Chromatography.

INTRODUCTION

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. Flash chromatography differs from the conventional Technique in two ways: first, 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 [5].

All chromatographic methods -with the exception of TLC- use columns for the separation process. Column chromatography has found its place in many laboratories for preparative purposes as well as for reaction control in organic syntheses. The importance of column chromatography is mainly due to following factors [3,9,10]:

- Simple packing procedure
- Low operating pressure
- Low expense for instrumentation

Column chromatography is separated 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, a "state of the art" method currently used in organic chemistry research laboratories.

Automated flash chromatography systems include components normally found on more expensive HPLC systems such as a gradient pump, sample injection ports, a UV detector and a fraction collector to collect the eluent. Typically these automated systems separate samples from a few milligrams up to an industrial kg scale and offer much cheaper and quicker solution to doing multiple injections on prep-HPLC systems. The software controlling an automated system coordinate the components, allow a user to only collect the factions that contain their target compound (assuming they are detectable on the system's detector) and help the user to find the resulting purified material within the fraction collector. The software also saves the resulting chromatograph from the process for archival and/or later recall purposes. Main is all process controllers by computerized System, Software & sensor.

PRINCIPLE [8]

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 of

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defined particle size. 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. Originally only unmodified silica was used as the stationary phase, so that only normal phase chromatography was possible. In the meantime, however, and parallel to HPLC, reversed phase materials are used more frequently in flash chromatography [8]. The computerized system control the Working of Flash chromatography.

INSTRUMENTATION OF ADVANCE FLASH CHROMATOGRAPHY [1,9,10]

Flash chromatography General consist of following parts

Pump Systems

Pump Controller

Type of pump

Vaccum Pump/peristaltic Pump

Sample Injection Systems

Glass Columns, Filling Sets & Column Valves

Precolumns

Fraction Collector

Detectors and Chart Recorders

Computerize LCD Display

Fig.1. Instrumentation of Flash Chromatography

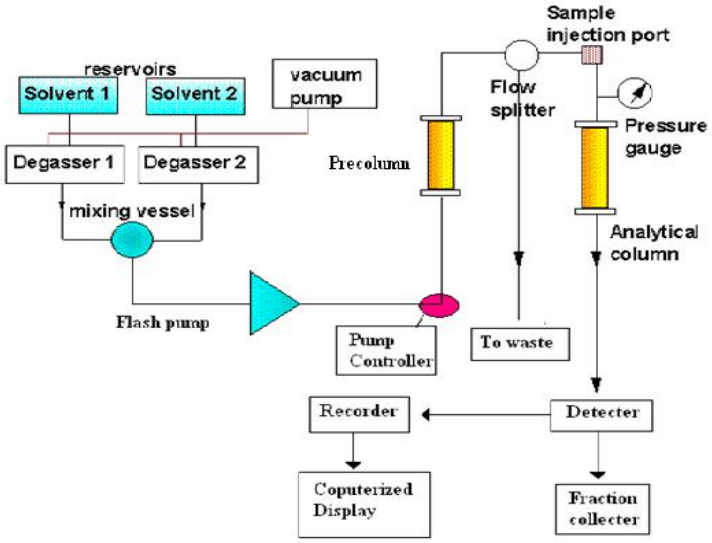
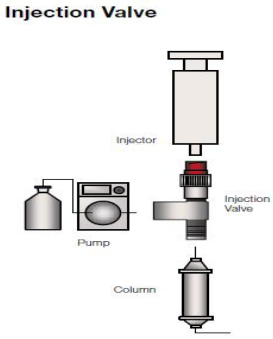


Fig. 2. Injection Valve

Fig. 3. Elute Glass Column

Fig. 4. Sample Chamber 100 ml

Fig. 5. Way of injecting sample



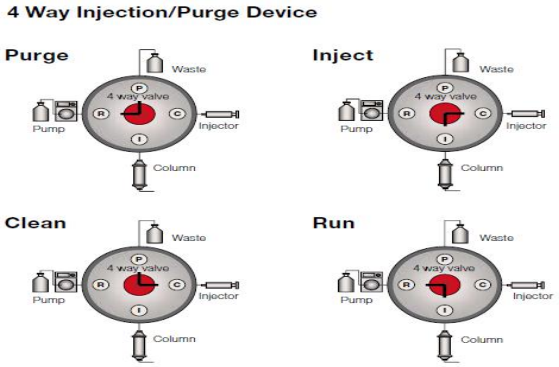


Fig. 6. Glass Flash Column

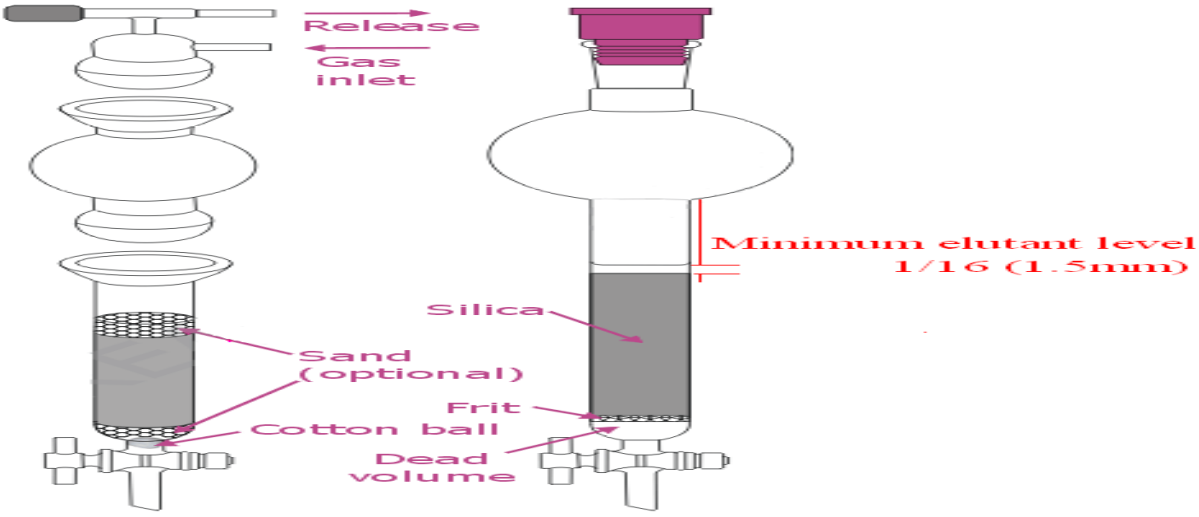


Table 1. Column Size, pressure Range Sample to Apply

Column Diameter (mm)	Pressure Range(bar/psi)	Volume eluent (ml)	Sample Load (mg)		Fraction Size(ml)
			Rf>0.2	Rf>0.1	
10	0-50/725	100	100	40	5
20	0-40/580	200	400	160	10
30	0-30/435	400	900	360	20
40	0-20/290	600	1600	600	30
50	0-10/145	1000	2500	1000	50

Table 2. Pressure Range for Various Column Size

Size(mm)	Pressure rang (bar/psi)	Size(mm)
Small	**0-50 bar/725psi**	**Small**
Large	**0-20 bar/290psi**	**Large**

Fig. 7. UV Monitor C-630

Fig. 8. UV Photometer C-635

Fig. 9. Differential Refractometer

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 overpressure 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

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.

Vaccum 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 10–100 ml including N2 gas valve (on/off). Glass parts with larger volumes 250 ml, 500 ml and 1000 ml on demand.

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.

Plastic+Glass Column [1,11]

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 50 bar 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 – 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 Syncore Racks Optional: Waste Diverter valve and Level sensor.

Detectors and Recorders/Software

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 C-630

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 C-635

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 [11].

APPLICATIONS [3,9,10]

- 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.

CONCLUSION & SUMARY

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.

AKNOLEDGEMENT

Respective Author Wish express their thanks to Mr. Prasad Tanpure President of S.V.N.H.T'S college of B.Pharmacy Shrishivajinagar (Rahuri factory), Tal-Rahuri, Dist-Ahemadnagar, MS, India-413706 and Buchi Preparative Chromatography For their support and encouragement.

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