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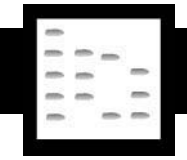
FLASH CHROMATOGRAPHY



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Flash chromatography and related techniques are widely used for laboratory-scale fractionation of mixtures from organic synthesis or for analysis when only a modest increase in resolution over conventional column liquid chromatography is required. These techniques employ short columns packed with particles of an intermediate size (typically 40–60 μm) combined with accelerated solvent flow achieved through modest pressure or suction. Compared to conventional column liquid chromatography, separations are obtained in less time; isolated compounds are often purer because resolution between bands is increased and band tailing is reduced; and compounds that are degraded or altered during chromatography are recovered in higher purity because of the shorter contact time with the chromatographic system.

The main applications of flash chromatography are purification of synthetic products, isolation of target compounds from natural products, the simplification of mixtures prior to high resolution preparative (usually) liquid chromatography and the fractionation of complex mixtures into simpler groups for analysis. Its primary virtue is low cost, since virtually no special equipment is required, and the stationary and mobile phases are inexpensive enough to be discarded after a single use, or can be recycled. Resolution is less than that obtained by medium and high pressure liquid chromatography but the operational costs and equipment needs are greater for these techniques. Flash chromatography is often employed as a pre-separation technique to remove particulate matter and sample components that are either weakly or strongly retained on the separation column in medium and high pressure liquid chromatography. This allows higher sample loads to be separated under more selective separation conditions and avoids column contamination and regeneration problems. The production costs of the isolated products are thus rendered more favourable.

Dry-column Chromatography

Dry-column chromatography (**Figure 1**) is a variant of preparative thin-layer chromatography with similar resolution but a higher sample loading capacity. A glass column or nylon tube is packed with thin-layer chromatographic grade sorbent, usually silica gel, to a height of 10–15 cm. Sample is added as

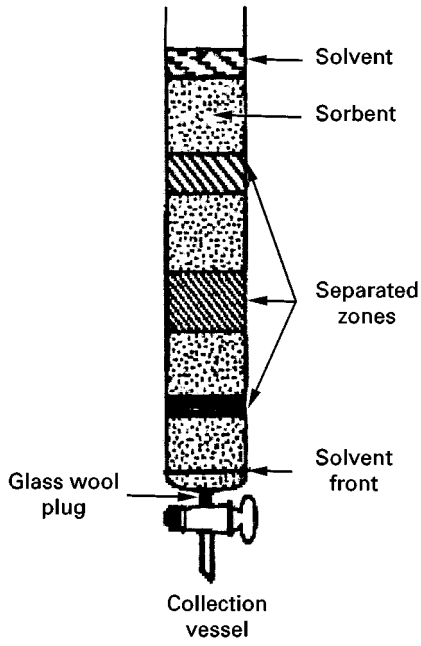

Figure 1 Apparatus for dry-column chromatography.

a concentrated solution or preadsorbed on to a small amount of sorbent. Separation is achieved by developing with a suitable volume of solvent to reach the lower end of the bed. Suction at the bottom of the column and/or slight overpressure at the top may be required to supplement capillary forces in moving the mobile phase down the column. Separated bands are removed by extrusion, slicing (if a nylon column is used) or by digging out, and the products freed from the sorbent by solvent extraction. The separation is fast, requires very little solvent and provides higher resolution than classical column techniques due to the use of sorbents with a smaller average particle size. It is suitable for the recovery of small quantities of material since the loading capacity is only about 0.2–1.0% w/w of the sorbent used, depending on the difficulty of separating the bands of interest.

Thin-layer chromatography provides a suitable technique for method development in most cases, although significant differences in separations can arise for mixed solvents, particularly when the solvent components differ in polarity and/or volatility. These differences result from the absence of pre-equilibrium with a vapour phase in the dry-column technique. Nylon columns can be more difficult to pack than glass columns, particularly when longer lengths are used, but nylon columns are easier to section and allow colourless bands to be observed with a UV lamp. Glass columns built up of segments connected by ground-glass joints can be useful for simplifying the extrusion process.

Dry-column chromatography is not a widely used technique. Preparative thin-layer chromatography or flash chromatography has generally been preferred. Although separations are fast, the recovery of separated zones is slow and labour-intensive compared to elution methods.

Vacuum Chromatography

Vacuum chromatography can be taken to mean the operation of a short column under suction to accelerate solvent migration. Either a short column or a Büchner filter funnel fitted with a glass frit is dry-packed with sorbent. The sorbent bed is consolidated initially by tapping the side of the column during filling and pressing the top layer of the sorbent bed with a flat object, such as a stopper, while suction is applied at the other end. Consolidation is completed by releasing the vacuum and pouring a solvent of low polarity over the surface of the sorbent bed followed by restitution of the vacuum. If the column is packed correctly the solvent front will descend the column in a horizontal line; otherwise the column should be sucked dry, repacked and tested again. When all the solvent has passed through the column, residual solvent trapped between particles is removed by suction. A solution of the sample in a suitable (weak) solvent or preadsorbed on to a small amount of sorbent or inert material, such as Celite, is applied to the top of the column (**Figure 2**). The sample solvent, if used, is sucked gently into the column packing. A piece of filter paper with the same diameter as the inside diameter of the column or funnel is placed on top of the sorbent bed to prevent disruption of the bed during addition of solvent. The column is then eluted with appropriate solvent mixtures of gradually increasing solvent strength. Between solvent applications the column is sucked dry and the eluent collected in test tubes or round-bottom flasks. Using a multiport manifold (similar to a pig adapter for distillation) or a separatory funnel allows sequential fraction collection without having to disassemble the apparatus after each fraction is collected.

Vacuum chromatography is simple, rapid and convenient. Optimum sample loads are similar to flash chromatography. However, it is not unusual to use sample overload conditions to separate simple mixtures by stepwise gradient elution or to simplify mixtures for further separation. Under these conditions the sample loads may reach 10% (w/w), or even higher, of the bed mass. Compared to flash chromatography, solvent changes are easy because the head of the column is at atmospheric pressure.

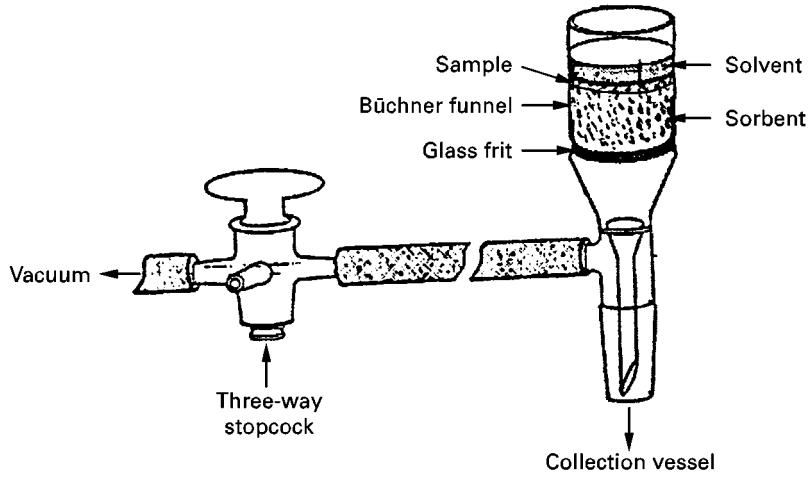

Figure 2 Apparatus for vacuum chromatography. (Reproduced from Pelletier SW, Chokshi HP and Desai HK (1986) Separation of diterpenoid alkaloid mixtures using vacuum liquid chromatography. *Journal of Natural Products* 49: 892, with permission from the American Chemical Society.)

Flash Chromatography

A glass column of a suitable length containing a small glass-wool plug and a layer of acid-washed sand or glass frit at its base is partially filled with sorbent using the dry-packing or slurry-packing technique. Incremental addition of the sorbent followed by tapping of the column with a hard object generally gives etter results for dry packing than bulk filling of the column. After packing, the column is freed from trapped air and further consolidated by forcing several column volumes of a weak solvent through the sorbent bed until no further air bubbles are seen exiting the column and the bed is stable. It is difficult to pack wide-diameter columns (> 5 cm) by dry-packing procedures and in this case slurry packing is nearly always used. In this case, the column is partially filled with a small volume of weak solvent and a dilute suspension of the sorbent in the same solvent is added slowly in increments, with excess solvent intermittently drained away. Periodic pressurization of the sorbent bed is used to aid consolidation. Tapping the sides of the column is not normally employed. The sample is added to the column in a small volume of solvent or adsorbed to a small amount of packing material.

Finally, a thin layer of glass beads, acid-washed sand or other inert material is added to the top of the column to prevent disturbance of the column bed by solvent added for elution. The amount of free space above the sorbent bed must be sufficient to hold the volume of solvent equivalent to the fraction size collected, or a solvent reservoir must be inserted between the column and the pressure regulation valve. The flow rate is adjusted to about 5 cm min^{-1} by application of gas pressure and controlled by the regulation valve. Pressures employed are typically less than 1–2 atm, with the various parts of the apparatus (**Figure 3**) held in place by springs, clamps or screw-thread connectors. It is a wise precaution to use plastic-coated glass columns or a safety shield to minimize the possibility of accidents. The column should not be allowed to run dry during the elution sequence.

Flash chromatography is simple to perform and is widely used in many laboratories. The main disadvantage is that the apparatus requires constant disassembly and reassembly of the air pressure inlet adapter in order to introduce new solvent into the column. Potter described an apparatus with a lateral solvent reservoir to overcome this problem. The essential feature of this apparatus is that a solvent reservoir with tap at the reservoir-to-column inlet is attached to the side of the column with the inlet situated above the height of the sorbent bed. A second tap at the top of the column allows air pressures to be equalized for rapid solvent addition to the column, without having to disassemble the apparatus. Radial compression columns have also been used with some commercial flash chromatography apparatus.

Stationary Phases

Silica, and to a lesser extent alumina, are the most common stationary phases used for the separation of low molecular weight organic compounds. Chemically bonded silica sorbents are used for the separation of polar organic compounds in the normal and reversed-phase modes. Wide-pore, chemically bonded phases are used for the separation of biopolymers. There is no technical reason why any

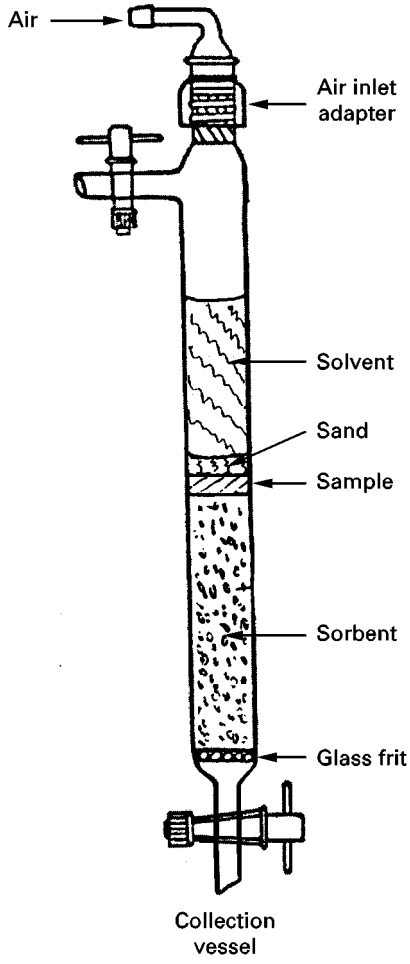

Figure 3 Apparatus for flash chromatography.

moderately rigid chromatographic sorbent, stable to solvent changes and available in the required particle size range, could not be used. In practice, the cost of the sorbent has to be set against the value of the product isolated, since the sorbent is often used for a single sample application and regeneration may be impossible, or tedious, costly and uncertain. Chemically bonded phases are more expensive than silica, or have to be synthesized form silica prior to use, and for this reason are less popular. For low molecular weight neutral organic compounds, small-pore silicas with a high surface area and high loading capacity are preferred, in particle size ranges of 20–40 or 40–63 μm. The smaller particle size materials provide higher resolution per unit length but generate greater back-pressure. Since longer columns can be used for the larger particle size sorbent, differences in resolution are often not great. Because of the limited operating pressure, columns are rarely longer than 30 cm, and 10–15 cm is recommended, unless longer columns are required to provide additional resolution. The optimum mobile-phase velocity for these particle sizes is about 5 cm min^{-1}. At this velocity well-packed columns are expected to provide about 5–20 theoretical plates per centimetre of bed height, depending on the column packing density and the quality of the sorbent material.

Some separations demand specially prepared stationary phases. A method has been described for impregnating silica with silver nitrate for the isolation of compounds with unsaturated groups capable of forming charge transfer complexes with silver. Silica was impregnated with phosphoric acid and the calcium salt of ethylenediaminetetraacetic acid to isolate microtoxins that were either unstable or produced tailing bands on normal silica gel. Thin-layer chromatography is generally a suitable technique to identify suitable additives for improving chromatographic properties in flash chromatography. Silica and chemically bonded phases coated with cellulose *tris*(3,5-dimethylphenyl carbamate) were used to isolate 10–100 mg amounts of pure enantiomers from racemic mixtures. The selection of mobile phases for this application is conveniently optimized using high pressure liquid chromatography.

Sample Loading

The sample is usually added to the column in a small volume of a weak solvent and the solution forced into the sorbent bed, forming a narrow sample application zone. For samples of low solubility in weak solvents, the sample is taken up in a strong solvent and added to a small amount of column packing material or other inert support. The solvent is then stripped from the slurry under vacuum to produce a dry free-flowing powder that can be added to the top of the column. Sorbent (1–2 g) may be required for each gram of sample. It is important that the sample is completely dry (high vacuum is used to remove the last traces of solvent) and free of lumps to obtain symmetrical separated zones. If the sample layer is relatively long compared to the column bed length, then a stepwise solvent gradient must be used for elution to minimize zone broadening.

There are no simple relationships between the sample amount that can be separated, the dimensions of the sorbent bed and the volume and number of collected fractions. The loading capacity depends on the ease of separation of neighbouring zones, the sorption capacity of the sorbent and the method of sample elution. It can be increased by using wider columns and sorbents with a larger specific surface area. A rough empirical guide is presented in **Table 1**. For stepwise gradient elution it has been assumed that the sample can be separated into fractions of different polarity when estimating the typical sample load.

Even for difficult samples it is often more productive to use column overload conditions, combining fractions containing pure materials, and recycle those containing mixtures. Flash chromatography may lack the resolving power needed to separate the components of interest. In this case a higher resolution technique, such as medium or high pressure liquid chromatography, would be a better choice, perhaps using flash chromatography to isolate fractions containing the components of interest from other sample components.

Method Development

Thin-layer chromatography is widely used to optimize the separation conditions for silica gel flash chromatography. For isocratic separations a mobile phase which provides an R_F of about 0.35 for the zone of interest is chosen. If several zones are to be separated, then the solvent strength is adjusted such that the centre zone has an R_F of 0.35. If all zones of interest are well separated from each other and from impurities ($\Delta R_F \geq 0.2$), then the solvent strength can be adjusted so that the most retained zone of interest has a $R_F \approx 0.35$. For fractionation and large sample loads it is critical that the most selective solvent composition for the separation is used. This can be quickly identified using the PRISMA model, a guided trial-and-error procedure using thin-layer chromatography and parallel separations with different solvents. The same process can be used to identify the composition of solvents suitable for the recovery of individual sample zones in order of increasing polarity by stepwise gradient elution.

For samples of wide polarity a useful gradient is to start from a weak solvent, such as hexane, and add to this various volume increments of a strong dipolar solvent (such as ethyl acetate, dichloromethane, chloroform or acetone), terminating with the strong dipolar solvent. Then continue adding volume increments of a strong hydrogen-bond solvent (methanol, ethanol, 2-propanol) to the strong dipolar solvent, terminating with the strong hydrogen-bond solvent. Monitoring the separation by thin-layer chromatography allows the solvent gradient to be trimmed and optimized to suit the requirements for individual separations. Predicting the number of fractions required at each step remains quite arbitrary and is best conducted by monitoring the composition of each fraction as it is collected. When adding a strong solvent in a binary mobile phase for a silica gel sorbent, it is important to note that the solvent strength for the mixture has a steep curved profile. For compositions containing low volume fractions of strong solvent, the volume fraction of strong solvent should be incremented by small changes, resulting in relatively large changes in retention, for example, 1%, 3%, 5%, 10% (v/v). At higher volume fractions of strong solvent, the changes in volume fraction should be larger to produce a significant change in retention, for example, 30%, 40%, 60%, 80%, 100% (v/v).

Silica gel (or alumina) is the most suitable sorbent for the separation of low molecular weight organic compounds soluble in organic solvents and for separations of geometric isomers and diastereomers. For compounds at the extreme end of the general adsorption scale (**Figure 4**), separations are difficult because

Table 1 Approximate sample-loading conditions for flash chromatography (density of silica ≈ 0.45 g mL^{-1})

Column diameter (cm)	*Amount of silica gel (g)*	*Sample loading for a particular TLC resolution (g)*		*Sample loading (g)*	*Typical fraction volume (ml)*
		$\Delta R_F \geq 0.2$	$\Delta R_F \geq 0.1$		
Isocratic elution (bed height=15 cm)					
1	5	0.1	0.04		5
2	20	0.4	0.16		10
3	45	0.9	0.36		20
4	80	1.6	0.6		30
5	130	2.5	1.0		50
Stepwise gradient elution (bed height=10 cm)					
3	30			1–3	50–100
4	55			3–8	100–200
6	125			8–35	200–300
8	250			35–60	200–300
10	350			60–80	300–500
14	700			80–150	300–500

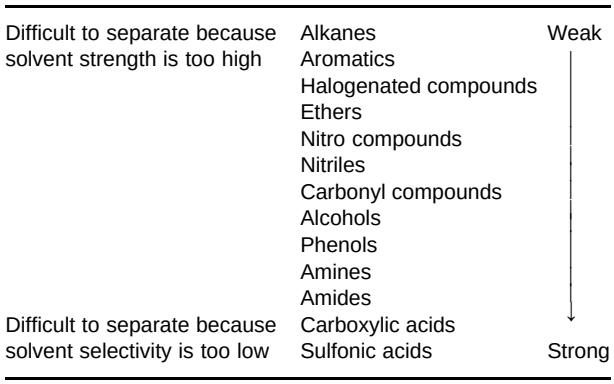

Figure 4 General adsorption scale for silica gel chromatography.

of inadequate selectivity. Water-soluble compounds, including biopolymers and easily ionized compounds, are generally better handled by reversed-phase chromatography. Compounds of low polarity that are weakly retained on silica gel with hexane as a solvent can be separated on chemically bonded phases in the normal or reversed-phase modes. For reversed-phase separations, chemically bonded phases with water as the weak solvent are used, and the solvent strength and selectivity of the eluting solvent changed by adding different volumes of water-miscible organic solvents, such as acetone, methanol, acetonitrile, tetrahydrofuran, etc. Optimization of solvent composition by thin-layer chromatography is possible but predictions may be unreliable due to differences in sorption properties between the column and layers. A better solution is to pack a short (10 cm) metal column with the sorbent for flash chromatography and use high pressure liquid chromatography to optimize separation conditions. Ideally, for isocratic elution a solvent composition should be chosen that provides a retention factor of 2–3 for the component of interest or those components of a mixture that are the most difficult to separate. For mixtures of wide polarity, stepwise solvent gradients are easily constructed and optimized by the same approach.

Detection

Monitoring separations by flash chromatography can be online and continuous using standard liquid chromatographic detectors (e.g. UV-visible, refractive index, or evaporative light scattering) but is more commonly done offline by collecting fractions that are subsequently combined, based on the similarity of their composition. Suitable monitoring techniques are thin-layer, gas or liquid chromatography, electrophoresis, bioassays, immunoassays and spectroscopy (e.g. infrared and nuclear magnetic resonance). For neutral organic compounds, thin-layer chromatography is widely used. Microscope slide-sized plates are suitable to screen individual fractions as they are obtained and larger plates for the grouping of multiple fractions. A wide range of selective and universal visualizing reagents are available to meet most detection requirements. Compounds with UV absorption can be visualized by fluorescence diminution using layers containing an inorganic fluorescent indicator. But most of all, thin-layer chromatography is used because it is quick, portable, inexpensive and generally adequate for the task.

Future Developments

Flash chromatography and related laboratory-scale techniques are already widely used for preparative chromatography when only modest resolution is required. The virtues of these techniques are favourable cost considerations and minimal instrumentation requirements. They are not a substitute for high resolution, preparative-scale techniques but a complement to them. Consequently, radical changes in how flash chromatography is carried out are not expected. The most likely future development is the wider use of sorbents other than silica gel in generally optimized separation schemes, made possible by the declining cost of chemically modified and other selective sorbents.

See also: **II/Chromatogrphy: Thin-Layer (Planar):** Modes of Development: Conventional; Spray Reagents.

Further Reading

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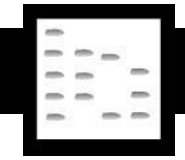
FLAVOURS: GAS CHROMATOGRAPHY



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Flavours are composed from a wide variety of materials such as essential oils, extracts of natural products, individual aroma chemicals and many other materials having an organoleptic impact producing a desired effect. Flavour analysis by gas chromatography (GC) is only associated with volatile and semi-volatile compounds. From an early stage in the development of GC, the analysis of flavours has played an important part. Up until the 1980s, the emphasis was on using GC to identify individual flavour molecules at trace levels (parts per million (ppm) to parts per trillion (ppt) range and sometimes less). This is still an important part of the job for flavour analysts, although today there is a greater tendency to correlate molecular structures with sensory attributes. GC methods that have been developed for flavour analysis include temperature-programmed capillary GC and GC combined with mass spectrometry (MS). Likewise, a technique such as GC-Olfactometry (GC-O), which was once the realm of the flavour and fragrance industry, is currently enjoying applications in the food and beverage, cosmetics, packaging, plastics, and pharmaceuticals industries.

This article provides a comprehensive review of the most important GC applications in flavour analysis. The Further Reading section lists some important literature sources that provide a useful overview of GC as applied to flavour analysis. In addition there is the Food Science & Technology Series from Elsevier Science Ltd. This series contains the proceedings from the International Flavor Conferences and the Weurman Flavour Research Symposia. Many GC methods have been used for a variety of purposes, including flavour and raw material quality control, flavour stability, identification of off-flavours and taints, studies of flavour biogenesis and metabolic pathways of plant volatiles, identification of new flavour molecules, consumer product development, and process optimization. The flavour analysis techniques covered here are: headspace GC including solid-phase microextraction (SPME) combined with GC thermal desorption techniques, pyrolysis-GC-MS, multidimensional GC, GC-MS and GC with selective detectors, chiral separations, GC-O and the recently developed fast GC process. Of course the quality of an analysis depends on the extraction techniques and sample preparation procedures. These areas are covered in other chapters in this encyclopedia. Headspace analysis and pyrolysis are also considered to be sampling techniques, but they are included here as they can be used in a coupled mode.

Headspace Gas Chromatography and Thermal Desorption Techniques

Since the early application of headspace GC analysis to flavours the technique has undergone a considerable degree of automation. Now it is possible to perform high throughput analysis, and reduce variability by automating the sampling and injection process. Basically there are two forms of headspace analysis: static and dynamic. In both forms volatiles that could be a source of interferences for the GC separation are removed from a complex sample matrix. It is important to note that the headspace contains the part of the flavour that one perceives first. Static