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AN OVERVIEW ON FLASH CHROMATOGRAPHY

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Abstract:

In earlier days, Column chromatography is used for preparative purposes, qualitative and quantitative analysis in many laboratories but it is an extremely time-consuming process. This led to the development of novel preparative liquid chromatography called as flash chromatography in which the mobile phase runs down by positive air pressure. Flash chromatography is a purification technique obtained by the blend of medium, short column chromatography, which results in quick separation of mixture of components. This technique is used as preceding step to highly sophisticated methods like HPLC, NMR, FT-IR to obtain pure samples. It is a simple, fast, economical approach to preparative liquid chromatography. This review focuses on the different aspects of flash chromatographic technique.

Keywords: *Flash chromatography, Preparative liquid chromatography, Highly sophisticated, Purification technique*

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INTRODUCTION

Column chromatography is used in the industries for the purification purpose and it has many advantages like being inexpensive, simple packing and easy operation. But it also has some drawbacks like being time consuming and low resolution. Column chromatography is a lengthy stage and can promptly turn into the hurdle for any process lab. This pressurized the demand for the development of a new and rapid liquid chromatographic technique, where in the eluent flows down the column rapidly with the help of positive pressure.[1]

Column chromatography is categorized into two classes, based on how the solvent flows down the column. If the solvent runs down the column by gravity, it is called Gravity column chromatography. If the solvent flows down the column by positive air pressure, it is called Flash chromatography.

Flash chromatography is an air pressure driven hybrid of medium pressure and shorter column chromatography which has been developed for particularly rapid separation.

Flash chromatography is also known as medium pressure chromatography that is used to separate mixtures of molecules into the individual constituents, it is frequently used in the drug discovery process [2]. Flash chromatography vary from the conventional technique in two ways:

1)Slightly small silica gel particles (250-400 mesh) are used

Restricted flow of solvent is caused by the small silica gel particles so the pressurized gas (10-15 psi) is used to drive the solvent through the column of stationary phase. The overall result is a rapid ("over in a flash") and high-resolution chromatography [3]. Various manufacturers have developed automated flash chromatography systems. It classified into two types 2

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

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

Automated flash chromatography systems contain the components like gradient pump, sample injection ports, a UV detector and a fraction collector to gather the eluent. Often, these automated systems separate sample mixtures from a couple of few milligrams to a kg scale and provides less expensive and quicker solution to do multiple injections on prep-HPLC systems. The software controlling an automated system coordinate the components, permits the user to collect only the factions that contain their target compound and helps them to find the resulting purified material within the fraction collector. This software saves the resulting chromatograph from the method of archival and later recall purposes.[4]

HISTORY

In 1978, an American organic chemist, Dr. W. Clark Still, working at Columbia University, developed the technique flash chromatography as an alternative to inefficient gravity-fed chromatography. This led to increased rate of solvent elution and reduced the run time.[5]

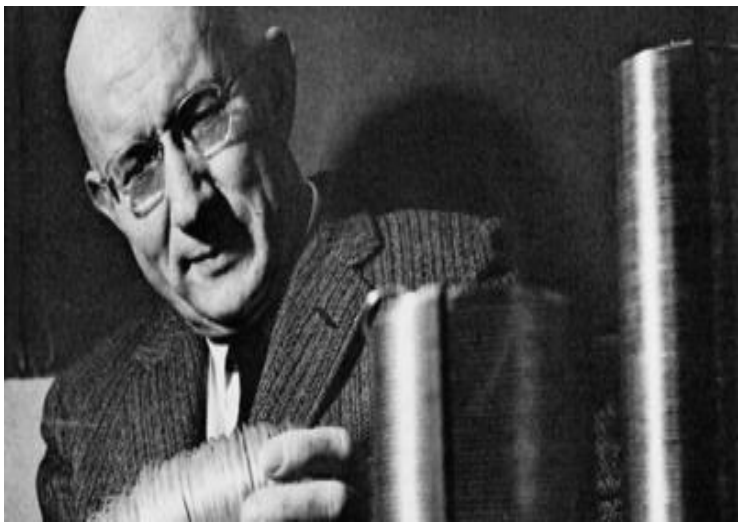

Fig -1: DR. W. CLARK STILL

PRINCIPLE

The principle is that the eluent which is a liquid, under gas pressure (usually nitrogen or compressed air) is quickly pushed through a short glass column. The glass column is packed with an adsorbent of definite particle size and large inner diameter. The most used stationary phase is silica gel 40 – 63 µm. Particles lower than 25 µm should be used with very low viscosity mobile phases, otherwise the flow rate would be very low. Initially only unmodified silica is used as the stationary phase, so that only normal phase chromatography was possible. However, reversed phase materials are used commonly in flash chromatography [6] Flash chromatography is based on the principle of adsorption chromatography. The components of the mixture are separated based on the differences in their affinity towards stationary phase and mobile phase. The component which has high affinity towards the stationary phase travels slower and the component which has high affinity towards the mobile phase travels faster. But the special characteristic feature of flash chromatography is the usage of positive air pressure to run the mobile phase across the column

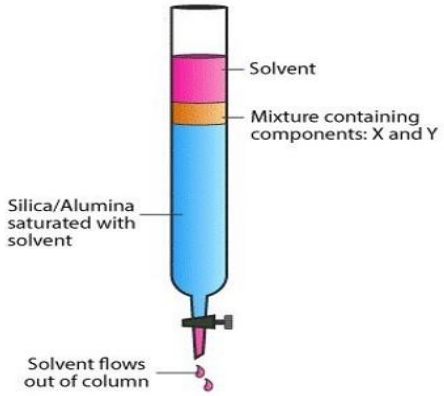

FIG - 2: PRINCIPLE - ADSORPTION

VARIOUS COMPONENTS OF FLASH CHROMATOGRAPHIC SYSTEM

Sorbent Selection [2,7]:

The basic requisite for successful separation of components is the choice of the proper adsorbent. The foremost stationary phase used in this chromatography is silica. Silica gel (SiO2) and alumina (Al2O3) are two adsorbents regularly used for the chromatography. These adsorbents are sold in different mesh sizes, that indicated by a number on the bottle label like “silica gel 60” or “silica gel 230-400”. This number indicates the mesh of the sieve that is used to size the silica, especially, the number of holes in the mesh through which the crude silica particle mixture is passed during the manufacturing process. The particle size of the adsorbent affects the solvent movement through the column. Small particles (high mesh values) are used for flash chromatography. The amount of silica gel depends on the Rf difference of the compounds to be segregated, and on the quantity of sample. For n grams of sample, 30 to 100 n grams of silica gel is to be used. For easier separations, 30: 1 ratio are effective, for difficult separations, more silica gel is usually required. But, by using more silica gel, the length of time required for chromatography is prolonged

These are some adsorbents that are mostly used in flash chromatography: -

- **Silica**: Slightly acidic medium. Best suitable for ordinary compounds, good separation is obtained
- **Florisil:** Mild, neutral medium. 200 mesh is effective for easy separations. Some compounds stick on florisil, so test them first
- **Alumina:** Basic or neutral medium. Effective for easier separations, and purification of amines.
- **Reverse phase silica**: The highly polar compounds elute fast; the highly nonpolar compounds elute slow

The properties of commonly used flash solvents:

- ★ The selected compound should have a TLC Rf of ≈0.15 to 0.20 in the solvent system you choose.
- ★ Binary (two component) solvent systems with one high polarity solvent are generally best as 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 components to be segregated.
- ★ High polarity of solvent increases rate of elution for all compounds.
- ★ If your Rf is a≈0.2, you require a volume of solvent ≈5 X the volume of the dry silica gel to run the column. (table)

Solvent Systems [2,8]

Flash column chromatography is normally performed with a mixture of two solvents,

One is polar and the other is nonpolar component**.**

One-component solvent systems

1)Hydrocarbons: pentane, petroleum ether hexanes

2) Ether and dichloromethane (nearly similar polarity)

3) Ethyl acetate

Two-component solvent systems

1)Ether/Petroleum Ether, Ether/Hexane, and Ether/Pentane: Choice of hydrocarbon component depends on the availability and requirements for boiling range. Pentane is costly and low-boiling, petroleum ether is low-boiling, and hexane is easily available.

2)Ethyl Acetate/Hexane: The standard, suitable for ordinary compounds and best for difficult separations. Methanol/Dichloromethane: for separation of polar compounds

3)10 % Ammonia in Methanol Solution/Dichloromethane: Sometimes moves the stubborn amines

4)For basic i.e., nitrogen containing compounds, sometimes it is necessary to add about 0.1% of triethylamine or pyridine to the solvent mixture

5) For acidic compounds, a small amount of acetic acid is sometimes helpful. In this case, care should be taken while concentrating the solvent as trace amounts of acids are dangerous when they are concentrated with a product.

Table 1. The Properties of Commonly Used Flash Solvents [9]

Solvent	Density(g/ml)	Elution strength	Solvent Group	Boiling Point(^{0}c)	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
112-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	-

Column Selection [10]

- Select a column that is 10, 20, 40 mm ID depending on the preparative requirements. In fact, Professor Still et al offered this selection Table 2. Single Step Flash Columns represent an innovative step

- Thomson flash columns available in a wide variety of sizes ranging from 4g to 300g silica-based for easy scalability of synthetic reactions.

- Thomson also provides other packing material like Amine and C18 flash columns which enable the end-user to use these flash columns for a broad range of reactions

Solvent Selectivity

★ Solvent selectivity is defined as the solvent to selectively affect the retention of one compound in the mixture relative to the others, thus affect Δ Rf and Colum volume

★ Solvent selectivity is to be adjusted to get Δ Rf > 0.20. Different solvent combinations to attain desired TLC separation generally reveals appropriate conditions for effective flash chromatography separation. Different solvent mixtures can also reverse the elution order of some of the components in the sample. Column volume difference ΔCV predicts the capacity of column the amount of material that can be successfully separated in a single column loading. The greater the ΔCV, the greater will be the effective capacity of the column.

TABLE NO.2: -TYPICAL VOLUME OF ELUANT REQUIRED FOR PACKING AND ELUTION

Column Diameter (mm)	Volume of eluent* (ml)	Sample Load (mg)		Fraction Size (ml)
		Rf>0.2	Rf>0.1	
10	100	100	40	5
20	200	400	160	10
30	400	900	360	20
40	600	1600	600	30
50	1000	2500	1000	50

*Normal volume required for equilibrium of column and elution

Data of Silica gel Column Grade Adsorbents [7].

- Iron Content :<0.02%
- Chloride Content: <0.10%
- Loss on Drying: <3%
- pH (10% suspension): 7±0.5
- Surface Area: 400–600m2/gm.8

INSTRUMENTATION [11,12]

Flash chromatography General consist of following parts:

Pump Systems

Type of pump

Vacuum Pump/peristaltic Pump

Sample Injection Systems

Columns

Filling sets for glass columns

Fraction Collector

Detectors and Chart Recorders

Computerize LCD display

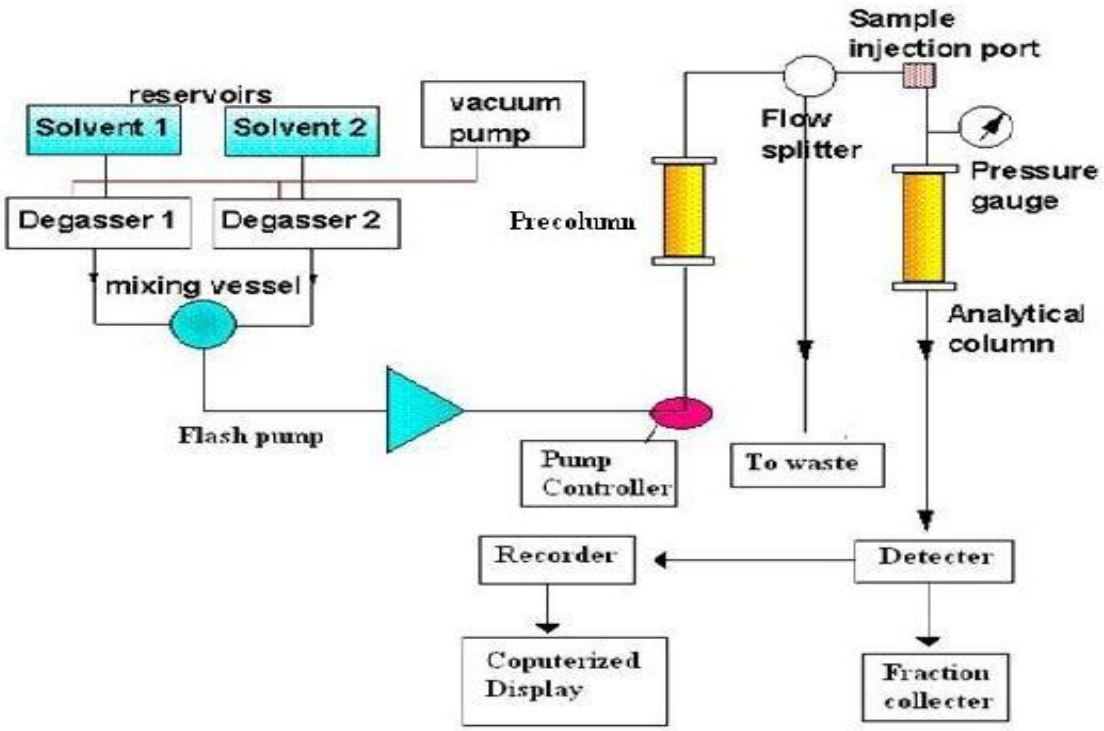

FIG - 3: SCHEMATIC DIAGRAM OF FLASH CHROMATOGRAPHY

Pump Systems:

Pump Controller

A pressure ranging from 10 bar or 50 bar gives optimum separation. The pump modules are controlled by three units. They are:

- Pump Controller C610 (for isocratic segregation up to 10 bar),
- Pump Manager C615 (for isocratic and gradient segregation up to 50 bar)
- Control UnitC620.

Pump Controller C-610

The Pump Controller C-610 for Pump Module C-601 is designed for isocratic segregation. The flow rate is adjusted by turning a knob and it is indicated by a large illuminated LCD-display. Delivered with a overpressure sensor for high safety.

Pump Manager C-615

The Pump Manager C-615 is developed for both isocratic and gradient separations. Quick operation, easy programming and a huge graphical display facilitates a rapid and easy set up. Running time, solvent consumption and actual pressure are displayed during a separation for maximum optimization. The unit contain Input and Outputs for solvent valves and level sensors and consists a pressure sensor and mixing chamber.

Control Unit C-620

The Control Unit C-620 along with Sepacore Control facilitates precise control of the chromatography system. These components can be connected to the Control Unit C-620: 2 to 4 Pump Modules C-601 or C- 605, 2 Fraction Collectors, 8 Detectors e. g. UV, RI Sequential Modules C-623 or C-625 for automatic sequential chromatography on 5 c cartridges. The control unit C-620 is added in the sepacore control package

Type of pump

Pump Module C-601, 10 bars

The Pump Controller C-610 along with a Pump RI Sequential Modules C-623 or C-625 for automatic sequential chromatography on 5 columns or Module C-601 is used for rapid isocratic Flash separations. No programming is required. The system runs by two buttons and one knob. Pump Module C-601, 10 bar Silent operating 3-piston Pump Module C-601is used for flash chromatography. The pump module supplies a constant, pulse-free flow from 2.5 to 250 ml/min and ensures reproducible, rapid separation at a maximum working pressure of 10 bar/145 psi. For sample sizes up to 5 g, pre-packed cartridges can be utilized for the rapid, secure implementation of normal phase and reversed phase applications.

Pump Module C-605, 50 bars

The Pump Manager C-615 along with a Pump Module C-601/C-605 is used for isocratic Flash separations. This combination facilitate search into the features of the Pump Manager C-615 for solvent selection and solvent level control. Pump Module C-605, 50 bar is closer to the Pump Module C-601, the pump module C- 601 possess high working pressure of 50 bar/725 psi. By using the Pump Module C-605, rapid separation with reversed phase is carried for samples of 100g size and this module is suitable to use with glass and plunger columns

Pump Manager C-615

The Pump Manager C-615 with two Pump Modules C-601/C-605 is useful for binary solvent gradients. The effective solvent mixing under pressure and the pulsation free solvent flow removes vapors bubbles and result in maximum separation performance.

Vacuum Pump/peristaltic Pump

Useful t**o** transfer Solvent from Mobile phase Reservoir to Flash Pump.

Sample Injection Systems

Injection systems facilitate column loading with liquids and low solubility oils, solids

Injection Valve

Used for the sample injection of 0–5 ml.

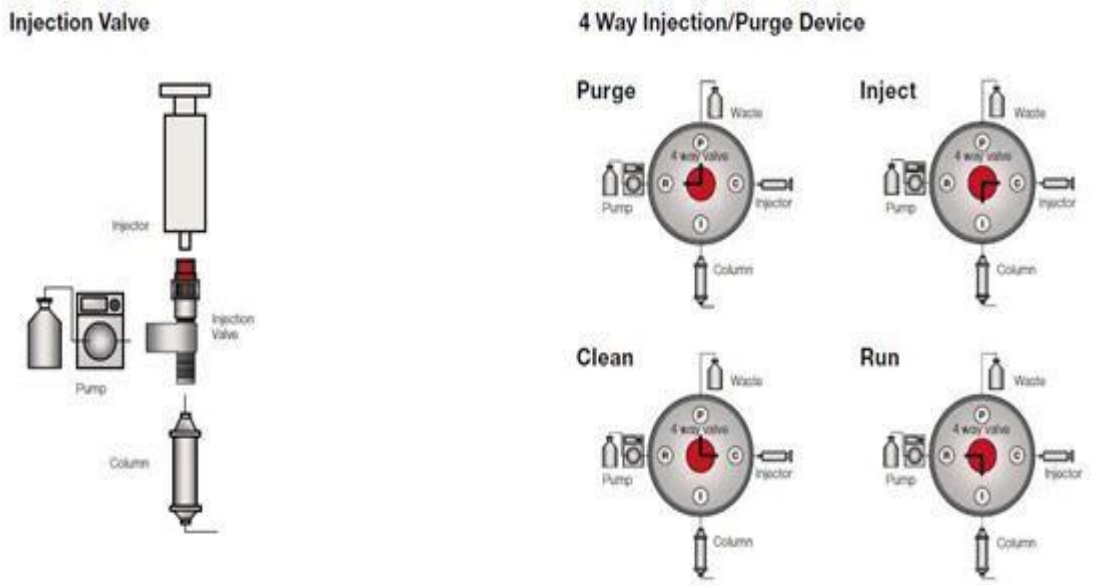

Fig- 4: SAMPLE INJECTION SYSTEM

Elute Glass Column

Elute Glass Column is used in combination with the Injection Unit for loading dry or narrowly soluble samples up to 18 ml or 53 ml

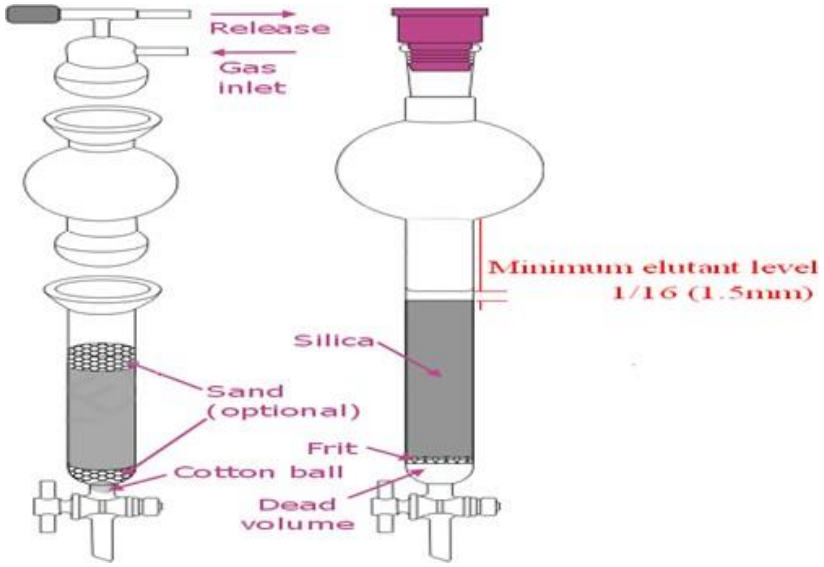

Fig - 5: ELUTE GLASS COLUMN

Sample Chamber 100 ml

Sample Chamber 100 ml along with the Injection Unit is utilized for loading sample volumes from 10 to 100 ml

FIG -6: INJECTION VALVE, ELUTE GLASS COLUMN, SAMPLE CHAMBER 100 ML

Columns

Glass Columns

A wide range of columns provide maximum flexibility for every situation. Based on the nature and the amount of the sample a series of column types vary in form, size and performance.

Plastic Glass Column

Plastic + Glass-coated Glass Columns are now available for large sample amounts from1 – 100 g and pressures up to 50 bar during preparative segregations. Easily fixed on a support rod by pivoting clamp.

Plunger Column C-695

Plunger columns that exhibit chemical resistance and biocompatible are developed for optimum operational performance and safety. Volume changes in soft gel is equalized and dead volume will be eliminated. 1 – 100 g and pressures up to 50 bar during preparative segregations. Easy fixation on a support rod with the help of pivoting clamp. An integrated cooling jacket offers separations under constant conditions at a top-quality level. Column Length is 460 mm.

Precolumn

Precolumn reduce the dead volumes and increase the life time of the main column by trapping contaminants. The smaller Precolumn, is fitted into to Glass Columns of inner diameter of ID 15, 26, 36 and 49 mm. The larger Precolumn is fitted in to Glass Columns of ID 70- and 100-mm inner diameter.

Filling Sets for Glass Columns

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

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

Fig -7: FILLING SET

Fraction Collector: For simple segregations a column, pump and pump controller are sufficient. For a greater level of automation with precision, use of Fraction Collector can be included into most setups.

Fraction Collector C-660
The height-adjustable and intelligence Fraction Collector are preferred mostly for preparative chromatography. The C-660 collects the segregated components according to time, volume or peak.

DETECTORS:
detectors deliver precise analysis of the separation results. For many applications UV/Vis detectors is enough. Both detectors are delivered in addition with a preparative flow cell. In the absence of adequate UV/Visible absorption, for sugars or polymers, a Differential Refractometer (RI Detector) is used in combination with a UV/Vis detector in the setup.

UV Monitor: It is filter Photometer with four standards built in filters at 200 nm, 220 nm, 254 nm and 280 nm. Delivered by a Deuterium Lamp and a preparative flow cell.

UV Photometer: It is Spectral Photometer with a wavelength range between 190 nm and 740 nm. Delivered by a Deuterium Lamp and a preparative flow cell.

Fig- 8: UV PHOTOMETER

Differential Refractometer: Refractive Index detector mainly used in combination with a UV/Vis detector to analyze low UV/Vis absorbing substances. Delivered with a preparative cell for a utmost flow rate of 100 ml/min

Fig -9: DIFFERENTIAL REFRACTOMETER

PROCEDURE

Packing the Column

*Get a glass column and make sure that it has either a glass frit or a plug of cotton wool directly above the stopcock to stop the escaping of silica gel from the column through the stopcock.

* Next, put a ~1/2-inch layer of clean sand above the plug of glass wool to obtain a flat surface, with the same diameter as that of the body of the column. Later dry silica gel adsorbent, 230-400 mesh generally the jar is labelled "for flash chromatography" is added. There are two ways of filling the column one is by inverting the column into the jar of silica gel and scoop it out & then tamp it down before scooping more out [2]

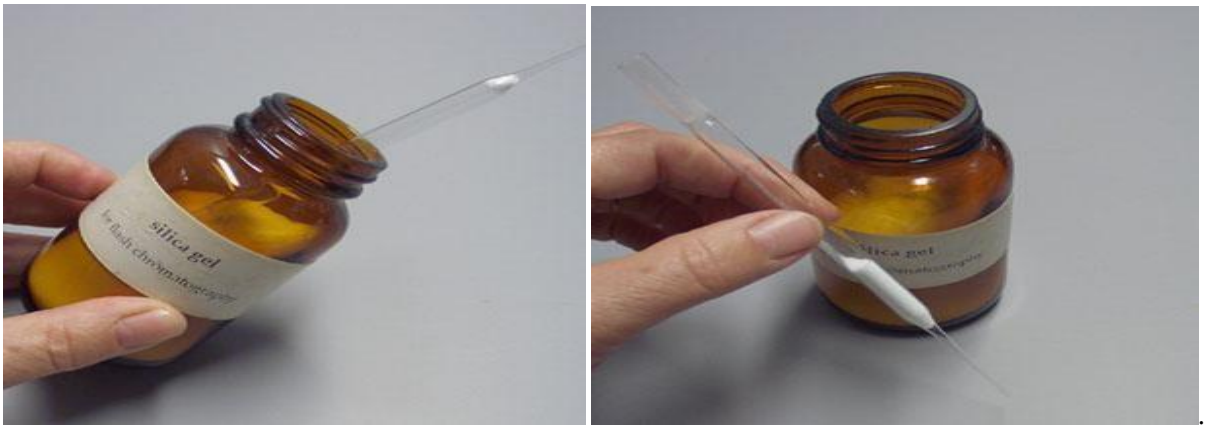

Fig -10: PACKING THE COLUMN (1)

The second way of filling the column is to pour the silica gel into the column by a 10 mL beaker. Whatever the method we use for filling the column, we should tamp it down on the bench top to pack the silica gel. Pipette bulb is also used to force air into the column and pack the silica gel. When accurately packed, the silica gel fills the column to just beneath the indent on the pipette. And this leaves a space of 4-5 cm on top of the adsorbent for adding solvent. Clamp the filled column firmly to a ring stand by small 3-pronged clamp.

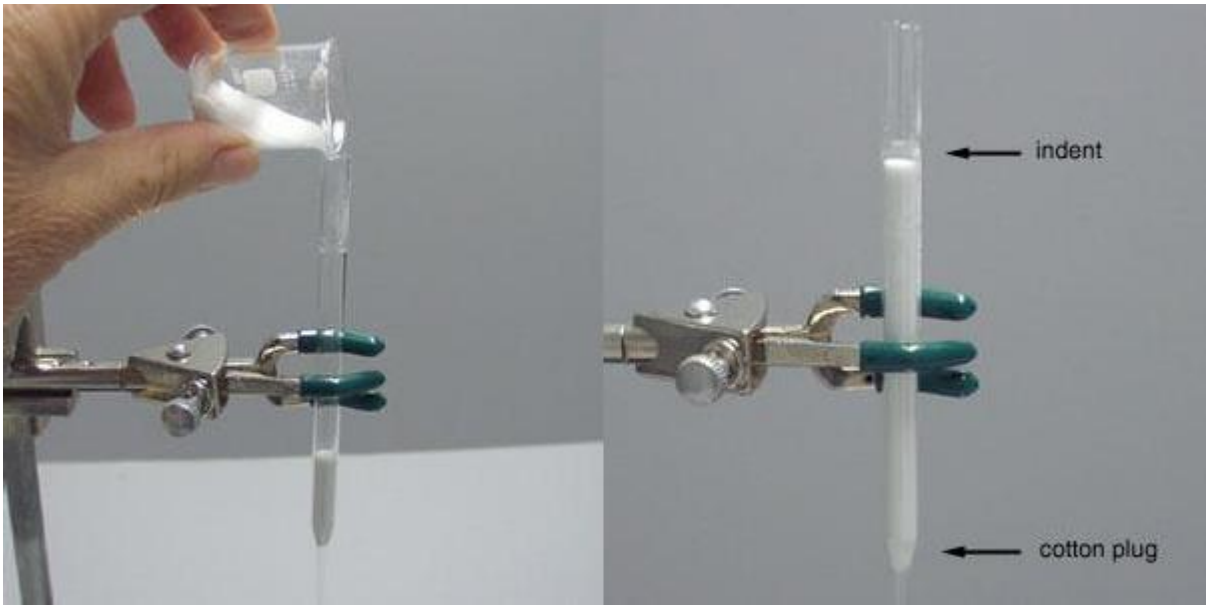

Fig -11: PACKING THE COLUMN (2)

Solvating the Silica Gel Column:
*Next, tap tenderly and uniformly the sides of the column with a piece of rubber tubing to set the silica gel.

Pour some amount of elution solvent onto the silica gel. The pressurized gas is used to force the solvent through the silica. Continue the solvent flushing till the whole silica plug becomes homogeneous in appearance. We must recycle the solvent that comes from the column onto the top of the column many times prior to all the silica gel isolated.[2]

Pre-elute the column
-Add hexanes or any other solvent, as mentioned by the procedure on the top of the silica gel.

-The solvent runs gradually down the column; on the column above, it has flowed down to the point marked by the arrow.

-Regulate the solvent level, both as it flows through the silica gel and the level at the top.
-When the bottom solvent level reaches bottom of the column, the pre-elution process is completed, and the column is ready for loading the sample

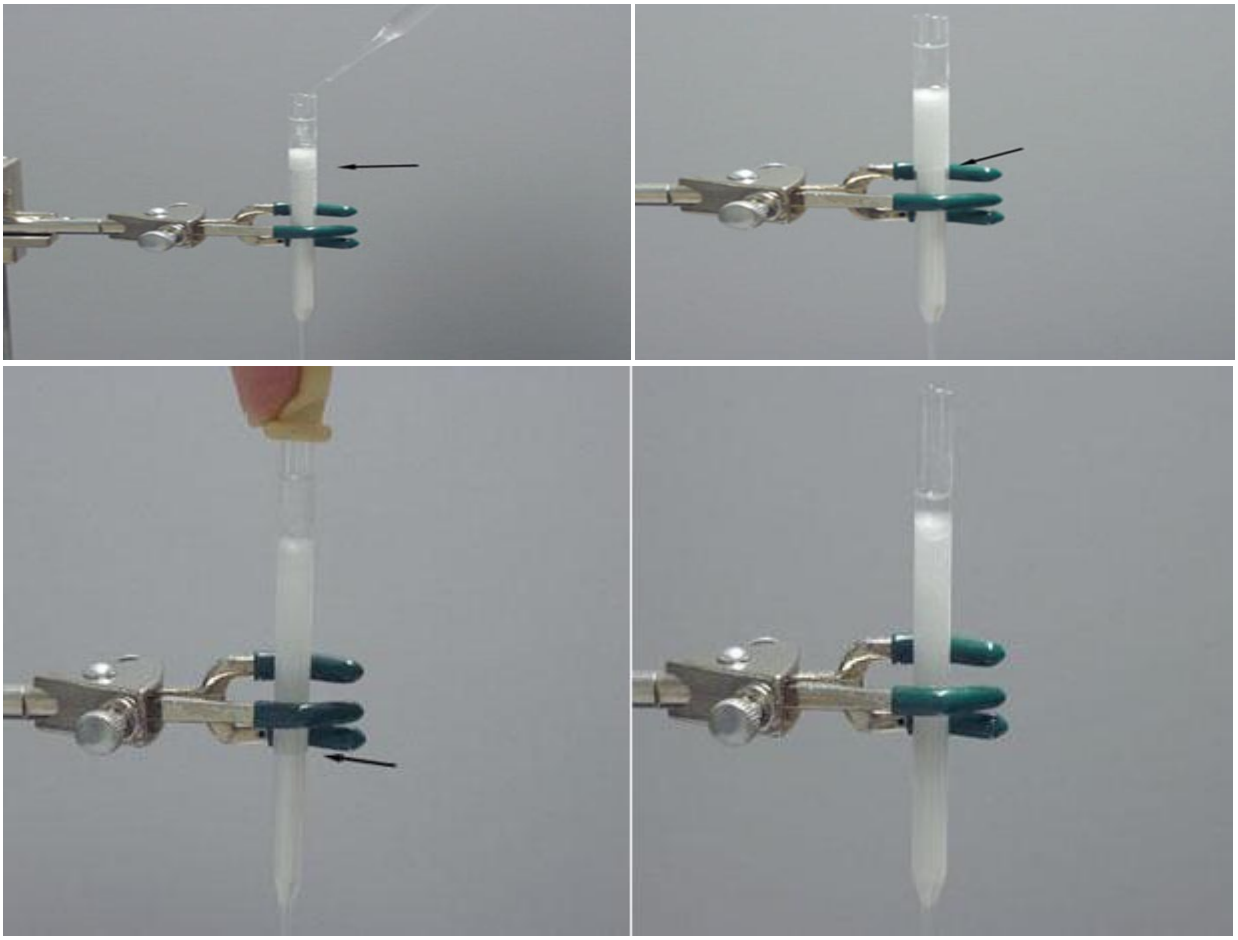

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Fig -12: PRE-ELUTING THE COLUMN

Loading the sample on the silica gel column: -
Two methods are used for loading the column:

Wet loading method

Dry loading method [13]

Wet Loading Method

In this method, the sample is dissolved in little amount of solvent, like hexanes, acetone, or any other solvent and solution is loaded on the column. If the solvent used is more polar than the eluting solvents. In this case, if we use the wet loading method, it is hard to use a few drops of solvent to load the sample. If we use high amounts of solvent, the loading solvent will intrude with the elution. In such cases, the dry loading method is recommended.

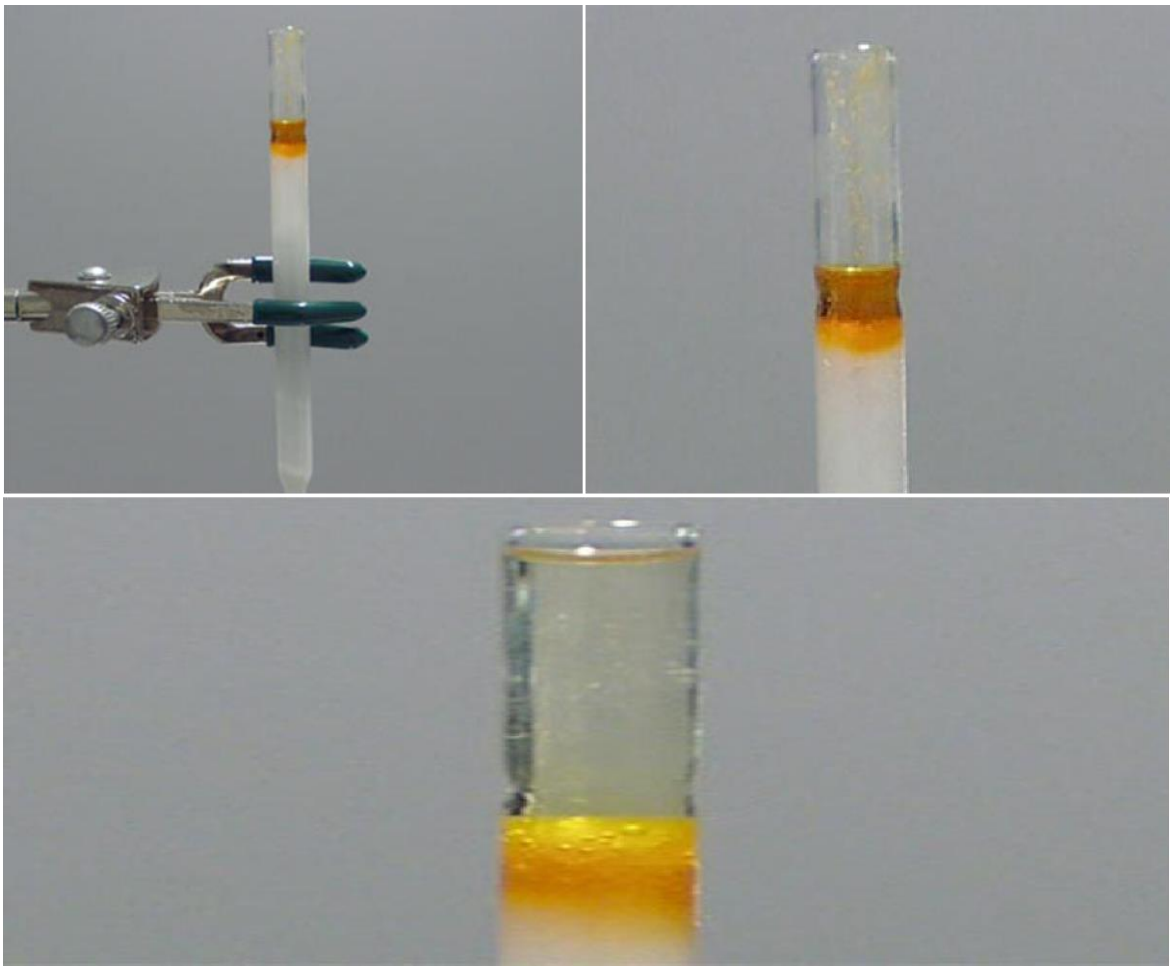

Fig -13: WET LOADING METHOD

Dry Loading Method:
Dissolve the sample in small amount of solvent and add 100 mg of silica gel. Agitate the mixture till the solvent evaporates completely and only the dry powder

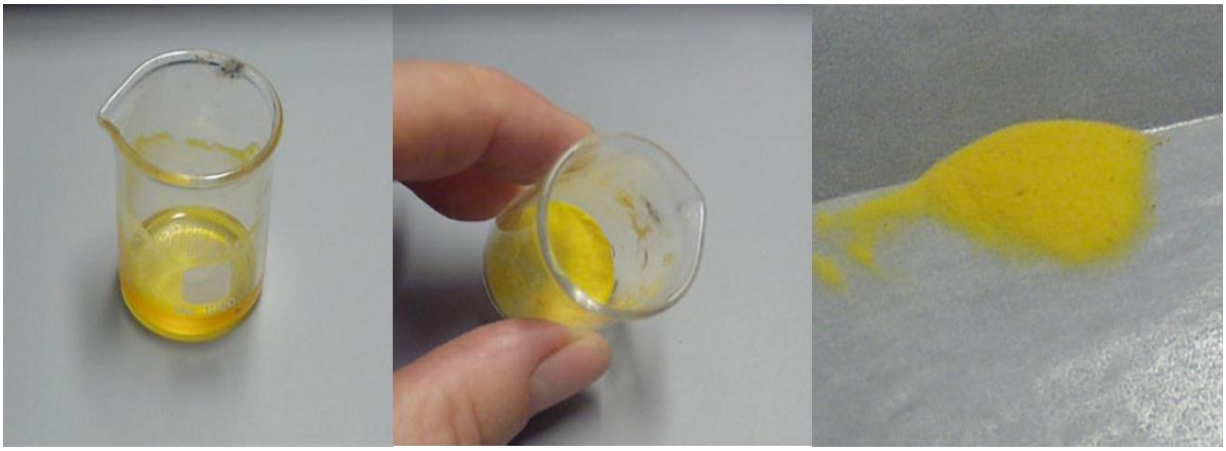

Fig- 14: DISSOLVING THE SAMPLE

remains. Place the dry powder on weighing paper and then transfer it to the prepared column. Add fresh eluting solvent onto the top of column begin the elution process.[13]

Fig- 15: DRY LOADING METHOD

Elute the column:
*Add a best part of elution solvent to the prepared column.
* Pressure is applied to force the solvent through the column by pressing on the top of the Pasteur pipette with a pipette bulb. The solvent is forced only to the very top of the silica: do not let the silica go dry. Add fresh solvent as required
* The pressure should be required to keep a steady stream coming out of the column. Figure below exhibit the colored compound as it moves through the column after successive applications of the pipette bulb process.
* The collection beaker is changed immediately when the colored compound begins to elute. The process is tricky if the compound is not colored. In such experiments, the
Subsequent equal sized fractions are collected and carefully labelled for later analysis.[13]

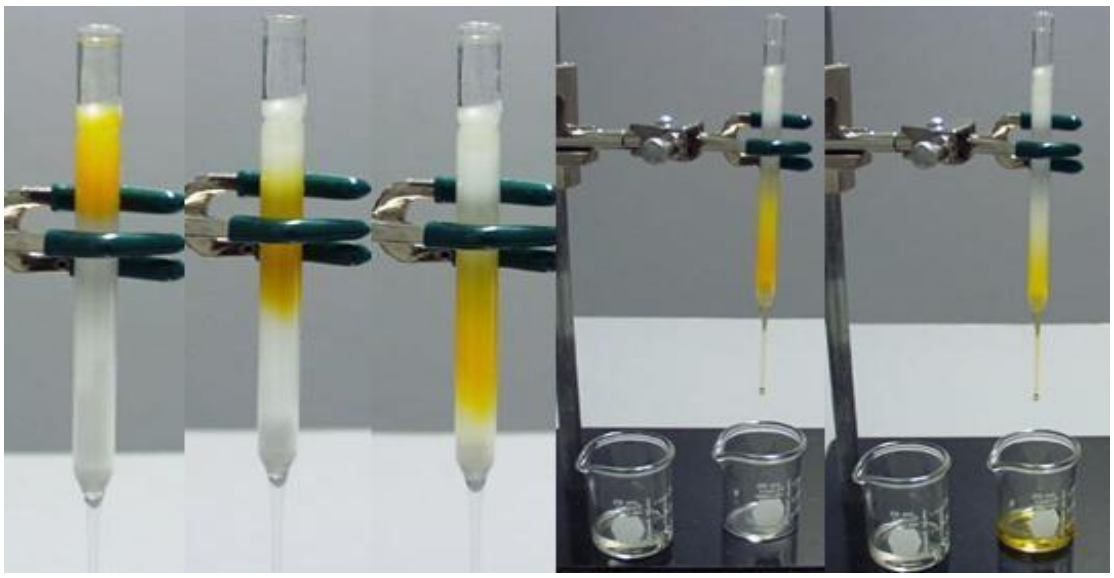

FIG -16: ELUTING THE SAMPLE

Analyze the sample

* If the fractions are colored, we can directly combine like-colored fractions, TLC before combination is generally performed. If the fractions are not colored, they are detected by TLC If the composition of each fraction is known, the fractions containing the desired components are combined.[13]

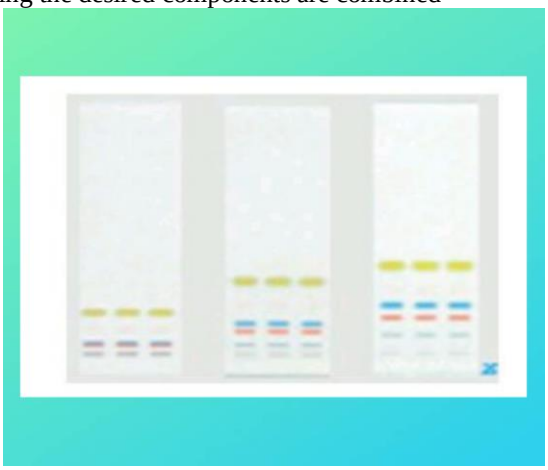

Fig - 17: ANALYZE THE SAMPLE

Cleaning the Column:

Flush all the remaining solvent out of the column by pressurized gas. Flow air through the column for two hours to get dry, free flowing silica gel.

Pour out the contents of the column into the silica waste container. Usually, washing the column with water and acetone is enough

If required, a small amount of liquid soap is used. When all liquid solvent is removed from the reservoir, remove the last residues of solvent by application of vacuum from aspirator to the bottom of the prepared column. Avoid

scratching the columns with abrasive brushes or soaps.[2] [8]

Modern flash chromatographic techniques

Pre-packed plastic cartridges [14]

In the modern Flash Chromatography techniques, the glass columns are replaced with pre-packed plastic cartridges which are safer, reproducible. Solvent is flushed through the cartridge, which is much faster and more reproducible. Systems are linked with detectors and fraction collectors which provides automation. Gradient pumps facilitate quicker separations, less solvent usage and higher flexibility.

Fig -18: PRE-PACKED PLASTIC CARTRIDGES

Column characteristics:

1. Disposable plastic cartridges will save time and improve reproducibility
2. Cartridges of different size are easy scale-up
3. Solid sample module and injection valve facilitate easy sample loading
4. Pressure up to 100 psi provide fast separation
5. Narrow particle distribution gives Low backpressure and greater efficiency

Advanced Detection Techniques for Flash Chromatography [15]

UV detection is the conventional method used in Flash chromatography to regulate and fractionate peaks during the purification. Compounds that lack chromophores, are not detected by UV. In some other cases, the compound's absorption spectrum may be unfamiliar or detection was at a sub- optimal wavelength. Besides, the UV absorption of the necessary mobile phase may interfere with the λ-max of the compound in process. In other cases, the absorption spectrum of the compound of interest or co- eluting impurities is not known and cannot detected. These advanced detection techniques permit the users to easily fractionate components without the need for follow-up TLC and followed by staining to determine where the purified compound eluted.

Evaporative Light Scattering Detection (ELSD) is used for High Performance Liquid Chromatography and is been recently employed for Flash chromatography. All- Wavelength Collection permits the collection of compounds.

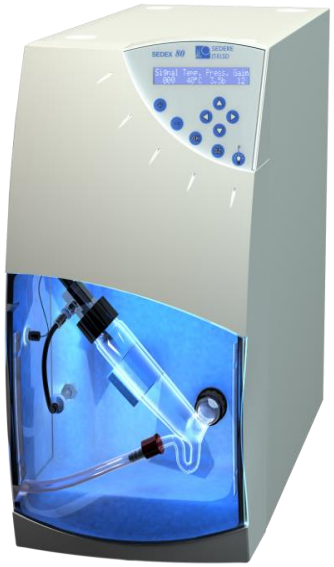

Fig – 19: EVAPORATIVE LIGHT SCATTERING DETECTION (ELSD)

Green Flash Chromatography [16]

Green Flash Chromatography is the ideal flash chromatographic technology that achieves most effective sample purification. The sample flush is always carried out with minimum eluting volume. It reduces run time and solvent use and achieves a good separation. It is Eco- friendly.

Features of Green Flash Chromatography

- Parameters for flow rate, run time, fraction volume, are calculated and set automatically by selecting a column on "Green Flash" software. The default parameters are shown in System Setting window.
- Software gives the maximum sample load information for the selected column.
- State-Of-The-Art Software depend on True Theory of Chromatography.
- Sample Eluting Position and Resolution is Fully Controlled for Systems.
- Automatic Method Setup - Reverse Phase Chromatography.
- Parallel Detection of UV Detector and Refractive index Detector or ELSD.

Flash chromatography with TLC image reader. [17]

The system is provided with a UV light source and a camera. By shooting the TLC plate and by clicking the target compound on the TLC plate, the Rf value of the target component will be calculated and the improved chromatography method is developed automatically. The TLC plate is exhibited on the screen during run. Compound spots on the TLC plate and their peaks both are displayed on the screen. The two- photographic image of the TLC plate and the purification are saved as a data file. Press on the target component and its nearer impurity on the TLC plate, and the highest sample load for every column will be automatically calculated. Thus, allowing the chemists to select the best suited column for their sample.

Advantages

* Fast and economic method in the synthesis laboratory.
* Ideal for the separation of components up to gram quantities.
* No expensive equipment is needed
* In an ideal way shifts results from TLC to CLC

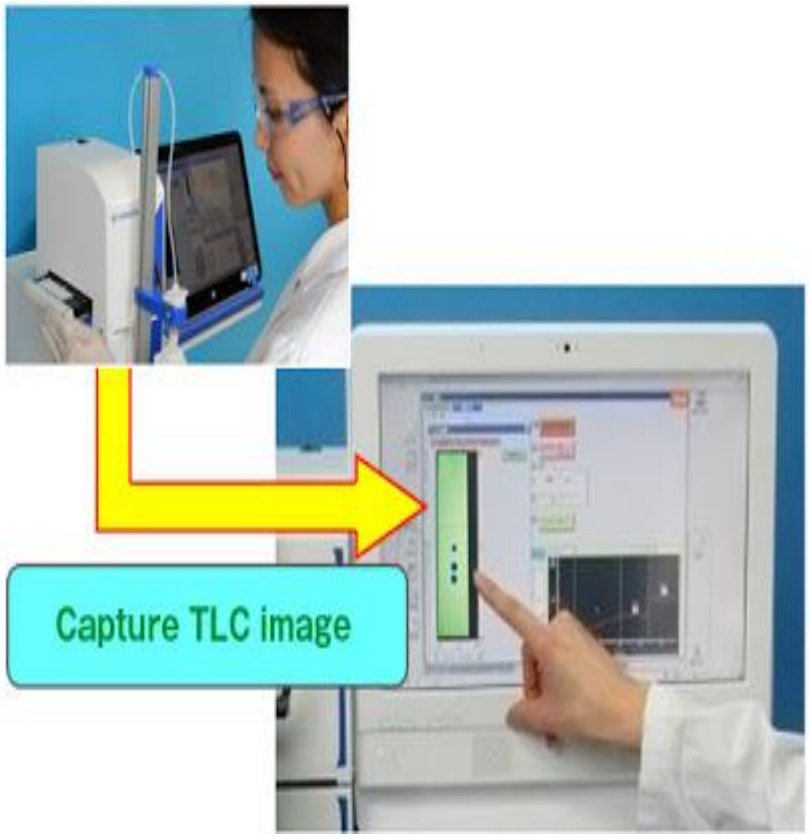

FIG – 20: FLASH CHROMATOGRAPHY WITH TLC IMAGE READER

Association between TLC and flash chromatography [18]

In the method development of flash chromatography, the sample is firstly subjected to TLC to improve different conditions. As TLC is a quick and inexpensive technique, many trials can be performed and it also gives an idea regarding solvent system composition, amount of adsorbent that has to be used.

- The solvent system which has a Rf equal to 0.15 or 0.2 in TLC for the analyte is then selected. The retention factor should be less because the surface area of adsorbent in TLC is twice to that of the silica gel surface area in a column chromatography.
- It is used to calculate column volume. Column volume (CV) is the volume inside the column which is not occupied by any media and includes internal porosity, interstitial volume and the empty volume in the column. This CV indicates the loading capacity and separation efficiency of a column in column chromatography. Column volume is the inverse value of retention factor.

Consideration between HPLC and flash chromatography: [19,20]

HPLC is an extensively used technique in various laboratories and industries& its main applications are separation of little quantities of sample, their identification and quantification. The HPLC instrument is very expensive for installation and operation. Flash chromatography is a technique extensively used for purification of desired sample& segregation of large quantity of samples within less time. Flash chromatography is preferred over the regularly used high cost preparative HPLC, if the compounds to be separated are in high concentration.

Preparative chromatography Vs Flash chromatography [19]

Preparative Chromatography	Flash Chromatography
0.1-2.0g of samples can be separated	0.5-2.0 g of samples can be separated
Separation time is 10 min to 1 Hour	Separation time is about 10-15 min
Complicated equipment and the purchase of expensive equipment is necessary	Complicated equipment and the purchase of expensive equipment is not necessary
Column is very Expensive	Cartridges can be reused for nearly 8 times and Cheaper than Preparative columns.
Sample is solubilized in mobile phase	No need to solubilize of sample in Mobile phase
It is useful in Agro chemistry, Synthetic chemistry, Natural products separation.	It is More useful in separation of various antibiotics, Impurities, Peptides.
This technique will save the time.	This technique saves both time and solvents
It is Reliable	It is Reliable and cost effective

Table – 3: Preparative chromatography Vs Flash chromatography

Column Chromatography vs. Flash Chromatography:[9]
Here in the below example, an 8 hours glass column chromatography run was separated in just 50 minutes by using a 40mm ID flash cartridge and in this case, there were no mixed fractions observed

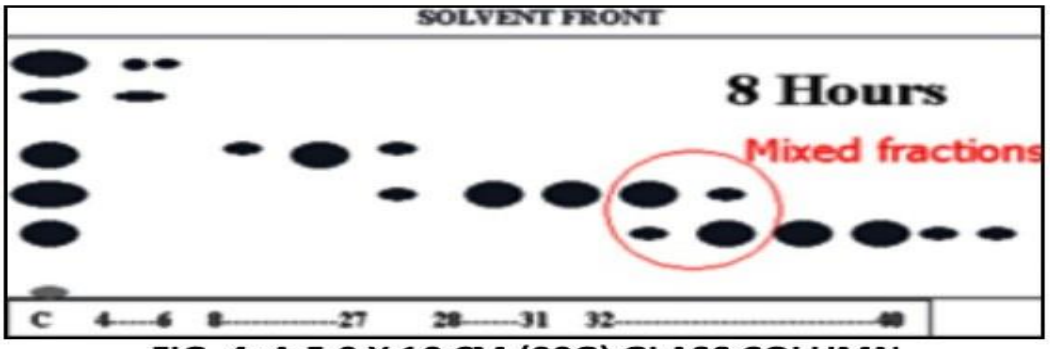

FIG. 4: A 5.0 X 10 CM (90G) GLASS COLUMN

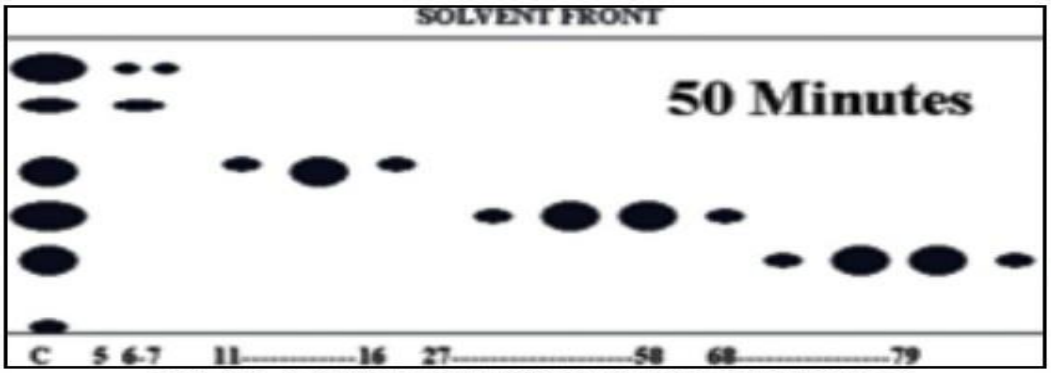

FIG.4 B 4.0 X 15 CM (90G) FLASH CARTRIDGE

Fig- 21: column chromatography Vs flash chromatography

Advantages of flash chromatography [21]

Flash chromatography has various advantages.

- It is a rapid method and the elution completes in a flash.
- It is a cost -effective technique.
- The compounds can be separated and collected up to few grams.
- This method can be used in low scale and also in high scale.
- The method is automated.
- Compounds which usually degrade in the column can be eluted in purified state in flash chromatography, as the contact time is very less.
- It is ecofriendly, as the solvent usage is less.
- The column can be reprocessed several times and saves the time for preparing the column.
- The hazardous health effects on the operating personnel are less, because of less time of operation [5,6]

Applications [20,22.23.24]

Phytochemistry

★ Separation and Isolation of α-Santalol and β-Santalol in Sandalwood Extraction
Isolation and Purification of:
★ Chromophoric and Nonchromophoric Compounds from Giant Knotweed Rhizome.
★ Flavonoids from Ginkgo Biloba Leaves Extract.
★ Ginsenosides from Red Panax Ginseng Extract.
★ Catechins from Green Tea Extract.
★ In Purification of GallaChinensis, Ferulic Acid in RhizomaChuanxiong Extract.
★ Flash chromatography is utilized for High-Speed Flash Fractionation of Natural Products

Lipids Application:

Purification of:
★ Fatty Acid Methyl Esters (FAMEs), sterols
★ Mixture of Glycerides, Mono-Di-, and Tristearin.

Pharmaceutical Application:

★ Bile Acid Purification during Lead Generation in the Drug Discovery process
★ Mestranol Purification during the Chemical Synthesis.
★ In Anti-malarial Drug Purification during Drug Discovery process
★ In Impurity Isolation in drug Purification.
★ Flash chromatography is used in isolation of strongly linked drug intermediates.
★ Flash chromatography is frequently used during drug discovery process

Carbohydrate Application

★ Impurity Isolation of Valproic Acid from Cyclodextrin During the Encapsulation process
★ Isolation of Amino sugar and Acarbose, Aminoglycoside Antibiotics.
★ In purification of Flavanone Glycoside
★ In Purification of Conjugated Quercetin and Rutinose.

Other applications

★ Flash chromatography is utilized to decontaminate, accumulate and recognize a variety of aromatic constituents from a semi-synthetic extract
★ Flash chromatography is used in refinement of mixture of peptides, antibiotics.
★ Flash chromatography is employed in agricultural chemistry.
★ Flash chromatography is utilized during refinement and processing of petroleum products.
★ It is used in Purification of Protected Peptide
★ It is used in the Separation of Closely Related Organic Compounds
★ Flash systems are strong tools for purification of trace compounds from organic mixture. It is used as a tool to regulate the reaction progress and to isolate and identify a mixture's compounds.
★ It is employed to purify, collect and identify the various ivermectin components in a semisynthetic extract.
★ The Flash system are used by many pharmaceutical companies to purify compounds in the drug discovery process
★ Continuous Gradient Purification of Closely Related Drug Intermediates is performed using Flash Chromatography
★ It attained attention as a lead investigation and optimization tool in drug discovery
★ Isolation of 4-Methoxyacetophenone, benzoin from a crude reaction mixture

CONCLUSION:

Flash Chromatography is an unsophisticated, simple, rapid, cost effective preparative liquid chromatography approach with minimal instrumentation requirements. It is very excellent preparative chromatographic tool for separation and purification of components of a mixture for more advanced use. It is frequently used in many laboratories, mainly during the drug discovery process.

Extrapolation of TLC results on preparative scale can be attained by Flash chromatography. This technique provides many advantages like easy installation and operation, usage of less amounts of solvents, purification of large quantities of samples, consumption of less time. Modern Flash chromatography with disposable cartridges and advanced detection techniques are available and are applicable to various compounds. Hence, flash chromatography is a low- cost chromatographic technique which can be used when modest resolution is required.

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