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A REVIEW ON A FLASH CHROMATOGRAPHY

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ABSTRACT

Flash chromatography is rapid form of preparative column chromatography- preparative liquid chromatography based upon an air pressure driven hybrid of medium and short column chromatography optimized for rapid separation of organic compounds. As technology has evolved available guidelines for normal-phase flash chromatography have become less relevant. Years of experience performing chromatography with disposable columns have been condensed into simple guidelines useful for translating TLC results into either isocratic- or gradient-flash chromatography. The described studies should provide researchers with a means of selecting adequate columns and guidelines to reduce the waste of solvents, silica, time, and money. Modern flash chromatography systems are sold as pre-packed plastic cartridges and the solvent is pumped through the cartridge. These systems may also be linked with detectors and fraction collectors providing automation. The introduction of gradient pumps has resulted in quicker separations and less solvent usage.

Keywords: Silica gel, Flash chromatography, TLC.

INTRODUCTION

Silica gel flash chromatography has become ubiquitous within organic chemistry and since its formal introduction in 1978 [1]. 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 are given below i. Simple packing procedure, ii. Low operating pressure, iii. Low expense for instrumentation [2].

Flash chromatography is basically an air pressure driven hybrid of medium pressure and shorter column chromatography which has been optimized for particularly rapid separation. Flash chromatography is a technique used to separate mixtures of molecules into their individual constituents, frequently used in the drug discovery process. 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 [3]. The net result is a rapid ("over in a flash") and high resolution chromatography. Several manufacturers have developed automated flash chromatography systems. It classified into two types 2: 1. LPLC - Low pressure liquid chromatography (LPLC) system which operate around 50 -75 psi 2. MPLC - Medium pressure liquid chromatography (MPLC) systems which operate above 150 psi. 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.

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 of defined particle size [4]. 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.

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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.

THEORY

Chromatography exploits the differences in partitioning behavior between a mobile phase and a stationary phase to separate the components in a mixture. 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. In a continuous development system like HPLC or GC where the compounds are eluted with the eluents, the retention is usually measured as the retention time (rt), the time between the injection and detection. In un-interrupted development system like TLC, the retention is measured as the retention factor (Rf), the run length of the compound divided by the run length of the eluent front. Rf = Distance traveled by the solvent front [5].

Various components 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. This number refers to the mesh of the sieve used to size the silica, specifically, the number of holes in the mesh or sieve through which the crude silica particle mixture is passed in the manufacturing process. Adsorbent particle size affects how the solvent flows through the column. Smaller particles (higher mesh values) are used for flash chromatography; larger particles (lower mesh values) are used for gravity chromatography. For example, 70-230 silica gels are used for gravity columns and 230-400 mesh for flash columns. The amount of silica gel depends on the Rf 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. These are some adsorbents which are mainly used in flash chromatography: [6].

- Silica: Slightly acidic medium. Best for ordinary compounds, good separation is achieved.
- 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.
- Alumina: Basic or neutral medium. Can be effective for easy separations, and purification of amines.
- 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 nonpolar component [7].

One-component solvent systems

1. Hydrocarbons: pentane, petroleum ether, hexanes
2. Ether and dichloromethane (very similar polarity)
3. Ethyl acetate

Two-component solvent systems

1. Ether/Petroleum Ether, Ether/Hexane, and Ether/Pentane: Choice of hydrocarbon component depends upon availability and requirements for boiling range. Pentane is expensive and low-boiling, petroleum ether can be low-boiling, and hexane is readily available.
2. Ethyl Acetate/Hexane: The standard, good for ordinary compounds and best for difficult separations.
3. Methanol/Dichloromethane: For polar compounds.
4. 10 % Ammonia in Methanol Solution/Dichloromethane: Sometimes moves stubborn amines off the baseline.
5. For basic (i.e. nitrogen containing) compounds, it is sometimes useful or necessary to add a small amount of triethylamine or pyridine to the solvent mixture (about 0.1%).
6. For acidic compounds, a small amount of acetic acid is sometimes useful. In this case, be very careful in concentrating the solvent as trace amounts of acids can be very dangerous when they are concentrated with a product. In these cases, the acetic acid can often be safely rotavaped away by adding portions of toluene and concentrating to a few mL volumes and repeating this several times. As acetic acid boils at a lower BP than toluene, this will remove the acid without exposing the neat compound to it.

The properties of commonly used flash solvents

The compound of interest should have a TLC Rf 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. 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 Table 2: 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.

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 affecting ΔRf and CV. Solvent selectivity should be adjusted to provide ΔRf > 0.20. Different solvent combinations to obtain desired TLC separation usually reveals appropriate conditions for effective flash chromatography separation. Different solvent mixtures can even reverse the elution order of some of the components in the sample. Column volume difference ΔCV predicts column capacity or the amount of material that can be effectively separated in a single column loading. The greater the ΔCV, the greater the effective capacity of the column.

PROCEDURE

Packing the Column

A chromatography column is plugged with a small piece of cotton wool, just enough to fill to stopcock hole. Sand, about 2 cm is added so that the diameter of the sand is approximately the same as the column. Silica gel is added dry. Usually, it is best if the silica is not too long, about 6 to 10 inches is best in most cases. Attach the house vacuum to the bottom of the column via the stopcock. Open the vacuum and the stopcock; this will compresses the silica gel and hold it tight for the next steps. Add sand to the top of the column, about 1-2 cm is enough. With the vacuum still applied, pour the solvent (premixed, i.e. 4:1 hexanes/ethyl acetate). Allow the solvent to flow though the column until it is almost eluting. At this point, close the stopcock and remove the vacuum line. Make sure enough solvent is in the column for 5-6 column volumes worth to flow though, to ensure complete packing. Now elute all of the solvent with air pressure, taking care not the let the column run dry. Stop with the solvent level parallel with the sand. A well-packed column should not have any cracks or patches. The solvent eluding from the stopcock should not be warm or hot [8].

Loading the Column

Prepare a solution of your reaction or compound mixture in the minimal amount of methylene chloride possible. Using a pipette, add this carefully to the top of the silica, washing the flask 3-4 times with methylene chloride or the chromatography solvent. After each addition, allow the solvent level to descend into the very top of the silica gel (below the sand).Carefully added 2-3 pipettes of chromatography solvent and push this into the column (repeat 3-4x).Now, carefully fill the remaining column space with the chromatography solvent and elute using compressed air. A flow rate of about 2 inches/minute is ideal. This is measured by how fast the solvent column descends in the straight part of column, above the silica gel. It is most convenient to measure and adjust the flow rate before adding the compound. In cases where a reaction mixture or compound is not soluble in a suitable solvent for loading, it can be absorbed onto silica gel. This is done by dissolving the compound in acetone, adding silica gel and carefully concentrating the silica gel to dryness (careful: it bumps!). The dry silica is then added to the top of the packed silica column. In this case, sand should not be added to the column until after the silica-compound mixture is added. This method is recommended only as a last resort as separations are often inferior to solution loading [3].

Running the column

Column fractions are collected in test tubes, of a size appropriate for the type of column and polarity. Use the 13 mm test tubes for small scale (i.e. 5-50 mg) and larger test tubes for bigger columns. Refer to the guidelines in Still's paper for choosing fraction sizes.Start collecting the fraction immediately after adding your compound; it does not take long for very non-polar compounds to elute from the column.Once you have loaded a column, it is best not to stop it for any length of time. This is due to slow diffusion of the compounds on the silica gel resulting in poor separation and diminished yields. To find your product, spot each fraction or so on a TLC plate and check which fractions contain compounds. Fractions containing the same compounds are combined, the test tubes washed with methylene chloride or (probably better for the environment), distilled ethyl acetate, and the solvent concentrated under reduced pressure. Do not let a column run dry or elute the solvent until after you are sure all of the compounds have eluted [9].

After the column-cleaning up

After you have finished, elute all of the solvent from the column using compressed air. Flowing air through the column for ~2 hours will give dry, free flowing silica gel. Pour out the contents of the column into the silica waste container. In most cases, washing the column with water and acetone is sufficient. If necessary, a small amount of liquid soap can be used. Try to avoid scratching the columns with abrasive brushes or soaps.

GENERAL PROCEDURE FOR FLASH CHROMATOGRAPHY FOR 100 TO 300 MG OF A MIXTURE

- Obtain a small flash chromatography column and use a 1-mL pipet to push a smallwad of cotton or glass wool into the narrow part of the valve stem [9].
- Clamp the column high in a ring stand and add about a 1/2" sand bed through a powder funnel. Make up 100 mL of your starting eluant determined by TLC, (for example 10/90 CH_2Cl_2/Hexanes mixture) in a 250-mL Erlenmeyer flask. Mix thoroughly by swirling. Pour 1 cm deep amount of this into a TLC development jar, cap and set aside for later use. Pour enough of this into the column so there is ~ 1" of solvent above the sand.
- In a 50 mL beaker, obtain 20 mL of 200mesh silica gel from the blue supply bucket. Fill the beaker to the 40 mL mark with your mobile phase and stir with a wide metal scoopula to make a slurry. Figure 1.
- With stirring, pour and scoop the silica gel slurry slowly into the column through a powder funnel. Use the scoopula to stir and help transfer the slurry. Tap the column gently with you finger tips to help the silica gel settle. Use additional solvent to rinse any remaining silica gel out of the beaker and into the column**.** Figure 2.
- Place the pressure Tee/rubber stopper loosely in the top of the column and connect one hose to the nitrogen supply and turn on. Put a Hoffman screw clamp on the end of the other hose and tighten to pressurize the system.
- Place the slurry beaker you just emptied under the column and open the stopcock to allow a stream of solvent to flow into it. Allow the solvent to flow out until the liquid level in the column is just at the top of the silica gel bed. To speed up the elution, press the tee/runner stopper into the top of the column to pressurize it and increase the solvent flow. Rinse the sides of the walls with solvent to wash down the silica gel. Drain solvent until it is about 1/2" above the top of the silica gel. Figure 3.
- Normally you will use the Wet Loading Method starting at step 9. If you are going to use the Dry Loading Method, place one or two scoopulas of silica gel into a 25-mL 19/22 RB flask. Dissolve ~250 mg of your mixture in 10 mL CH2Cl2 and add this to the silica gel. Remove the CH2Cl2 on a rotovap or with a stream of nitrogen so that you obtain a dry powder. Carefully pour the dry powder onto the top of the column to obtain a even layer at the top. Using a Pasteur pipet, add eluant by draining it onto the glass wall just above the silica gel until it is just covered. Try not to disturb the silica gel too much.

Table 1. The Properties of Commonly Used Flash Solvents

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	-

Table 2. Typical Volume of Eluant Required For Packing and Elution

Column Diameter (mm)	Volume of eluant* (ml)	Sample Load (mg) Rf > 0.2	Rf >0.1	Fraction Size (ml)
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

- Gently sprinkle about 1/2" of sand through the powder funnel. If necessary, add a little more solvent to cover the sand.
- **Wet Loading Method -** Dissolve ~250 mg of your mixture in a minimum amount of mobile phase (less than 1 mL of solvent if possible. Using a Pasteur pipet, draw up the mixture solution. Insert the pipet into the column so it is touching the inside wall just above the silica gel surface. Gently flow the solution onto the top surface of the silica gel so that you hardly disturb the surface. Drain until the solvent level is just at the top of the silica gel. Gently sprinkle about 1/2" of sand through the powder funnel. If necessary, add a little more solvent to cover the sand. Drain until solvent level is at top of sand. Apply pressure if necessary.
- Using Pasteur pipette, very gently add about 1" of your elution solvent that you collected in the beaker when preparing the column. Let the solvent run down the glass wall so that the sand is disturbed as little as possible. Open the stopcock to allow eluant to drain into the beaker until the liquid level is again just at the top of the sand surface. Gently add more eluant washing down the walls of the column just above the silica. Again drain out just enough to bring the liquid to the top of the surface. Repeat this two more times until you have washed the band of your mixture into the column. You want to get as thin and compact a band of your mixture as possible. Now pipette in about 3" of solvent in the column, and then pour in more solvent until the column is 3/4 full.
- Place about 8 to 12 numbered test-tubes in a rack and place the rack below the column so that you can collect column eluant in them. Figure 4.
- Now open the stopcock and pressurize the column. Fill consecutive test-tubes 3/4's full of eluant. Collect about 5 fractions, then, if necessary, increase your solvent polarity (to 30/70 CH_2Cl_2/Hexanes, for example).
- Do TLC analysis on each of the fractions, checking spots before development under a U V light to make sure there is enough material to see. Develop in the corresponding eluant solvent and examine under UV light or by iodine to see which fractions contain material. If you do not see any spots, then let the solvent evaporate from your test tubes (Label the rack of tubes with your name and leave in the hood.) You should see material in some tubes. Combine similar fractions in a flask or beaker, label and put in your hood to evaporate.
- Clean out the column by blowing out all solvent and leaving upside down in a 5 gallon bucket to dry [11].

APPLICATION OF FLASH CHROMATOGRAPHY

Natural compounds are more and more evaluated as alternatives to classical drugs and therefore the needs for the separation of such complex mixtures are also growing. Flash chromatography is a very valuable technique in the field of natural compounds research because it provides a fast and economical way to separate the main components of complex plant extracts.

Flash chromatography is the most often used purification tool after organic synthesis. The following "Short Notes" illustrate the flexibility of the Sepacore system for the optimization of the flash purification of various synthesis reaction mixtures. Isolation of 4-Methoxyacetophenone from a crude reaction mixture, Isocratic elution, using an additional polar solvent for sample loading, Isolation of Benzoin from a crude reaction mixture, Isolation of 2,2'- Furoin from a crude reaction mixture, Cleaning up of ααα- Methylstyrene by flash chromatography, Isolation of 3-Nitro-4-ethoxybenzaldehyde from a synthesis mixture, Isolation of Benzylideneacetophenone from a crude reaction mixture.

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