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Review

Preparative enantioseparation by simulated moving bed chromatography

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Abstract

Simulated moving bed (SMB) chromatography was invented in the 1960s in the petrochemical industry and has since then been widely used to produce petrochemicals and sugars at the multi-ton scale. In the early 1990s its principle could be successfully adapted to chromatographic enantioseparation, due to developments in system design, chiral stationary phase synthesis and improvements in modelling and simulation of non-linear chromatographic behaviour. Since then a lot of separation systems have been brought into production, which are reviewed. In addition new developments are outlined in the field of system design and stationary phase development.

Keywords: Reviews; Simulated moving bed chromatography; Chiral stationary phases, LC; Non-linear chromatography; Preparative chromatography; Enantiomer separation

Contents

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1. Introduction

Chromatography on achiral as well as on chiral stationary phases (CSPs) has been right from the beginning a method more to prepare pure compounds than to analyse them. Nevertheless, chromatography as a production tool has long been recognised as a very expensive tool and therefore limited to special applications, e.g., the isolation of bio-molecules, where no other production methods were suitable. On the other hand, there have been processes in the petrochemical and sugar industries which proved that with adsorption or chromatographic principles even low-value products can be produced with very good economy. These processes based on the continuous counter-current movement of stationary and mobile phases and were implemented as simulated moving bed (SMB) processes. The counter-current movement shows some decisive features which makes the process highly productive. Therefore with the access to sufficient amounts of stable and selective CSPs the application of SMB chromatography to chromatographic enantioseparations showed that it can be highly competitive in terms of price, reliability, prediction and scaleability.

The history SMB chromatography can be divided into three main periods: (i) early developments in the petrochemical and sugar industries, (ii) first applications to chromatographic enantioseparations and (iii) full-scale processes and future trends.

2. History (1968–1992)

Although the idea of continuous chromatography is rather old and was first discussed for the separation of enantiomers [1] it took several decades until the first systems were established. The fundamental work of Broughton [2,3] opened the field of SMB chromatography in the petrochemical industry, where today several million tons of product/year are produced using mainly zeolites as the stationary phase. Only a few years later the system design was successfully applied to the separation of monosaccharides. Today SMB chromatography is used in the sugar industry for the production of several mono- and oligosaccharides [4].

2.1. Basic principle and benefits of continuous counter-current chromatography

Several disadvantages of batch elution chromatography, e.g., discontinuous operation, non-effective adsorbent utilisation and high product dilution, have been overcome by continuous SMB chromatography. Its principle is illustrated in Fig. 1.

An SMB system consists of several columns (in most cases 6–12) which are connected in series and between each of them four valves are placed which can be individually opened and closed. A recycling pump inside the column circle delivers the mobile phase flow through all columns. Two additional pumps constantly inject the feed and fresh eluent and two pumps withdraw the raffinate and extract flows. The five pumps of the system allow one to control the internal recycle fluid flow stream as well as all the four external fluid flow streams. The valve system between the columns allows one to open or close inlet and outlet streams of each column simultaneously at the switch time intervals. The counter-current movement of stationary and mobile phase is simulated by switching the four external fluid flow

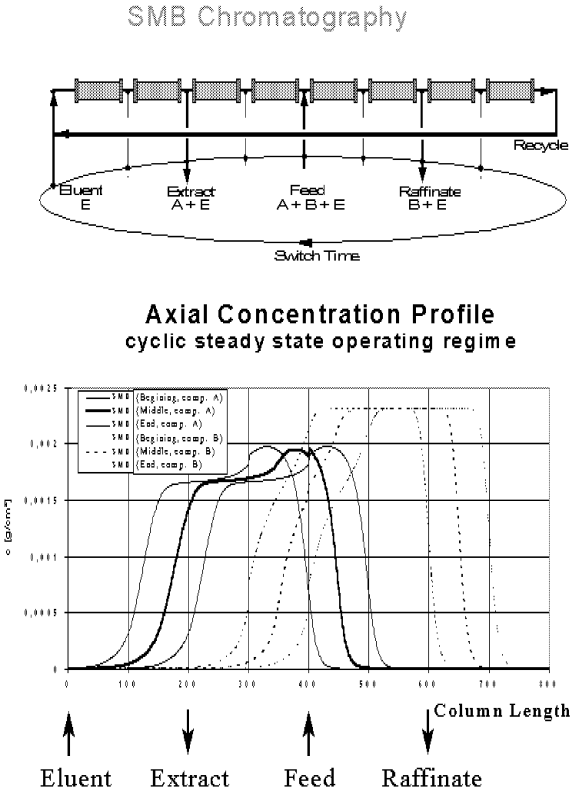

Fig. 1. System design of an SMB separation and axial concentration profile.

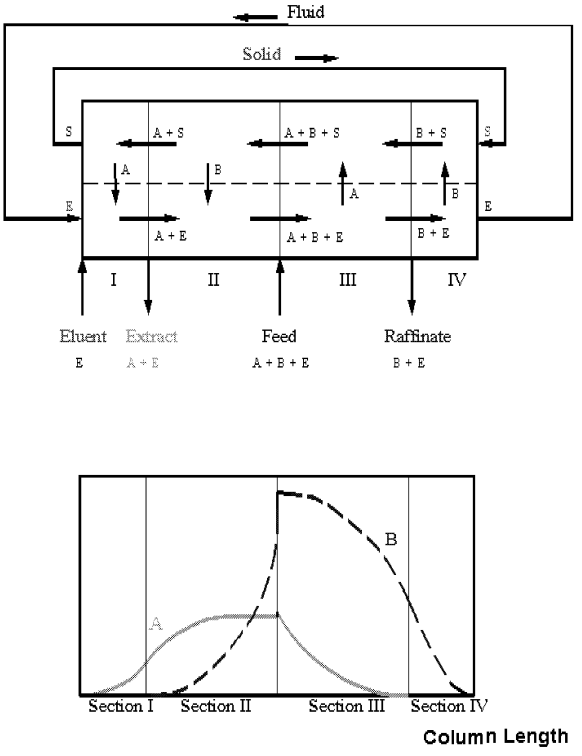

Fig. 2. Definition of zones in SMB chromatography.

streams and the recycle fluid flow stream at discrete switch time intervals, simultaneously one valve position further in the direction of the internal fluid flow stream. Due to that movement of the external streams and the internal flow-rates of the four sections the counter-current flow of the adsorbent is ''simulated'', this led to the name simulated moving bed chromatography, see Fig. 2.

To operate SMB chromatography a lot of parameters (column diameter, column length, total column number and number of columns per section, eluent, feed, raffinate, extract and recycle fluid flow and switch time interval) have to be chosen correctly. Therefore, design and process optimisation should be done by computer simulations. Totally empirical approaches are too time consuming, expensive – and in most cases even impossible [5].

From the beginning of SMB technology [3] it was designed by modelling approaches. In the last 5 years various simulation tools on the basis of differently detailed modelling approaches and simulation programs have been written and established by SMB plant vendors or by universities [6–9]. The HELP software of Novasep proved to be reliable for fast method development in the stage of product development up to pilot plant and production unit operation [6]. The operating diagram methodology based on equilibrium theory proposed by Masi et al. has been accepted by all users to be the most efficient short cut method of hand [10]. Detailed modelling approaches aim to support process optimisation of larger pilot plant and production units [7,8,11,12].

3. Enantioseparations (1992–1999)

3.1. System development

In 1992 the first successful chromatographic enantioseparation by applying the SMB principle was

published by Negawa and Shoji [57] who separated 1-phenylethanol on Chiralcel OD. Already this pioneering publication showed the superiority of SMB chromatography over batch chromatography with regard to increased productivity (61:1 SMB:batch) and decreased eluent consumption (1:87 SMB:batch). Although the principles had been shown in this publication three main obstacles had to be overcome before the SMB technology could show all its benefits: (i) the problem of a suitable instrument which allows the down-sizing from the petrochemical and sugar multi-ton dimensions; (ii) the lack of suitable chiral stationary phases; and (iii) the simulation of chromatographic separations under non-linear conditions.

The scale-down problem of SMB systems is inherent in the relation of column volume and extra-column (=dead) volume. The smaller the column scale the higher the contribution of the extra-column volume of pipes and pump heads. In 1993 the small French company Separex[1], which was founded to apply the large-scale SMB technology of IFP to the pharmaceutical and fine-chemical market, developed an SMB system (Licosep 8*200) whose column switching scheme compensated the high percentage of extra-column volume in the system [13–15]. With the asynchronic column switching regime [16] it is possible to scale down the system to a suitable size for enantioseparations. The Licosep 8*200 (Fig. 3) consisted of eight columns of 200 mm I.D. which required around 15 kg of chiral stationary phase. With a productivity of 250 g enantiomer/kg CSP d, which was a quite good productivity for the first enantioseparations in 1993, the system was able to produce 3.75 kg of each enantiomer per day. Using this system the necessary amount for a first validation batch of a pharmaceutical compound of 80–100 kg can be prepared within 1 month.

The first separation on the Licosep 8*200 was the diastereomer separation of *cis*- and *trans*-phythol a precursor of vitamin E and K on LiChroprep Si 60 [17]. The productivity was very high with about 550 g isomer/g kg stationary phase. In the framework of a Brite-Euram project enantioseparations have been set-up for 1,1′-binaphthyl-2,2′-diol on Chirasep DNBPG and D/L-threonine on Chirosolv L-Prolin [18].

Chiral stationary phases, as well as chromatography in general, have been developed to isolate compounds rather than to analyse them. The first natural polymers (cellulose-triacetate, developed by Hesse and Hagel in 1973 [19]) and synthetic polymers (poly-trisphenyl-methylmethacrylate [20] and poly-aminoacidester-acrylamides [21,22]) were used in big glass columns for the isolation of pure enantiomers of pharmacologically active substances. With the pioneering works of Pirkle et al. (brush-type CSPs) [23] and Okamoto and co-workers (cellulose- and amylose-carbamates and -esters) [24,25] which attached the chiral discriminating principle onto silica it was possible to generate columns which could be used in high-performance chromatography with an efficiency high enough to use them for analytical purposes. Using this stationary phases, different pharmacokinetic, pharmacologic and pharmacodynamic behaviour of the enantiomers of new and already used drug substances could be analysed. The first convincing results showing the different behaviour of drug enantiomers [26] promoted the field of analytical chiral HPLC and made it a million-dollar market [27]. In parallel there have been always attempts to use the new silica-based CSPs for preparative chromatography under optimised economical conditions [28,29]. The greatest drawback for this work was given by the fact that the CSPs were not commercially available or only at an extraordinary price. Only the first striking calculations and then real examples of CSPs used in SMB processes, which showed that the cost contribution of the CSP is marginal in a multi-ton separation, opened the field for large-scale enantioseparations on CSPs [30].

The simulation of preparative chromatography under non-linear conditions started about 40 years ago with the basic works of Hellferich and Klein [31] and Rhee et al. [32]. Analytical solutions of differential equation systems which describe the mass balances of chromatography on the basis of equilibrium theory had to be established. To design and to predict operating conditions more exactly it is necessary to take non-idealities of fluid dynamics

[1]In 1996 the chromatography part of Separex was integrated into the newly founded company Novasep, which is a joint venture of IFP, Paris, France and Merck KGaA, Darmstadt, Germany.

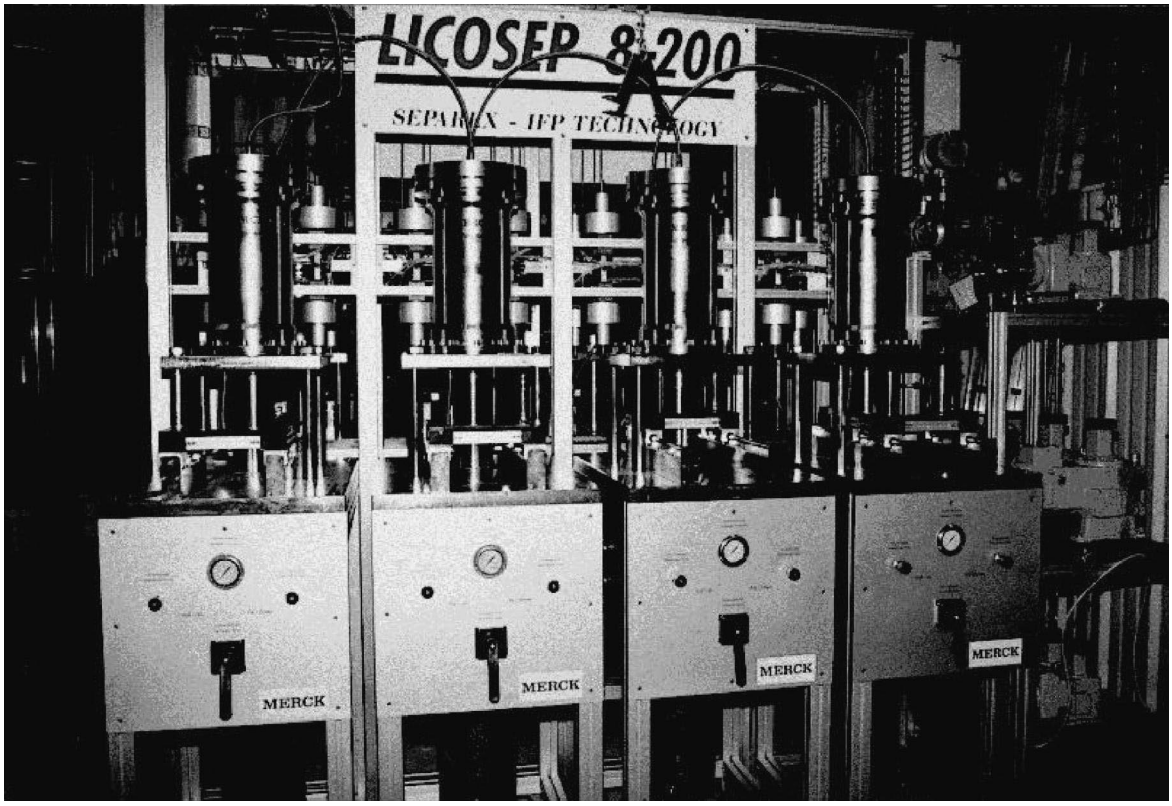

Fig. 3. Licosep 8*200 SMB system. First SMB system for separation of racemates and fine chemicals (Separex, 1993).

and mass transfer resistances into account. The resulting system of non-linear partial differential equations has been solved numerically [33]. During the last 10 years various new approaches to solve these equations more or less exactly and efficiently have been developed [7,8,34–36]. Today it is possible to calculate exact numerical results in 10 to 1% of original time.

In addition to the computational implementation and solution an exact measurement methodology of all model parameters is necessary. A methodology to determine multicomponent isotherms efficiently with acceptable efforts and less needed amounts of pure products is still a challenge to be developed further. For binary separations, e.g., enantioseparations, different approaches to determine isotherms based on the elution at characteristic point method (ECP) [15] or the perturbation method (PM) [37,38] have been established with accepted results.

3.2. Usability of CSPs for SMB separations

Today over 100 different chiral stationary phases are available on the market for analytical enantioseparation by HPLC. From these multitude of phases only a few of them have yet been used for preparative enantioseparations by SMB chromatography. There are three main factors which make a CSP especially suitable for preparative use. They all together result in the general benchmark for preparative chromatography which is the productivity, defined as amount of product separated per time unit and amount or volume of stationary phase.

3.2.1. Selectivity

The selectivity requirement for a preparative enantioseparation is different compared to an analytical separation. In the early 1990s SMB was promoted with the argument that even with very low selec-

tivities a separation can be realized, because of the lower requirements for the stationary phases in terms of selectivity and efficiency. While this is definitely true because of the counter-current nature of the SMB separation process, today most systems which are brought into production show, in the linear range, a medium selectivity for the two enantiomers between 1.3 and 2.0. On the other hand, a too high selectivity will give a negative contribution to the eluent consumption especially for the extract enantiomer. While in analytical chromatography a CSP should show good selectivities for a wide range of compounds, it could be an economic way for a large-scale preparative separation to develop or optimise a CSP for only one large-scale separation. Several approaches have been made to generate CSPs especially for a given separation problem. One approach is the reciprocal design of a chiral ligand which was developed by Pirkle and Däppen [39]: the target enantiomer is fixed on a silica support and a lot of different racemates are chromatographed over this CSP. From the racemates which show a good selectivity on this CSP one enantiomer is in a second step attached to the silica and this CSP is now used to separate the wanted enantiomers. Following this strategy special designed CSPs for 3-phenylpropionic acids, e.g., naproxen or ibuprofen, have been developed. A second approach uses the variety of different derivatives of a series of chiral selectors. In a more or less automated screening system the best selectivity for a racemate is screened. Such strategies have been applied to the poly-aminoacidester-acrylamides [40], the cellulose- or amylose-esters and -carbamates [41] and the tartardiamides [42].

There have even been approaches to predict or optimise such separations by molecular modelling [43] or correspondence analysis of chiral molecular database data [44] but up to now no prediction of chiral separation is possible. Most screening strategies are still based on trial and error approach.

Today several approaches are made to structure and optimise such screening strategies by methods of combinatorial chemistry and high throughput screening [45,46].

3.2.2. Saturation capacity (including solubility)

While selectivity is of importance in analytical chromatography it drops drastically when the linear range of the adsorption isotherm is ignored. The crucial point is therefore a certain selectivity plus a high saturation capacity.

Because the saturation capacity (which is given as g enantiomer per g sorbent) can only be high at high concentrations of compounds under separation in the mobile phase, solubility of the feed compound in the mobile phase is one of the most important points in preparative chromatography. In a strategy to optimise a preparative separation it is therefore a good starting point to determine the solubility of the feed compound in different solvents first. Then a screening of different CSPs can be performed using only the solvents with the highest solubility. Because the productivity is also determined by the feed flow-rate and the general flow-rates in the system, the high saturation capacity has not only to be shown under static but also under dynamic conditions (high flow-rates). These pre-requisites limit the use of pure natural or polymeric materials (cellulose beads or poly-aminoacidacrylamide beads) which show their high saturation capacities only at low flow-rates. An optimised preparative separation system however is operated at the highest possible pressure drop, which is reached at the highest possible flow-rates [47].

3.2.3. Mechanical and chemical stability

The economy of an SMB separation is determined by the speed of a separation and the time span it can be operated without downtime. In addition, the lifetime of the stationary phase is an important point for the economy of the separation. To operate a production system with minimum downtime the mechanical and chemical stability of the chiral stationary phase packed into preparative columns has to be high.

Today most production-scale SMB systems are operated with stainless steel columns with axial compression (dynamic or static). To pack a sorbent especially into dynamic axially compressed columns the mechanical stability of the sorbent has to be very high. A good alternative is the use of static axially compressed columns. Even semi-rigid polymeric materials can be packed into these columns with high efficiency. The vacuum packing method used to pack such columns results in very equal columns. Table 1 shows the k' value and the axial dispersion coeffi-

Table 1
k'-value and axial dispersion of an UCB-Pharma developmental substance on Chiralpak AD (20 μm particle size) packed into Selfpacker NW 50 columns[a]

Column	$\bar{K}_1$	D_L (m^2/s)
1	3.83	$2.64 \cdot 10^{-3}$
2	3.79	$2.88 \cdot 10^{-3}$
3	3.81	$2.66 \cdot 10^{-3}$
4	3.80	$2.72 \cdot 10^{-3}$
5	3.79	$2.55 \cdot 10^{-3}$
6	3.89	$2.55 \cdot 10^{-3}$
7	3.87	$2.43 \cdot 10^{-3}$
8	3.86	$2.55 \cdot 10^{-3}$
9	3.81	$2.47 \cdot 10^{-3}$
10	3.86	$2.51 \cdot 10^{-3}$
11	3.85	$2.61 \cdot 10^{-3}$
12	3.81	$2.52 \cdot 10^{-3}$
Mean	3.83	$2.59 \cdot 10^{-3}$
SD	0.04	$1.39 \cdot 10^{-4}$
RSD (%)	0.96	5.35

[a] Chromatographic conditions: column Selfpacker 200×50 mm, bed height 148 mm, Chiralpak AD (20 μm), mobile phase: ethanol, flow-rate: 148 ml/min, detection: UV at 254 nm. Explanation of terms: $\bar{K}_i = ((t_i/t_0) - 1) \cdot ((1 - \epsilon)/\epsilon)$ = capacity factor of substance i; $D_L = (Lu/N2)$ = axial dispersion (m^2/s). Parameters: t_i = retention time of substance i (min), t_0 = retention time of an unretained compound (min), L = column length (m), u = linear velocity (m/s), N = plate number.

cient for 12 columns (Selfpacker NW 50) packed with Chiralpak AD [48].

The relative standard deviation (RSD) of the relevant column parameters is less than 2%. This is an excellent value compared to series of column packings into other glass type or stainless steel columns. Table 2 shows the RSD of the k' value for the more retained compound. The columns packed with the vacuum packing technology show a very good uniformity compared to glass columns without exact compression.

A well packed column bed is a prerequisite not only for a successful SMB separation but also for a high flow-rate and long lifetime of the CSP. The downtime of the system due to alterations in column performance can be significantly decreased by packing high-performance columns.

Despite the mechanical stability of the columns chemical stability is another critical factor. The sources of chemical contaminations can stem from the eluent, the feed substance and the chiral stationary phase itself. The robustness of the optimised separation should be tested in the method development study. The influence against small additives in the eluent has to be checked too. In some cases even very small contaminations of the mobile phase with water (0.1%) can destroy the separation totally. Especially separations on cellulose- and amylose-packings are sometimes sensitive to small amounts of impurities [50]. Other problems can be created by a changing feed composition. The reason for variable feed compositions can result from different impurity profiles of the synthesised batches or impurities can be created in the feed tank. Fig. 4 shows an analytical chromatogram of a freshly prepared feed solution of EMD 53986 and a solution after 15 days of storage in a stainless steel vessel. After analysing the feed compounds it turned out that a degredation took place which only occurred when a certain batch of the feed compound which contains traces of sulfur was dissolved in ethanol (the mobile phase) and stored in a stainless steel vessel. After preparing fresh feed once a day in a glass vessel no more degradation took place.

A type of impurity which can cause serious problems are compounds which show a very strong retention on the sorbent, because they can accumulate on the columns and can destroy the selectivity after a while or decrease the saturation capacity by

Table 2
Relative standard deviation of k' values for different series of glass and stainless steel columns packed for SMB chromatography

Substance	Columns	Diameter (mm)	RSD (%) for k'_1	Ref.
EMD 53986	Superformance glass	26	13.98	[50]
EMD 77697	Superformance glass	26	8.34	[50]
Sandoz-epoxide	Superformance glass	26	3.11	[49]
EMD 53986	Selfpacker stainless steel	50	0.84	[50]
UCB developmental substance	Selfpacker stainless steel	50	1.53	[48]
Phytol	Selfpacker stainless steel	200	1.78	[17]

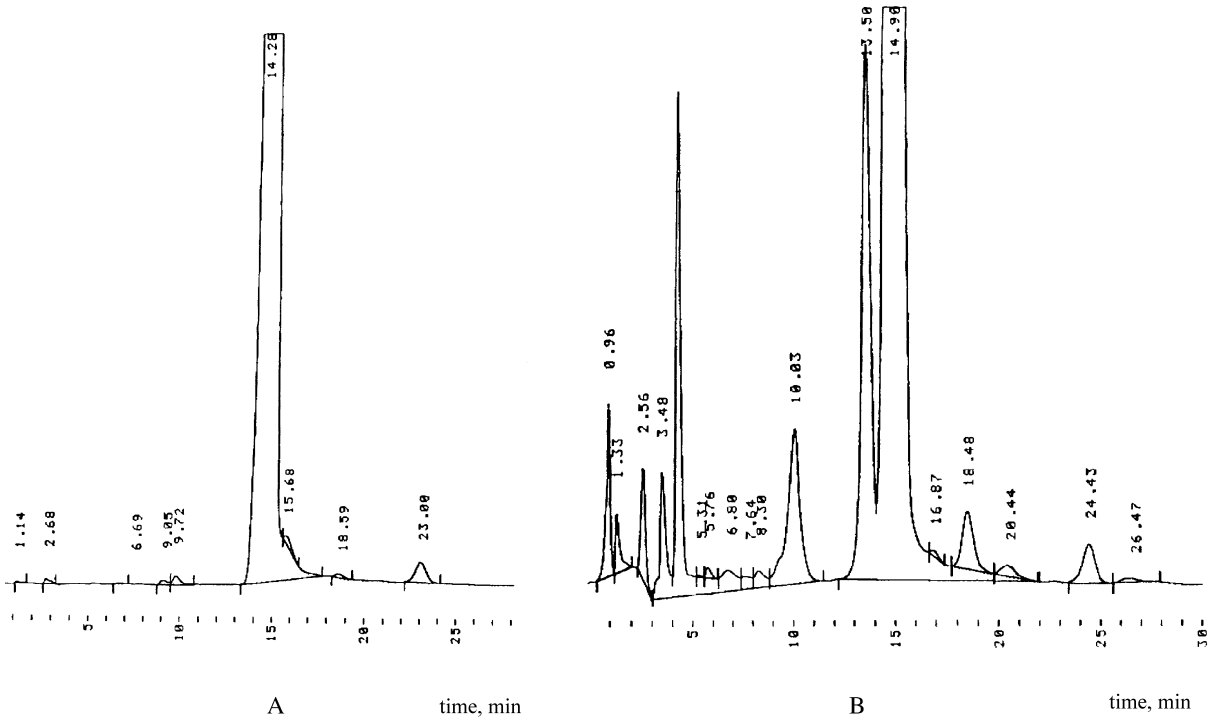

Fig. 4. Determination of chemical purity of EMD 53986-feed; concentration: 8 mg EMD 53986/ml EtOH, (a) freshly prepared solution, (b) solution after 15 days incubation at 25°C. Chromatographic conditions: column: LiChroCART 250×4 mm LiChrospher RP-select B, 5 μm, mobile phase: acetonitrile–water (35:65), flow-rate 1.0 ml/min, detection: UV at 254 nm.

occupying the adsorption sites. The feed should be carefully checked for such substances and if they are present a small pre-column with high capacity should be implemented into the system.

Furthermore, pollution of the separated compounds can be generated from leachables from the packing material (e.g., the chiral selector). The amount of leachables has to be carefully monitored when flushing the columns before use and checking the overall purity of the system. A determination of the dry residue is necessary before injecting the feed solution into the system.

3.3. Strategy to develop an SMB separation

The strategy to develop a separation system is demonstrated by the example of the enantioseparation of 1,1′-binaphthyl-2,2′-diol [51].

The method development can be divided into several steps:

- Setting of requirements
- Determination of solubility
- Selectivity screening
- Optimisation of mobile phase/stationary phase combinations (retention time, elution order, temperature)
- Separation of possible intermediates
- Modelling and simulation of separation parameters
- Pilot-scale study (optimisation of throughput, checking of robustness)

3.3.1. Setting of process requirements

The most important but sometimes also most difficult point is the correct setting of the process requirements. Before starting a method development the following points should be clarified:

(1) Desired purity (enantiopurity and chemical purity): the lower the purity requirements the higher the throughput of the system. Sometimes it is a good alternative to increase the purity in an additional crystallisation step, which is sometimes very efficient

with an enantiomerically enriched sample. It has to be decided, if only one enantiomer or both have to be obtained in pure form.

(2) The yield of the target enantiomer is another crucial point. If a low yield can be tolerated and if the undesired enantiomer can be racemised the internal concentration profile can be optimised in favour for the target enantiomer, which can be recovered than in high purity and with a good productivity.

(3) The further processing of the target enantiomer has to be taken into account.

Chromatography is by its nature a diluting process, but SMB chromatography minimises the dilution and can in some cases for the raffinate product even result in an enrichment. The further work-up procedure for the target product has to be examined, because a major contribution of the overall separation costs will come from the work-up of the product. The temperature sensitivity of the product has to be checked too because it will limit the choice of solvents further or increase the efforts for product work-up.

3.3.2. Determination of solubility

As pointed out before an economic enantioseparation process can only be achieved when the solubility of the racemate in the mobile phase is high enough. A rough estimation of the minimum solubility is in the range of about 10 g racemate/l mobile phase. Therefore every optimisation study should start with the determination of the solubility. Table 3 shows the solubility of 1,1′-binaphthyl-2,2′-diol in different solvents. This racemate is difficult to separate on a large scale because of its very limited solubilities in solvents which are very common in chiral chromatography. The solubility in alkanes and most alcohols is below 10 g/l. A high solubility can only be found in solvents like tetrahydrofuran (THF), ethylacetate and ethylmethylketone which cannot be used with some chiral stationary phases, especially not with those based on cellulose- or amylose-derivatives.

Table 3
Solubility of 1,1′-binaphthyl-2,2′-diol in different solvents at 25°C

Solvent	Solubility (mg/ml)
Methanol	68
Ethanol	31
1-Propanol	31
2-Propanol	20
Acetonitrile	78
Acetone	91
Methyl-*tert*.-butyl-ether	41
Tetrahydrofuran (THF)	238
Dichloromethane	20
Ethylacetate	254
Ethylmethylketone	249
Toluene	6
Cyclohexane	<1
Isooctane	<1
n-Heptane	<1

3.3.3. Selectivity screening

Knowing the solubility of the racemate in different solvents a screening procedure has to be run to identify an appropriate enantioselectivity. Only the solvents with high solubility were used for this screening routine. Several procedures are described in literature to automize a stationary phase screening [52] but still finding and optimising an enantioselectivity needs a lot of experience.

For 1,1′-binaphthyl-2,2′-diol selectivites on five different chiral stationary phases were known or could be found. Fig. 5 shows the separation on a tartaric acid-diamide CSP (Kromasil CHI-TBB), an amylose derivative (Chiralpak AS) and a poly-aminoacidester-acrylamide CSP.

3.3.4. Optimisation of the separation

On the five different CSPs the selectivities have been optimised with respect to retention time (target value for k_1': 2–6), solubility (>10 g/l) and selectivity (α>1.3). During this optimisation procedure seven combinations of CSP and solvent could be found which were further examined (Table 4).

3.3.5. Separation of intermediates

70% of all new drug substances in the pipeline are bearing one or more chiral centre [53]. Only 20% of those molecules are of natural origin and are manufactured by fermentation or extraction from natural sources. The majority has to be synthesised. During the synthetic route towards a new chemical entity in most cases a lot of intermediates are synthesised after introducing the chiral centre(s). All these intermediates are possible candidates for an enantioseparation. Several arguments have to be judged to

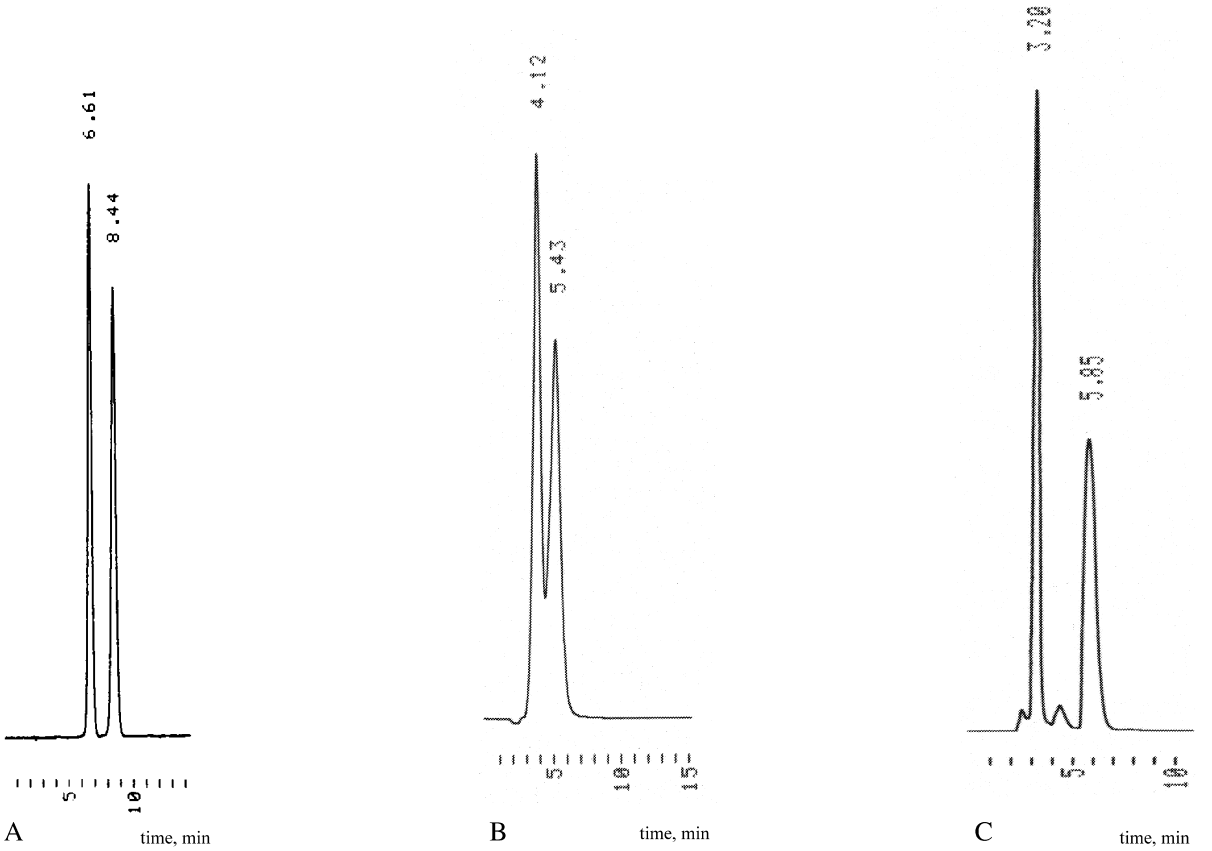

Fig. 5. Separation of 1,1′-binaphthyl-2,2′-diol on different CSPs (all columns: Hibar RT 250×4 mm, flow: 1.0 ml/min, detection: UV at 254 nm). (A) Kromasil CHI-TBB, *n*-heptane–MTBE (50:50, v/v). (B) Chiralpak AS, *n*-heptane–IPA (70:30, v/v). (C) exp. polyaminoacidester-acrylamide, 100% ethyl acetate.

find the optimum separation step. The most important argument is that a certain selectivity and solubility for the racemate is necessary as explained before. An argument for separating at the latest possible stage is given by the fact that at the last stage the amount to be separated is the lowest and therefore the chromatography step which is regarded as expensive can be kept at a smaller scale. On the other hand, if the separation is performed at one of the earlier stages only half of the substance (only one enantiomer) has to be moved further down the synthetic route which results in cutting the expenses

Table 4
Separation conditions for 1,1′-binaphthyl-2,2′-diol on different CSPs[a]

Chiral stationary phase	Mobile phase composition (%, v/v)	$k'_{1\ \text{extract compound}}$	Selectivity	Solubility (mg/ml)
Kromasil CHI-I	*n*-Heptane–MTBE (50:50)	2.96	1.59	7.5
Kromasil CHI-I	*n*-Heptane–ethylacetate (50:50)	4.22	1.36	5.5
Chiralpak AS	*n*-Heptane–IPA (70:30)	2.60	1.47	8.0
Polyacrylamide CSP	*n*-Heptane–EMK (50:50)	3.83	2.44	100
Polyacrylamide CSP	Ethylacetate (100)	2.44	2.36	250
Chiraspher-amide	THF (100)	1.01	1.36	240
Chirasep DNPG	*n*-Heptane–IPA (70:30)	5.92	1.24	8.0

[a] IPA = Isopropanol, EMK = Ethylmethylketene, THF = Tetrahydrofuran, MTBE = Methyl-tertbutyl-ether.

for all synthetic materials to the half. These arguments can only be seen as general guidelines, because every new molecule is a single case which has to be regarded individually. In an ideal combination the medicinal chemist, the process engineer and the expert in process-scale chromatography work together in a very early stage to find the optimum synthetic strategy.

3.3.6. Modelling and simulation of the process parameters

After a limited number of stationary/mobile phase combinations could be identified the process parameters for this combinations have to be simulated. As a basis for the calculations the adsorption isotherms of the compounds have to be known. Several methods to determine the isotherms in an accurate and automated way have been described in the literature [8,34,38,54]. Based on the isotherms plus several other system parameters the chromatographic behaviour in an SMB system can be simulated. Different strategies for process optimisation have been published recently [55,56].

The simulation results (column dimensions, flow-rates of the pumps, switching time, feed concentration and product stream concentrations) for the 1,1′-binaphthol-2,2′-diol-example can be found in Table 5. The simulation results are the basis for a decision which system should be used for further scale-up.

3.3.7. Pilot-scale study

The best stationary/mobile phase combination for the optimal intermediate is chosen for a pilot-scale study. Depending on the size of the final process this pilot-scale study is performed on a laboratory system (column I.D. 25 or 50 mm) or a pilot-plant system (column I.D. 100 or 200 mm). The columns have to be packed with high accuracy to achieve a stable system. The simulated data are checked by a pilot-pulse injection on the SMB system (pulse injection over all columns of the system) to determine the plate number of the system and the axial dispersion. The SMB system is then operated with the calculated parameters. Samples are withdrawn from the system, an internal concentration profile is constructed and the flow parameters are adjusted to achieve the target purities (Fig. 6).

During this pilot-scale study the SMB plant should be operated with a feed whose impurity profile is close to the final feed composition, to check the robustness of the process. All used materials should be similar to those used in the final process, e.g., technical-grade solvents, CSP from the same batch as used in the large-scale system, same materials for solvent and feed storage tanks, same principle of product work-up to make sure that all technical problems can be identified in that early process stage.

With the results from the pilot-scale study the final process can be designed. Using the scale-up formulas for process-scale chromatography it is one big advantage of chromatographic processes that scale-up is straight forward with high accuracy and reliability.

3.4. Published SMB examples

Following the strategy outlined before a lot of processes have been brought into production during the last few years. Not all of them (and for sure not the most interesting ones) are published in the

Table 5
Calculations of different SMB systems for the separation of 1,1′-binaphthyl-2,2′-diol[a]

Chiral stationary phase	Mobile phase composition (%, v/v)	Feed concentration (g racemate/l)	Productivity (g enantiomer/d kg CSP)	Eluent consumption (l/g enantiomer)
Kromasil CHI-I	*n*-Heptane–MTBE (50:50)	6	980	0.73
Kromasil CHI-I	*n*-Heptane–ethylacetate (50:50)	4.4	350	1.02
Chiralpak AS	*n*-Heptane–IPA (70:30)	6.4	433	0.77
Polyacrylamide CSP	*n*-Heptane–EMK (50:50)	40	1289	0.90
Polyacrylamide CSP	Ethylacetate (100)	78	2227	0.26
Chiraspher-amide	THF (100)	42	313	0.19
Chirasep DNPG	*n*-heptane–IPA (70:30)	5	431	3.18

[a] Calculations performed with the simulation software HELP from NOVASEP [6].

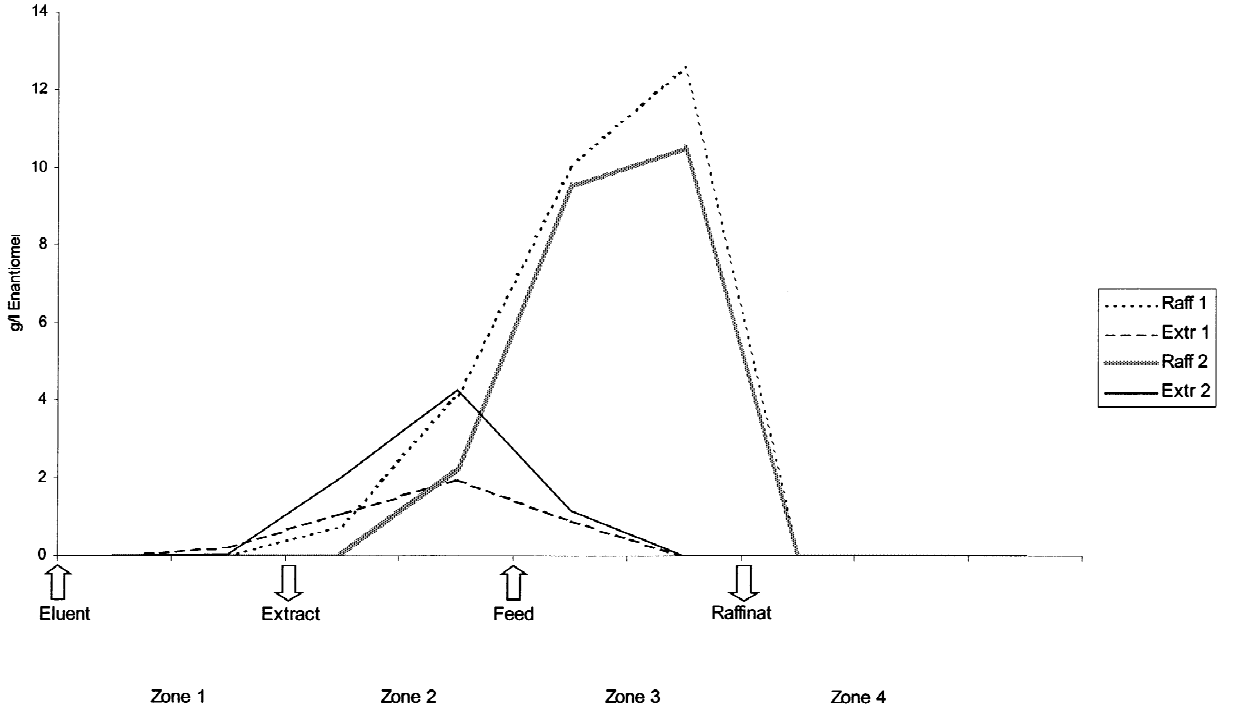

Fig. 6. Internal concentration profile. SMB system: Licosep Lab; column number and dimension: 8, 114×25 mm; stationary phase: Chiralpak AD; mobile phase: methanol; compound: Merck developmental substance; system parameters 1 (before optimisation): flow-rates: feed: 12.12 ml/min, raffinate 44.31 ml/min, eluent 122.47 ml/min, extract 90.28 ml/min, recycling 172.37 ml/min, period time 0.83 min, purities: raffinate>99%, extract 85.24%; system parameters 2 (after optimisation): flow-rates: feed: 11.52 ml/min, raffinate 45.25 ml/min, eluent 116.34 ml/min, extract 82.61 ml/min, recycling 163.75 ml/min, period time 0.96 min, purities: raffinate>99%, extract>99%.

literature, but from those who are public knowledge the importance of this new technology to the pharmaceutical industry can be seen. Table 6 summarises the SMB separations from the literature with their process properties.

The majority of SMB separations are performed on cellulose- and amylose-based CSPs (18 out of 25 published separations). As it is in analytical chiral chromatography Chiralcel OD and Chiralpak AD are the dominating ones. Other CSPs used are the polyaminoacidacrylamides (Chiraspher), microcrystalline cellulose-triacetate, dinitrobenzyl-phenylglycin (Chiralsep DNBPG) and a ligand-exchange phase (Chirosolv L-Proline). Most of the published separations were performed on a laboratory- to pilot-scale with column diameters from 16 to 200 mm I.D. The productivities of those separations are very varying due to the development stage of the project. It is a big difference if the reported data stems from the first experiences a company generates with SMB technology or from a system which is optimised to produce the single enantiomer with maximum economy. Therefore productivities between 10 g enantiomer/d kg CSP and over 1000 g/d kg can be found.

A trend can be seen in the latest published data: a series of separations with very high productivities are reported on single solvent systems (mostly alcohols) on cellulose- and amylose-based CSPs. While the separation of EMD 53986 on a Licosep 8*200 on Chiralpak AD resulted in a productivity of 374 g/d kg CSP one of our latest results in SMB separations shows a productivity of >1000 g/d kg CSP with the system Chiralcel OC and 100% ethanol [71]. Nevertheless, the argument given by Guest in 1997 is still valid:

Table 6
Summary of SMB separations reported in the literature

Substance	Company	Stationary phase e.g.	Selectivity (linear range)	Column (No., length ×diameter, mm)	Feed concentration (g racemate/l)	Feed-flow (ml/min)	Productivity (G/enantiomer/d kg CSP)	Eluent consumption (l/g enantiomer)	SMB system	Ref.
Sandoz-epoxide	Sandoz	CTA	1.28	12, 110×26	10.0	1.52	35.3	0.80	Novasep	[14]
D/L-Threonin	Novasep	Chirosol Prolin	1.60	8, 1000×26	5.0	4.20	4.01	0.99	Novasep	[15]
WEB 2170	Boehringer Ingelheim	CTA		8, 100×90	100.0	23.20	1453	0.18	Novasep	[15]
cis/*trans*-Phytol	Novasep	LiChrospher Si	1.16	8, 400×200	105.0	130.0	549	0.08	Novasep	[17]
1-Phenylethanol	Daicel	Chiralcel OD		8, 150×20	39.1	0.5	70	3.33	Laboratory made	[57]
Praziquantel	University of Singapore	CTA	3.42	4, 445×12.5	50.0	0.30	123	0.59	Laboratory made	[58]
Sandoz-epoxide	Sandoz	Chiralcel OD	1.53	12, 100×16	20.0	0.40	37	0.50	Laboratory made	[59]
1,1′-Binaphthyl-2,2′-diol	Novasep/Merck	Chirasep DNBPG	1.24	8, 80×200	0.30	157.5	2.13	41.1	Novasep	[60]
Aminoglutethimide	Ciba	Chiralcel OJ	2.07	8, 80×26	16.0	2.64	160	1.55	UOP	[30]
Guafenesin	Ciba	Chiralcel OD	2.43	16, 60×21	30.0	0.75	77	0.67	UOP	[60]
Formoterol	Ciba	Chiralcel OJ	1.43	16, 60×16	2.50	0.52	11	10.25	UOP	[60]
CGS 26214	Ciba	Chiralcel OJ		8, 80×26	5.0	2.5	47.4	4.56	UOP	[61]
EMD 53986	Merck	Polyacrylamide	2.82	8, 54×26	12.0	3.32	319	2.54	Novasep	[62]
EMD 53986	Merck	Chiralpak AD	3.1	8, 50×26	6.0	10.0	432	2.60	Novasep	[62]
Seebach-oxazolidinone	ETH Zürich/Merck	Chiraspher	1.87	8, 131×26	15.0	1.90	103	1.82	Novasep	[63]
Chloropropiophenone	UOP	Chiralcel OD	1.3					8.27	UOP	[64]
EMD 53986	Merck	Chiralpak AD	3.1	6, 79×200	7.60	416.6	375	1.48	Novasep	[65]
EMD 77697	Merck	Chiralcel OD	2.3	8, 54×26	30.0	1.90	451	1.64	Novasep	[65]
SB202026	SmithKline	Chiralpak AD	1.8	8, 105×26	20.0	4.30	258	0.65	Novasep	[66]
Tramadol	UCB Pharma	Chiralpak AD		12, 100×21.25	20.0	10.0	600	0.29	Laboratory made	[67]
Cyclosporine A	AWD	LiChrospher Si		8, 100×50	5.80	5.30	44	4.18	Novasep	[68]
Cyclosporine A	AWD	LiChrospher RP		8, 100×50	1.0	12.70	19	8.40	Novasep	[68]
DOLE	Daicel	Chiralcel OF	1.23	8, 100×100	24.0	59.3	272	0.44	Novasep	[69]
DOLE	Daicel	Chiralcel OF	1.23	16, 150×30	30.0	1.66	35	0.99	UOP	[69]
EMD 122 347	Merck	Chiralcel AD	1.47	8, 93×50	20.31	17.0	311	0.59	Novasep	[70]
Cycloalkanone	Merck	Chiralcel OC	5.26	8, 103×25	20.0	16.0	1082	0.28	Novasep	[71]
Wieland-Mieschler-Ketone	FH Nürnberg	Chiralpak AD	1.30	8, 82×26	40.0	0.63	84	0.49	Novasep	[72]
Sandoz-epoxide	University of Porto	CTA		8, 100×26	10.0	1.52	59	0.80	Novasep	[49]

SMB chromatography can be operated in a way that is advantageous to a pharmaceutical development department. With the support of appropriate software, a system can be set up to produce useful quantities of material with good purity and recovery in a very short time. The efficiency of the system compared to conventional isomer separations has an impact on the resources required for drug development by allowing correspondingly smaller amounts of precursors to be synthesised [66].

4. Future (beyond 2000)

4.1. Large-scale separation systems

The development time of SMB for enantioseparations on a laboratory- and pilot-scale took about 5 years (1992–1997). After the first examples have been shown on that scale the story of enantioseparation by SMB technology moved further on and systems in the production scale were set up. A first system was installed at UCB-Pharma in Belgium to produce multi-tons per year of a pharmaceutical compound [73,74]. The system is operated with six columns of 450 mm I.D., which gives a scale-up factor of five compared to the Licosep 200 mm system. In 1999 Novasep announced the building of an even bigger system with 800-mm columns which is installed at Aerojet (Sacramento, CA, USA) [73,74].

As pointed out above separations which advance to the production scale have to be as economic as possible. While in the earlier days SMB was used just as a tool to demonstrate that chromatographic enantioseparation works and was operated with productivities as low as 10 g enantiomer/kg CSP d, today it is one possibility of manufacturing chiral drugs which has had to compete with other techniques such as crystallisation, asymmetric synthesis and biotransformation. Therefore only SMB systems with high productivities can be brought into production. Those system are often based on a CSP with medium selectivity (α between 1.3 and 2.0) but high capacity and a single solvent which is easy to handle and reuse. With such a system set-up SMB separations with productivities higher than 1500 g/d kg are possible. Such systems show production costs below US$100 per kg enantiomer on a multi-ton level [75]. The race is on and SMB chromatography is right on the track to win it in more and more cases.

4.2. On-line detection and optimisation

Up to now SMB plants are manually controlled by determining the set points of the operating parameters volume flow-rates and switch time. The internal concentration profile or extract and raffinate specifications are analysed in order to show the status of the process performance. If any corrections to the given set of operating parameters have to be made, this is done by manual parameter setting. As chromatographic processes are very slow in process dynamics some skill and experience is needed to follow abnormal process behaviour and to predict proper corrections of the operating parameters to keep the process within the necessary purity requirements.

First approaches to control chromatographic processes were implemented about 10 years ago [76–78]. Recent research activities to develop online process control devices focus on enantioseparations with the goal to operate that processes as close to the theoretical optimal operating point as possible. By online detection methods the internal concentration profiles parameters are estimated and fitted to an underlying process model. Model-based process control alogarithms are combined with the online process optimisation to control automatically all operating parameters simultaneously in order to keep the process performance as close as possible to an optimised operating point. Instabilities of the process can be detected and corrected by the control method immediately [79]. Important precondition for these approaches are reliable and fast multi-component process analytics [80,81]. Multiwave diode array UV and chiral polarimeter detectors open up recently new perspectives for the future [82].

4.3. SMB in other modes: supercritical fluid chromatography SMB

Continuous chromatographic systems are not only limited to liquid chromatography as the separating principle they can also be applied to other modes of operation. One of the most promising ones is the use

of supercritical fluids as chromatographic driving force. Supercritical fluid chromatography (SFC) is in most cases performed with CO_2 as the mobile phase, which offers several advantages: it is cheap, non-toxic and non-flammable. The most impressing benefit of SFC-SMB is given by the fact that under SFC conditions it is possible to apply a pressure gradient throughout the certain zones of the simulated moving bed. In SFC the adsorption is depending not only on the mobile phase composition but also on the applied pressure. By varying the pressure in the zones it is therefore possible to change the adsorption strength of the mobile phase. With the highest pressure in zone I (where the desorption of the extract compound takes place) and the lowest in zone IV (where the raffinate compound is desorbed) much steeper fronts of the internal concentration profile can be achieved. As a consequence the feed introduction can be increased and the total productivity is increased. Clavier et al. showed this principle for the separation of fatty acids [83]. They increased the productivity from 33 g/d kg SP to 122 g/d kg SP by applying a pressure gradient from 174 bar (zone I) to 138 bar (zone IV). The most serious drawback for SFC is the limited solubility of the feed compounds in the mobile phase. Only lipophilic compounds show a high solubility in pure CO_2 which is in most cases used as the mobile phase. By adding different modifiers, e.g., alcohols, the solubility can be influenced but still the type of compounds which are separable is limited. So most examples shown on SFC-SMB system separated lipophilic compounds such as fatty acids [83,84] and *cis*/*trans*-phytol [85]. As for some chiral stationary phases the superior behaviour under SFC conditions was shown [86] the use of SFC-SMB for enantioseparations can be expected in the near future.

4.4. New stationary phase developments

New developments with regard to chiral stationary phases can be expected from two different directions: new or better chiral modifications and new basic materials as supports for the chiral modifications.

4.4.1. New chiral modifications

New chiral modifications are introduced with regularity into the market. While most of them are first used as analytical CSPs they are applied to preparative chromatography if they show a sufficient saturation capacity and stability. The latest chiral phases which have been made commercially available and used for preparative chromatography or will be used in the future are the tartardiamide derivatives (Kromasil CHI) [87], chiral phases based on natural occurring antibiotics, e.g., vancomycin and teicoplanin [88], and brush-type phases based on diphenylethanediame (DPEDA, ULMO) [89].

Not only new phases are necessary also more stable phases with known selectors are developed for preparative chromatography. There have been numerous approaches to improve the most commonly used cellulose and amylose derivatives by binding the selector onto the support. Okamoto et al. bound the chiral selectors via diisocyanate [90], as did the group of Oliveros et al. via 10-undecenyl-spacers [91]. Enomoto et al. also developed a strategy of enzymatic in-situ synthesis of the chiral selector on the support by using a potato phosporylase [92]. A very recent development is the cross-linking of the cellulose and amylose polymers which are adsorbed on the silica support by radical polymerisation [93]. The CSPs prepared by Francotte can be operated even with dichloromethane as the mobile phase which offers for a wide variety of different drug molecules a good solubility and therefore a high productivity. The technology has recently been licensed to Daicel and CSPs will be made commercially available in future [94]. But nevertheless all the bonded cellulose and amylose phases show a different selectivity compared to the known CSPs with adsorbed selectors.

4.4.2. New basic materials

Not only improvements in chiral selector synthesis can be expected, also the basic support on which the selector is attached, can be optimised. To maximise the economy of a preparative separation process the feed and the overall system flow-rate has to be maximised. This can only be achieved unless the total system pressure reaches a certain limit. On the other hand when chiral phases based on coarser particles are used the overall system efficiency decreases and therefore the productivity is lower. Several approaches are known in literature to break this vicious circle and to develop chiral phases with a low back pressure but still good efficiency. Lindner has developed selectors based on quinine derivatives

which have an extraordinary high selectivity (α between 15 and 50) for amino acid derivatives. Those selectors can be used as supported liquid membranes which promise to have a very high productivity because of the operating principle as a chiral filtration step [95].

Another approach uses monolithic silica materials with double pore structure [96]. The mesopores generate a high surface area which results in a high saturation capacity. The macropores, which can be independently controlled in the range from 1 to 6 μm, allow a very high flow-rate and give good adsorption characteristics due to short diffusion distances. First examples of SMB separations using very high flow-rates have been shown for this sorbents. The separation of χ- and δ-tocopherol could be achieved with a productivity of 1500 g product/d l column volume [97].

5. Conclusions

Over the last 6 years chromatographic enantioseparations by SMB chromatography have gained a tremendous interest. They have shown for the first time that preparative chromatography, which had the stigma of being extremely expensive and therefore to be avoided wherever possible, can be the most economic way to produce pure enantiomers. From the first laboratory- and pilot-plant-scale experiments which today have been considered and carried out in numerous pharmaceutical companies now the first multi-ton production systems are under full-scale operation. New developments in system design and chiral stationary phase design will result in even better economics so that chiral chromatography on SMB systems will be one of the most powerful tools in manufacturing enantiomerically pure drugs.

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