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SOME EMPIRICAL ASPECTS ON THE PRACTICAL USE OF FLASH CHROMATOGRAPHY AND MEDIUM PRESSURE LIQUID CHROMATOGRAPHY FOR THE ISOLATION OF BIOLOGICALLY ACTIVE COMPOUNDS FROM PLANTS*

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ABSTRACT

The applications of flash chromatography and medium pressure liquid chromatography (MPLC) for the isolation of pure compounds from plants are presented with the emphasis on the practical aspects of both techniques. The pressure for flash chromatography, which operates at about 1-2 bars, is provided by an aquarium pump or several pumps in parallel. The simple necessary glass apparatus, which can be constructed in a glass blowing workshop, are described diagrammatically. The practical operation of the technique is included. Up to 200 grams of crude plant extract can be readily separated in a single run. The approximate relationship among sizes of samples, columns and fractions collected is given.

MPLC can be used as a complement to flash chromatography. The pressure required is in the range of 5-20 bars and it can be provided by a piston pump with a variable flow rate. Crude plant extract fractionated by flash chromatography can be further purified by MPLC to give pure materials. Up to 50 grams of semipurified fraction can be conveniently chromatographed in a single run.

The use of gradient elution is essential for the success of both flash chromatography and MPLC. A gradient can be constructed between any two miscible organic solvents. Initially, the polarity is increased slowly and more steeply toward the end of the separation.

A brief guideline for the use of various normal phase column packing materials is given.

An example illustrating the utilization of the two techniques in a multigram scale isolation of pure compounds from the plant Curcuma xanthorrhiza *Roxb. (Zingiberaceae) is described.*

INTRODUCTION

The different preparative liquid chromatographic techniques are commonly classified according to the pressure employed for the separation (1,2):

- flash chromatography, *ca* 1 - 2 bar
- low pressure LC, < 5 bar
- medium pressure LC, 5 - 20 bar
- high pressure (high performance) LC, > 20 bar

These figures are not absolutely fixed and there is a considerable overlap between the different techniques. Flash chromatography and Medium Pressure Liquid Chromatography (MPLC) are two chromatographic techniques that are often employed in the isolation of constituents from plant sources. The basic experimental procedures of preparative column chromatography are very rarely described in detail in regular research articles. The understanding of the detailed operations of these techniques is, however, essential for successful isolation work. This article summarizes how the techniques have been practically applied in large laboratory scale isolation of compounds from plant extracts. An example of recent isolation work with these techniques is also reviewed.

A GENERAL STRATEGY

The plant material under investigation is exhaustively percolated sequentially at room temperature with hexane, chloroform (or ethyl acetate) and methanol. Up to 15 - 20 kg of plant material can easily be handled in the laboratory using modified stainless steel drinking water containers. Normally approximately 300 - 500 g each of the crude extracts are obtained. These extracts are subsequently assayed for biological activity and the active ones are fractionated by flash chromatography to achieve a rough separation of the compounds present in the extracts. Fractions thus obtained, which exhibit biological activity, are then further fractionated either by repeated flash chromatography or by MPLC, depending on the complexity and the amount of material. Occasionally pure compounds are isolated by flash chromatography, but usually MPLC, HPLC, recrystallization, or a planar chromatographic technique is necessary to obtain pure compounds. The progress of the separation is monitored by a combination of bioassay, TLC, direct UV-detection, HPLC, ^{1}H-NMR, and colour reactions. The 60 MHz ^{1}H-NMR spectra of crude extracts and fractions are often very informative, and should routinely be recorded. For the initial separation by flash chromatography, up to 200 g of the crude extract can be fractionated in a single run as described in the subsequent sections. This approach is particularly important if the target compound is present in a low concentration. Furthermore, it also minimizes the tedious process of combining various small fractions at the later stages of the separation procedure. A general guideline for the selection of a chromatographic technique, with respect to the amount and the type of the sample, for a single run, is given in Table 1.

Table 1. A general guideline for selection of chromatographic technique with reference to the amount of sample to be processed.

Amount of sample (g)	Type of sample	Chromatographic technique
0.5 - 1 (or less)	Semipurified	Preparative TLC, Centrifugal TLC, Preparative HPLC, MPLC
1 - 50	Semipurified	MPLC
1 - 200	Crude, semipurified	Flash chromatography

FLASH CHROMATOGRAPHY

The concept of flash chromatography was originally described by Still, Kahn and Mitra (3). The technique was a considerable step forward in preparative chromatography compared to the previously used open columns, driven by gravity. The resolution was improved and the separation times were drastically shortened. Briefly, the original method typically employed columns with a diameter of 1 - 5 cm and a sample loading of 0.1 - 2.5 g. The columns were dry-filled with silica gel (40 - 63 μm) to a length of about 15 cm and flow rates of up to about 100 ml/min were obtained by application of compressed air *via* a needle valve. Samples were introduced in solution and the eluents (ethyl acetate/petroleum ether) were selected with the aid of TLC. A solvent mixture giving the compound of interest an R_f-value of 0.35 was used for isocratic elution.

The flash chromatographic technique was originally described for the separation of synthetic products but during work with natural products we have found it useful and necessary to modify the technique to a certain extent; apparatus, packing materials, and operational procedure, as described in the sections below.

Apparatus

The apparatus required for the modified flash chromatography consists of four major components (Fig. 1). A glass column (A) with a length of 30 - 40 cm. The shorter length (30 cm) is recommended for columns with diameters of 8 - 14 cm. Ground joints B29 and B34 are used with column diameters of 3 - 6 cm and 8 - 14 cm, respectively. The air pressure is introduced through a T-way stopcock (B) with the ground joint B29 or B34. Component B is strapped to the column with rubber bands through the glass hooks on the outer side walls. The eluting solvent is introduced into the column with a sealed-end glass funnel (C) with small orifices near the sealed end, so that the introduced solvent will not disturb the surface of the packing material. The air pressure is provided by an aquarium pump (or several in parallel) fitted with the T-way stopcock. The air flow from the aquarium pump is adjusted to give the required pressure for a suitable elution speed.

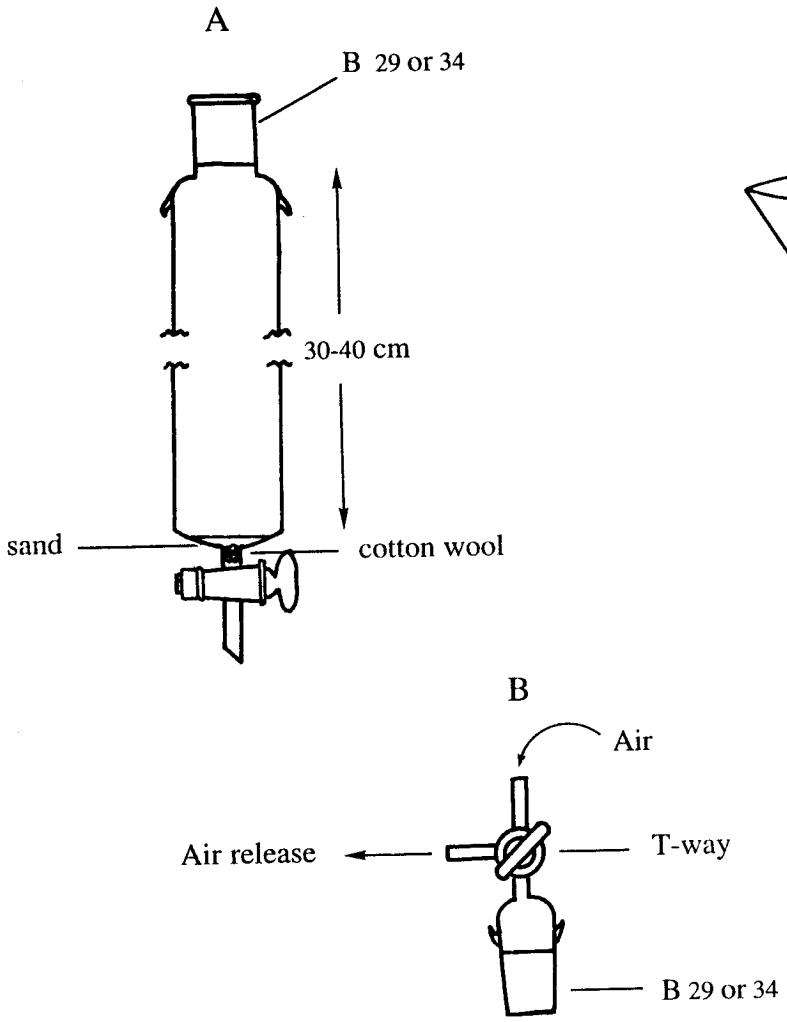


Fig.1. Diagramatic details of the apparatus for modified flash chromatography.

Packing materials

Several different types of packing materials can be employed in flash chromatography. Often used are Merck LiChroprep Si 60 (25 - 40 μm), Merck silica gel 60 (40 - 63 μm) or Merck silica gel 60 (63 - 200 μm) (1). The Merck silica gel 60 H, which is a TLC grade (5 - 40 μm) silica gel without a binder, also works well. It has a comparatively low price and can be purchased in bulk quantities of 25 kg. The resolution using this silica gel is not inferior to other considerably more expensive silica gel qualities.

Alumina is a useful column packing material, particularly for removing undesired polyphenolic compounds, and the Merck aluminium oxide 90 (63 - 200 μm) can be employed for this purpose. A very large column may accommodate up to 200 g of a crude extract and an adequate flow rate is obtained by simply filtering the extract through the adsorbent under gravity. If deactivation of the alumina is necessary, this can be achieved easily by prior elution with ethyl acetate.

An interesting application of flash chromatography with reversed phase (RP-18) support is the separation of compounds that are too nonpolar to be resolved on normal silica gel (R_f > 0.6 in TLC with hexane as eluent). Such compounds have conveniently been separated on reversed phase adsorbent eluted with mixtures of dichloromethane and acetonitrile (4).

Packing technique

For the packing of silica gel columns, slurry packing is easier to perform and gives a better packing than the dry filling method. This is especially true for columns with wide diameters (> 6 cm), which are quite difficult to dry-pack satisfactorily. For the slurry packing the employment of a very dilute silica gel-solvent slurry helps minimizing the formation of air bubbles. Sufficient solvent (about five centimetres in height) is placed in the column (with the stopcock closed) to provide a head into which the slurry is poured. The fine "snow flakes" fall under gravity to give a homogeneous packing which should not be disturbed by stirring. After a few centimetres of the slurry have settled, the column stopcock is opened to speed up sedimentation. Pressure can then be applied on the column using an aquarium pump. Further portions of the slurry are added in the same way until the desired height of the packing material is obtained. The column is conditioned with at least five to eight column volumes of solvent before use.

Sample application

The sample to be chromatographed can either be introduced as a solution or preadsorbed on the packing material. The latter method is found to be superior, especially for plant extracts which often are not completely soluble in the initial eluent. The sample is preadsorbed by dissolving it in a suitable low boiling point solvent (*e.g.* dichloromethane) and silica gel is added (about 1 - 2 g for every 1 g of sample). The solvent is then removed on a rotary evaporator until the mixture is dry and can move freely in the flask. The last trace of solvent is removed by placing the flask under high vacuum for 15 - 30 minutes. It is important to obtain a completely "dry" and freely flowing mixture. Depending on the nature of the sample, it may be necessary to increase the proportion of silica gel to sample

to 3:1 or 4 : 1. In some cases, the layer with the sample preadsorbed on silica gel may be even higher than the silica gel layer (the effective column length) where the actual separation takes place. It may be anticipated that this will lead to large band broadening during the separation, but experience has shown that by avoiding isocratic elution, and instead, employing gradient elution (see below), the expected band broadening can be drastically reduced. The advantage of diluting the sample with a large proportion of silica gel is that the overloading at the top of the column, which frequently occurs when applying a concentrated sample, is minimized. It is thus possible to load a considerably larger amount of sample onto the column than otherwise would be possible.

The preadsorbed sample is added to the top of the column, carefully not to perturb the surface of the silica gel layer. The sample can be either added directly on the column top or allowed to "fall" through approximately three centimetres of solvents, which are left on top of the silica gel. Usually the latter is preferred. In both cases, the sample layer is levelled by gentle tapping of the column. On top of this layer about 0.5 - 1 cm of sand is placed and the column is now ready to run.

Selection of solvent system for elution

In the original method (3), a mixture of solvents which moved the desired component in the sample to R_f 0.35 on analytical TLC was selected as the column eluent. The elution was carried out isocratically. For our modified procedure it is essential to use stepwise gradient elution with binary solvent mixtures of gradually increasing polarity. Empirically, the solvent components that give the best separation in TLC are selected. Commonly used solvent components for intermediately polar compounds are mixtures of hexane with ethyl acetate, chloroform, dichloromethane or acetone. For more polar compounds gradients of increasing amounts of methanol in chloroform, dichloromethane or ethyl acetate are often used. The profile of the stepwise gradient mainly depends on the sample composition and the column size. Exactly how rapidly the polarity of the eluent should be increased and how large eluent volumes of each step that should be used is still a matter of experience. Generally, it is recommended to increase the polarity of the eluent only with small increments in the beginning of the gradient. At the later stages of the elution, larger polarity increments can be employed. An increment from 1% to 2% of ethyl acetate in hexane represents approximately a 100% increase of relative polarity and eluting power of the eluent, whereas an increase from *e.g.* 50% to 60% of ethyl acetate in hexane only represents a relative polarity increase of 20%. Normally, 2 - 4 column volumes of eluent is needed for each polarity step of the gradient.

When fractionating crude plant extracts containing many compounds of different polarity, it is usually necessary to employ two consecutive gradients *e.g.* increasing amounts of chloroform in hexane up to pure chloroform, followed by increasing amounts of methanol in chloroform up to pure methanol.

Relationship between column diameter, effective length and sample size

It is possible to apply a considerably larger amount of sample when using gradient elution as compared to the originally described isocratic flash elution. The gradient elution,

furthermore, gives a better separation on a column of a shorter length than the originally described 15 cm. For rather nonpolar samples, effective column lengths of about 10 - 12 cm are employed and for more polar samples, effective column lengths of 6 - 8 cm are recommended. For the largest column (diameter 14 cm), however, usually lengths of 14 - 16 cm (intermediately polar samples) and 8 - 10 cm (polar samples) are employed. An approximate guideline for the choice of column diameter according to sample size is given in Table 2.

Table 2. Approximate relationship between sizes of sample, column and fractions collected for flash chromatography.

Sample size (g)	Column diameter (cm)	Amount silica gel (g)	Fraction volume (ml)
1 - 2	3	25	50 - 100
3 - 6	4	50	100 - 200
7 - 30	6	100	200 - 300
30 - 50	8	200	200 - 300
50 - 80	10	400	300 - 500
80 - 200	14	600-1000	300 - 500

It should be noted that the amount of sample that can be loaded on the column depends on the difficulty of the separation, but this approximate guideline has been used successfully for hundreds of columns run in our laboratories. The resolution on the small diameter columns is generally better than that on the ones with larger diameter, the latters being used mainly to obtain a rough fractionation of crude extracts.

MEDIUM PRESSURE LIQUID CHROMATOGRAPHY (MPLC)

MPLC appears to be a column chromatographic separation technique best suited for large laboratory scale isolation of pure compounds from semipurified plant extracts. The technique is thus complimentary to the flash chromatography. In favourable cases, it can be stated that the MPLC combines a resolution which approaches that of HPLC with a preparative capacity and a simplicity in operation similar to that of low pressure LC.

MPLC equipment from several manufacturers are available on the market. The general principles outlined below should be applicable to any such equipment, although our experience is mainly from the Büchi B-680 system (Flawil, Switzerland). The principle components of an MPLC system are outlined in Figure 2.

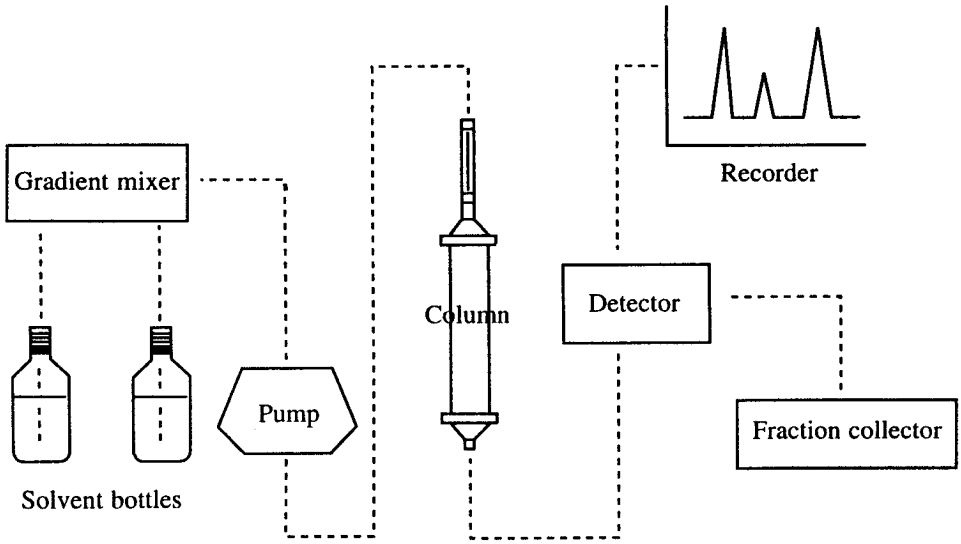


Fig.2. Principle components of an MPLC-system.

Solvents

MPLC generally consumes considerable amounts of solvents and therefore the costs of different solvents is an important factor. Most of the common technical grade solvents (*e.g.* hexane, ethyl acetate, methanol, dichloromethane, chloroform) are perfectly adequate for MPLC after a simple distillation. It is not necessary to degas or membrane filter these solvents prior to use. One precaution on chloroform should be noted. This solvent often contains traces of HCl, which can cause considerable damage to the equipment and to acid sensitive substances. After using chloroform as an eluent, the equipment should never be left standing in this solvent. The HCl can largely be removed by filtering the solvent through sodium carbonate before use.

The gradient mixer

The gradient mixer is a technical device, which mixes two (or more) solvents according to a predetermined elution program. The technical details of how the gradient mixer is programmed varies with different MPLC systems, but generally the working principle of a gradient mixer for MPLC does not differ from that of a conventional HPLC gradient mixer.

How the program is constructed depends on the sample composition and the column size. Some guidance can be obtained from TLC experiments as to what type of solvents to be employed in the separation, but since gradient elution normally is employed, no simple direct "transfer rule" can be given. Generally, solvent polarity should be increased slowly

in the beginning of the program, but in the end it can be increased more rapidly. It is often useful to include segments of isocratic elution between the gradient segments.

The gradient mixer is also very useful for making a program for washing and regenerating the column after use. Such a program for a silica gel column may consist of a washing segment (100 % methanol), followed by two gradients; increasing amounts of ethyl acetate in methanol and increasing amounts of hexane in ethyl acetate, and finally a long segment with pure hexane. To shorten this procedure a high flow rate can be employed (*e.g.* 100 ml/min).

The pump

A chromatographic pump which can stand back-pressures up to *ca* 30 bar and deliver solvent flow rates up to *ca* 100 ml/min is adequate for MPLC. With silica gels (*cf.* below) and solvent flow rates of *ca* 20 - 50 ml/min, the pressure usually is about 5 - 15 bar. Pressures above 15 bar, which can occur *e.g.* after heavy sample loading, are difficult to handle practically because the tubing system is somewhat prone to leak at these high pressures. The general problem of leakage at connecting joints can be alleviated by careful tapering of the teflon tubing ends and by keeping these tapers free from dust and packing material.

Sample introduction

As in flash chromatography, the sample to be chromatographed can be introduced either in solution or preadsorbed on the column packing material. When using the liquid sample introduction it is recommended to dilute the sample with a high proportion of solvent (up to 1:5) to avoid overloading at the column top and consequently impaired separation. The introduction of a sample in solution requires that the sample is soluble in the initial solvent composition of the elution program. This is very rarely the case when working with natural product isolation, especially if gradient elution is employed. The most suitable way of introducing a sample, in our experience, is to use the technique of preadsorption on silica gel. When applying this technique to MPLC, the sample (preadsorbed on silica gel) can be packed into a column, which is mounted in series prior to a second column. The mobile phase thus first passes the "loaded" column, before entering the second column where the actual separation takes place. As in flash chromatography it is important not to overload the silica gel that is used for the preadsorption of the sample (see discussion above).

Columns

Glass columns with a wide range of diameters and lengths are available from different manufacturers. The columns from Buchi have diameters ranging from 1.5 - 10 cm and lengths of either 23 or 46 cm. The most suitable columns for rapid gradient elution are those with a length of 23 cm. The columns can be equipped with precolumns (guard columns), which easily can be emptied and refilled again. This prolongs the life time of the separation columns, which normally can be reused many times. With relatively clean samples, we have sometimes been able to carry out as many as 10 - 15 preparative separations on a single column without any noticeable impairment of resolution.

For columns with diameters of 5 cm or less, silica gels with fine particles [*e.g.* Ueticon, (15 - 35 μm) or Merck LiChroprep Si 60 (25 - 40 μm)] are suitable. For columns with wider diameters the Merck silica gel 60 (40 - 63 μm) is preferred, mainly because it is easier to pack the large columns with gels of larger granulometry, but also for economical reasons. The silica gels with finer particle sizes generally give a better separation (resolution) than the coarser ones.

As an approximate guideline for the relationship between the amount of sample (for a single run) and the diameter of the column (silica gel, 23 cm in length) the figures given in Table 3 are recommended. It should be noted that the amount of sample that can be loaded also depends on the difficulty of the separation. Sometimes it is necessary to reduce the column loading considerably in order to obtain an acceptable separation.

Table 3. Approximate relationship between sample size and recommended column diameter for MPLC, with silica gel as column support and a fixed column length of 23 cm.

Sample size (g)	Column diameter (cm)
<0.5	1.5
0.5 - 5	2.6 or 3.6
5 - 10	4.9
10 - 20	7
20 - 50	10

Column packing

The technique for dry-packing columns described in reference (5) is simple and gives reproducible results. It should be stressed that tapping and particularly the use of a vibrator during the dry filling of the column should be avoided in order to prevent separation of particles with different granulometry in the packing material. The best result is obtained by gently filling the column with an even stream of adsorbent.

Detection and fraction collection

The compounds eluting from the column are detected by a UV-detector, a chromatogram is recorded, and fractions are collected. In principle any suitable combination of a UV-detector with a high flow cell, a chart recorder, and a high speed-high volume fraction collector, can be employed. It is very convenient to have a marker, which appears directly on the chromatogram showing where the different fractions are positioned. This greatly facilitates the localization of the fractions corresponding to a particular peak in the chromatogram. Additionally, TLC and a spray reagent are employed to analyze the fractions in order to get more detailed information on the composition and to detect UV-inactive compounds.

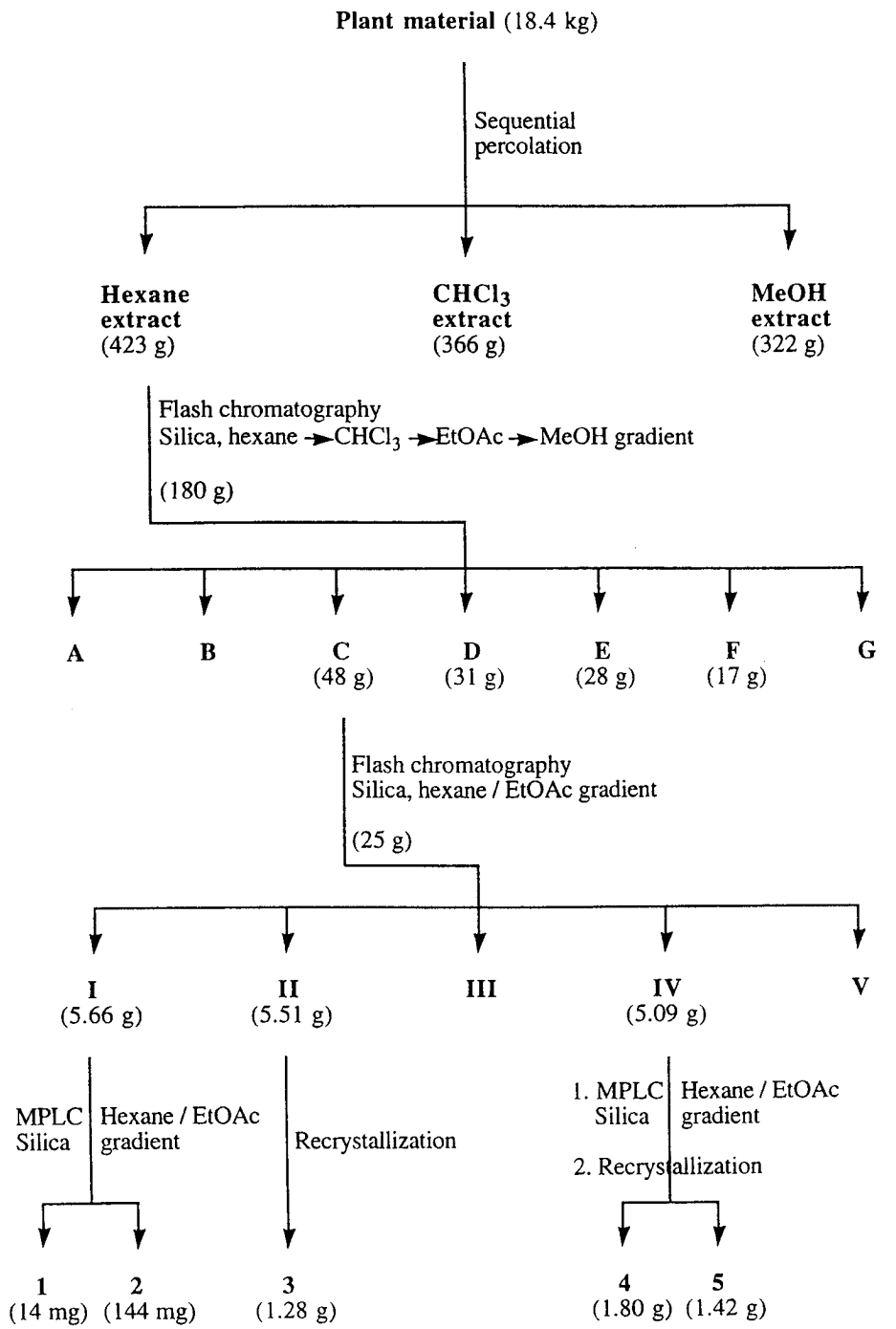
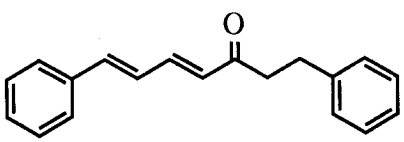


Fig.3. Isolation of sesquiterpenes and antiinflammatory diarylheptanoids from rhizomes of *Curcuma xanthorrhiza* Roxb. (Zingiberaceae).

AN EXAMPLE

Recently, we isolated two sesquiterpenes and three antiinflammatory diarylheptanoids from the rhizomes of *Curcuma xanthorrhiza* Roxb.(Zingiberaceae) (6). This research problem may serve as an illustration of the application of flash chromatography and MPLC in natural product chemistry.

The plant material was percolated, at room temperature, sequentially with hexane, chloroform and methanol (Fig. 3). The hexane and chloroform extracts exhibited significant antiinflammatory activity in the test model of carrageenin-induced hind paw edema in rats. The methanol extract was inactive. The crude hexane extract was separated by flash chromatography, with three consecutive gradients, into seven fractions (A - G). Fractions C and D were active in the bioassay. ^{1}H-NMR and TLC of fractions D, E and F were practically identical and thus it was not necessary to submit fractions E and F to the quite elaborate bioassay. Fraction C was further flash chromatographed over silica gel with a stepwise hexane - ethyl acetate gradient to yield five fractions (I - V). Fraction I was subjected to MPLC (silica gel, combined gradient and isocratic elution, hexane - ethyl acetate) to yield the sesquiterpenes germacrone **1** and curzerenone **2**. Fraction II solidified after evaporation of the solvent and recrystallization afforded the diarylheptanoid **3** as yellow needles. Compound **3** exhibited significant antiinflammatory activity in the bioassay. Fraction



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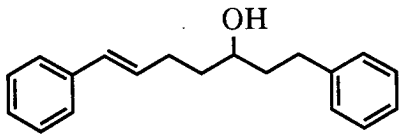

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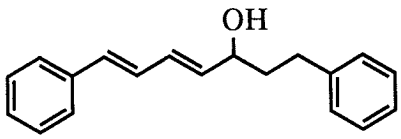

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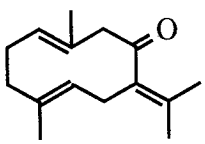

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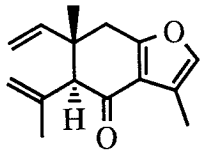

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IV appeared as a solid, homogeneous in TLC, and was initially thought to be one compound. However, analytical HPLC [Column dimensions: 250 x 4.6 mm; packing material: Partisil 5 (silica gel, 5 μm); mobile phase: 5 % ethyl acetate in hexane; flow rate: 4.5 ml/min] of the fraction gave a chromatogram with two peaks (Retention times: 13.5 and 16.2 min, respectively). Furthermore, the ^{1}H-NMR spectrum of fraction IV also indicated that the solid was a mixture. The two components of the solid could not be separated by further flash chromatography, but repeated MPLC (silica gel, combined gradient and isocratic elution, hexane - ethyl acetate), followed by recrystallization, yielded compounds **4** and **5**. Both compounds showed significant antiinflammatory activity. Fractions D, E and F (from the first column) were then subjected to repeated MPLC, employing the same conditions as for fraction IV, to yield additional quantities of **4** (11.1 g) and **5** (8.9 g), which subsequently were used for extended pharmacological studies. It appears impossible for most laboratories to acquire facilities to isolate such large quantities of pure compounds by means of preparative HPLC.

CONCLUSIONS

The following list summarizes and compares some of the main characteristics of the two techniques flash chromatography and MPLC.

Flash Chromatography	**MPLC**
- Low or moderate resolution	Moderate or high resolution
- Simple technology, easy trouble shooting	Moderate technology, requires access to reasonable service facilities for common electronics
- Moderate solvent consumption	Moderate solvent consumption
- High or very high loading capacity	High loading capacity
- Good packing requires experience	Columns easy to pack
- Low reproducibility of packing and separation	High reproducibility of packing and separation
- Column support (silica gel) not reused	Column support normally reused
- TLC analysis of fractions necessary	UV-detection may be sufficient
- Column length flexible	Column length fixed
- Low technology - low price	Medium technology - moderate price

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บทคัดย่อ

บทความปริทัศน์นี้ได้บรรยายถึงเทคนิค 2 ชนิดคือ Flash Chromatography และ Medium Pressure Chromatography (MPLC) ที่ใช้ในการแยกสารให้บริสุทธิ์จากส่วนสกัดของพืช ในเทคนิคเหล่านี้ต้องใช้ปั๊มชนิดต่างๆ ในการให้ความดันแก่ระบบของเครื่องมือ ความดันที่ใช้ใน Flash Chromatography จะอยู่ระหว่าง 1-2 บาร์ และปั๊มที่เหมาะสมที่สามารถทำให้เกิดความดันในระดับนี้คือ ปั๊มที่ใช้ในการให้อากาศกับตู้เลี้ยงปลาทั่วไป ซึ่งอาจใช้ปั๊มเครื่องเดียวหรือหลายเครื่องต่อกันก็ได้ เครื่องแก้วที่ใช้ในการแยกสารด้วยเทคนิคนี้ สามารถสร้างขึ้นในห้องเป่าแก้ว ในบทความนี้ได้แสดงรูปเครื่องแก้วเหล่านี้และมีคำบรรยายประกอบไว้อย่างละเอียดในเนื้อหา ซึ่งเขียนขึ้นจากประสบการณ์ของผู้เขียน บทความยังได้กล่าวถึงความสัมพันธ์ระหว่างปริมาณของสารที่จะนำมาแยก ขนาดของคอลัมน์ และปริมาตรของสารละลายที่จะเก็บในขวดแต่ละใบด้วย ข้อดีของเทคนิคนี้ก็คือ สามารถทำการแยกส่วนสกัดจากพืชได้ปริมาณสูงถึง 200 กรัมต่อการแยกแต่ละครั้ง

MPLC เป็นเทคนิคที่ใช้ในการแยกสารให้บริสุทธิ์หลังจากสารสกัดจากพืชได้ผ่านการแยกขั้นต้นด้วยวิธี Flash Chromatography ความดันที่ใช้กับระบบในเทคนิคนี้จะอยู่ในระดับ 5-20 บาร์ ซึ่งตรงกับความสามารถของปั๊มชนิดลูกสูบที่ผลิตขึ้นจากหลายบริษัทเพื่อการนี้โดยเฉพาะ ด้วยเทคนิคของ MPLC นี้เราสามารถทำการแยกสารได้ปริมาณสูงสุด 50 กรัมต่อการแยกแต่ละครั้ง

องค์ประกอบที่สำคัญที่ทำให้เทคนิคทั้งสองใช้ได้ผลดีในการแยกสารให้บริสุทธิ์คือ การใช้สารละลายผสมของตัวทำละลายอินทรีย์ที่มีสภาพขั้วต่างกัน ซึ่งในทางเทคนิคเรียกว่า เกรเดียนท์ โดยทั่วไปนั้นการสร้างเกรเดียนท์จะทำโดยเพิ่มสภาพขั้วของสารละลายผสมในตอนต้นอย่างช้าๆ และในตอนท้ายของการแยกจะเพิ่มสภาพขั้วของสารละลายผสมให้เร็วขึ้น

บทความนี้ได้มีข้อแนะนำเบื้องต้นในการเลือกใช้ซิลิกาเจลชนิดต่างๆ ในการแยกสาร และยังได้แสดงถึงการประยุกต์ใช้เทคนิคทั้งสองชนิดนี้ในการแยกสารบริสุทธิ์ปริมาณมากๆ จากส่วนที่สกัดได้จากว่านชักมดลูก (*Curcuma xanthorrhiza* Roxb.)