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Flash Chromatography—A Simple Technique of Potential Value to the Food Chemist

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ABSTRACT

The technique of flash chromatography, a simple and inexpensive hybrid of gravity column chromatography and conventional HPLC, is described. The use of the technique in food-related studies is considered and its potential value to food chemists is discussed.

INTRODUCTION

Over the last two decades there has been a plethora of chromatographic techniques described in the scientific literature. Many of these, although undoubtedly effective in particular areas, are limited by solvent characteristics, throughput, cost and commercial availability. In recent years there have been considerable advances in the development, availability and utilisation of high pressure (>300 psi) and medium pressure (50–300 psi) liquid chromatography and most advanced analytical laboratories currently contain at least one such instrument (MacRae, 1982). In addition to routine operation in the analytical mode, these techniques have also found application in the preparative mode and impressive results have been reported.

Preparative HPLC is especially suited to repetitive, large-scale (≥ 10 g) operation where solvent systems may be standardised; however, the cost of large, preparative columns often precludes their use in university and

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research laboratories. In comparison, MPLC is well suited to the latter requirements, with individual columns being able to be repacked and used in conjunction with any available pump/fraction collector. In situations where 1–5 g of material is required and continually different solvent mixtures preclude standardisation, MPLC may be considered to have advantages in both convenience and cost.

Still *et al.* (1978) described a method called flash chromatography (presumably indicative of its speed) in which the pressures applied to the column were even lower than for MPLC, typically of the order of ≤ 20 psi; the technique may thus be seen to combine the advantages of conventional gravity column chromatography and HPLC/MPLC. Flash chromatography found initial applications in synthetic and pharmaceutical chemistry and, until recently, was little used by chemists working in the food area. The technique was initially introduced to provide a fast chromatographic separation with only a limited resolution ($\Delta R_F \geq 0{\cdot}15$) using a short bed of silica gel. However, the advent of modified silica supports (for example, SiO_2–CN; SiO_2–C_8; SiO_2–C_{18} and SiO_2–NH_2) has greatly extended its potential. In particular, benefits have accrued from the lower flow rates imposed by the higher viscosities of the aqueous solvent systems necessary for the use of such reversed-phase silica supports.

In the course of recent investigations on water-soluble plant glycosides the present authors have made extensive use of this chromatographic technique, in both reversed phase and normal phase modes, and additional applications have been described in the course of recent years. This suggests that the method may have considerably wider potential for use in food research. In this paper, the operation of flash chromatography is briefly described, some examples of its application are given and suggestions are made for additional areas in which it might be used with advantage.

FLASH CHROMATOGRAPHY

Apparatus

The apparatus required is shown in Fig. 1. The original apparatus described by Still *et al.* (1978) has been modified by various workers, to facilitate operation with air-sensitive species (Kremer & Helquist, 1984), volatile components (Hajji *et al.*, 1984) and its use by students (Thompson & Handson, 1984; Feigenbaum, 1984; Bell & Edmondson, 1986; Hanson & Smith, 1986). The apparatus shown in the Figure is that used by the present authors.

The apparatus consists of a heavy walled glass column (J. T. Baker Flash

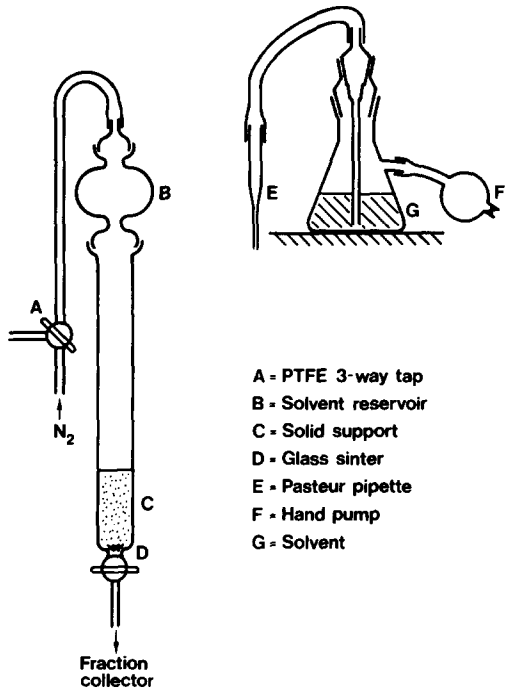

Fig. 1. Modified apparatus for flash chromatography.

chromatography columns) with a ball and socket joint and teflon stopcock, modified by the addition of a sintered glass disc (D) at the lower end of the column. The latter prevents tracking of silica when vapour bubbles pass through due to the heating effect of some solvent gradient systems. A 500 ml solvent reservoir (B, with ball and socket joint) is attached to the top of the column secured by a pinch clamp. A second pinch clamp secures a ball joint to which is attached, via plastic tubings, a three-way tap (A) connected to a supply of compressed gas (N_2).

Solvent is added to the column by means of a hand-pressurised reservoir consisting of a QQ Buchner flask, to the side arm of which is connected a rubber hand pump (F). An insertion tube and adapter are attached to the flask and via tubing to a pasteur pipette (E).

Preparation of column

The appropriate amount of column packing (Baker Silica gel or bonded phases for flash chromatography) is poured into the column dry to form a level bed (C, Fig. 1). The average particle diameter of the packing is 40 μm with a minimum diameter of 25 μm for both normal and reversed-phase systems. The column is then carefully filled with the chosen solvent without

disturbing the surface of the packing. The stopcock is opened, the pinch clamps secured and the three-way tap opened to allow a stream of gas ($\sim$ 10 psi) into the column. The solvent is forced rapidly through the column expelling all the air and compressing the packing. The column must not be allowed to run dry or cracks may occur in the packing and channelling of the solvent may result. If the solvent used to pack the column is not the same as the solvent/solvent mixture to be used as the eluting solvent, the column should be conditioned with the latter prior to use. A 12 cm long packing of silica gel will have a resolving power of up to 200 theoretical plates.

Column operation

The sample is applied to the column by pipette in the minimum volume of eluting solvent and forced into the column under pressure, a small volume of solvent is used to wash the walls of the column and again forced into the column under pressure. The column is then topped up with solvent (using the solvent pump) and connected to a fraction collector. The gas pressure is adjusted to give a suitable flow rate through the column and fractions are collected as required. Flow rates achieved and back pressures required will depend upon the viscosity of the solvents being used. At one extreme the reversed-phase systems using aqueous solvents require pressures of 10–15 psi to achieve flow rates of 15–25 ml min^{-1} whereas non-polar solvents such as hexane will produce flow rates in the order of 100 ml min^{-1} when used in conjunction with silica gel and require pressures of 2–5 psi.

Detection

The column eluate/fractions can be monitored by TLC (analytical plates are available in modified silica gel to match column packings) or spectroscopically

Regeneration of column packing

It is generally possible to reuse a flash chromatography column after a suitable clean-up procedure. For reversed-phase packings this can be achieved by flushing with a less polar solvent followed by the eluting solvent, and for normal phase packings with a more polar solvent followed by the eluting solvent. Some crude plant extracts leave almost irreversibly-bound coloured residues on the column which require more intensive treatment. In some cases it may be possible to remove and replace the top section of packing. In extreme cases, however, where there is a possibility of slow leaching out of coloured material during subsequent elutions, it will be necessary to repack the column with a fresh quantity of packing.

APPLICATION

Flash chromatography has been used in a number of areas, as shown in Table 1. More particularly related to food chemistry, Cohen *et al.* (1985) have applied the technique, in combination with semi-preparative reversed-phase liquid chromatography, to the isolation and purification of gram amounts of the trichothecene fungal metabolite, deoxynivalenol (vomitoxin), required for toxicological investigations. The authors used large (60 cm × 7·5 cm inside diameter) columns of silica gel for the purification of the extract from >1 kg sample (corn or rice fermented by *Fusarium graminearum*). Sequential elutions with dichloromethane, hexane and ethyl acetate–hexane were necessary to remove impurities. Losses of deoxynivalenol were ≤4% based upon that present in the original samples.

The tetranorterpenoid, azadirachtin, obtained from the neem tree is currently being evaluated for its effectiveness in insect control. Problems of obtaining sufficient material from this, and other, studies have been overcome (Yamasaki *et al.*, 1986) by employing flash chromatography (both normal and reversed phase) and HPLC (both analytical and preparative). The initial flash chromatography readily removes pigments and other

TABLE 1
Application of Flash Chromatography to the Separation of Organic Compounds

Support	*Species separated*	*Reference*
NP	Substituted cyclohexanol epimers	Still *et al.* (1978)
RP	Aromatic aliphatic alcohols	Crane *et al.* (1981)
NP	2,2′-bis(Bromoacetyl)biphenyl oxidation products	Dobler (1983)
RP	Hydroxybenzenes	Kühler & Lindsten (1983)
RP	Methylisothiazolone derivatives	Matissek *et al.* (1986)
RP	Na^+, K^+-ATPase inhibitors	Wainer *et al.* (1985)
NP	Organometallics	Kremer & Helquist (1984)
NP	Essential oils	Hajji *et al.* (1984)
NP	Chiral stationary phases	Pirkle *et al.* (1985)
NP	Deoxynivalenol	Cohen *et al.* (1985)
NP	Saponins	Nonaka (1986)
RP	Saponins	Curl *et al.* (1985)
RP	Saponins	Price *et al.* (1986)
RP/NP	Saponin	Polacheck *et al.* (1986)
NP	Fluorene, fluorenone	Hanson & Smith (1986)
NP/RP	Azadirachtin	Yamasaki *et al.* (1986)

RP = Reversed phase
NP = Normal phase

impurities which bind to silica gel columns and enables gram amounts of partially (25%) purified azadirachtin to be processed in less than 1 h. Further purification was achieved by analytical HPLC (85%) and, finally, preparative HPLC (99%). The authors considered that only single runs necessary in each of the HPLC steps were a consequence of the effectiveness of the clean-up by flash chromatography.

Independently, Price and coworkers (Price & Fenwick, 1984; Curl *et al.*, 1985; Price *et al.*, 1986), Nonaka (1986) and Polacheck *et al.* (1986) have used flash chromatography for the separation of biologically-active saponins. The former workers have developed a three-step system for the isolation of purified soyasaponins from legumes: first, reversed phase flash chromatography using SiO_2–C_{18} to effect clean-up of the food extract, then conventional column chromatography with silica gel and chloroform-based solvent elution and, finally, reversed-phase flash chromatography of the separated column fractions. The importance of the initial clean-up step of this combined procedure should not be underestimated. The procedure has been successfully employed in the isolation of soyasaponin I from pea flour (Price & Fenwick, 1984), soyasaponins I–IV from soybean flour (Burrows *et al.*, 1987) and the bisdesmosidic saponins from guar meal (Curl *et al.*, 1986).

The same group has also employed flash chromatography in the clean up of plant foodstuffs prior to quantitative and qualitative analysis. Aqueous extracts of plants and foodstuffs can be directly applied to the reversed-phase flash chromatography column. Successive elutions with water, then methanol, remove neutral sugars and saponins, respectively. Any additional less-polar material may be removed as required by elution with hexane. Using such techniques, extracts from species of *Gypsophila* and *Saponaria* have been rapidly cleaned up, prior to further structural analysis and inclusion in animal feeding trials. Such a clean up technique, in combination with FAB–mass spectrometry, has been effectively employed for the rapid screening of legume flours for soyasaponins and the technique is currently being extended to other classes of food saponins (Price *et al.*, 1987). The investigations on guar and *Gypsophila* saponins also revealed flash chromatography to be preferable to biological filtration for the separation of alkali-released ester-linked oligosaccharide products and for the clean-up (desalting) of de-esterified prosaponins.

Nonaka (1986) has adapted normal phase flash chromatography to the extraction of saponins from alfalfa root extracts. The extraction procedure was rapid and removed all the saponin-species (as evidenced in bioassays using the fungus, *Trichoderma viride*). The root extract was partially purified by sequential elution from the silica gel support with dimethyl ether/ethyl acetate, ethanol, methanol, aqueous methanol and water. Eluates obtained with ethanol, methanol and aqueous methanol included saponins con-

taining both medicagenic acid and hederagenin, which were further purified by preparative TLC. Polacheck *et al.* (1986) have summarized the use of normal phase (ethyl acetate:methanol mixtures) and reversed-phase (water:methanol mixtures) flash chromatography for the separation and purification of medicagenic acid-3-*O*-*β*-D-glucopyranoside from alfalfa roots.

The prefractionation of the essential oils of black spruce (*Picea mariana*) into hydrocarbon and oxygen-containing species has been achieved by Hajji *et al.* (1984) using a combination of flash chromatography and liquid–solid chromatography. In some instances individual purified components were obtained. Complete separation of neutral hydrocarbons and oxygenated species was effected in less than 60 min using hexane/ether solvent systems and a deactivated silica gel support. 2 ml of spruce extract could be analysed per batch. The authors refer to, but do not describe in detail, the effectiveness of flash chromatography for the separation of unspecified alkaloids and saponins.

Wainer *et al.* (1985) have reported the routine use of reversed phase (C_{18}) flash chromatography for the concentration and purification of endogenous Na^+, K^+-ATPase inhibitors of human plasma and urine. 1–2 litres of urine may be handled in a single run, the column being subjected to an acetonitrile step gradient elution. It is possible, given these results, that the method might find wider applicability in the concentration and purification of xenobiotic conjugates and metabolites.

The industrial application of flash chromatography in the refining of edible and natural oils has been reported by Leigh & Coupland (1984).

POTENTIAL

As part of researches on food quality and food safety there is a need to obtain relatively large amounts (grams) of biologically-active components, including fungal metabolites, glycosides, alkaloids and polyhydroxylated species for toxicological evaluation, sensory assessment and animal feeding trials. Procedures for the isolation and purification of many such compounds are, if available, necessarily lengthy and often severely limited in terms of the amount of sample which can be handled. In such circumstances flash chromatography, alone or in combination with other techniques, would appear to offer advantages, as evidenced by its application to vomitoxin, azadirachtin and various saponins, above. Keeling & James (1986) have recently emphasised the importance of desalting as a preliminary to the analysis of sugars, polyhydric alcohols and other species by HPLC. These authors advocate the use of Amberlite mixed-bed resins and freeze-concentration but it is possible that flash chromatography has a role to play here.

Interest in the fate of ingested chemicals, whether naturally occurring in food, added purposely (additives) or by chance (contaminants) is increasing. The problems of sample concentration, separation, purification and isolation may be facilitated, as in the example reported by Wainer *et al.* (1986), by the use of flash chromatography. The technique may also enable the isolation of sufficiently large quantities of xenobiotic conjugates and metabolites to allow rigorous biological investigation.

Researchers aimed at identifying naturally occurring compounds of value to the food industry (e.g. sweeteners, colourants, bittering compounds, preservatives) are increasingly utilising tissue culture and bioengineering techniques. Such programmes have need of simple procedures affording the rapid clean up, concentration and purification of the, frequently complex, mixtures resulting therefrom. The simplicity and capability of flash chromatography would appear to offer possibilities in this direction.

The purpose of this paper has been to direct the attention of food scientists, and food chemists in particular, to a relatively new technique which has been used very successfully in other branches of chemistry. The envisaged programme of food safety research, to be undertaken by the authors, will rely significantly upon the use of this technique, but the advantages of the method are such as to make it a useful adjunct to other chromatographic and isolative techniques generally available in the food laboratory.

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