Large-Scale Liquid Chromatography

H. Colin and G. B. Cox, Prochrom R&D, Champigneulles, France

Introduction

The 1990s have seen the birth of high performance preparative liquid chromatography (HPPLC) and its recognition by the pharmaceutical and fine chemical industries as a very powerful and versatile purification technique. In contrast to traditional low perforamance/low pressure preparative chromatography, HPPLC is based on the use of columns of medium to high efficiency, operated at high mobile phase velocities, and consequently at moderate to high pressure – conditions quite similar to those met in analytical chromatography.

The very early work carried out in liquid chromatography at the beginning of the twentieth century (at a time when liquid chromatography was used for preparative purposes only) made use of particles 1–15 μm in diameter. For several reasons, preparative liquid chromatography (PLC) was later carried out with large particles (>100 μm) packed in columns of very low efficiencies operated at pressures close to atmospheric. Under such conditions, the kinetic properties of the columns are obviously very poor; the preparative separations require large quantities of mobile phase, long separation times and are expensive. For this reason, for a long time PLC was considered to be inappropriate for industrial purifications and was the choice of last resort. There were three reasons for this poor image: first, the misunderstanding of the effect of the column efficiency and the lack of a theoretical framework to describe the behaviour of columns under highly overloaded conditions; second, the use of low-quality packing materials (large particle size and large size distribution); and third, the lack of proper equipment in general, and adequate column technology in particular. Now that these problems have been addressed, PLC has many advantages.

Particular Effects Related to Column Overloading

For the sake of simplicity, the following discussion is limited to a binary mixture, but the phenomena described are the same for more complex mixtures. It is also assumed that the adsorption isotherms are Langmuirian. This is the case for most practical situations in chromatography. The total band broadening occurring in the column is the combination of two contributions: that of the intrinsic column efficiency (kinetic term – the column efficiency under an infinitely small injected quantity) and that of the finite mass of product injected (thermodynamic term – related to the amount injected). The equipment itself, besides the column, can also contribute to band broadening, but this is not considered in the present discussion.

When a sufficiently large quantity of product is injected, the thermodynamic term (which is proportional to the injected quantity, to a first approximation) becomes larger than the kinetic term and the total band broadening is primarily controlled by the quantity injected. It has been concluded from this observation that, under high mass overload, there is no reason to use efficient columns in PLC since the contribution of the intrinsic column efficiency is masked by the thermodynamic contribution. This is true as long as pure products are injected, which is obviously not the case when PLC is used.

When a mixture of two products is injected, the elution bands occupy the same section of the column during a certain fraction of their migration through the bed. When this happens, the molecules of each component compete for the same retention sites. When the injected quantity becomes sufficiently large, the molecules that have more affinity for the stationary phase prevent the other ones from interacting with the adsorbent. As a result, the weakly retained product is eluted faster than expected. This is known as a displacement effect. This effect is particularly strong when the weakly retained product is present at a smaller concentration than the strongly retained one. Under such conditions, the peak for the weakly retained component is not only eluted more quickly than if it were injected alone, but it is also narrower and has the typical shape shown in **Figure 1**A. Better separation is achieved than would be found if the displacement effect were not effective. When the more strongly retained compound is at lower concentration than the weakly retained one, the peak for the second compound is broader than it would be if injected alone. It also starts to be eluted earlier, an effect known as the tag-along effect (see Figure 1B).

It is clearly preferable to optimize the choice of the chromatographic conditions so that the first product is the one at the smaller concentration (typically, the impurity to be removed). Under these conditions, the region where the two products interfere is strongly

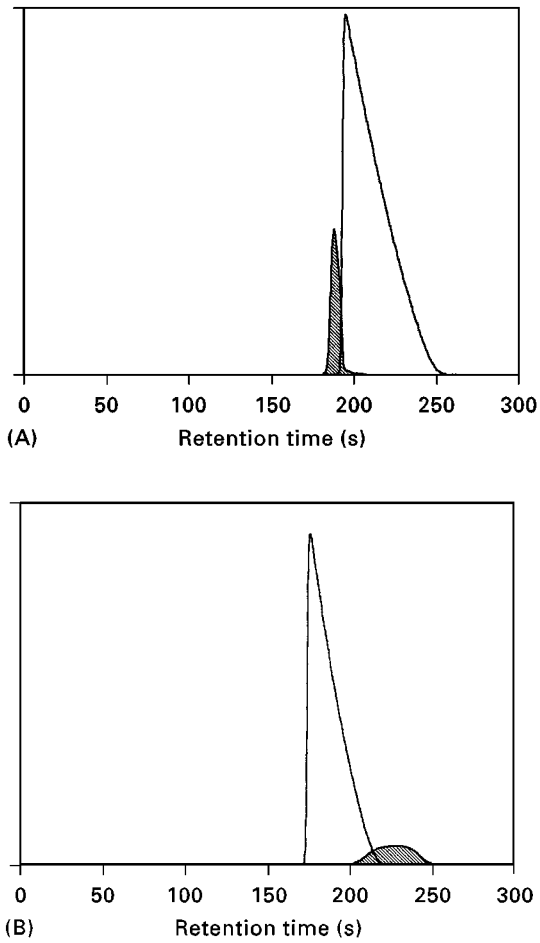

Figure 1

controlled by the column efficiency (see **Figure 2**) and increasing the column efficiency results in the possibility of injecting (much) more product or recovering more product for a given injected quantity. The possibility of using displacement effects and injecting large quantities is thus associated with columns of sufficient efficiency. Such columns enable higher production rates, reduce solvent consumption and purification times, and thus decrease purification costs.

The displacement and tag-along effects, as well as the role of the various operating parameters, have been modelled by several groups. Unfortunately, it is not yet possible (and it is questionable if it will be in a near future) to make accurate predictions of the properties of a given chromatographic system under moderate and severe overloading conditions, and thus optimize the operating conditions to minimize the purification cost, for instance. The rigorous treatment of Guiochon and co-workers, based on the simultaneous resolution of the mass balance equations for the various species involved in the chromatographic process, involves numerical integration of a set of differential equations. This treatment is based on the model of competitive Langmuir isotherms and the approach is not simple since it requires knowledge of the composite adsorption isotherms. Major difficulties lie in experiments to measure these composite isotherms and the lack of suitable models to describe them with sufficient accuracy.

Column Technology

In order to get efficient columns of large diameter (i.e. >5 cm) for preparative purposes, it is necessary to have a packing material of adequate quality and also the appropriate column technology. Column technology is a very critical issue, since it is known that above a certain diameter columns tend to be unstable because of bed settling and the resulting formation of voids in the packing. These voids are very detrimental to the column efficiency and are responsible for band distortion. In addition, large columns are potentially more difficult to pack than small ones, particularly when small particles (<50 μm) are used. Indeed, the high pressure slurry-packing technique used to pack

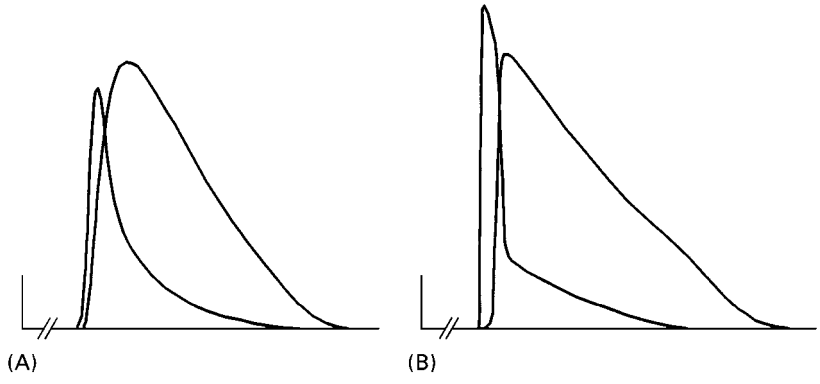

Figure 2

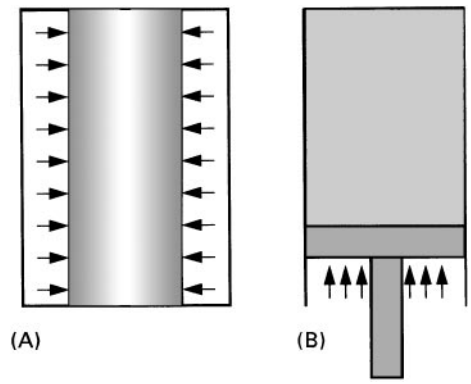

Figure 3 Methods of dynamic compression. (A) Radial compression; (B) axial compression.

analytical columns cannot be used for large diameter columns for both practical and technical reasons.

Various solutions have been proposed to solve the problem of bed stability. It seems that the best approach is to compress the bed of particles, either radially (using columns with flexible walls immersed in a pressurized fluid) or, preferably, axially using a piston (see **Figure 3**). This bed compression must be dynamic so that voids are eliminated as soon as they form. Using dynamic axial compression, it is possible to pack columns of large diameter with small particles offering the same efficiency as analytical columns (see **Figure 4**). This technology is particularly versatile because it gives the operator the possibility of packing the column very easily and quickly with any type of material. This is clearly important for equipment used in production where downtime has to be minimized. In addition, the length of the bed can be easily adjusted to optimize the column efficiency. Finally, whatever the compression technique used, it is very important to design the column ends properly. Indeed, in order to generate piston flow and avoid band distortion due to unequal flow distribution, it is necessary to spread the flow of liquid evenly across the cross-section of the column.

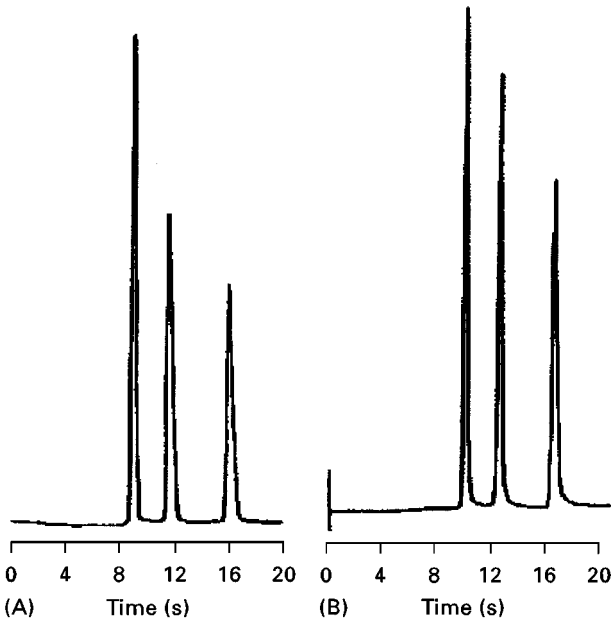

Figure 4 Comparison of chromatograms from columns. (A) 25 cm × 4.6 mm and (B) 25 cm × 150 mm.

Packing Materials

The best column technology is useless without the proper packing material. There are two main aspects to consider when selecting the packing material: chemical and physical. The chemical aspect is related to the chemistry of the purification problem and is not discussed here since it is very much product dependent. A material well suited for a given application may be inadequate for another. The physical aspects, however, can be discussed in general terms. The various models of HPPLC have shown that there is an optimum value of the ratio d_p^2/L for any separation problem (d_p is the particle size and L the column length). In typical conditions, for columns operated at a pressure of 30 to 60 bar, an 'optimum' particle size is about 10–20 μm with a column length between 20 and 50 cm. Accordingly, rather small particles have to be used for PLC.

It is also very important to keep the particle size distribution as narrow as possible, since small particles in the distribution have a very strong effect on the column permeability and large particles strongly influence the column efficiency. In other words, a material with a large size distribution tends to produce low-efficiency columns with low permeability. It is usually considered that the ratio d_{p10}/d_{p90} should be less than 1.5 for a good material (d_{p10} and d_{p90} being the particle sizes corresponding to 10% and 90% of the cumulated volume-based size distribution).

Besides the average particle size and the size distribution, other physical properties of the packing material are important, particularly the specific surface area and the pore size. It is desirable to maximize the surface area of the material in order to increase its adsorption capacity. Increasing the surface area, however, usually means decreasing the pore size and thus the access of the molecules to the internal pores of the particles. There exists an optimum pore size and specific surface for each compound to be purified.

Among the other important physical parameters, one should also mention the mechanical strength of the particles, since they must resist the stress of the compression technique used.

Optimization of Separation Conditions

The optimization of PLC is not a simple task since many parameters have to be taken into account: kinetics (column efficiency and column design in terms of d_p and L), thermodynamics (choice of the chromatographic system, temperature, degree of column overloading, etc.) and economics. The necessity to take into account economic aspects is important since the most favourable conditions in terms of purification cost do not necessarily correspond to the best chromatographic conditions in terms of quality of separation. Indeed, a better chromatographic mobile phase providing more selectivity may turn out to be more expensive to buy or to recycle (mobile phase recycling is often essential to keep purification costs low). This means that such parameters as the heat of vaporization of the mobile phase and its cost have to be considered. The mobile phase viscosity is also very important since it controls not only the velocity of the eluent at a given operating pressure (and thus the time required for the separation), but also the values of the diffusion coefficients of the products in the mobile phase, and accordingly the column efficiency. Very viscous eluents (such as propanol–water mixtures, for example) should be avoided in preparative chromatography.

A general economic analysis of a purification process by PLC indicates that there are typically two situations to be considered. When the quantity to be purified is low, the optimization should be aimed at maximizing the production rate because time (labour) is the largest contribution to the purification cost. When large quantities have to be processed, the optimization should be aimed at reducing the solvent cost (solvent consumption and regeneration). Many strategies can be envisaged to reduce the cost of PLC, depending on the conditions of the purification in terms of expected production, required purity and recovery ratio, etc. For instance, a purification can be made in several steps. This is often advisable when the concentration of the product of interest in the crude feedstock is low (i.e. less than 50%). In this respect, it is important to clean up the mixture to be purified as much as possible before injecting it (using a preliminary treatment such as flash chromatography, for example).

Various techniques can be used to reduce time and solvent consumption, and thus purification cost. Among them are shave-recycling and flip-flop. Shave-recycling consists of injecting (very) large quantities of feedstock – too large to provide satisfactory recovery of the product of interest at the expected purity. The fraction of the product peak where the purity is acceptable is collected and returned to the column inlet (typically by using the solvent pump – adequate valving is required). During the second passage through the column, the separation between the product of interest and the impurities is further improved. The pure fraction of the product peak is then collected and, if necessary, the contaminated fraction of the peak is returned to the column for a third cycle. Typically, three or four cycles are made before a new large injection is performed. This shave-recycling approach saves on solvent (no fresh solvent is consumed when the column effluent is returned to the column inlet), reduces product dilution and decreases purification costs.

The flip-flop technique is an optimized back flush operation. It is very common for several impurities to be eluted after the product of interest. If these impurities are not removed from the column before the next injection is made, they can contaminate the product of interest in subsequent purification runs. Backflushing the column is often done to remove the strongly retained impurities. Flip-flop consists of performing the next injection a certain time after the backflush process has been initiated. During this time the weakly retained impurities of the next injection are eluted. Accordingly, during each elution run the flow direction in the column is reversed (hence the name flip-flop). This technique requires very good column stability because of these changes of flow direction.

Another powerful approach employed to reduce purification costs and solvent consumption is the recently 'rediscovered' technique of simulated moving bed (SMB), which is of particular interest for binary separation.

Simulated Moving Bed

When working on a large production scale, there are many arguments in favour of continuous processes. The majority of chromatographic processes are, however, batch processes that are incompatible with continuous production. Some years ago, continuous chromatographic processes were developed for large-scale separations in the food and petroleum industries. These processes solved some of the problems of implementation of large-scale chromatography. They are based upon the technique that is now known as simulated moving bed chromatography. The original application areas for this process were in the purification of *p*-xylene (a precursor of polyester plastics) and the separation of glucose from fructose to make high-fructose corn syrups, which are used in the soft drinks industry as a sucrose replacement. A few other applications also based upon this technology exist. There are perhaps 150 of these large-scale, low

pressure SMB systems in the world, responsible for the production of many thousands of tonnes of product each year. More recently, the combination of the new regulatory atmosphere in the pharmaceuticals industry, where pure enantiomers, of racemic drugs are becoming more and more in demand, together with the development of new media for the chromatographic resolution of enantiomers, has resulted in new areas of opportunity for SMB applications. These applications, by virtue of the high value of the products and the requirements for high purity in the industry, are for the most part operated using small particles in high pressure SMB equipment; this parallels the extensive use of HPLC purification methods in production for the pharmaceutical industry.

The basic principle of a true moving bed (TMB) unit is shown in **Figure 5**. The basis of TMB lies in the idea that in a chromatographic system the 'stationary' phase may be moved as well as the mobile phase. In a binary separation, the two components move at different velocities through the column. If one were to move the packing material in a direction opposite to that of the mobile phase in the column, one could choose a velocity of the packing material relative to that of the mobile phase such that one of the components moved in the original direction, carried by the mobile phase, while the other component, which spends a greater proportion of its time in the stationary phase, would be transported by the packing material in the opposite direction. By injecting in the middle of such a column, one product would emerge from each end of the column (the more retained product is often called the extract and the less retained product the raffinate, see Figure 5). Thus one could inject and collect product in a continuous fashion.

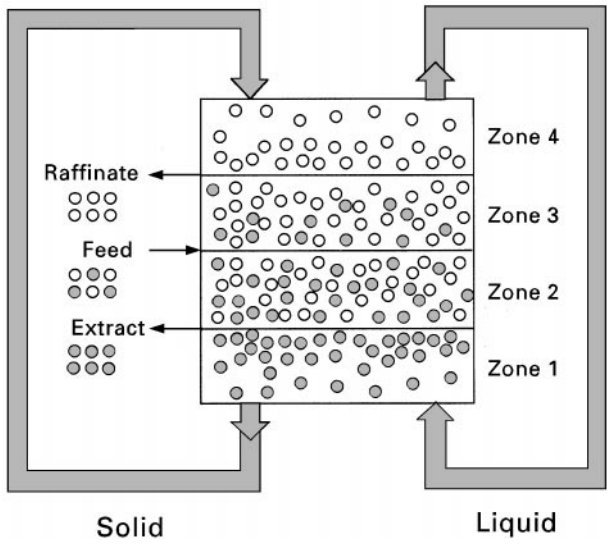

Figure 5 TMB unit.

In practice, many attempts have been made to realize such a countercurrent flow of packing material and mobile phase. None has been really successful, due mainly to the incompatibility of the needs of chromatography (tightly packed beds) and those of the transport of the solid particles (loose, fluid beds). The solution to the problem is to move the entire column, but in this case a column cut into short sections. In this way, rather than moving the bed in a continuous fashion, the individual sections of the column are moved periodically, such that the average bed speed remains that required for continuous separation. The feed inlet and the product outlets are arranged at the interfaces between the sections of the column, facilitating the entry and removal of materials. This principle operation simulates a true moving bed system and is thus called a simulated moving bed, SMB. It has been shown that when the number of columns exceeds about eight (this number depends on the separation problem considered), the performance of SMB is very close to that of TMB.

The removal of the products requires some special arrangements of flow rates in the system. The slower moving product (extract) moves during the separation phase in the direction of the packing material. If, in the section between the solvent inlet and the product outlet, the solvent flow is increased, a velocity will eventually be attained at which the product slows and becomes stationary. At a higher velocity, the product will reverse direction in the system and move with the solvent. This extra flow is removed from the product port; downstream of this port the velocities in the system remain the same as before. This means that in the first part of the column the product is moving toward the exit port in the same direction as the solvent. In the second section, the product is still moving with the packing material, also in the direction of the exit port. The only possible route for this product to take is out of the port, along with the extra solvent used to increase the flow rate beyond that critical for the change in direction of the product. In certain cases, this extra flow is not very large, and can be close in value to that of the feed.

At the other end of the system, the raffinate is removed by bleeding off some of the flow rate at the exit port. This reduces the solvent velocity in the final section of the column set such that the product moves with the packing material rather than the solvent. This mans that, in this final section, the product is moved toward the exit port. In the penultimate section, the flow continues as before and the product again is moved in the direction of the outlet port. Thus, this product, too, is eluted from the system at the appropriate product port.

This arrangement for the removal of the products allows the solvent to be recycled. The solvent that elutes from the end of the column set does not (if the flow rates, etc., are controlled correctly) contain any product. It can then be redirected back to the solvent pump with the addition of a make-up flow to compensate for the excess flow removed in the raffinate and extract streams in comparison with that added by the feed. By the same token, the columns that pass the solvent inlet contain no product and so these can be added back at the other end of the column set for reuse. In this way a limited number of columns – usually between 8 and 16 – are used for the separations and the solvent use is also reduced in comparison with the batch operation. It should be noted that, in the majority of SMB system, the columns themselves remain stationary. Their movement is simulated by the use of switching valves, which are used to move the entry and exit ports around the column set.

In comparison with batch chromatography, SMB separations can be performed using smaller columns and less solvent than the equivalent batch separation. This is the result of the reduction in solvent required, the increased efficiency of use of the column and also the reduced need for column efficiency in terms of the number of theoretical plates in the system. Thus, although one requires a greater number of columns, the column set does not have to be as long as for a batch separation using the same column packing. This can lead to some bizarre solutions, in that batch HPLC separations are usually optimized for column lengths of 25 to 50 cm, using particle sizes of around 10 to 25 μm. The equivalent SMB separation, using the same particle size, would require column lengths of only 1 or 2 cm. This is not difficult to realize for a small-scale system, but becomes difficult if large diameter columns – of 50 to 100 cm internal diameter, for example – are envisaged. In this case, larger particle sizes are required in order that the columns can have reasonable product distribution to eliminate band deformation. The optimum size of the particles, columns, etc., for the separation then becomes a question of economics rather than of science.

Conclusions

HPPLC is certainly one of the most powerful purification techniques available today, and as such must be considered by process and production engineers with the same attention as distillation, crystallization and other more traditional techniques. Provided it is properly used, HPPLC can be cheaper than traditional techniques, provide higher yields, higher degrees of purity and save on purification times, a particularly critical aspect in the highly competitive world of the modern pharmaceutical industry. Properly using HPPLC means understanding the behaviour of columns under highly overloaded conditions and using such beneficial effects as displacement effects. It also means using the right packing material, the right column efficiency and the appropriate column technology, as well as considering alternative strategies such as shave-recycling, backflush, SMB, etc. SMB is of particular interest for binary separations (but not only) and in the near future is expected to become the technique of choice for such separations at large and very large scales.

See Colour Plate 23.

See also: **II/Chromatography: Liquid:** Column Technology. **III/Chiral Separations:** Liquid Chromatography. **Flash Chromatography. Medium-pressure Liquid Chromatography.**

Further Reading

Bidlingmeyer BA (ed.) (1987) *Preparative Liquid Chromatography* Elsevier: Amsterdam.

Golshan-Shirazi S and Guiochon G (1988) *Analytical Chemistry* 60: 2364.

Gviochon G, Shirazi SG and Katti AM (1994) *Fundamentals of Preparative and Nonlinear Chromatography* Boston: Academic Press.

Knox JH and Pyper HM (1986) *Journal of Chromatography* 363: 1.

Miller L, Orihuela C, Fronek R, Honda D and Dapremont O (1999) Chromatographic resolution of the enantiomers of a pharmaceutical intermediate from the milligram to the kilogram scale. *Journal of Chromatography A* 849: 309–317.

Nicoud RM (1992) *LC-GC INTL*, 5: 43.

Pynnonen B (1998) Simulated moving bed processing: escape from the high-cost box. *Journal of Chromatography A* 827: 143–160.

Snyder LR, Dolan JW, Antle DE and Cox GB (1987) *Chromatographia* 24: 82.

Zhang GM and Guiochon G (1998) Fundamentals of simulated moving bed chromatography under linear conditions. *Advances in Chromatography* 39: 351–400.

Mass Spectrometry Detection in Liquid Chromatography

See **II / CHROMATOGRAPHY: LIQUID / Detectors: Mass Spectrometry**