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BRIEF 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.

INTRODUCTION: 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:

- 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 [1].

In traditional column chromatography a sample to be purified 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.

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 [1].

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.

The absorbent has a much smaller particle size (about the same as that on TLC plate). There are many different of kinds of chromatographic columns. If the column contains a porous plate to support packing, no additional support such as cotton, glasswool and sand is necessary [1].

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 [3]: 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. 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.

Various components of Flash Chromatographic System:

Sorbent Selection [1, 4]: 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 *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 [1].

These are some adsorbents which are mainly used in flash chromatography [5]:

- 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 [1, 5]: Flash column chromatography is usually carried out with a mixture of two solvents, with a polar and a nonpolar component. Occasionally, just one solvent can be use. The *only* appropriate one-component solvent systems (listed from the least polar to the most polar):

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

The most common two-component solvent systems (listed from the least polar to the most polar):

4. 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.
5. Ethyl Acetate/Hexane: The standard, good for ordinary compounds and best for difficult separations.
6. Methanol/Dichloromethane: For polar compounds.
7. 10 percent Ammonia in Methanol Solution/Dichloromethane: Sometimes moves stubborn amines off the baseline.
8. 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%).
9. 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.
10. **Table 1** summarizes the properties of commonly used flash solvents.

TABLE 1: THE PROPERTIES OF COMMONLY USED FLASH SOLVENTS [6]

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	-1

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.

Column Selection [7]: Select a column that is 10, 20, 40 mm ID based upon preparative requirements. Indeed, Professor Still et al offered this selection **Table 2**:

TABLE 2: TYPICAL VOLUME OF ELUANT REQUIRED FOR PACKING AND ELUTION

Column Diameter (mm)	Volume of eluant* (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

* Typical Volume required for equilibrium of the column and elution

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 [8].

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

- Iron Content : <0.02%
- Chloride Content : <0.10%
- Loss on Drying : <3%
- pH (10% suspension) : 7±0.5
- Surface Area : 400–600 m^2/gm

Packing the Column [1]: Obtain a glass column and make sure that it has either a glass frit or a plug of cotton wool directly above the stopcock to prevent the silica gel from escaping from the column through the stopcock. (IF it doesn't have either, we have to put in a somewhat loosely stuffed plug of cotton wool; if we stuff it too much; solvent flow becomes painfully slow even with air pressure above the column).

Next, put a ~1/2 inch 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. Add dry silica gel adsorbent, 230-400 mesh usually the jar is labeled "for flash chromatography." One way to fill the column is to invert it into the jar of silica gel and scoop it out & then tamps it down before scooping more out **(fig.1).**

FIG.1: METHOD OF PACKING THE COLUMN

Another way to fill the column is to pour the gel into the column using a 10 mL beaker **(fig.2).** Whichever method we use to fill the column, we must tamp it down on the bench top to pack the silica gel. We can also use a pipette bulb to force air into the column and pack the silica gel. When properly packed, the silica gel fills the column to just below the indent on the pipette. This leaves a space of 4-5 cm on top of the adsorbent for the addition of solvent. Clamp the filled column securely to a ring stand using a small 3-pronged clamp.

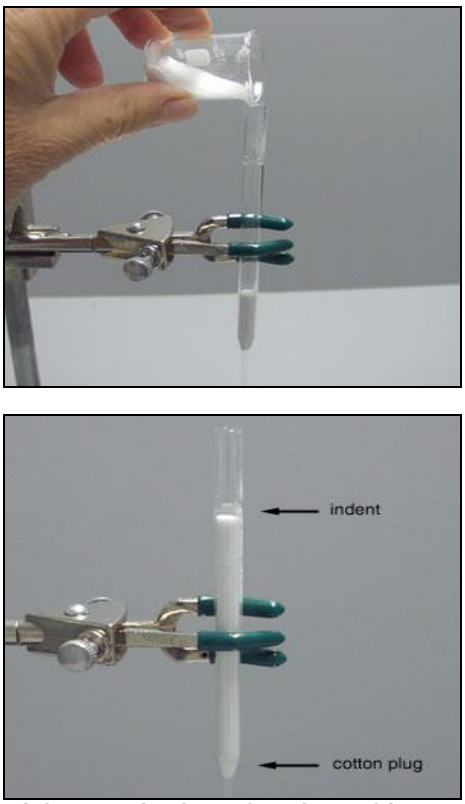

FIG.2: METHOD OF PACKING THE COLUMN

Solvating the Silica Gel Column [1]: Next, tap gently and evenly the sides of the column with a piece of rubber tubing to settle the silica gel. Pour a good amount of elution solvent onto the silica gel. Use pressurized gas to force the solvent through the silica. As we force through a few hundred milliliters, we should see the top part of the silica become more homogeneous. This is because we are forcing out air that was entrapped in the silica gel. Continue to flush solvent through the silica gel until the entire silica plug becomes homogeneous in appearance. We may have to recycle the solvent coming through the column onto the top of the column several times before all the silica gel is solvated. Do not let the top of the column run dry, otherwise we will force air back into the top of the silica, and we will be back where you started.

Load the sample onto the silica gel column [9]: Two different methods are used to load the column: the wet method and the dry method: wet and dry. Below are illustrations of both methods of loading a crude sample of ferrocene onto a column.

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

Dry Loading Method: First dissolve the sample to be analyzed in the minimum amount of solvent and add about 100 mg of silica gel. Swirl the mixture until the solvent evaporates and only a dry powder remains. Place the dry powder on a folded piece of weighing paper and transfer it to the top of the prepared column. Add fresh eluting solvent to the top now we are ready to begin the elution process.

Elute the column [9]: Add a good part of our elution solvent to the column. Apply pressure to force the solvent through the column by pressing on the top of the Pasteur pipette with a pipette bulb. Only force the solvent to the very top of the silica: do not let the silica go dry. Add fresh solvent as necessary. The pressure should be the minimum necessary to keep a steady stream coming out of the column. **Fig. 3** shows the colored compound as it moves through the column after successive applications of the pipette bulb process. The last two photos illustrate collection of the colored sample. Note that the collection beaker is changed as soon as the colored compound begins to elute. The process is complicated if the compound is not colored. In such experiments, equal sized fractions are collected sequentially and carefully labeled for later analysis.

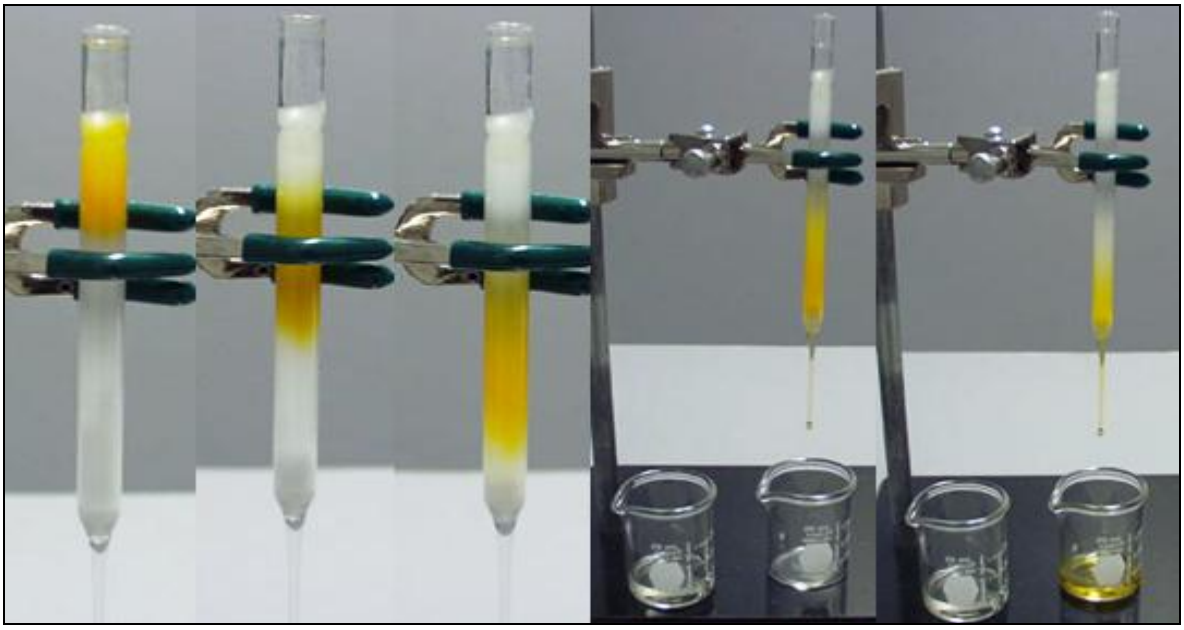

FIG. 3: ELUTION PROCESS IN FLASH CHROMATOGRAPHY

Analyze the fractions [9]**:** If the fractions are colored, we can simply combine like-colored fractions, although TLC before combination is usually advisable. If the fractions are not colored, they are analyzed by TLC (usually). Once the composition of each fraction is known, the fractions containing the desired compound(s) are combined.

Cleaning the Column [1, 5]**:** Flush all the remaining solvent out of the column using pressurized gas. 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. 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. Try to avoid scratching the columns with abrasive brushes or soaps.

General procedure [1]**:** First a low viscosity solvent system (e.g. ethyl acetate/30-60°C petroleum ether) is found which separates the mixture and moves the desired component on analytical TLC to an R*f* of 0.35. If several compounds are to be separated which run very close on TLC, adjust the solvent to put the midpoint between the components at R*f* = 0.35. If the compounds are widely separated, adjust the R*f* of the less mobile component to 0.35.

Having chosen the solvent, a column of the appropriate diameter (Table 1) is selected. Dry 40-63 mm silica gel is poured into the column in a single portion to give a depth of 5.5-6 in. With the stopcock open, the column is gently tapped vertically on the bench top to pack the gel. Next a 1/8 [to 1/4] in. layer of sand is carefully placed on the flat top of the silica gel bed and the column is clamped for pressure packing and elution.

The solvent chosen above is then poured carefully over the sand to fill the column completely (Air pressure is then applied by holding the cork containing the air line on the top of the column). This cause the pressure above the absorbent bed to climb rapidly and compress the silica gel as solvent is rapidly forced through the column. It is important to maintain the pressure until all the air is expelled and the lower part of the column is cool; otherwise, the column will fragment and should be repacked unless the separation desired is a trivial one.

The pressure is then released and excess eluant is forced out of the column. The top of the silica gel should not be allowed to run dry. Next the sample is applied by pipette as a 20-25% solution in the eluant to the top of the adsorbent bed and the flow controller is briefly placed on top of the column to push the entire sample into the silica gel. The solvent used to pack the column is ordinarily reused to elute the column.

The walls of the column are washed down with a few milliliters of fresh eluant, the washings are pushed onto the column as before, and the column is carefully filled with eluant so as not to disturb the adsorbent bed (The cork is held securely onto the top of the column and the air regulator is carefully adjusted to cause the surface of the column to fall 2.0 in. /min.).

Fractions are collected until all the solvent has been used (see Table 1 to estimate the amount of solvent and fraction size). It is best not to let the column run dry since further elution is occasionally necessary. Purified components are identified by TLC and the appropriate fractions are combined and the solvent removed by rotary evaporation to yield the desired material. If the foregoing instructions are followed *exactly*, there is little opportunity for the separation to fail.

Column Chromatography vs. Flash Chromatography [6]:
In the example below, an 8 hours glass column chromatography run was separated in just 50 minutes using a 40mm ID flash cartridge. In this case there were no mixed fractions either **(fig.4).**

FIG.4 COMPARISON STUDIES OF COLUMN CHROMATOGRAPHY VS. FLASH CHROMATOGRAPHY

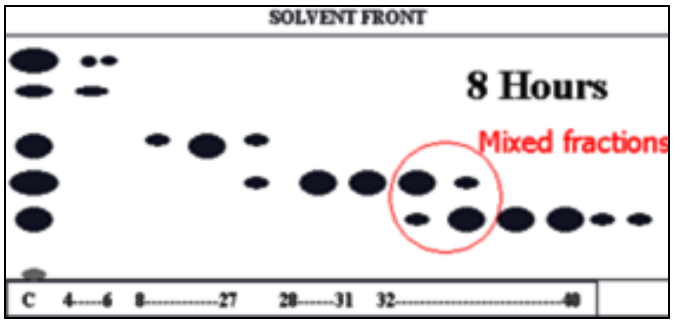

FIG. 4: A 5.0 X 10 CM (90G) GLASS COLUMN

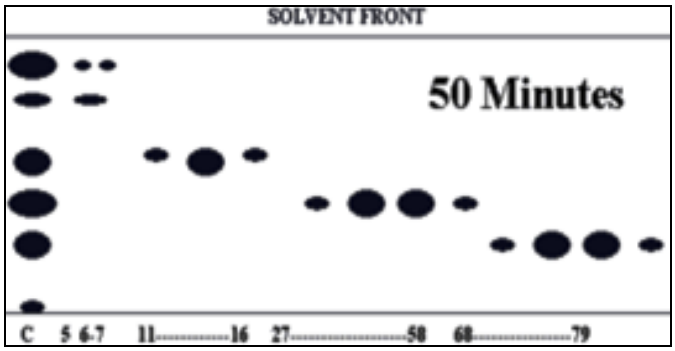

FIG.4 B 4.0 X 15 CM (90G) FLASH CARTRIDGE

Advantages [3]:

- Fast and economic methods for the synthesis laboratory.
- Ideal for the separation of compounds up to gram quantities.
- No expensive equipment required.
- In an ideal way transfers results from TLC to CLC.
- Automated changes between normal phase and reversed phase chromatography

Application [10]:

- It is used for Purification of Protected Peptide
- It is used for Separation of Closely Related Organic Compounds
- Flash systems are powerful tools for purification of trace compounds from organic mixtures.
- It is used as a tool to monitor the reaction progress and to isolate and identify a mixture's compounds.
- 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.
- Improving Natural Product Purity by Orthogonal FLASH Purification
  In this application, several solvent systems were evaluated by TLC. No solvent system was capable of resolving capsaicin, dihydrocapsaicin and lutein from each other. The best solvent mixture for this TLC separation was 90:10 methylene chloride (DCM)/ acetonitrile (ACN).
- It is used to purify, collect and identify the various avermectin components in a semi-synthetic extract.
- The Flex system has been used by many pharmaceutical companies to purify compounds in the process of drug discovery.
- It is used to purify, identify and collect the isomers of an aqueous modified antibiotic precursor. The goal for this work was to isolate each isomer with > 98% purity.

- Continuous Gradient Purification of Closely Related Drug Intermediates Using Flash Chromatography
- Amino modified silica is used with normal-phase solvents and is better suited for nitrogen heterocyclic purification because the surface chemistry is slightly alkaline.
- It has received increased attention as a lead investigation and optimization tool in drug discovery.

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

REFERENCES:

1. Still WC. Kahn M., Mitra A, Flash chromatography, **J.Org.Chem**. 43(14)1978; 2923-2925.
2. www.wapedia.mobi/en
3. www.pretech.nu/products
4. www.sorbeadindia.com
5. www.chem.rochester.edu/how to flash.html
6. www.labhut.com
7. www.saiadsorbants.com
8. www.htslabs.com
9. www.orgchem.colorado.edu
10. www.Biotage.com

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