Simulated moving-bed chromatography and its application to chirotechnology

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The increased awareness of the differences in biological activity of the two enantiomers of a chiral drug has raised the demand for enantiomerically pure products, particularly in the pharmaceutical industry. Simulated moving-bed chromatography can be used for the separation of the two enantiomers of a chiral molecule, which is feasible at all production scales, from laboratory to pilot to production plant. The use of non-enantioselective synthesis of racemic mixtures and simulated moving-bed enantiomer separation might make the development process of a new chiral drug substantially shorter and cheaper.

Chirality is ubiquitous in nature. Our hands, as well as the horns of an ibex and the wings of a white dove, are chiral because they are each other's mirror image; no matter how much we twist and turn them, we cannot superimpose the right hand image onto the left one. In addition, many organic molecules exhibit chirality, for example, whenever four different groups are attached to a tetrahedral carbon atom.

A chiral molecule and its mirror image form a pair of enantiomers. They share the same physical properties except their effect on the rotation of polarized light ('handedness'), that is, they are optically active, and are chemically identical except when reacting with other chiral compounds. All amino acids, with the exception of glycine, are chiral; the essential components of life itself (proteins, carbohydrates and DNA) are constructed from optically active building blocks. However, it is remarkable (although not yet fully understood) that, contrary to artificially synthesized molecules, all naturally occurring amino acids in living organisms have the same handedness.

This implies that just as a left shoe does not fit on both the right and the left foot, chiral biological receptors might interact differently with the two enantiomers of a chiral flavor, fragrance or drug, thus resulting in a different biological activity. For example, one enantiomer of limonene tastes like an orange, whereas the other tastes like lemon. One enantiomer of vitamin C is an antioxidant, whereas the other has almost no effects on humans. Thalidomide is a chiral drug that, in the 1960s, was administered to pregnant women as a racemic mixture (i.e. a mixture of enantiomers in equal proportions). Tragically, it was realized that only one enantiomer is beneficial; the other is believed to be responsible for major limb malformations in fetuses and other birth defects. At present, the authorities responsible for drug administration impose stringent rules on tests and chiral purity of the final product; this has a big impact on the development of new chiral drugs.

During the research stage, a new chiral molecule (as the corresponding racemic mixture) is discovered that exhibits biological activity. Small amounts (from milligrams to grams) of the pure enantiomers for biological tests are obtained using different techniques, for example, preparative high-pressure liquid chromatography (HPLC). In all cases, their enantiomeric purity is checked using analytical chiral chromatography. These two chromatographic techniques exploit the well-known property of a class of stationary phases to be selective for the two enantiomers. These are called chiral stationary phases (CSP) and are often based on polysaccharide compounds such as cellulose and amylose derivatives (for HPLC) and cyclodextrin derivatives [for gas chromatography (GC)].

Only ~0.1–0.2% of the molecules discovered fulfill the bioactivity requirements and enter the so-called phase I of the drug-development process, where larger amounts (100–1000g) of both enantiomers are prepared for large-scale pharmacological and toxicological tests. Already at this stage, the pharmaceutical intermediates leading to the target molecule are obtained via a synthesis and separation route. This route is similar to the final production process because changes in the impurity profile and in the enantiomeric purity could cause misleading results to be obtained during toxicological screening. In phase I, the biological activity of both enantiomers has to be carefully monitored under conditions in which the presence of one does not mask the effect of the other. Hence, enantiomeric purity is already a key issue at this stage of the development process.

Finally, when a new active pharmaceutical substance enters the clinical testing or production stage, and reaches the market either as a pure enantiomer or a racemic mixture, the production scale is in the order of kilograms to tons. This stage is called phase II when the testing involves healthy volunteers, and phase III when it extends to voluntary patients. If the drug to be marketed is a pure enantiomer, chiral chromatography is used; this is one of the three main techniques for obtaining enantiomerically pure chemicals in large quantities. The other two methods are: (1) enantioselective chemical or enzymatic synthesis, and (2) chiral resolution through derivatization and the formation of a compound that can be easily separated, for example, a diastereomeric salt.

Following phase II, the productivity of preparative HPLC is often insufficient; it must be decided whether

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Box 1. Simulated moving-bed technology

The operation of a simulated moving-bed (SMB) unit can be best understood by examining the concept of batch elution chromatography in a continuous fashion (Fig. ia).

Consider a single chromatographic column and two species, blue component A and yellow component B, having a greater and lesser affinity for the stationary phase, respectively. A pulse of a mixture of A and B diluted in the correct mobile phase is fed to the column. As a result of their different affinities, A and B travel slower and faster, respectively, along the column and provided the column is long enough they are collected separately at the column outlet.

This situation is similar to that of a swift cat and a sluggish turtle running a race on a straight track. They will both reach the track, but at different times. If the two animals run on a moving belt, whose velocity is intermediate but opposite to theirs (Fig. ib), the faster animal will fall off on the right-hand side, and the slower on the left-hand side. With this principle in mind, a device can be constructed that can separate cats and turtles, which are fed continuously to the center of the moving belt, that is, a turtle–cat continuous separator.

The same principle can be applied to chromatography in the countercurrent column illustrated in Fig. ii. Here the adsorbent solid particles flow along the column countercurrently to the fluid stream, at a velocity that renders the propagation rates of A and B negative and positive, respectively. A and B can be continuously fed in the middle of the column. The concentration profiles (Fig. ii) develop, and pure samples of A and B can be collected at the extract and raffinate port, respectively.

The moving bed enables the achievement of high purity even if the resolution of the two peaks is not excellent because only the purity at the two tails of the concentration profiles, where the withdrawal ports are located, is of interest. This is contrary to batch chromatography where high resolution is vital in order to achieve high purity. However, it is also evident that a larger portion of the stationary phase is loaded in the moving bed than in the fixed bed. This will ultimately lead to a higher productivity per unit mass of stationary phase. In principle, this moving-bed concept can be fully exploited in the true moving-bed (TMB) unit (Fig. iii). Four countercurrent sections similar to that in Fig. ii are used, each playing a specific role in the separation. The mixture to be separated is fed between sections 2 and 3 where A and B are separated. The more-retained A and the less-retained B are collected in the extract and raffinate, respectively. A desorbent or eluant is fed to section 1 to regenerate the solid phase before recycling and the fluid phase is regenerated in turn in section 4.

However, the movement of the adsorbent particles causes problems as a result of attrition and mixing. Therefore, in practice this is simulated by using fixed beds of adsorbents (chromatographic columns) and periodically moving the inlet and outlet ports to the unit in the same direction as the fluid flow (with a period indicated as t^*). This is illustrated in Fig. iv, where the continuous movement of the solid in the TMB unit is simulated in a discrete fashion. In order to closely mimic this continuous movement, each section of the TMB unit is divided into several subsections. In this case, there are eight subsections, which is a typical number for small-scale applications; large-scale units for hydrocarbon separation can involve up to 24 subsections.

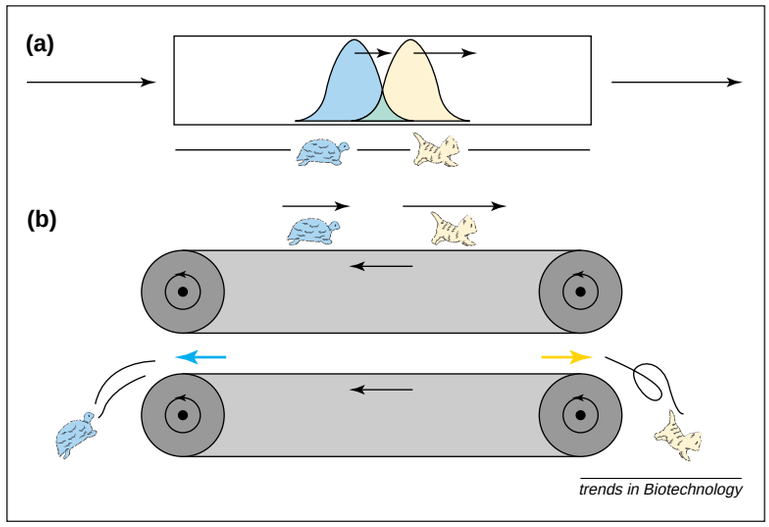


Figure i

(a) A single chromatographic column and its zoological metaphor; the sluggish turtle and the more-retained species A (blue) travel more slowly than the swift cat and the less-retained species B (yellow). **(b)** The continuous turtle–cat separator. The velocity of the moving belt is opposite and intermediate to the that of the cat and the turtle.

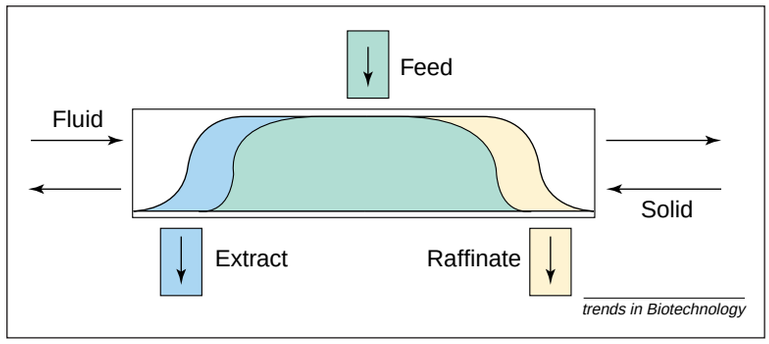


Figure ii

The continuous countercurrent chromatographic column (the chromatographic metaphor of the turtle–cat separator of Fig. ia). A stream of clean, solid particles flows countercurrently to the fluid phase. The mixture of A and B to be separated is fed to the middle of the column and the two components are collected pure at the two withdrawal ports.

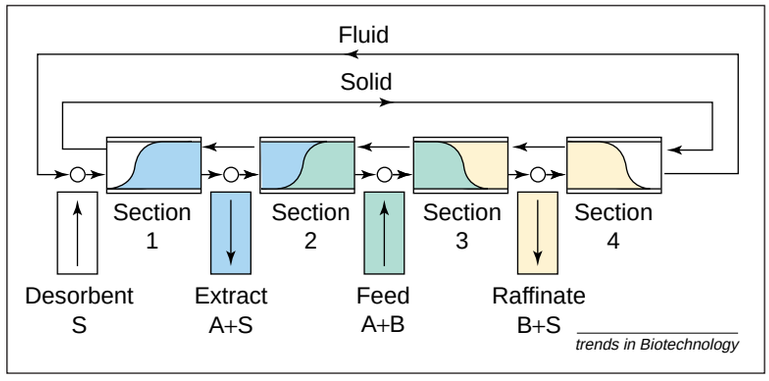


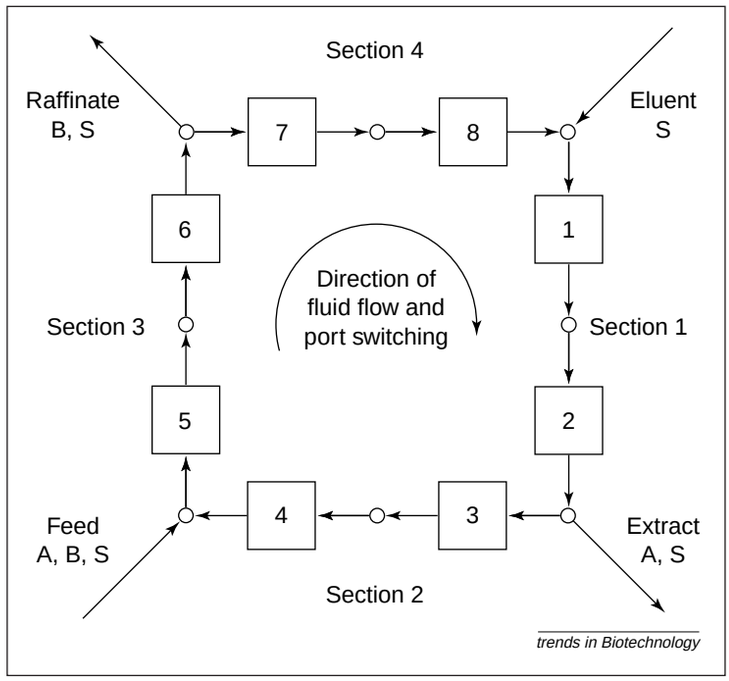
Figure iii

A four-section true moving-bed (TMB) unit. Each section comprises a continuous countercurrent chromatographic column and plays a specific role in the separation of A and B. This is accomplished in sections 2 and 3, whereas section 1 regenerates the solid phase and section 4 cleans up the solvent; in both cases before recycling. The more-retained species A and the less-retained species B are collected in the extract and raffinate stream, respectively.

Box 1. Simulated moving-bed technology (continued)

Figure iv

A four-section simulated moving-bed (SMB) unit, the practical implementation of the TMB unit in Fig. iii. The continuous movement of the solid phase of the TMB unit is simulated by periodically switching the inlet and outlet ports of the unit in the same direction as the fluid flow. In order to make the discrete movement closely mimic the continuous movement, each section of the TMB unit is divided into several subsections; two subsections are present in this example.



or not the chiral drug should be produced through enantioselective synthesis or as a racemic mixture, and then separated through either chiral resolution or chiral chromatography. On the one hand, the development of a new method for asymmetric, enantioselective chemical or enzymatic synthesis is a lengthy, expensive procedure, which might result in a process with a relatively low enantioselectivity. On the other hand, chiral resolution is often labor-intensive.

The alternative solution to these two strategies is to use chromatographic techniques to separate the racemic mixture obtained via non-enantioselective synthesis at all stages of chiral drug development. This can be done using simulated moving-bed (SMB) technology[1] (Box 1), which exploits the same chromatographic method adopted for analytical chiral chromatography (i.e. the same CSP and mobile phase) but achieves greater productivity than preparative column chromatography. Moreover, SMBs are multipurpose units that can be applied to different separations in all stages of the drug-development cycle. This can also be beneficial for the acceptance procedure of a new product and its process by Federal Drug Administrations.

This mode of operation has attracted considerable attention by several pharmaceutical companies; indeed, most of them are now evaluating SMB technology for their processes. Two companies in particular (UCB Pharma, Belgium; Daicel Chemical, Japan) have been operating production SMB units since 1998, with a capacity of several tons per year of pure enantiomers from two different chiral drugs[2]. Moreover, there are currently several companies that, in multipurpose SMB units, offer the service of carrying out the chiral separation of batches of the racemic mixture provided by the customer. These are operated on different separations for relatively short times using the same equipment but with different columns, stationary and mobile phases.

Simulated moving-bed technology

Industrial applications of SMBs involve widely different scales; SMB technology (Box 1) was developed in the early 1960s (under the generic name of Sorbex) for large-scale hydrocarbon separations in plants with capacities of up to several tens of thousands of tons per year. Approximately 100 Sorbex units have been licensed to date, half of them for *p*-xylene separation from the alkyl-aromatic C_8 fraction using Y-zeolites as an adsorbent and toluene or *p*-di-ethylbenzene as a desorbent[1,3]. One third of all commercial Sorbex units are devoted to linear and nonlinear paraffin separation, exploiting the molecular-sieving effect of 5A-zeolite. Several manufacturers have also commercialized a number of large-scale separations involving sugars.

Using SMBs for chiral separations

Within the past decade, the possibility of scaling down SMB technology for the separation of fine chemicals, particularly chiral molecules, has been investigated. In fact, at the beginning of the 1990s, analytical chiral chromatography could be extended to preparative and SMB separations as a result of the possibility of using new stable and relatively inexpensive chiral stationary phases[4–19].

When SMBs are scaled down, the Sorbex configuration is no longer convenient and a set of chromatographic columns, normally between 6 and 12 [i.e. one column for each subsection of the SMB unit and from one to three columns for each section of the SMB (Box 1)], together with standard chromatographic equipment, such as multiposition, multiway valves and HPLC pumps, are used. SMB units ranging in size from the laboratory scale to the pilot and production scale are all based on the same design and technology; they only differ in the size of the columns and the ancillary equipment.

All commercial applications operate in the liquid phase, but different reports have shown the feasibility of other operating modes[16,20–22]. In general, gas-phase operation offers improved mass-transfer efficiency (i.e. a larger number of theoretical plates) and reduced hold-up in the nonselective fluid phase compared with liquid-phase operation. This might improve the process economics for the separation of volatile compounds.

Several features of SMB technology make it highly suitable for the resolution of racemates, particularly in the pharmaceutical industry. Indeed, SMB technology allows a fast and reliable scale-up of enantiomer separation from analytical chromatography. Thus, data provided by analytical chromatography (e.g. solubility, retention times and selectivity) can be used to directly evaluate the feasibility of large-scale SMB chromatographic separations. Using the design and simulation tools available today, SMB separations can be developed from analytical data in a time frame that is much shorter than the time needed to develop an enantioselective chemical route[12,13].

Moreover, SMB technology has several advantages over other preparative chromatographic techniques. First, the process is continuous, thus enabling unattended operation and stable product quality. Also, the solvent requirement and the productivity per mass of the chiral stationary phase is significantly lower, as previously mentioned. The process is designed to enable the collection of products with the desired degree of purity and enantiomeric excess, together with automatic recycling of the desorbent (or solvent), thus minimizing its consumption. This has been confirmed by several studies, which have demonstrated that compared with classical batch preparative chromatography, SMB units often exhibit improved performance in terms of solvent consumption and productivity per unit mass of stationary phase. Küsters *et al.* demonstrated a 33% increase in productivity for SMB compared with batch preparative chromatography[9]. Furthermore, a 20-fold increase in productivity in the resolution of the chiral DOLE (Ref. 19; an ester of quinoline mevalonic acid) and a 170% and 530% increase in productivity for the separation of the enantiomers of guaifenesin and formoterol, respectively, have been reported[12]. These studies attested solvent savings ranging from 84% to 95%.

Moreover, SMB technology is significantly more robust than preparative chromatography because it requires a smaller number of theoretical plates to achieve the same product purity. In fact, Nicoud *et al.* demonstrated with the separation of the isomers of phytol that a 20% decrease in column efficiency resulted in a 10% decrease in SMB productivity, compared with a 50% decrease in preparative chromatography productivity[23]. Although these results are not applicable to all chiral separations, they appear to be extremely promising; however, it is advisable when considering a new separation to evaluate the economics of the different separation alternatives before proceeding with the final selection.

It is worth noting that the adsorption columns are operated under overload conditions, that is, in the nonlinear region of the adsorption equilibria of the components to be separated, which is always avoided in analytical chromatography. This is favorable for preparative applications because it enables the most-efficient use of the stationary phase. However, under these conditions the retention behavior of the enantiomers depends on their concentration in the stationary phase and has to be described by competitive adsorption isotherms. This makes the design of the operating conditions of SMBs demanding; special criteria have been developed for this purpose (Box 2). SMB operation has only one major drawback compared with batch preparative chromatography. In fact, batch preparative chromatography enables the recovery of all the pure components of a multicomponent mixture, whereas SMBs produce only two fractions, one in the raffinate and the other in the extract stream (Box 1). This disadvantage has no effect on binary separations, such as the resolution of racemates.

Thus, in summary, when racemate synthesis is coupled with racemate resolution using a SMB unit, this is typically an economical and relatively simple process resulting in the production of both enantiomers with high enantiomeric excess and high recovery. This feature is particularly attractive when both enantiomers are needed with a high degree of optical purity to assess their biological activity, toxicity and pharmacokinetics. Alternatively, if only one of the enantiomers exhibits the desired activity (in this case, the 'eutomer'), the other enantiomer (the 'distomer') can be either racemized and fed again to the separation unit or disposed of.

Therefore, the application of SMB technology to production-scale enantiomer separation might enable the reduction of development times and production costs, and the availability of both enantiomers with high purity and product recovery, at each stage of chiral drug development.

Chiral stationary phases

A prerequisite for the scale-up of a chromatographic analytical chiral separation is that the CSP is available in large amounts, with reproducible batch-to-batch properties and at relatively low cost with respect to the value of the enantiomers to be separated. If this is fulfilled, the economical feasibility of the SMB process will be dictated by the key properties of the CSP, namely its selectivity, loading capacity and efficiency, which control the size of the unit and the achievable specific productivity of the process per unit mass of stationary phase. Besides these, there are other important issues that are related to the behavior of the CSP. These refer to: (1) chemical stability, which limits the number of compatible mobile phases and, indirectly, the maximum solubility of the solute; (2) mechanical stability, which is particularly important in HPLC applications where small particle sizes are adopted and large pressure drops are imposed; and (3) its lifetime. All these characteristics have to be taken into account when selecting the CSP for a specific SMB separation. The CSPs most commonly used for preparative chromatography and SMB applications can be grouped as shown in Table 1; Table 2 shows examples of chiral SMB applications.

Natural products such as cellulose and amylose have proved to be of great versatility for enantiomer separation. Although natural products are poor chiral selectors in their native state, they become highly effective when their hydroxyl functions are derivatized, particularly with aromatic moieties via ester or carbamate linking,

Box 2. Design of operating conditions for SMBs

Simulated moving beds are complex units, whose operation requires the choice of several parameters. With reference to Fig. iv, these are the flow rates in the four sections, the switch time and the feed composition, that is, the overall feed concentration of the components to be separated. These parameters have to be chosen to achieve given process specifications, such as productivity per day, minimum purity of the product streams and minimum recovery of the species to be separated[8,26]. This complexity can be tackled in two different ways. The first is based on using a detailed model of the SMB unit and carrying out numerical simulations. The model of an SMB comprises column models for each of the SMB subsections, coupled through mass-balance equations at every node of the unit[27]. Moreover, the port switching mechanism, with period t^*, has to be implemented. The detailed column model consists of the following material balance equations (i = A,B),

$$\epsilon \frac{\partial c_i}{\partial t} + (1-\epsilon)\frac{\partial n_i}{\partial t} + u\frac{\partial c_i}{\partial z} = \epsilon D_L \frac{\partial^2 c_i}{\partial z^2} \qquad (1)$$

$$\frac{\partial n_i}{\partial t} = a_p k_i \left(n_i^* - n_i\right) \qquad (2)$$

$$n_i^* = f_i^{eq}(\underline{c}) = \frac{a_i c_i}{1 + \sum_j b_j c_j} \qquad (3)$$

where c_i and n_i are the fluid and adsorbed phase concentration, respectively; u is the superficial velocity of the fluid (the volume-flow rate divided by the empty column section); ϵ is the overall void fraction of the bed; D_L and $a_p k_i$ are the axial dispersion coefficient, and the mass-transfer coefficient multiplied by the specific mass-transfer surface, respectively; n_i^* represents the adsorbed-phase concentration at equilibrium with the fluid-phase composition, which can be given by the multicomponent Langmuir isotherm, as in Eqn 3 (f_i^{eq} is the function defining the adsorbed-phase concentration in terms of the whole fluid composition). These equations account for accumulation in the fluid and the adsorbed phase, the convection and axial dispersion in the fluid phase, and the mass transfer through the linear driving-force approximation.

For design purposes, a second strategy is more useful, which involves a simplified version of this model. This is the 'equilibrium theory' model, where axial dispersion and mass-transfer resistance are neglected, hence infinite column efficiency is assumed. This leads to the 'triangle theory', where the separation performance is controlled by the flow-rate ratios, m_j, one for each section of the SMB unit.

$$m_j = \frac{u_j t^* - \epsilon L}{(1-\epsilon)L} \qquad j = 1,\ldots,4 \qquad (4)$$

Because they are defined in terms of flow velocity, u_j; switch time, t^*; column length, L; and overall bed void fraction, ϵ; all process parameters are brought together in these four dimensionless groups. The triangle theory enables one to derive conditions on the flow-rate ratios to achieve complete separation of the two species. These conditions define the triangle-shaped separation region in the operating parameter plane spanned by the flow-rate ratios in the central sections of the unit (m_2, m_3) (Fig. i; Ref. 44). The boundaries of this region are given by explicit relationships, again in terms of isotherm parameters and feed composition. The three surrounding regions attain different separation regimes, where either the extract or the raffinate is pure, or where both components distribute in the two product streams. The vertex of the triangle represents optimal operating conditions in terms of separation performance. The relative location of the operating point in the operating parameter plane with respect to the four regions of separation gives a prediction of the separation performance.

The triangle theory approach is acknowledged as one of the most effective design tools for SMBs. In practice, it can be used in two ways: (1) to explain experimental results and (2) to design optimal operating conditions. In both cases, it is necessary to have at least an empirical approximation of the competitive adsorption isotherm of the components to be separated.

As an example of the first case (the explanation of experimental results), a significant application is the separation of guaifenesin, as carried out by Francotte and Richert[12] (case 4 in Table 2). They were able to fine-tune the separation performance by adjusting the flow rates but were not able to explain why the behavior of the SMB unit was not symmetric after changes in the feed and extract flow rates[45].

Application of the triangle theory to the design and optimization of a new separation has been well illustrated[18,29]. Using the model described previously, the non-ideal effects, mass-transfer resistance and axial dispersion, which are neglected in the frame of the triangle theory can be accounted for. Running simulations at different levels of column efficiency (where the triangle theory assumes infinite column efficiency) demonstrates that the complete separation region becomes smaller and smaller as the efficiency is reduced[27]. This is expected because both phenomena are unfavorable for separation. However, the qualitative features and the topology of the separation regions in the operating parameter plane remain unchanged. In addition, this implies that in the presence of non-ideal effects, the information obtained through the idealized triangle theory provides a useful approximation of the real SMB behavior.

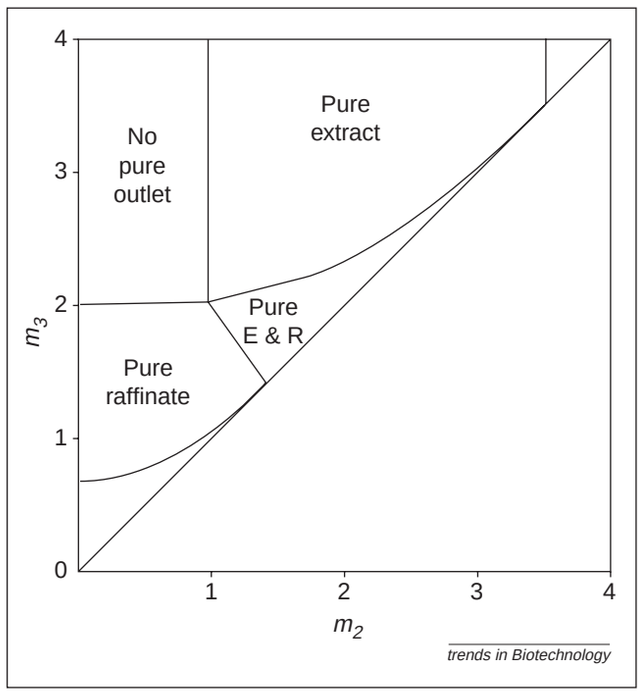


Figure i

Regions attaining different separation regimes in the operating plane spanned by the flow-rate ratios in the two central sections of the SMB unit, that is, m_2 and m_3. The triangle-shaped region is made up of points corresponding to operating conditions that enable complete separation of A and B (100% purity of both A and B) in the extract and raffinate, respectively. The flow-rate ratio, m_j, is calculated in terms of the SMB-operating conditions according to Eqn 4. The boundaries of the complete separation region are given by explicit relationships in terms of the parameters of the Langmuir isotherm and the feed concentration.

Table 1. Chiral selectors applicable in SMB units for enantiomer separations

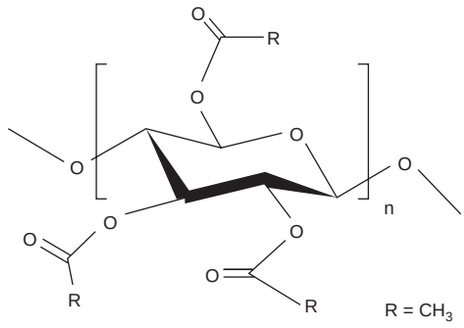
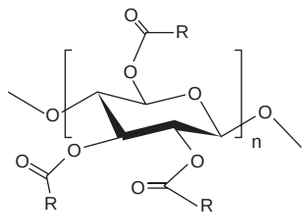
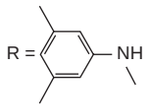
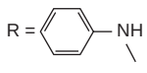
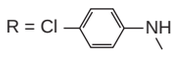
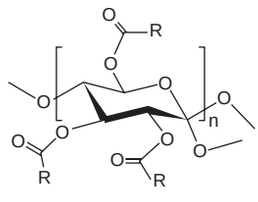
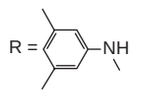
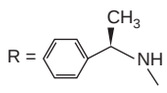
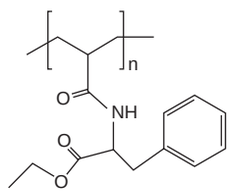
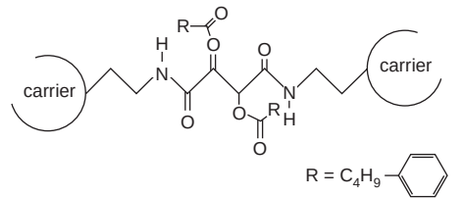
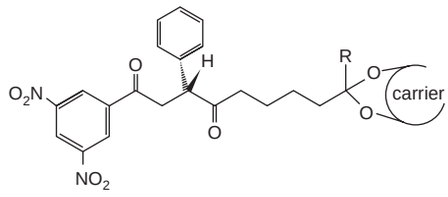
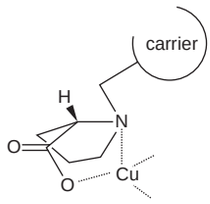
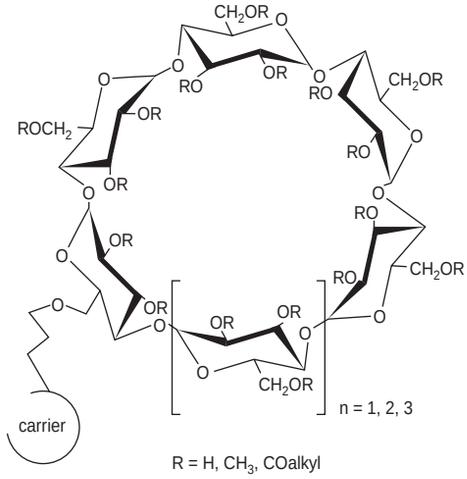
Type of CSP	Structure of CSP	Chiral selector	Compatible solvents (examples)	Trade name	Supplier
A		Microcrystalline cellulose-triacetate	Hexane/ethanol (100/0–0/100) Hexane/2-propanol (100/0–0/100)	MCTA or CTA-I	Merck
		Cellulose tribenzoate		Chiralcel OB	Daicel
B	 	Cellulose tris(3,5-dimethylphenyl-carbamate)	Hexane/ethanol (100/0–0/100) Hexane/2-propanol (100/0–0/100)	Chiralcel OD	Daicel
		Cellulose tris(phenyl-carbamate)		Chiralcel OJ	Daicel
		Cellulose tris(4-chloro-phenyl-carbamate)		Chiralcel OF	Daicel
	  	Amylose tris(3,5-dimethylphenyl-carbamate) Amylose tris[(S)-methylbenzyl-carbamate]	Hexane/ethanol (100/0–0/100) Hexane/2-propanol (100/0–0/100)	Chiralpak AD Chiralpak AS-V	Daicel Daicel
C		Poly[(*S*)-*N*-acryloylphenyl-alanine ethyl ester]	Hexane/2-propanol Hexane/tetra-hydrofurane Hexane/dioxane Tetrahydrofurane Dichloromethane Toluene	Chiraspher	Merck
D		O,O′-bis(4-tert-butyl-benzoyl)-N,N′-diallyl-L-tartardiamide	Hexane/2-propanol Hexane/tetrahydro-furane Hexane/dioxane	Kromasil CHI-TBB	Akzo Nobel

Table 1. Chiral selectors applicable in SMB units for enantiomer separations (continued)

Type of CSP	Structure of CSP	Chiral selector	Compatible solvents (examples)	Trade name	Supplier
E		3,5-Dinitrobenzoyl-phenylglycine (either ionic or covalent bonding)	Hexane/2-propanol Hexane/acetonitrile Hexane/dichloro-methane Acetonitrile Ethyl acetate	DNBPG	Regis
F		L-Proline bound to polyacrylamide	Copper(II) acetate buffer/acetic acid	Chirosolve-pro	JPS Chimie
G		Cyclodextrin derivatives bound to silica	Water/methanol Water/acetonitrile	Cyclose Nucleodex	Chiralsep Macherey–Nagel

Abbreviations: SMB, simulated moving bed; CSP, chiral stationary phase.

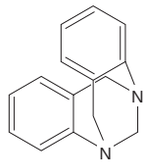
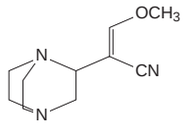
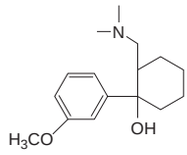
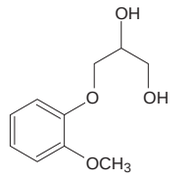
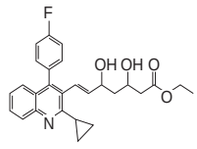
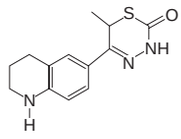
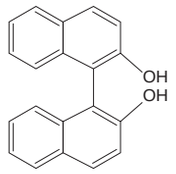
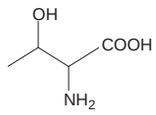
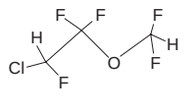
and are immobilized on silica supports. These materials are composed of small chiral units regularly repeating along the polymeric chain, so that the density of active sites capable of chiral recognition is high and results in a high loading capacity. Microcrystalline cellulose triacetate and cellulose tribenzoate (group A) have in some cases been used for chiral SMB separations as neat, non-coated particles. However, they are inconvenient to pack, have a restricted chemical stability and show low efficiency. To overcome these limitations, a family of derivatized cellulose and derivatized amylose CSPs (group B) have been produced. These chiral polymers are not covalently attached to the support but are coated on a silanized, wide-pore silica gel. Consequently, some caution has to be exercised when choosing mobile-phase solvents to be used with such CSPs; they are most commonly used in the normal-phase mode (i.e. with solvents such as hexane and ethanol). However, it is possible that soon the range of usable solvents will extend to organic eluents such as ethyl acetate, chloroform and tetrahydrofurane[24].

Polymers based on cross-linked, optically active polyacrylamides and polymethacrylamides (group C) constitute a class of synthetic CSPs with high loadability and efficiency. However, the gel structure of these polymers prevents their use at high pressure. Improvement of the mechanical performance of these CSPs was achieved by polymerization of the acrylic monomer to the silica-gel surface, thus producing a grafted polymer.

Immobilized network polymers based on O,O′-diaroyl-derivatives of (+)−(2R,3R)-N,N′-diallyl tartrardiamide represent a further class of synthetic CSPs obtained from the C_2 symmetric material from the chiral pool (group D). The covalent bonding to functionalized silica gives these tartrate phases a high stability to mobile phase additives, such as alcohols or ethers. Because only incomplete information on the use of this type of CSP for SMB chiral separations has been reported to date, this is not included among the SMB applications presented in Table 2.

The best known CSPs of the π-acidic and π-basic type are the Pirkle phases (group E), which are classified

Table 2. Examples of chiral SMB applications

Number	Structure of racemate	Systematic name, 'trade name', therapeutic or substance class	Number and size of columns [length × ID (mm)]	CSP and mobile phase	Amount of CSP (g)	Selectivity (approx. value)	ee (%) in extract	ee (%) in raffinate	Specific productivity[a] (kg kg^{-1} day^{-1})	Refs
1		5,6,11,12-Tetrahydro-2,8-dimethyl-5,11-methano-dibenzo[b,f][1,5]diazocin, 'Tröger's base', chiral nitrogen model compound	8 (250 × 4.6)	MCTA Ethanol	15	2.0	97.4	96.4	0.006	17
2		(1-Aza-bicyclo-[2,2,2]-oct-3-yl)-methoxyimino-acetonitril, agonist at muscarinic receptors	8 (105 × 26)	Chiralpak AD Hexane/ isopropanol (95:5, v:v)	240	1.9	97.8	99.5	0.260	13
3		2-[(Dimethyl-amino)-methyl]-1-(3-methoxy-phenyl)-cyclohexanol, 'Tramadol', analgesic	12 (100 × 21.2)	Chiralpak AD Benzine/ isopropanol/ diethylamine (95:5:0.1, v:v:v)	240	2.1	99	>99.8	0.600	10
4		3-(2-Methoxy-phenoxy)-1,2-propane-diol, 'Guaifenesin', antitussive	16 (60 × 21)	Chiralcel OD Heptane/ ethanol (65:35, v:v)	201	2.4	98.8	99.2	0.080	12
5		(E)-(3R,5S,6E)-7-[2-Cyclopropyl-4-(4-fluoro-phenyl)-quinolin-3-yl]-3,5-dihydroxy-6-heptenoic acid, 'DOLE', pharma-ceutical intermediate	8 (100 × 100)	Chiralcel OF Hexane/ isopropanol (50:50, v:v)	3770	1.35	94.4	99.4	0.270	19
6		5-(1,2,3,4-Tetra-hydro-quinoline-6-yl)-6-methyl-3,6-dihydro-1,3,4-thiadiazine-2-one, 'EMD 53986', pharmaceutical intermediate	8 (54 × 26)	Chiraspher Ethylacetate/ ethanol (95:5, v:v)	90	3.3	99	73.8	0.17	11
7		2,2′-Dihydroxy-1, 1′-binaphthol, intermediate for chiral catalysts	8 (105 × 26)	DNBPG Heptane/ isopropanol (72:28, v:v)	250 ml	1.4	89	97.8	0.03 kg l^{-1} day^{-1}	14
8		D,L-Threonine, amino acid	12 (1000 × 25.4)	Chirosolve-L-proline Acetic acid (0.05 M) Copper acetate (0.125 μM)	2800 ml	1.6	98	98	0.005 kg l^{-1} day^{-1}	4
9		2-Choloro-1-(difluoromethoxy)-1,1,2-trifluoro ethane, 'Enflurane', 'Ethrane' inhalation anesthetic	8 (800 × 15)	γ-Cyclodextrin, nitrogen	280 ml	1.34	98.4	98.4	0.026 kg l^{-1} day^{-1}	16

[a]The specific productivity is defined as the amount of enantiomer of interest produced at the stated purity per unit time and unit mass of CSP.
Abbreviations: SMB, simulated moving bed; ID, internal diameter; CSP, chiral stationary phase; ee, enantiomeric excess (a positive quantity defined as the difference between the concentrations of the two enantiomers divided by their sum and multiplied by 100).

into π-acceptor and π-donor phases. The most frequently used π-acceptor phases are derived from the amino acids phenylglycine (DNBPG) or leucine (DNBLeu), covalently or ionically bonded to 3-aminopropyl silica gel. The π-donor phases (e.g. with naphthylalanine as the chiral moiety) enable the separation of π-acceptor analytes as a result of the reciprocity concept. Usually these CSPs are used under normal-phase conditions, but it has also been demonstrated that racemate resolutions can be achieved in the reversed-phase mode. They exhibit a high efficiency and significant loadability, but are intrinsically limited to π-interactions for chiral resolution.

Ligand-exchange chromatography is based on the reversible formation of complexes between metal ions (e.g. Cu^{2+}) and chiral complexing agents carrying functional groups capable of interacting as ligands. This type of CSP (group F) is particularly suited for the resolution of racemates of chiral compounds with chelating functionalities such as amino and hydroxy acids.

Finally, cyclodextrins are cyclic oligosaccharides comprising α-D-glucose units linked through the 1,4-position (group G). Cyclodextrins possess a conical structure with an interior cavity that is relatively hydrophobic. A variety of compounds fit into this cavity to form inclusion complexes; however, until recently the application of silica-bonded cyclodextrins was limited by their low loading capacity and limited availability. Cyclodextrin derivatives can also be used for enantioselective gas chromatography; for this application, cyclodextrins are often dissolved in polysiloxane, which is then coated onto an inert porous support[25].

Chiral SMB separations

Although many chiral SMB separations have been developed, it is likely that some of them will never be disclosed for proprietary reasons. It is readily observed (Table 2) that the scale of the separation is widely different, from the few grams of CSP loaded in the case of Tröger's base separation[18], to the few kilograms in the case of DOLE separation[19]. This indicates that SMB technology can be adopted at different scales, with essentially similar equipment because only the column size and pump performance are different. The productivity values depend on several factors: selectivity, loadability and efficiency of the stationary phase; these properties depend in turn on the enantiomers to be separated. However, it is worth noting that the same orders of magnitude in productivity have been achieved in examples 2 and 3 (0.26 and 0.60 $kg\,kg^{-1}\,d^{-1}$, respectively) where the same Chiralpak AD has been used. However, because SMB optimization is still unresolved, it is likely that optimal performances have not been achieved in all the examples in Table 2.

Let us now consider certain examples from Table 2 in greater detail. Tröger's base (case 1) has a special type of chirality, where the inversion at the chiral nitrogen is hindered by the presence of the N–CH_2–N-bridge. This separation proves the additional applicability of the design criteria (Box 2) to SMB units with a CSP with low efficiency. In fact, SMBs are robust unit operations with respect to mass-transfer efficiency; if the operating conditions are correctly chosen, high purity performance can always be achieved, albeit at relatively low productivity.

The second application (case 2) was developed with the aim of developing a high-throughput separation with the help of software produced by the SMB manufacturer[13]. The racemates of three different chiral drugs: the anti-tussive guaifenesin (case 4), the anti-cancer aminoglutethimide and the anti-asthmatic formoterol, were resolved after only a few days of development work[12] (Box 2). A specific objective was the comparison between SMB and batch preparative chromatography performance. A 2.5–5.0-fold improvement in both productivity and solvent consumption was reported in the resolution of both guaifenesin and formoterol. This is significant even though there is no indication of how the batch chromatography has been optimized. This is a frequent limitation of this kind of investigation and is ultimately a result of the fact that there is no well-defined optimization procedure for either SMB or batch chromatography.

The separation of the enantiomers of the analgesic Tramadol (case 3; Ref. 10), DOLE (case 5; Ref. 19) and EMD 53986, a precursor in the synthesis of a Calcium-sensitizing agent[11] (case 6), have been carried out by scale-up of the chiral SMB technology to production levels. The separation of binaphthol (case 7) was attempted[14] but, unfortunately, a satisfactory enantiomeric excess level could not be achieved, probably because of suboptimal operating conditions[27]. The separation of the two enantiomers of threonine was carried out on Chirosolve-L-proline, involving ligand-exchange chromatography (case 8), and was one of the first SMB chiral separations to be performed[4]. It was shown that as a result of the overload operation of the chromatographic columns, a significant enrichment of the less retained enantiomer in the raffinate could be achieved. In fact, its concentration in the raffinate was 25% higher than in the feed. This effect is well known to be possible in principle but is seldom observed in practice.

The last example (case 9) is the separation of enflurane[9] using a gas-phase SMB. This is a widely used chiral inhalation anesthetic whose enantiomers cannot be separated except using gas chromatography. There is some evidence that the two enantiomers have different biological activities, but amounts of pure enantiomers sufficient for clinical tests have never been obtained. The chiral selector was produced from octakis(3-*O*-butanoyl-2,6-di-*O*-n-pentyl)-γ-cyclodextrin diluted in polysiloxane SE-54 and then coated onto inert Chromosorb particles[25,28].

Other SMB applications including bioseparations

SMBs have been applied not only to hydrocarbons, sugars and enantiomers, but also to many other forms of separations. The driving force has always been the difficulty or even the impossibility of using distillation or other traditional unit operations and the need for operating the separation under relatively mild conditions, which are also feasible for thermolabile compounds. One example is the fractionation of isotopes, which has been studied particularly in tight connection with the nuclear power industry. The isotopes separated include H and D, D and T, ^{16}O and ^{17}O, ^{14}N and ^{15}N, and ^{40}Ar and ^{41}Ar (Refs 30,31); the stationary phases used for these applications were cation-exchange resins, zeolites and a palladium-based adsorbent.

SMBs promise to also have an impact on the field of bioseparations, as demonstrated by the purification of a monoclonal antibody from a cell-culture supernatant with a yield ⩾90% (Ref. 32). Ion-exchange resins can be used for the recovery of amino acids such as lysine (purity ⩾98.5%) from microbial cultures, for example, *Corynebacterium glutamicum*, using corn-steep liquor as the protein source[33]. Wu *et al.* separated two amino acids, L-phenylalanine and L-tryptophan, on a PVP resin (poly-4-vinylpyridine cross-linked, Reillex HP polymer) using a ten column SMB unit that yielded 96.7% L-phenylalanine purity in the raffinate and 99.7% L-tryptophan purity in the extract[34].

SMB chromatography has also been used for the entire or partial replacement of conventional batch chromatography in the purification of cyclosporin A (Ref. 35). This process could fulfill the quality requirements of drug-administration regulations with high yields and lesser amounts of solvent than previously used methods.

A further extension of the SMB technology involves the coupling of the chemical reaction and the continuous chromatographic separation process in a single apparatus, the SMB reactor (SMBR). This is a hybrid process, conceptually similar to reactive distillation, which is not energy-intensive and is competitive with traditional processes wherein the reaction and separation are carried out in different devices. In reactions limited by chemical equilibrium where more than one product is formed, conversion can be enhanced in this kind of hybrid apparatus because the products are separated as they are formed. Here, SMBRs can simultaneously achieve the goals of complete conversion and separation of the products. Candidate reactions include esterifications, transesterifications, formation of ethers, acetylations, some isomerizations, hydrogenations and various enzyme reactions.

The simplest application of this concept involves reactions producing water, such as esterifications and acetylations, and the use of a strong cation-exchange resin in the hydrogen form, which plays the dual role of being a selective sorbent of water and a heterogeneous catalyst. In the case of esterification, the alcohol and the acid are fed to the SMBR, where they react, and the ester and water are chromatographically separated and collected individually in the raffinate and extract, respectively. Either a solvent or one of the reactants itself, for example, the alcohol, can play the role of desorbent (Fig. i, Box 1). Achieving complete conversion of the limiting reactant in the synthesis of ethylacetate[36,37] and bisphenol A (Ref. 38) has proved this concept.

In other cases, two different solid materials must be used as the catalyst and the adsorbent. The different materials can be placed in different columns, therefore each section of the SMBR unit forms a reaction column and a chromatographic column, in series. Alternatively, they can be mixed in granular form and the mixture can be used to pack the SMBR columns. Tonkovich and Carr have applied the former approach to the oxidative coupling of methane[39] and the latter method to the hydrogenation of mesitylene[40] using metal or metal-oxide catalysts and activated charcoal as an adsorbent.

SMBRs have also been used effectively to carry out enzymatic reactions, where the enzyme is immobilized on a support matrix and an ion-exchange resin is used to separate the reaction products. Glucose isomerization using isomerase and glucose–fructose separation has been combined to produce a fructose-rich syrup[41]. Sucrose inversion using invertase and glucose–fructose separation have been carried out in the same SMBR unit[42]. Finally, lipase-catalyzed formation of isoamyl propionate in a hexane solvent with the selective removal of water, thus avoiding enzyme deactivation, has also been demonstrated[43].

Concluding remarks

Recently, SMB separation technology has been successfully extended from hydrocarbons and sugars to fine chemicals, particularly enantiomers. The driving force has been the increasing need for pure enantiomeric products, particularly in the pharmaceutical industry, and for the rapid and reliable development of new chiral drugs. Conditions that have enabled this development to be sustained have been the availability of an established technology for SMB units of different scales, from grams per day to tons per year, and stable, efficient and relatively inexpensive chiral stationary phases. It is thought that both the technology and the materials are no longer of primary concern. Even though new chiral stationary phases will be introduced, thus broadening the application spectrum and improving the economics of the technology, SMBs for chiral separations are an established, reliable and robust technology. However, from an engineering perspective, SMBs are a new unit operation for the chemical and fine-chemical industry, whose utilization will become standard in the future.

An important contribution to the broadening of the use of SMBs has been an improved understanding of SMB behavior and the development of user-friendly criteria for the design and optimization of SMB operation (Box 2). It is now possible to develop a new SMB separation procedure in a few days as a result of the use of robust procedures for isotherm determination, the available criteria for the selection of operating conditions and software packages for process optimization.

However, several further developments can still be envisaged. The two most important being multifraction separations and gradient-mode operations. Multifraction separations are necessary whenever the desired product has to be purified from more or less retained impurities or when the mixture to be separated comprises more than two components; for example, non-selective synthesis of a species with more than one chiral center. In both cases, a single SMB unit is not sufficient. Likewise, multicomponent separations by distillation and SMB multifraction separations require a sequence of SMB units. This configuration presents no theoretical or practical difficulties and will be a natural evolution of this technology.

Analytical chromatography often exploits temperature gradients or gradient elution operation modes, to increase the resolution and productivity of the technique. Likewise, these gradient mode operations can in principle be exploited within a SMB unit, where each section is optimized in an independent manner relative to the other sections. In reality, SMB units operate all

sections under the same conditions, but this could be improved by operating different sections at different temperatures or feeding solvents with different elution strengths to different sections (Fig. i, Box 2). So far, the only experimental implementation of this concept has been the operation of an SMB using supercritical carbon dioxide as an eluent and enforcing a pressure gradient along the SMB unit so as to exploit the different elution strengths of supercritical carbon dioxide at different density levels[22]. However, gradient-mode SMBs continue to be an area of active research.

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