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IMPACT OF A MODIFICATION IN THE PRODUCTION PROCESS OF AN AMYLOSE DERIVED STATIONARY PHASE ON THE SMB SEPARATION OF A PHARMACEUTICAL INTERMEDIATE

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ABSTRACT

The propensity of helical homochiral polymers derived from amylose to resolve optical isomers is being exploited intensively for the production of active pharmaceutical ingredients (APIs), using either high performance lipid chromatography (HPLC) or the simulated moving bed (SMB) principle. Several lots of an amylose derived stationary phase, Chiralpak® AS™, and its successor, Chiralpak® AS-V™, were compared by pulse injections with increasing amounts of various racemates in order to determine the loading capacity and the competitive adsorption isotherms. Software simulations allowed to assess the possible effects due to the observed variations between the chiral stationary phases (CSPs) on the performance of a pilot SMB unit.

The obtained results were verified by two multi-kilogram separations performed under current Good Manufacturing Practice (cGMP) guidelines employing the two CSPs. The results

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of the two production runs are discussed in the light of the recently introduced "triangle theory," which allows to account for the overload conditions prevailing under preparative chromatographic conditions and to predict optimal operating conditions.

Under optimized conditions the enantiomer separation of 1.4 kg racemate/kg stationary phase per day with purities >99.6% for the target enantiomer has been achieved.

Key Words: Simulated moving bed chromatography; Preparative chromatography; Chiral separation; Enantiomer discrimination; Amylose carbamate

INTRODUCTION

Single enantiomers of active pharmaceutical ingredients (APIs) often provide unique and improved properties compared to the racemic mixture and can possess profoundly different physiological activities (1). In addition, regulatory pressures regarding racemic compounds (2) force companies to look into ways and means of producing pure enantiomers economically. Various strategies for either the enantioselective synthesis or the chiral resolution are used in the pharmaceutical industry (cf. Fig. 1).

In the recent years, simulated moving bed (SMB) chromatography on chiral stationary phases (CSPs), especially helical homochiral polymers derived from naturally occurring macromolecules, such as cellulose or amylose, has become an essential tool for the chromatographic resolution of racemates on a preparative scale (3), and has shown in some cases distinct advantages over synthetic routes involving chiral or prochiral precursors and "classical" resolutions.

Efficient criteria for the optimal design of SMB systems have been developed, which allow to account for the nonlinear character of the involved adsorption equilibria and to optimize the productivity per kg CSP easily (4). Following the so-called "triangle theory," constrains on these criteria have been derived, which allows the complete separation of a binary mixture following the

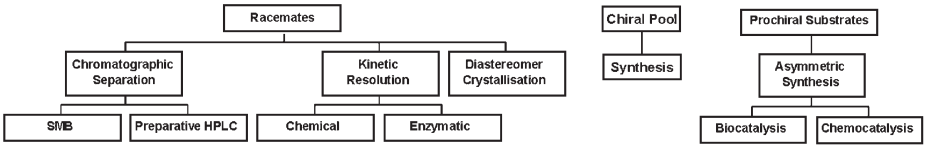


Figure 1. Methods for obtaining optically active APIs.

Langmuir and the modified Langmuir isotherm (5) and the most general case of a bi-Langmuir multi-component adsorption isotherm (6).

Among the commercially available CSPs various polysaccharide derivatives have proven their great versatility for enantiomer separations in analytical chromatography (7,8) and for preparative separations using batch chromatography (9) and SMB (10,11). The impact of enantioselective chromatography on the development of pharmaceuticals has been reviewed recently by Francotte (12,13) and others (14,15). Recently, amylose carbamates coated on silica particles have gained increasing importance for the separation of racemates not resolved on microcrystalline cellulose triacetate (16), cellulose tribenzoate (17), or cellulose carbamates (18). These amylose-derived CSPs allow the separation of amounts typically ranging from 0.2 to 2 kg racemate/kg CSP per day (19) through SMB chromatography. For industrial applications in the pharmaceutical field very narrow specifications for the CSPs and the products separated on them have to be met and the consequences of nonsatisfactory separations or production delays can have considerable impact on the registration process and the time to market for an API and the market supply.

Thus, the recent modification in the production process of the CSP Chiralpak AS (now marketed in Europe under the name Chiralpak AS-V) made it necessary to study the properties of this stationary phase in detail and to compare its properties with previous production lots of this CSP.

EXPERIMENTAL

Analytical Chromatography

Analysis of the product streams and overload measurements were carried out on five 250 × 4.6 mm I.D. columns, containing Chiralpak AS and Chiralpak AS-V with a pore size of 1500 Å and a nominal particle size of 20 μm. Columns 1 and 2 were packed with Chiralpak AS material provided by Chiral Technologies, Strassbourg, France by Grom (Herrenberg, Germany); columns 3, 4, and 5 were obtained from Chiral Technologies, Strasbourg, France. Columns 1–3 contained CSP with the lot No. HB001, column 4 contained material with the Lot No. VIK001, column 5 was packed with Lot No. AC002. All columns had a bed porosity of $\varepsilon^* = 0.4$ (determined via injection of a nonretained compound, e.g., *n*-hexane). Injection loop volume and extra-column dead volume were 250 and 220 μL, respectively. The mobile phase consisted of *n*-hexane/isopropanol (99:1; v:v, both high performance lipid chromatography (HPLC) grade, J. T. Baker, Deventer, The Netherlands) for the separation of *trans*-stilbene oxide (Aldrich, Buchs, Switzerland), *n*-hexane/ethanol (95:5; v:v, both HPLC grade, J. T. Baker, Deventer, The Netherlands) for the separation of 1,1′-bi-2-naphthol (Aldrich,

Buchs, Switzerland) and acetonitrile (HPLC grade, J. T. Baker, Deventer, The Netherlands) for the separation of Tröger's Base (Aldrich, Buchs, Switzerland) and compound A (synthesized in-house) at a flow rate of 1.00 mL/min. The operating temperature was 30°C for all measurements. All chromatograms were measured on an HP 1090 system (Hewlett-Packard, Basel, Switzerland) equipped with an 6 mm flow cell and connected to a Kajak XA HP Chemstation (Hewlett-Packard, Basel, Switzerland). The DAD UV-detector was operated simultaneously at 234, 254, 280, and 330 nm.

Simulated Moving Bed Separation

Compound A, synthesized under cGMP guidelines in our laboratories, was separated employing pure acetonitrile (Schweizerhall, Basel, Switzerland) as eluent (cf. "Analysis of the Preparative Separation"). The bulk stationary phases were Chiralpak AS and Chiralpak AS-V (20 μm) purchased from Chiral Technologies (Strassbourg, France). Analytical assay of extract and raffinate stream was performed without dilution employing the same stationary phase and eluent as for the preparative separation.

Column Packing and Testing

Bulk Chiralpak AS and Chiralpak AS-V were packed into eight NW-50 columns purchased from Merck (Darmstadt, Germany). The bed length of the eight columns used ranged from 107 to 114 mm; the I.D. of the columns was 48 mm. Each column contained exactly 110.00 g dry mass of the stationary phase and gave an averaged bed length of about 107 mm. All columns were tested with a preparative HPLC system provided by Knauer (Berlin, Germany), which consisted out of a K-1800 pump with a 1000 mL/min pump head, a HPLC-Box and a K-2500 UV detector. In the case of Chiralpak AS, the retention times for compound A, measured at a flow rate of 50 mL/min, were (averaged over the eight columns) 3.074 $\pm$ 0.076 and 3.590 $\pm$ 0.114 min S.D.; for Chiralpak AS-V, the retention times for compound A, measured at an flow rate of 50 mL/min, were (averaged over the eight columns) 3.478 $\pm$ 0.029 and 4.391 $\pm$ 0.096 min S.D.

Determination of Adsorption Isotherms

Five analytical HPLC columns (cf. "Analytical Chromatography" section), containing various lots of Chiralpak AS and Chiralpak AS-V, were

installed into a HP 1090 system equipped with a Jasco CO-1560 oven (Omnilab, Mettmenstetten, Switzerland) and thermostated at 30 ± 0.1°C, the operating temperature of the SMB unit. Racemates of 1,1′-bi-2-naphthol, *trans*-stilbene oxide, Tröger's base, and compound A were injected at increasing concentrations (cf. Table 4). The obtained data was entered into the NOVASEP software (cf. "Simulated Moving Bed Hard- and Software") to determine the modified competitive Langmuir adsorption isotherms and SMB operating conditions.

Simulated Moving Bed Unit

A Licosep 10 × 50, produced by NOVASEP (Vandœuvre les Nancy, France) was used for the experiments. A detailed description of the unit has been given recently (20). It was equipped with eight NW-50 (nonjacketed) columns produced by Merck (Darmstadt, Germany) with a variable bed length ranging from 15 mm up to 119 mm and an inner diameter of 48 mm that can be self-packed easily. A schematic overview of the system components is given in Fig. 2.

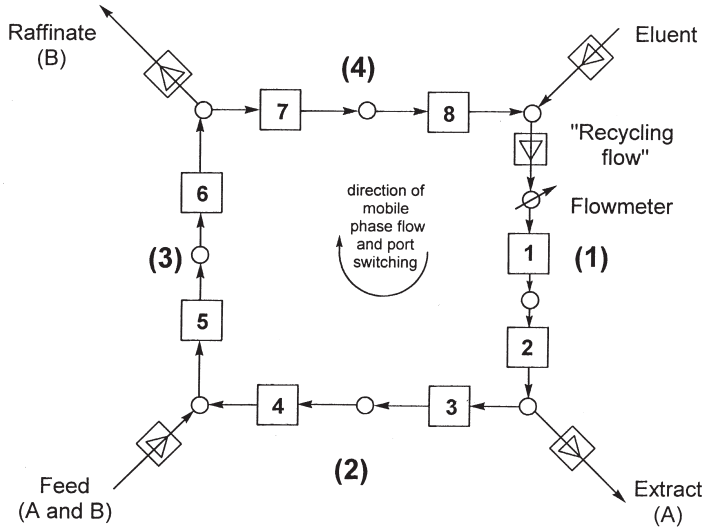


Figure 2. Schematic flow diagram of the Licosep 10 × 50 unit with a 2-2-2-2 column configuration in the first period of a cycle. Flow direction and location of the five pumps are indicated by triangles.

Simulated Moving Bed Hard- and Software

The Licosep 10 × 50 SMB unit from NOVASEP (Vandœuvre les Nancy, France) is controlled by a central system composed of a Siemens PLC (type S7-300) and a PC (P200, Siemens-Nixdorf, Stuttgart, Germany) as the user interface. The supervision software works under DOS and allows the full control of the unit parameters (valves, pumps, flow rates, pressures) when the unit is running or under test. All relevant parameters and data are continuously stored in files for quality control. The software allows an easy access to real time curves (flow rates, temperature, pressure). All measurements are transmitted from the Licosep 10 × 50 through a control board, containing the PLC and all required interfaces and supplies.

A simulation software called "softSMB" is supplied with the system. It works under Windows 95 and allows to find a competitive adsorption isotherm based on overload injections and to optimize the operating parameters before starting the unit itself.

BACKGROUND

Standard SMB units contain four discrete sections (cf. Fig. 2), each composed of at least one chromatographic column. The time between two shifts of the injection and collection points after a predefined period is called switch time, t^*. It must be understood that a column can appear in any of these sections (1–4), depending on the time at which it is observed. The duration of t^* is determined by the flow rate of the solid phase Q_s and its volume V_s in a hypothetical true moving bed (TMB) unit:

$$t^* = \frac{V_s}{Q_s} \tag{1}$$

The key to the successful operation of the simulated moving bed are the four internal volumetric flow rates, $Q_j, j = 1, \ldots 4$, in these sections, which have to be controlled rigorously. The internal flow rates are related to the four external fluid streams through simple mass-balance relations:

$$Q_1 = Q_{\text{Eluent}} + Q_4 \tag{2}$$

$$Q_2 = Q_1 - Q_{\text{Ex}} \tag{3}$$

$$Q_3 = Q_2 + Q_{\text{Feed}} \tag{4}$$

$$Q_4 = Q_3 - Q_{\text{Ra}} \tag{5}$$

Whenever four of the flow rates are given (one of them being an internal flow rate), the other four are defined also. Together with the overall void fraction of the columns, ε^*, the switch time t^*, and the single column volume V the internal flow rates determine the so-called flow-rate ratios, m_j, which are defined as the ratio of the net fluid flow rate over the solid phase flow rate in each of the four sections of the TMB unit. Exploiting the equivalence between SMB and TMB (21) one obtains:

$$m_j = \frac{\text{net fluid flow rate}}{\text{solid flow rate}} = \frac{Q_j t^* - V\varepsilon^*}{V(1-\varepsilon^*)} \quad (j = 1, \ldots 4) \tag{6}$$

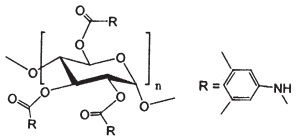
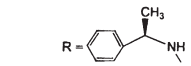
Based on these flow rate ratios m_j, the experimental performances of SMBs can be designed properly, interpreted, and compared following a recently presented approach, which leads to criteria for the choice of optimal and robust operating conditions of such units (4). It should be mentioned that already small changes in the internal flow rates result in deviations from the optimal operating conditions (described by a set of four m_j values and t^*) and cause impure extract and raffinate streams. Another *conditio sine qua non* for optimal operation are minimized dead volumes and a set of very similar and stable chromatographic columns.

RESULTS AND DISCUSSION

Natural products such as cellulose and amylose have proved to be of great versatility for enantiomer separation. Though poor chiral selectors in their native state, they become highly effective when their hydroxyl functions are derivatized, particularly with aromatic moieties through ester or carbamate linking (7,8), and they are properly coated on silica supports (cf. Table 1 for examples). These materials are composed of small chiral units regularly repeating along the polymeric chain; hence the density of active sites capable of chiral recognition is very high and results in a high loading capacity.

Table 1 lists the functional groups of two of the most used CSPs derived from amylose, together with compatible solvent mixtures. The *tris*(3,5-dimethylphenyl carbamate) derivative of amylose has been commercialized under the name Chiralpak AD, the *tris*[(*S*)-methylbenzylcarbamate] has been named Chiralpak AS. The latter derivative, as well as providing polar, polarizable sites, also contributes another chiral center to improve enantioselectivity. It should be noted that the (*S*)-configured derivative provides often a greater chiral recognition ability than the (*R*,*S*) and the (*R*)-derivative (22); however, there are exceptions from that rule for the racemic selector (23–25). The chiral polymers are not attached to the support covalently, but rather are coated on silanized, wide-pore silica gel.

Table 1. The Chiral Stationary Phases Chiralpak AD and Chiralpak AS

Structure of CSP	Chiral Selector	Compatible Solvents[a]	Trade Name
	Amylose *tris*(3,5-dimethyl-phenylcarbamate)	Hexane/ethanol (100/0–0/100)	Chiralpak AD
	Amylose *tris*[(*S*)-methyl-benzylcarbamate]	Hexane/2-propanol (100/0–0/100)	Chiralpak AS or AS-V

[a] Examples.

Chiralpak AS is one of the newest members of the CSP family commercialized by Daicel, Japan, first described by Okamoto and Hiroshi 10 years ago (26). It has been used, e.g., for the HPLC separation of methyl jasmonat (26) and amide conjugates of jasmonic acid (27), 1-azabicyclo[2.2.1] heptane-3-one, and pyrrolidin derivatives (28), protected α-amino acids (29), and chiral selenoxides (30) and has been employed for supercritical fluid chromatography (31). Also the semi-preparative purification of benzofuroxane derivatives (32) and the isolation of an API on a kilogram scale (9) have been described. The mechanisms governing the chiral discrimination have been studied in detail by Roussel and co-workers (33,34) for atropisomers and by Okamoto and Kaida for various other systems (35).

An example for changes in retention behavior depending on the manufacturing process of the CSP is the separation of 1,1′-bi-2-naphthol on Chiralpak AS and the recently introduced successor of that phase, Chiralpak AS-V. As can be seen in Fig. 3, the two enantiomers of 1,1′-bi-2-naphthol are hardly resolved (cf. Fig. 3, top; $\alpha = 1.08$; $R_s = 0.49$) on Chiralpak AS, whereas on Chiralpak AS-V (cf. Fig. 3, bottom; $\alpha = 1.28$; $R_s = 1.42$) a satisfactory separation can be obtained.

This unexpected observation prompted us to characterize some of the batches of this CSPs used in our laboratories in more detail, giving special regard to the modified retention behavior due to the new production process for the AS phase.

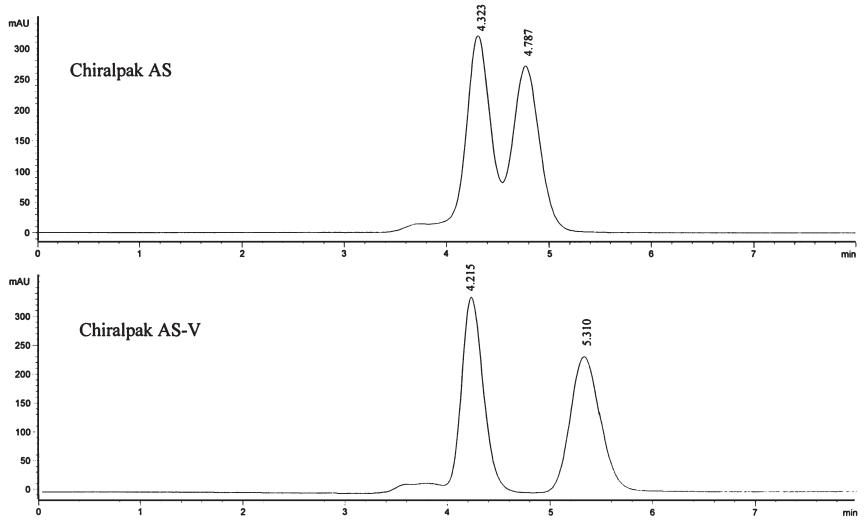


Figure 3. Separation of 1,1′-bi-2-naphthol on Chiralpak AS, column dimensions: 250 × 4.6 mm I.D.; $T = 30°C$, particle size: 20 μm, eluent: *n*-hexane/ethanol (95:5, v:v), detection: 250 nm, flow rate: 1.00 mL/min; top: Chiralpak AS, bottom: Chiralpak AS-V.

Determination of Competitive Adsorption Isotherms

In order to establish differences in the retention behavior of the two types of CSP under investigation and their concentration dependent adsorption properties three test substances, 1,1′-bi-2-naphthol, Tröger's base and *trans*-stilbene oxide, and a racemate synthesized in our laboratories (compound A, structure cannot be disclosed due to proprietary reasons) were injected in analytical amounts on four HPLC columns containing Chiralpak AS and AS-V (cf. Table 4 and "Analytical Chromatography" section). The four compounds show significant differences in the origin of their chirality. Tröger's base has a chiral nitrogen atom surrounded by three different substituents and a lone electron pair, 1,1′-bi-2-naphthol is a case of so-called atropisomerism, *trans*-stilbene oxide possesses an oxiran ring where the different orientation of two phenyl groups to the planar ring gives raise to chirality, whereas compound A has a tetrahedral carbon atom with four different substituents at the chiral center.

Table 2 gives an overview of the analytical pulse injections performed on the five different columns. Columns 1 and 2 were packed by a contract laboratory with bulk Chiralpak AS material, columns 3, 4, and 5 were obtained directly from the manufacturer of the CSP and contained Chiralpak AS and AS-V, respectively. Columns 1–4 were used only for the separation of the four test compounds, whereas column 5 was used for several different racemate resolutions before the four test compounds were injected.

Quite surprisingly, the differences between the various columns are very pronounced, reaching from base-line separation for all four compounds to almost no separation. The compound least affected by the properties of the various lots of the CSP is *trans*-stilbene oxide, which was resolved by using a small amount of isopropanol (1%, v:v) added to *n*-hexane as eluent. 1,1′-bi-2-naphthol was separated using an eluent consisting of *n*-hexane/ethanol (95:5, v:v). Tröger's base and compound A were resolved using pure acetonitrile as eluent (1,1′-bi-2-naphthol and *trans*-stilbene oxide could not be separated using pure acetonitrile). Columns 1 and 2 show a considerable fronting of the peaks, probably due to an unsuccessful packing procedure. However, the enantioselectivity for *trans*-stilbene oxide and Tröger's base is reasonably well. Column 2 shows acceptable enantioselectivity for compound A, Tröger's base, and 1,1′-bi-2-naphthol, but not for *trans*-stilbene oxide. Column 3 gives a poor resolution for *trans*-stilbene oxide and 1,1′-bi-2-naphthol, and a partial resolution for Tröger's base and compound A. Column 4 excels columns 1–3 dramatically, all four compounds are baseline-resolved and the eluted enantiomers show symmetrical peak shapes. Column 5 shows a similar behavior as column 4.

Summarizing, the best resolution and chromatographic behavior for all compounds could be obtained on columns packed with Chiralpak AS-V.

Table 2. Separation of Compound A, Tröger's Base, 1,1′-bi-2-Naphthol and *trans*-Stilbene Oxide on Chiralpak AS Columns

Compound	Column No.	CSP	t_{R1}	t_{R2}	α	R_S
Compound A	1	AS	3.493	3.835	1.18	0.63
	2	AS	3.538	3.972	1.23	0.72
	3	AS	3.604	4.03	1.21	0.84
	4	AS-V	3.590	4.45	1.44	1.74
	5	AS-V	3.529	4.263	1.39	1.39
Tröger's base	1	AS	4.632	5.402	1.25	1.04
	2	AS	4.187	4.574	1.15	0.67
	3	AS	4.313	4.766	1.17	0.97
	4	AS-V	4.221	5.301	1.42	2.19
	5	AS-V	4.045	5.316	1.51	2.39
1,1′-bi-2-naphthol	1	AS	18.657	20.806	1.12	0.59
	2	AS	15.547	18.404	1.20	0.88
	3	AS	18.770	20.138	1.07	0.49
	4	AS-V	17.327	21.765	1.28	1.41
	5	AS-V	17.206	22.006	1.30	1.52
trans-Stilbene oxide	1	AS	6.053	6.697	1.14	0.91
	2	AS	6.224	6.672	1.09	0.64
	3	AS	4.819	5.094	1.08	0.57
	4	AS-V	5.079	5.973	1.26	1.60
	5	AS-V	5.751	7.225	1.35	1.85

Column dimensions: 250×4.6 mm I.D.; $T = 30°C$, particle size: $20\,\mu$m, flow rate: 1.00 mL/min, eluents and detection wavelength see experimental part.

Among the most important mechanisms governing preparative and simulated moving bed chromatographic applications is the thermodynamic equilibrium of the separation system under overloaded conditions. Therefore, increasing amounts and volumes of the four racemates were injected on the five columns containing Chiralpak AS and AS-V and the resulting retention times were measured. As an example the chromatograms obtained for the resolution of compound A on column 4 are shown in Table 3 and Fig. 4.

Based on pulse injections on the five HPLC columns filled with Chiralpak AS and Chiralpak AS-V with increasing amounts of the four racemates (cf. Fig. 4 and Table 3) the NOVASEP software package "softSMB" allows to correlate through a curve-fitting procedure the equilibrium experimental results with a postulated modified Langmuir competitive isotherm, which takes the form:

$$n_i = \lambda c_i + \frac{\bar{N}_i K_i c_i}{1 + \sum_{k=1}^{2} K_k c_k} \tag{7}$$

Table 3. Retention Times of Analytical and Overloaded Injections of Compound A

Experiment No.	Concentration (g/L)	Injected Volume (μL)	Rt_1 (min)	Rt_1 (min)
1	Analytical	10	3.590	4.450
2	0.5	10	3.583	4.448
3	1.0	10	3.580	4.440
4	5.0	10	3.578	4.433
5	10.0	10	3.576	4.427
6	20.0	10	3.573	4.421
7	30.0	10	3.572	4.416
8	30.0	50	3.570	4.410
9	30.0	100	3.565	4.405

Flow rate: 1.00 mL/min; $T = 30$°C; detection at 254 nm.

In this equation n_i and c_i are the adsorbed and the fluid phase concentration, respectively; λ is a dimensionless coefficient; K_i is the equilibrium constant of the ith component, which accounts for the overload effects; the upper limit of n_i is given by the saturation capacity $\bar{N}_i$.

This isotherm is often applied to the modeling of competitive adsorption behavior of racemic mixtures taking into account the adsorption on a

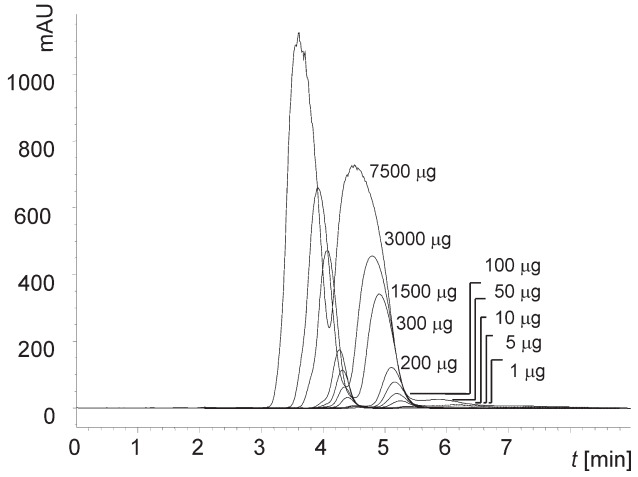


Figure 4. Overload injections of compound A on Chiralpak AS-V; column 4: 250 × 4.6 mm I.D., 20 μm; mobile phase: acetonitrile; detection: 254 nm; $T = 30$°C.

heterogeneous surface that consists of two different types of adsorption sites, e.g., a nonchiral interaction (linear term) and an enantioselective discrimination site with different affinity for the two chiral substances (Langmuirian term). The assumption that the non-chiral adsorption sites cannot be saturated is true only for low concentrations; at high concentrations, their adsorption behavior will also become dependent on the mobile phase concentration in a nonlinear fashion. Therefore, it can be assumed that the parameters obtained will provide only a rough description of the true competitive behavior of the two compounds. The results of the calculations are summarized in Table 4.

The retention times for the selected test compounds could be predicted with these isotherms surprisingly well; however, some deviations (± 5%) were observed, especially for the separation of 1,1′-bi-2-naphthol (± 10%). It should be noted that the isotherm parameters do not represent actual thermodynamic adsorption mechanisms, but should be considered as a working hypothesis that

Table 4. Competitive Adsorption Isotherm Parameters of Chiralpak AS and Chiralpak AS-V

Compound	Column No.	λ_1	N_1K_1	K_1	N_2K_2	K_2	$\bar{N}_i$
Compound A	1	0.7	0.033	0.0037	0.173	0.0192	9
	2	0.7	0.052	0.0021	0.227	0.0094	24
	3	0.7	0.079	0.0026	0.252	0.0084	30
	4	0.7	0.073	0.00073	0.418	0.0041	100
	5	0.7	0.048	0.00048	0.343	0.0034	100
Tröger's base	1	1.0	0.194	0.0176	0.503	0.0457	11
	2	0.9	0.114	0.0126	0.270	0.0300	9
	3	1.0	0.069	0.0098	0.255	0.0364	7
	4	0.9	0.0058	0.0002	0.563	0.0255	22
	5	0.9	0.0063	0.005	0.566	0.047	12
1,1′-bi-2-naphthol	1	—	—	—	—	—	—
	2	—	—	—	—	—	—
	3	—	—	—	—	—	—
	4	1.7	5.047	0.100	6.816	0.136	50
	5	3.75	2.485	0.024	4.410	0.044	100
trans-stilbene oxide	1	1.0	0.844	0.422	1.109	0.5545	2
	2	1.7	0.141	0.047	0.335	0.111	3
	3	1.2	0.068	0.0052	0.179	0.0137	13
	4	1.0	0.375	0.0197	0.734	0.0386	19
	5	1.3	0.340	0.068	0.931	0.1862	5

has to be refined when there is need for a further elucidation of the adsorption behavior under investigation.

In all cases the isotherms for Chiralpak AS-V (columns 4 and 5) are the most favorable-as can be seen when comparing the saturation capacity $\bar{N}_i$ for the separation of Tröger's base, *trans*-stilbene oxide and compound A. Surprisingly, for compound A the calculated saturation capacity is even in the range observed normally only for bulk polymeric chiral phases, such as cellulose triacetate (25–75 g/L) (36). The calculations imply that for the separation of compound A the material in columns 2 and 3 is almost identical and the material in column 1 is inferior for this separation, while columns 4 and 5 are almost identical. For the separation of Tröger's base columns 1, 2, and 3 can be considered as very similar and no significant differences have to be expected for an up-scaling of that separation. Columns 4 and 5 show some differences in regard to the loading capacity. The chromatographic resolution of 1,1′-bi-2-naphthol on a larger scale seems only to be possible with Chiralpak AS-V, which showed more than a partial resolution of the two enantiomers. However, the long retention times have to be considered as not very promising and the inaccuracy of the predicted retention times (columns 1–3) seems to indicate that the isotherm parameters derived for the separation of that compound on Chiralpak AS (data not given) cannot be described satisfactorily with the modified Langmuir isotherm. In the case of *trans*-stilbene oxide several differences can be observed. The results for columns 1 and 2 are prohibitive for any attempt to separate significant amounts of the compound. The material in columns 3 and 4, however, would allow satisfactory productivities in the SMB mode. Column 5 shows a drastic increase in the retention time of the second eluted enantiomer, but the calculated adsorption isotherm implies an inferior loading capacity in comparison to columns 3 and 4. These differences might arise from the "history" of the column, which was used for various other separations prior to the injection of the test compounds.

Without knowledge of the exact conditions for the synthesis and coating of the individual batches of Chiralpak AS and Chiralpak AS-V any attempt to explain the observed different characteristics will be a difficult, if not impossible task. However, it should be noted that amylose is a linear polymer obtained from natural sources (potatoes, wheat, corn, rice, etc.) and thus small variations in the relative molecular mass and branching can be expected, depending on the origin of the starting material for the CSP.

Impact of the Changes in Enantioselectivity on Productivity

To find satisfactory and optimal operating conditions for a SMB unit experimentally is very tedious and time consuming. Therefore NOVASEP

provides a software package, which allows to model nonlinear chromatography using laboratory results as described in "Determination of Competitive Adsorption Isotherms" section. Assuming a SMB unit with a configuration of 2-2-2-2 with eight chromatographic columns (cf. Fig. 2) it is possible to predict starting and operating parameters for a pilot-scale SMB unit. Table 5 shows a comparison of predicted productivities for the optimized separation of the four compounds on Chiralpak AS and Chiralpak AS-V.

The results of the calculations compiled in Table 5 show as a general trend the improved capability of Chiralpak AS-V to resolve racemates more efficiently than the "old" Chiralpak AS. We observed no case where the separation/resolution and loading capacity was worse for the new CSP. Due to the observed discrepancies in the experimental and calculated retention times for the separation of 1,1′-bi-2-naphthol on Chiralpak AS the predicted productivity can only be a rough estimate.

Interestingly, the already quite satisfactory separation of compound A should allow under optimized conditions the separation of more than 3 kg of racemate per day and kilogram stationary phase.

Analysis of the Preparative Separation

The racemic compound A was obtained through a multi-step synthesis in kilogram amounts and was subsequently separated using the Licosep 10×50 unit described in "Simulated Moving Bed Unit" section. One of the obtained enantiomers was converted in a further multi-step synthesis into a final drug intended for phase I studies (3). 1.07 kg racemates were separated (extract: 98.2% ee, raffinate: 99.9% ee) within 1 day/kg CSP (Chiralpak AS). Unfortunately, the separation was complicated by an increase in back-pressure in the unit from an original 39 to 105 bar, a value, which is far beyond the specifications of the unit. After the completion of the separation (~14 kg racemate) the CSP had to be replaced. During that process it was discovered that all frits of the eight columns

Table 5. Comparison of Predicted Productivities on Chiralpak AS and Chiralpak AS-V

	Chiralpak AS (kg/kg/day)	Chiralpak AS V (kg/kg/day)
Tröger's base	0.59	2.61
trans-stilbene oxide	0.28	0.90
1,1′-bi-2-naphthol	0.1[a]	0.21
Compound A	1.71	3.58

[a] Estimated.

were blocked by small particles, which had a similar composition as the packing material.

In order to understand the impact of replacing Chiralpak AS by Chiralpak AS-V on the separation, the so-called "triangle theory" proved to be very helpful. This theory allows an easy graphical description of the internal flow rates and the switch time, which are determining the flow rate ratios. The projection of the regions of separation on the m_2,m_3 plane spanned by the flow rate ratios of the two key sections is shown in Fig. 5.

Several areas in this plane can be distinguished. A triangular region describes an area where the flow rates in sections 2 and 3 of the SMB lead to a complete separation. This triangle is determined through the adsorption isotherm and the two points on the diagonal are equivalent to the Henry constants (see below). Above this triangle, a region is found where only the extract stream is pure, and on the left side of this triangle, a region can be found where only the raffinate stream is pure. Over the vertex of the triangle, another region is located, where none of the streams is pure. The area under the diagonal of the m_2,m_3 plane has no physical meaning. The interested reader is referred to the literature where the "triangle theory" is explained and applied in great detail (4–6).

The Henry constants give the slope of the component's adsorption isotherm under linear conditions, i.e., at infinitely small concentration:

$$n_i = H_i c_i \tag{8}$$

At low concentrations, the modified Langmuir isotherm (cf. Eq. (7)) allows a calculation of the Henry constants:

$$H_i = N_i K_i + \lambda \tag{9}$$

The ratio of the Henry constants is equal to the enantioselectivity α. It should be noted that the constants are affected by variations in the bed density (cf. Table 6), respectively, the resulting overall porosity ε and can be determined from simple experiments (37).

The larger the differences in the Henry constants will be, the larger the triangular region of complete separation (cf. Fig. 5) will become. A comparison of the H_i values in Table 6 shows significant differences between Chiralpak AS and Chiralpak AS-V and indicates that the adsorption isotherms derived for both CSPs through the pulse experiments (on 250 × 4.6 mm I.D. columns) described in "Determination of Competitive Adsorption Isotherms" section might differ for the preparative separation (107 × 48 mm I.D.). Chiralpak AS-V should allow for higher feed flow rates and therefore for a better productivity.

The NOVASEP software was used to generate a set of starting parameters for the separation of compound A on Chiralpak AS-V, which were as follows: feed concentration = 32 g/L; $Q_{\text{Feed}} = 23.9$ mL/min; $Q_{\text{Eluent}} = 116.0$ mL/min; $Q_{\text{Ex}} =$

Table 6. Henry Constants Obtained for Compound A on Analytical and Preparative HPLC Columns

	Analytical Columns		Preparative Columns	
	H_1	H_2	H_1	H_2
Chiralpak AS	0.77	0.95	0.63	0.85
Chiralpak AS-V	0.77	1.11	0.83	1.22

Column dimensions: analytical: 250 × 0.46 mm I.D.; preparative: 107 × 48 mm I.D.

108.9 mL/min; $Q_{Ra} = 31.0$ mL/min; $Q_1 = 352.0$ mL/min; and column shift period of $t^* = 0.64$ min (cf. Table 7, run A). Interestingly, the operating point proposed by the software was outside the triangular region of complete separation in Fig. 4. It should be noted that the Novasep software uses a value of $\varepsilon^* = 0.4$ for all calculations and allows the user to verify this value by a pulse injection on all eight columns installed in series in the Licosep 10 × 50. Based on a comparison of expected and observed retention times (at an identical temperature) the arbitrary

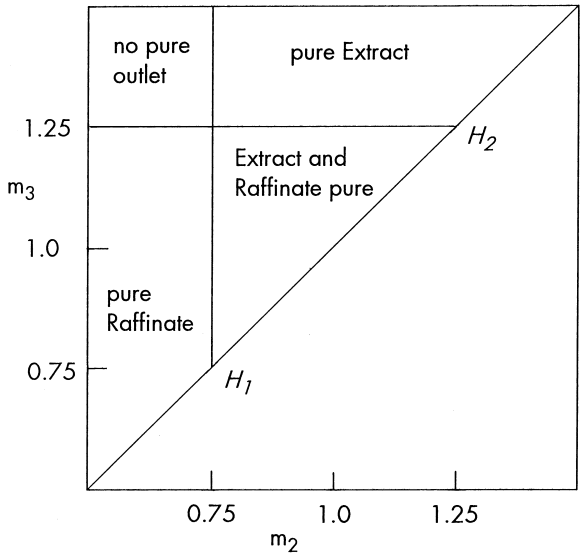


Figure 5. Regions in the m_2,m_3 plane with different separation regimes in terms of purity of the outlet streams, for a system described by the linear isotherm (cf. Eq. (8)): $H_1 = 0.75$, $H_2 = 1.25$.

Table 7. Operating Conditions and Purities of the Outlet Streams in the Experimental Runs of the Licosep 10 × 50 Simulated Moving Bed Unit Using Chiralpak AS-V

Run No.	Switch Time (min)	Flow Rates (mL/min)				Flow Rate Ratios[a]				Experimental ee (%)		Feed Concentration (g/L)
		Q_1	Q_2	Q_3	Q_4	m_1	m_2	m_3	m_4	Extract	Raffinate	
A	0.64	352.00	243.10	267.00	236.00	1.281	0.678	0.810	0.639	81.04	100	32
B	0.64	360.00	251.10	275.00	244.00	1.325	0.722	0.855	0.683	95.74	100	32
C	0.64	365.00	256.10	280.00	249.00	1.353	0.750	0.882	0.711	93.09	100	32
D	0.64	360.00	252.10	276.00	236.00	1.325	0.728	0.860	0.639	94.41	100	32
E	0.64	362.20	254.60	278.50	238.50	1.339	0.742	0.874	0.653	98.55	100	32
F	0.64	376.00	264.62	289.29	246.00	1.349	0.751	0.884	0.652	99.31	100	32
G	0.64	376.00	267.62	292.29	246.00	1.349	0.768	0.900	0.652	99.48	100	32
H	0.64	376.00	269.62	294.29	246.00	1.349	0.778	0.910	0.652	99.9	99.9	32
I	0.64	374.19	275.81	300.48	246.19	1.339	0.811	0.944	0.653	99.9	99.9	36

[a] For $\epsilon^* = 0.4$.

porosity is corrected through the software in order to compensate for the different porosity of the CSP in the preparative columns. In view of our results (see below) this correction had not the desired effect, most probably due to an unsuccessful temperature equilibration before the pulse injection.

The Licosep 10×50 was started with these parameters and after 10 complete cycles (50 min), the collection of extract and raffinate streams was started. The system was allowed to run for 3 hr and the streams produced were then analyzed. In the raffinate stream, no more retained compound could be detected, the extract stream had a purity of ee $= 81.0\%$.

The "triangle theory" suggests that by moving the operating point in a straight line parallel to the diagonal (i.e., without changing Q_{Feed} and Q_{Eluent}) one enters from the region of pure raffinate (run A) either into the triangle of complete separation or the region of pure extract. This was done in runs from B to H (cf. Table 7). As can be seen in Table 7, the purity in the extract stream is increasing

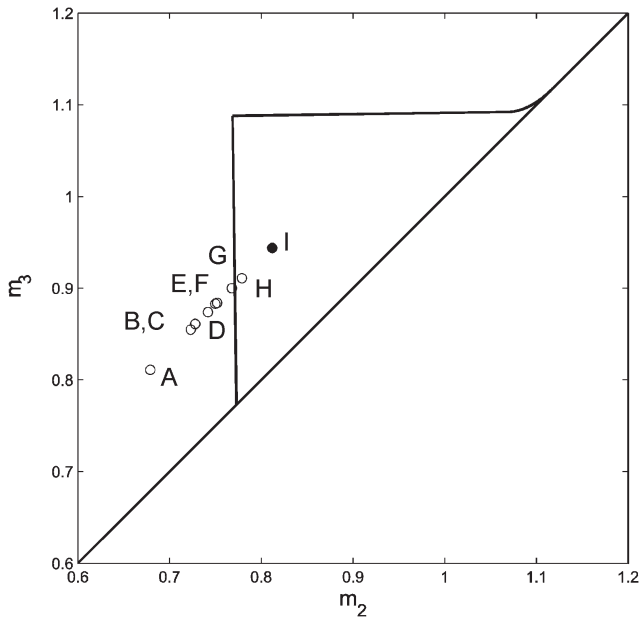


Figure 6. Separation of compound A in the Licosep 10×50 unit; regions in the m_2,m_3 plane with different separation regimes in terms of purity of the outlet streams. Predicted region of complete separation: (—) isotherm parameters: $\lambda = 0.7, H_1 = 0.77, H_2 = 1.11, K_1 = 0.0007\,\text{L/g}, \quad K_2 = 0.0041\,\text{L/g}, \quad c_1 = c_2 = 16\,\text{g/L}$. (○): Operating points corresponding to the runs A to H in Table 9. (●): Operating point corresponding to run I in Table 9.

steadily as the operating point nears the triangle. The raffinate purity remains unaffected during this increase parallel to the diagonal. The operating point H resulted in complete separation, which is a confirmation of this approach. Therefore, it can be assumed that the region of complete separation assumes a similar shape as the predicted triangle under nonlinear conditions (cf. Fig. 6). Point I shows the operating point for a higher feed concentration, 36 g/L, close to the solubility limit of the compound. This operating point was used for the separation of 104 kg of racemic compound A. The productivity was 1.45 kg racemate (extract: >99.6% ee, raffinate: >99.9% ee) per day/kg CSP (Chiralpak AS-V). No significant problems with an increase in back-pressure or changes in the purity of the product streams were observed. It should be noted that a further improvement of the productivity seems to be possible, but was not attempted in order not to change the master operation record of the separation, which was performed under cGMP guidelines.

CONCLUSION

The chromatographic properties of the commercial preparative (i.e., 20 μm particle size) CSP Chiralpak AS and its successor Chiralpak AS-V differ to a considerable degree. The impact of these differences on the SMB separation of various racemates can be assessed by determining the competitive adsorption isotherms and comparing the predicted productivities obtained through simple software simulation calculations. The calculated operating points can be refined through the recently introduced "method of triangles," which allows to find an optimal operating point for multi-kilogram separations within 1–2 days. The new production process for Chiralpak AS seems to have a beneficial effect on enantioselectivity and achievable productivity.

NOTATION

c	mobile phase concentration
ee	enantiomeric excess
H	Henry constant
K	adsorption equilibrium constant
m	flow rate ratio, defined by Eq. (6)
n	adsorbed phase concentration
$\bar{N}$	saturation capacity
Q	volumetric flow rate
Q_s	solid flow rate in an hypothetical TMB
t^*	switch Time in a SMB Unit

V volume of a single column of a SMB
V_S solid volume in an hypothetical TMB

Greek Letters
ε^* overall void fraction of the bed
λ linear coefficient of the modified Langmuir isotherm given by Eq. (7)

Subscripts
Eluent eluent or desorbent
Ex extract
Feed feed
i component index
j section index
Ra raffinate
s solid

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