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Development of an high-performance liquid chromatographic simulated moving bed separation from an industrial perspective

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Abstract

A binary test mixture consisting of cyclopentanone and cycloheptanone is used for the performance evaluation of a pilot-scale simulated moving bed unit. The involved adsorption equilibria and the kinetic behavior are discussed in detail. The results of the test runs are evaluated using the recently introduced ''triangle theory'' which allows to account for the overload conditions prevailing under preparative chromatographic conditions and to select optimal operating conditions. Under optimized conditions the separation of 735 g test mixture/kg stationary phase per day with purities >99.9% for extract and raffinate stream has been achieved.

Keywords: Simulated moving bed chromatography; Preparative chromatography; Enantiomer separation

1. Introduction

The development of continuous chromatography based on the simulated moving bed (SMB) principle and the introduction of turn-key units has led to numerous applications of enantiomer separations for pharmaceutical compounds based on this technique during the last decade [1]. SMB chromatography is now recognized as one of the fastest, most expedient processes for isolating single enantiomers, especially for research and early development stages (phase 1) of a new drug or agrochemical product candidate. This trend results from the combination of recent technological advances in the production of chiral stationary phases (CSPs) based on cellulose and amylose derivatives employed in high-performance liquid chromatography (HPLC) [2], the scale-up of HPLC process technology, and the adaptation of the simulated moving bed process to the scale required for the pharmaceutical products.

Another key issue for the recent success of the SMB technology as a tool for speeding up the design of chromatographic production processes is the fact that enantiomer separations can easily be scaled-up employing the same method (same type of CSP and mobile phase) as used for analytical purposes in the laboratory [3].

Compared to conventional batch chromatography, SMB chromatography has the benefits of a continuous process which allows for a constant product purity without permanent control of all collected fractions. Productivities of chiral separations trough SMB chromatography typically range from 100 to 1000 g pure enantiomer/kg CSP per day [4]. Less solvent is used as for a batch separation [5]. This advantage results from the overload conditions under which the columns of such units are usually operated in order to exploit the loading capacity of the CSP. Under these conditions the retention behavior of the enantiomers to be separated becomes dependent on their concentration in the stationary phase and has to be described by ''competitive adsorption isotherms''.

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During the last years 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 [6]. Following the so-called ''triangle theory'' constrains on these criteria can been derived which allow for complete separation of a binary mixture following the Langmuir and the modified Langmuir isotherm [7] and the most general case of a bi-Langmuir multicomponent adsorption isotherm [8].

The counter-current principle on which SMB units are based can be implemented in various ways (as will be described later), hence all units share some common properties which can be simply explained with reference to the equivalent true counter-current (TCC) unit schematically described in Fig. 1.

A true counter-current contact between mobile and solid-phase implies a flow of both the liquid and the stationary phase in opposite directions and involves the actual circulation of solid (true moving bed, TMB) with a flow-rate Q_S. For a chromatographic enantioselective separation four external streams are present: the racemic feed mixture, containing the two enantiomers A and B; the desorbent, i.e., the eluent or the mixture of eluents constituting the mobile phase; the extract stream enriched in the more retained enantiomer A; the raffinate stream enriched in the less retained enantiomer B. Each of the streams has a specific flow-rate (Q_{Eluent}, Q_{Ex}, Q_{Feed}, Q_{Ra}) that is maintained during the separation, and, of course, the inlet and outlet flow-rates are related through: $Q_{Eluent}+Q_{Feed}=Q_{Ex}+Q_{Ra}$. These streams divide the unit in four zones: zone 1 between the desorbent inlet and the extract port, zone 2 between the latter and the feed inlet, zone 3 between this and the raffinate outlet and zone 4 between the raffinate port and the desorbent inlet. Each of these zones plays a specific role in the process. The separation is performed in zones 2 and 3, where the less retained enantiomer B must be desorbed and carried by the mobile phase towards the raffinate, while A is retained by the stationary phase and carried towards the extract port through the solid movement. In zone 1 the stationary phase is regenerated by the fresh mobile phase stream and A is conveyed towards the extract port. Finally, in zone 4 the mobile phase is regenerated by adsorbing the amount of enantiomer B not collected in the raffinate. In this way both the stationary and the mobile phase can be recycled to zones 4 and 1, respectively. However, in fact, it is extremely difficult to operate a TCC unit because it involves the circulation of a solid stationary phase. This is the reason why another implementation is preferred: the simulated moving bed. Most of the benefits of a continuous counter-current of a TMB can be achieved in simulated moving bed chromatography [9] by using several chromatographic columns connected in series and an appropriate shift of the injection and collection points after a predefined time. SMB units have been operated for more than 30 years in the petrochemical field and for the purification of sugars [5]. Since the early 1990s, laboratory-scale, pilot-scale, and production-scale units have become available which allow the use of HPLC columns with an inner diameter range from 0.46 cm to 45 cm [4].

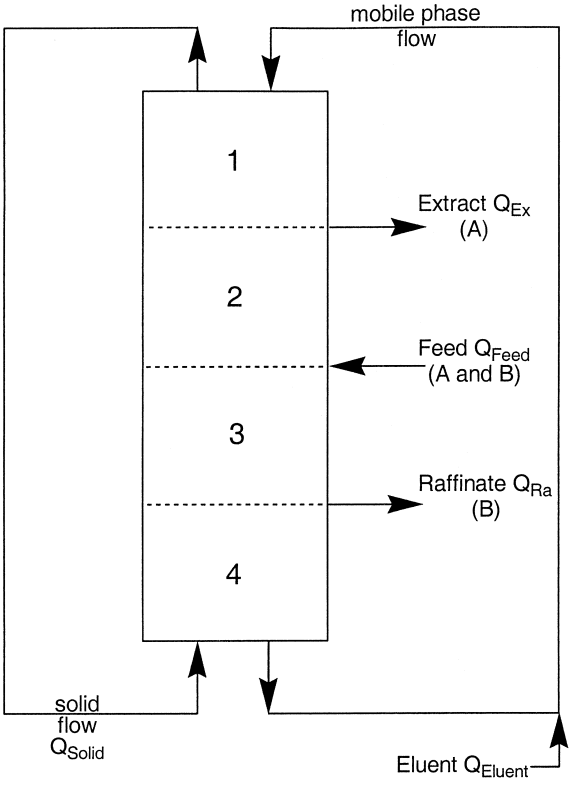

Fig. 1. Schematic diagram of a true counter-current unit.

CarboGen Laboratories (Aarau, Switzerland) is a

service provider for customers who desire kg amounts of pharmaceutically active compounds produced under cGMP conditions for phase 1 studies. Therefore it was decided to test SMB units which would ideally allow the purification of up to one kg of enantiomerically pure target compound per day. In order to assess the potential of such SMB units, an evaluation was arranged at CarboGen. Of the commercial systems considered, only those from Novasep and Knauer[1] were supported by software that could predict the optimum operating conditions without a lengthy, iterative change of the systems parameters and could comply with official regulations. We decided to perform a binary test separation consisting of cyclopentanone and cycloheptanone whose properties (adsorption isotherms, achievable purities and productivity) had been described in detail for a small-scale SMB separation in the open literature [10]. The results and conclusions drawn from the software tests and the experiments will be described in the following paragraphs. Special emphasis will be given to the approach used for the quick and straightforward optimization of the flow-rates using the recently introduced ''triangle theory'' [7].

2. Experimental

2.1. Analytical chromatography

Analysis of the product streams was carried out on a 250×4.6 mm I.D. YMC-Pack SIL column, containing 1.79 g YMC silica gel SL12S21 with a pore size of 120 Å and a particle size distribution of 15–30 μm (Stagroma, Wallisellen, Switzerland), the overload measurements were carried out on a 100×6.0 mm I.D. YMC-Pack SIL column, containing 1.21 g YMC silica gel SL12S21 from the same lot (Stagroma). Both analytical columns had a bed porosity of $\epsilon^*=0.815$. Injection loop volume and extra-column dead volume were 25 μl and 68 μl, respectively. The mobile phase consisted of hexane–ethyl acetate (85:15, v/v, HPLC grade, Machler, Reinach, Switzerland and Fluka, Buchs, Switzerland, respectively) at a flow-rate of 1.00 ml/min. The operating temperature was 26°C. The chromatograms were measured either 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) or an Contron system (pump type 322; autosampler type 360; UV detector type 332) with a Chrom Star data acquisition system (Bruker, Basel, Switzerland). The UV detectors were operated at 265 nm.

2.2. Test separation

The two compounds (cyclopentanone and cycloheptanone, both obtained from Fluka) were separated employing a binary eluent system consisting of hexane–ethyl acetate (85:15, v/v). The feed concentration was 10 g/l for each cycloalkanone in all experiments. The bulk stationary phase was a normal-phase spherical silica (YMC SL12S21, 120 Å, 15–30 μm) purchased from Stagroma. Analytical assay of extract and raffinate stream was performed without dilution employing the same stationary phase and eluent composition as for the preparative separation.

2.3. Column packing and testing

Bulk YMC SL12S21 was 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 85.00 g dry mass of the stationary phase and gave an averaged bed length of about 109 mm. Over the eight columns, the retention times for an analytical injection of the test mixture, measured at an flow-rate of 100 ml/min, were 3.398±0.084 min SD and 4.111±0.109 min SD; the retention time of an non-retained compound (*n*-hexane) was 1.812±0.019 min SD; the porosity of the columns was measured to $\epsilon^*=0.805$.

[1] A Csep 912 SMB unit from Knauer, Berlin, Germany, run in a 2-2-2-2 configuration with the same test separation at CarboGen showed considerable instability in the flow-rates (ranging from −69% to +434%) which precluded its use for the intended purpose.

2.4. Determination of adsorption isotherms

The Langmuir isotherms of the test system have been published recently [10]. For further measurements an analytical HPLC column (100 mm×6 mm I.D.), containing the same stationary phase as the eight preparative columns, was installed into the HP 1090 system and thermostated at 26±0.3°C, the operating temperature of the SMB unit. Binary mixtures of the two analytes (50:50, w/w), cyclopentanone and cycloheptanone, were injected at increasing concentrations (cf. Table 1).

The data were entered into the Novasep software (cf. Section 2.6) to determine the modified competitive Langmuir adsorption isotherms and SMB operating conditions.

2.5. SMB unit

The SMB unit tested successfully in our premises was the Licosep 10x50, produced by Novasep (Vandœuvre les Nancy, France). A detailed description of the unit is given in Section 4.1. It was equipped with eight NW-50 (non-jacketed) columns produced by Merck 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.

2.6. SMB hard- and software

The Licosep 10x50 SMB unit from Novasep is controlled by a central system composed of a Siemens PLC (type S7-300) and a personal computer (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 10x50 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 one to find a competitive adsorption isotherm based on overload injections and to optimize the operating parameters before starting the unit itself.

Table 1
Retention times of analytical and overloaded injections (flow-rate: 1.000 ml/min; T=26°C; detection at 265 nm)

Experiment No.	Concentration (g/l)[a]	t_{R1} (min)	t_{R2} (min)
1	Analytical	4.967	6.122
2	1	4.966	6.088
3	2	4.948	6.087
4	5	4.923	5.991
5	10	4.885	5.907
6	20	4.834	5.803
7	50	4.709	5.598
8	100	4.560	5.389
9	250	4.248	4.985

[a] Injected volume: 0.025 ml, cycloheptanone–cyclopentanone (50:50, w/w).

3. Background

The functions of the four zones of a TMB (cf. Introduction) are accomplished in SMB units by four discrete sections, 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 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,..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:

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

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

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

$$Q_4 = Q_3 - Q_{Ra} \quad (5)$$

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 [9] one obtains:

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

Based on these flow-rate ratios m_j, the experimental performances of SMBs can be properly designed, interpreted and compared following a recently presented approach, which leads to criteria for the choice of optimal and robust operating conditions of such units [6]. 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 prerequisite for optimal operation are minimized dead volumes and a set of very similar and stable chromatographic columns.

4. Results and discussion

4.1. Technical description of the tested SMB unit

Simulated moving bed units for preparative separations are composed of a given number of chromatographic columns (usually between 6 and 16), pumps (4 or 5) and valves allowing connection of the different inlet/outlet lines to the columns which are linked in series. The two inlet lines permit the continuous injection of the solution to be separated (feed) and the eluent (or desorbent). The two outlet lines (extract and raffinate) allow the continuous withdrawal of the pure products.

There are two basic principles known for simulating the counter-current movement: the first approach implies that the solid beds are fixed and the counter-current solid movement is simulated by periodically shifting for one column the points where feed, eluent, extract and raffinate are fed and withdrawn from the unit in the same direction as the mobile phase flow. Or, secondly, the ports for ingoing and withdrawal points remain in a constant position but the solid beds are actually moved periodically for one column in the opposite direction as the mobile phase flow. The former implementation of SMB is employed by Novasep [11] and has also been used by Prochrom (Nancy, France) [12] and UOP [13]. The latter principle is applied by Knauer (Berlin, Germany) (see Ref. [14] and footnote in the Introduction).

The system used by Novasep employs a five-pump arrangement, where all four external flows (feed, eluent, extract and raffinate) are directly controlled through pumps and the flow-rate of the mobile phase is defined by an internal ''recycling pump'' (cf. Fig. 2) which is kept motionless between two given columns. Depending on the injection and collection line positions and the number of columns, the recycling pump successively regulates the flows in sections 1, 2, 3 and 4.

Each inlet and outlet of the columns is connected through 48 two-way high-pressure pneumatic valves to the four lines (feed, eluent, extract and raffinate) leading to the external pumps. At a given time, only one line of each type is opened. The feed and injection lines and the extract and raffinate lines are shifted with t^*. The presence of a recycling pump (and the associated flow meter and pressure sensors) introduces a volume asymmetry into the system, which has to be compensated through an asynchronous shift of the inlet and outlets [15]. The unit is equipped with a heat exchanger and a cryostat which allows to adjust the temperature in the eluent-line from -20°C up to 70°C with a precision of ± 0.3°C. The maximum pressure in the system is 100 bar. In the Novasep unit the pressure is always highest in the column installed directly downstream behind the recycling pump and drops continuously to the point of lowest pressure, at the end the column upstream from the pump. This design implies that the column installed (physically) behind the so-called recycling pump will be the only one to experience the direct

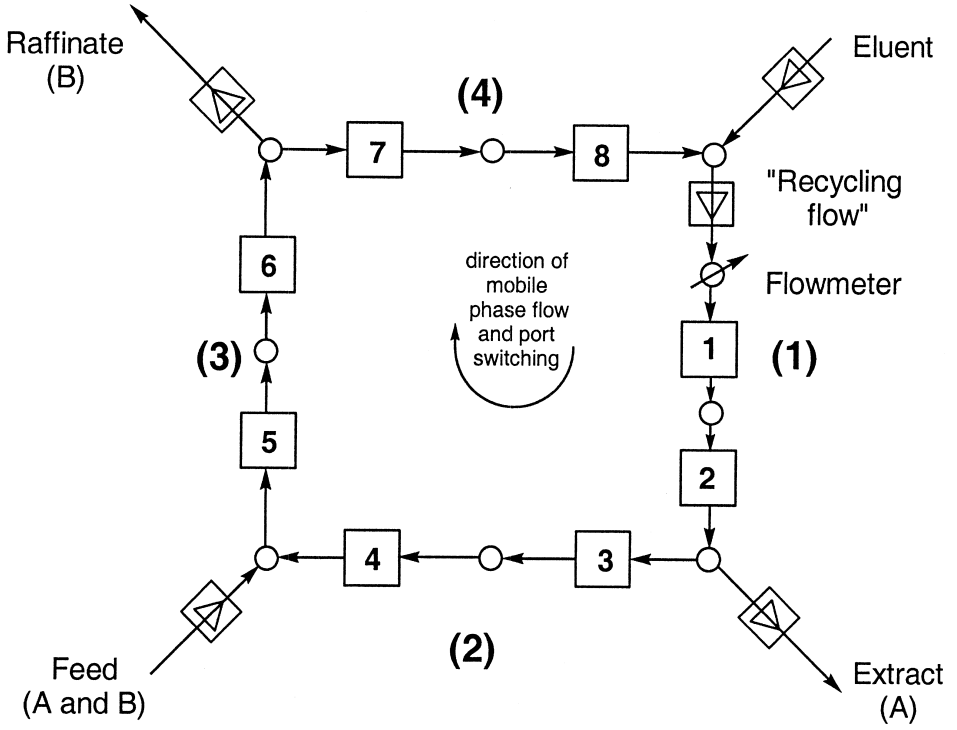

Fig. 2. Schematic flow diagram of the Licosep 10x50 unit with a 2-2-2-2 column configuration in the first period of a cycle. Flow direction and location of the five pumps are indicated by triangles.

impact of the mobile phase flow changes after it ''moves'' from one zone to the next. The system can be equipped with up to 12 columns. It was decided to restrict the experimental runs to eight columns (average bed length 109 mm, I.D. 4.8 cm; 85 g of packing each) in a ''classical'' four-zone SMB with a symmetric 2-2-2-2 configuration as illustrated in Fig. 2.

4.2. Test mixture and involved adsorption equilibria

All chromatographic analytical methods have been evaluated in the past by test mixtures which allow a standardized comparison of column performances, inertness and efficiencies obtained by coating procedures in gas chromatography (GC) [16], or packing and deactivation procedures in HPLC [17], or separation capabilities in capillary electrophoresis (CE) [18]. Today rarely any analytical column is sold without some kind of a test-chromatogram indicating selectivity and number of theoretical plates. Thus the idea of separating a test mixture in order to determine the system characteristics of a SMB unit suggests itself. Instead of a racemate to be resolved on a CSP a binary mixture (50:50, w/w) consisting of cyclic ketones was selected as bench mark for the separation capacity of the tested SMB unit.

The selected test mixture shows several advantages over the actual separation of an pharmaceutically active compound (which might be difficult to obtain or synthesize or might be a highly biological active substance) and other separations recently used for testing such units: The stationary phase is a normal-phase spherical silica with a moderate price compared to CSPs and shows a pressure drop comparable to 20 μm chiral stationary phases. The two compounds (cyclopentanone and cycloheptanone) and the binary eluent system consisting of hexane–ethyl acetate (85:15, v/v) can be considered as non-toxic and do not have to be disposed off as

chlorinated solvents. The solvent mixture could be stored for more than six month without any problems concerning volume ratios and up-take of water. Analytical assay of extract and raffinate stream can be performed without further dilution by direct injection of the collected outlet streams in an HPLC system, employing the same stationary phase and eluent composition as for the preparative separation. Since the test compounds are liquids no problems of precipitation in valves or connecting pipes have to be expected as in the case of e.g., a naphthalene–toluene separation.

Once the initial decision on the nature and the particle size of the stationary phase has been taken, parameters for the test separation to be determined are geometric quantities (column length and diameter), dead volumes (capillaries, valves, pumps, etc.) and porosity. With the knowledge of these parameters it is possible to determine the thermodynamic behavior of the system (adsorption isotherms) and to perform some predictions on the kinetic constraints (number of theoretical plates per column and involved pressure drop). These calculations are followed by a decision on the volumetric flow-rates and the feed concentration.

Among the most important mechanisms governing preparative chromatographic applications is the thermodynamic equilibrium of the separation system under overloaded conditions. The determination of the thermodynamic characteristics for the involved adsorption equilibria is a straightforward procedure, as the components to be separated are both liquids that are totally soluble in the eluent mixture. It should be mentioned in this context that the Langmuir adsorption isotherms given in the literature [10,14] have to be examined with some precaution.

As can be seen in Table 1 and Fig. 3 the retention times of the two compounds are decreasing significantly when larger amounts are injected.

Based on some injections at increasing concentration (cf. Fig. 3) on an analytical HPLC column filled with the identical stationary phase the Novasep software package ‘‘softSMB’’ correlates through a curve-fitting procedure the equilibrium experimental

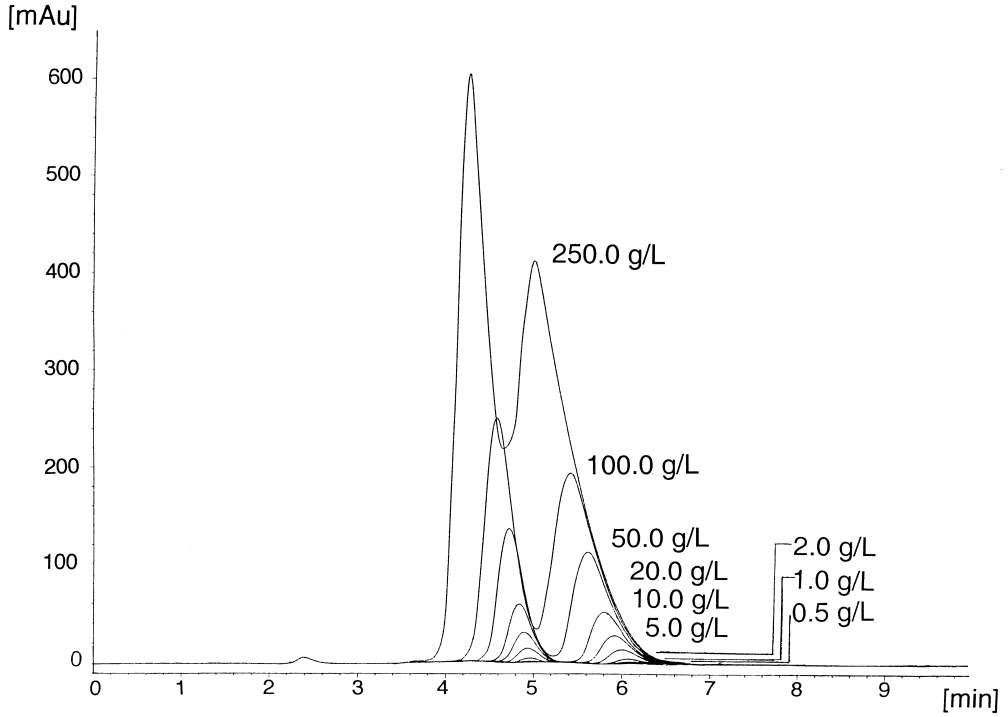

Fig. 3. Overload injections of cycloheptanone and cyclopentanone on YMC silica gel SL12S21; column: 10 cm×6.0 mm I.D., 120 Å, 15–30 μm; mobile phase hexane–ethyl acetate (85:15, v/v); detection: 265 nm; $T=26$°C.

results with a postulated modified Langmuir competitive isotherm which takes the form:

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

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 heterogeneous surface that consists of two different types of adsorption sites, e.g., a non-chiral interaction (linear term) and an enantioselective discrimination site with different affinity for the two chiral substances (Langmurian 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 non-linear fashion. Therefore it can be assumed that the parameters obtained will provide only a rough description of the true competitive behavior of the two non-chiral test compounds cycloheptanone and cyclopentanone. In order to improve the accuracy of the thermodynamic model more experiments and a more detailed fitting procedure would be needed.

The obtained isotherms are for cycloheptanone and cyclopentanone, respectively:

$$n_1 = 1.8c_1 + \frac{0.461c_1}{1 + 0.031c_1 + 0.076c_2} \quad (8)$$

$$n_2 = 1.8c_2 + \frac{1.142c_2}{1 + 0.031c_1 + 0.076c_2} \quad (9)$$

The retention times for the selected test compounds could be predicted with these two isotherms surprisingly well, however, some deviations ($\pm 5\%$) were observed. In Eqs. (8) and (9) the non-selective coefficient λ takes the value of 1.8 for both isotherms. Considering that at infinite dilution conditions the modified isotherm (cf. Eq. (7)) should reduce to a linear relation, referred to as linear adsorption isotherm:

$$n_i = H_i \cdot c_i \quad (10)$$

in which H_i is the dimensionless Henry constant of the ith component, i.e., the slope of the component's adsorption isotherm at infinite dilution, a calculation of these constants becomes possible:

$$H_i = N_i K_i + \lambda \quad (11)$$

The obtained values ($H_1^{\epsilon=0.4} = 2.26$ and $H_2^{\epsilon=0.4} = 2.94$) are based on the presumption that the overall porosity of the beds assumes an value of $\epsilon^* = 0.4$. Through one linear pulse injection on the preparative SMB columns installed in the Licosep 10x50 the arbitrarily value for ϵ^* is corrected through the Novasep software in order to compensate for the different porosity of the stationary phase. Variations in packing density are frequently encountered in scale-up in preparative chromatography and are caused by different packing techniques and variations in bed compression [19]. The corrected ϵ^* takes the value of 0.422 and the linear coefficient of the isotherm has to be adjusted to $\lambda = 1.75$, thus accounting for the measured retention times.

Following a recently described procedure for the determination of the Henry constants [20], the following values for H_i and ϵ^* have been obtained in separate measurements: $H_1 = 4.96$, $H_2 = 7.17$, $\epsilon^* = 0.815$ (analytical column) and $\epsilon^* = 0.805$ (preparative SMB columns). The ratio of the two constants (1.44) gives the selectivity, S, of the test separation under linear conditions.

In the view of the differences in the Henry constants and the porosity, some doubts about the isotherm given through the software remain, mostly due to the small number of experimental information on which it is based and due to the fact that the experimental measurement of ϵ^*, though difficult to be made precisely, indicates a significantly higher porosity. It should be noted that the isotherm Eqs. (8) and (9) do not represent actual thermodynamic adsorption mechanisms, but should be considered as a working hypothesis that has to be refined when there is need for a further elucidation of the adsorption behavior under investigation.

4.3. Column efficiency

Dispersive effects linked to mass transfer kinetics and to the non-ideality of the flow-profiles in liquid chromatography can be globally quantified by a single parameter, the number of theoretical plates N_p. It can be derived from the retention time of a pulse injection at low concentrations, $t_{R,i}$, using e.g., the half-height peak width, w_h:

$$N_p = 5.54 \cdot \left(\frac{t_{R,i}}{w_h}\right)^2 \quad (12)$$

Applying Eq. (12) for cyclopentanone a value of 5077 can be calculated for N_p based on an analytical pulse injection over the eight columns used in the SMB units connected in series (resulting bed length: 87.2 cm) at a flow-rate of 100 ml/min (e.g., $t_R = 33.3$ min, $w_{1/2} = 1.1$ min). This number of plates is much higher than generally necessary for the operation of a SMB unit. As a rule of thumb usually a limit of 20 to 30 theoretical plates per column, respectively for eight columns a minimum of approximately 200 plates [4], is necessary for non-sophisticated separations, as in the case of the test system.

The change of N_p as a function of the interstitial mobile phase velocity (or ''linear velocity'') is usually well fitted to the van Deemter [21] or Knox [22] equations. The number of plates allows one to calculate the height of a theoretical plate (HETP):

$$\text{HETP} = \frac{L}{N_p} \quad (13)$$

in which L is the column length. The van Deemter equation implies that there is a velocity which minimizes HETP and hence minimizes band spreading, but this velocity usually gives too low throughput in large scale separation. In the mobile phase velocity range used in preparative chromatography, the van Deemter curve is in its linear range, thus a linear relation is often suitable because molecular diffusion can be neglected:

$$N_p = \frac{L}{au + b} \quad (14)$$

where u is the fluid velocity of the eluent in the packed bed, and a and b are two coefficients depending on the diffusion coefficient, porosity and mass transfer. The van Deemter curve for both test compounds, based on a series of analytical chromatograms (cf. Fig. 4), shows a minimum in the

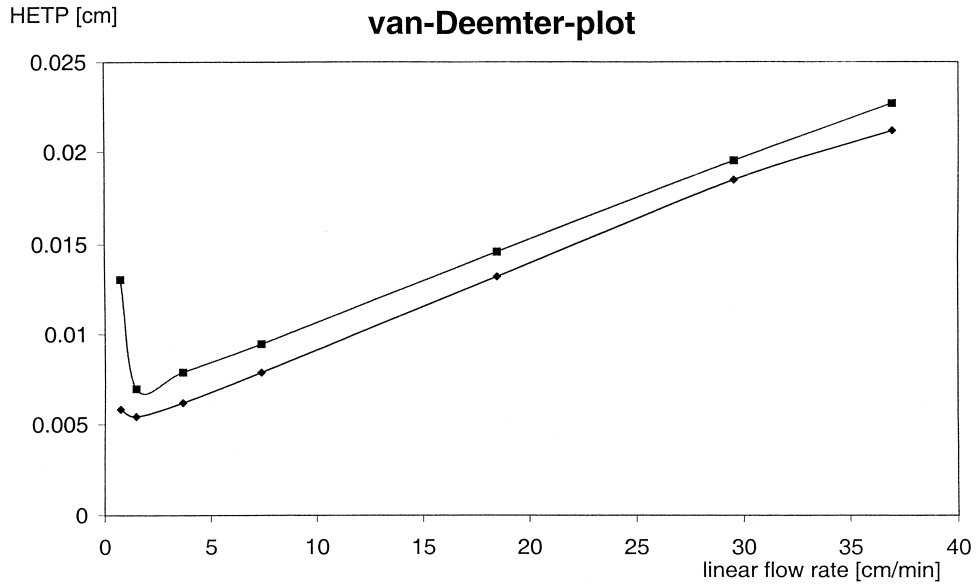

Fig. 4. van Deemter curve for analytical pulse injections of cycloheptanone (■) and cyclopentanone (♦) on YMC silica gel SL12S21; column: 25 cm×4.6 mm I.D., 120 Å, 15–30 μm; mobile phase hexane–ethyl acetate (85:15, v/v); detection: 265 nm; $T = 26$°C.

range of 3 cm/min (~0.4 ml/min) and continues linear within the experimental error.

The a and b parameters can be determined from chromatograms at two or more different velocities, but can also be estimated based on some simple considerations if no further data is available [23]. Through a linear regression estimates of $a = 0.005$ cm (± 0.001 cm) and $b = 0.0004$ min (± 0.0002 min) were obtained. In an SMB unit the internal flow-rates are different from one zone to another. Consequently, the number of plates for each column depends on its position (in zone 1, 2, 3 or 4). Combining Eqs. (13) and (14) to Eq. (15) allows one to quantify in a general way the available number of plates for an hypothetical averaged internal mobile phase flow-rate, e.g., 300 ml/min (20.6 cm/min), in a series of eight SMB columns (with a total bed length of 87.2 cm, a porosity of $\epsilon^* = 0.805$ and an inner diameter of 4.8 cm):

$$\mathrm{HETP} = a + b\mathrm{u} = \frac{L}{N_\mathrm{p}} \tag{15}$$

to a number of plates of 6500. However, the measured value is significantly lower ($N_\mathrm{p} = 5077$, see above), since the void volumes between the eight columns and the 16 frits will lead to considerable back mixing and therefore loss of plates. Nevertheless the parameters derived from the linear part of the van Deemter curve and the pulse injection over the eight SMB columns show that the available number of plates under non-overloaded conditions is at least one power of 10 higher than required.

4.4. Prediction of operating conditions

To find satisfactory and optimal operating conditions for a SMB unit experimentally is very tedious and time consuming. Therefore Novasep provides for the users of their SMBs a software package which allows to model non-linear chromatography using laboratory results for the prediction of flow-rates and achievable performance.

In Table 2 a comparison is given between the (proposed) optimal operating parameters and one set of optimal experimental results for the same feed concentration (40 g/l) which has been published recently [10]. It should be mentioned that the experimental values have been obtained for the same test system (cycloheptanone–cyclopentanone), but on a much smaller scale.

Table 2
Comparison of predicted optimal operating parameters and experimental data [10] for a feed concentration of 40 g/l

	Novasep[a] (“soft SMB”)	Experiment[a] [10]
V (ml)	197.25	2.83
ϵ^*	0.422/0.815	0.855
t^* (min)	1.34	1.22
Q_1 (ml/min)	325.50	4.405
Q_2 (ml/min)	246.66	3.522
Q_3 (ml/min)	257.09	3.722
Q_4 (ml/min)	220.72	3.444
m_1	7.211	7.305
m_2	4.464	4.654
m_3	4.828	5.254
m_4	3.561	4.420
Purity extract (%)	99.8	99.6
Purity raffinate (%)	100	100

[a] Feed concentration: 40 g/l cycloheptanone–cyclopentanone (50:50, w/w); configuration: 2-2-2-2.

Even though the numerical values of the switch time t^* and the internal mobile phase flow-rates Q_j are considerably different, a comparison of the underlying flow-rate ratios m_j shows that there are no significant differences between the predicted values and experimental results, event though the Novasep software leads to smaller values for the flow-rate ratios in all four sections. However, the proposed flow-rate ratios are based on a modified Langmuir adsorption isotherm (cf. Section 4.2) and therefore it can be expected that the calculated optimal operating conditions vary from those experimentally determined. Interestingly, the predicted purities of extract and raffinate stream and the experimental values are next to identical.

Table 2 highlights one of the advantages of the so-called “triangle theory” [7]: the possibility to compare experimental and predicted results on a basis of four dimensionless numbers, neglecting parameters like number of columns and even different isotherm models. This advantage will be exploited and exemplified further in the following paragraph.

4.5. Analysis of experimental results

To validate the predicted operating parameters,

several SMB experiments were carried out. It was decided to use a feed concentration of 10 g/l for each cycloalkanone as this implies that less of the two test compounds, which were not recovered, is consumed per day.

The Novasep software was used to generate a set of starting parameters which were as follows: feed concentration = 20 g/l; Q_{Feed} = 10.39 ml/min; Q_{Eluent} = 104.78 ml/min; Q_{Ex} = 78.84 ml/min; Q_{Ra} = 36.37 ml/min; Q_1 = 325.5 ml/min and column shift period of t^* = 1.34 min (cf. Table 3, run N).

In order to achieve a higher productivity the proposed set-points were modified without a predicted loss in performance to: Q_{Feed} = 17.35 ml/min; Q_{Eluent} = 123.1 ml/min; Q_{Ex} = 83.35 ml/min; Q_{Ra} = 57.1 ml/min; Q_1 = 346.4 ml/min and column shift period of t^* = 1.24 min. The Licosep 10x50 was started with these parameters (cf. Table 3, run A) and after three complete cycles (30 min), the collection of extract and raffinate streams was started. The system was allowed to run for 3 h and the streams produced were then analyzed. In the extract stream no cycloheptanone could be detected, the raffinate stream had a purity of 82.4%. Obviously the more retained component cyclopentanone was being carried too far into section 3 and could not be retained enough through the simulated counter flow of the solid. It was therefore decided to increase simultaneously the extract flow-rate (91.15 ml/min) and to lower the raffinate flow-rate (49.3 ml/min) without changing Q_{Feed} and Q_{Eluent} and also maintaining the recycling flow-rate (cf. Table 3, run B). After this adjustment both streams were pure (>99.9%), giving complete separation. A comparison of the flow-rate ratios in Table 3 shows that the described changes in extract and raffinate flow-rate result only in small decreases in zone 2 (m_2) and 3 (m_3), whereas the flow-rate ratios in zones 1 (m_1) and 4 (m_4) remain unchanged.

This behavior is best described by the before mentioned ''triangle theory'' [7] which allows a clear graphical description of the involved changes in the flow-rates. 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 drawn 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. 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 [6–8].

As can be seen in Fig. 5 both, the predicted operating point N and the experimental point A, are in the triangle which was obtained through the Novasep software for an overall porosity of ϵ^* = 0.422 and a linear coefficient of the modified Langmuir isotherm of λ = 1.75. The performance of the unit in run A confirms that the predicted isotherm is

Table 3
Operating conditions and purities of the outlet streams in the experimental runs of the Licosep 10x50 SMB unit for a feed concentration of 20 g/l

Run No.	Switch time (min)	Flow-rates (ml/min)				Flow-rate ratios				Experimental purities (%)	
		Q_1	Q_2	Q_3	Q_4	m_1	m_2	m_3	m_4	Extract	Raffinate
N	1.34	325.5	246.66	257.09	220.72	3.0958	2.1694	2.2918	1.8640	(100)[a]	(99.8)[a]
A	1.24	346.40	263.05	280.4	223.30	3.0376	2.1316	2.3197	1.6987	>99.9	82.4
B	1.24	346.40	255.25	272.6	223.30	3.0376	2.0467	2.2349	1.6987	>99.9	>99.9
C	1.24	346.40	258.25	275.6	223.30	3.0528	2.0174	2.3089	1.7041	>99.9	54
D	3.72	115.45	85.10	90.88	74.45	3.0387	2.0467	2.3360	1.7008	99.8	>99.9
E	4.96	86.60	64.19	68.53	55.83	2.9941	2.0326	2.2240	1.6758	79.3	>99.9
F	4.96	86.60	65.19	69.53	55.83	3.0376	2.1066	2.2936	1.6976	>99.9	89.4

[a] Predicted values.

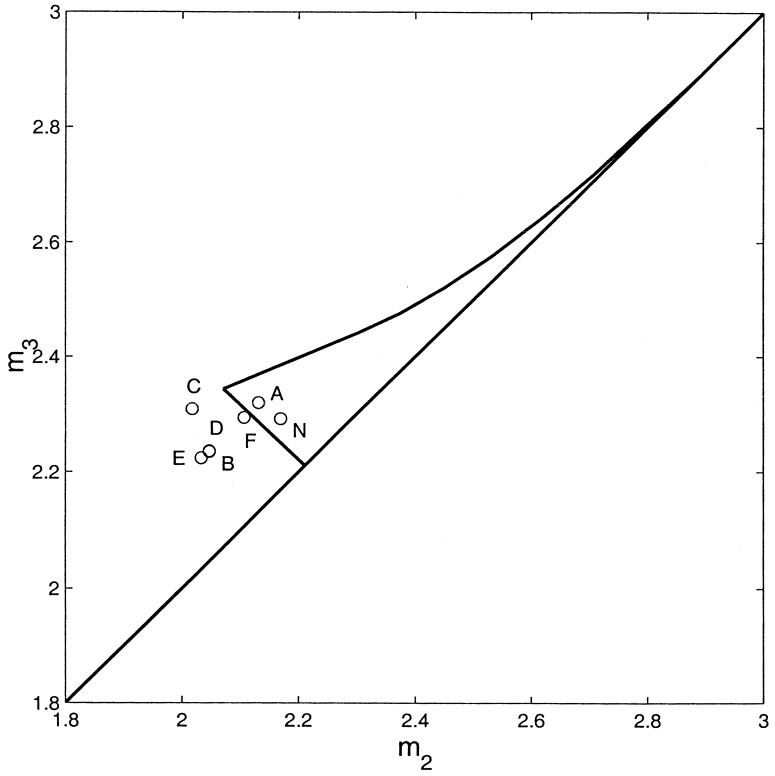

Fig. 5. Separation of cycloheptanone–cyclopentanone in the Licosep 10x50 unit; regions in the m_2,m_3 plane with different separation regimes in terms of purity of the outlet streams. Predicted region of complete separation: (——) isotherm parameters: $\lambda = 1.75$, $H_1 = 2.211$, $H_2 = 2.892$, $K_1 = 0.031$ l/g, $K_2 = 0.076$ l/g, $c_1 = c_2 = 10$ g/l. (○) Operating points corresponding to the runs A to F in Table 3.

inaccurate; the operating point A is situated in the region of pure extract.

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 extract either into the triangle of complete separation or the region of pure raffinate. This was done in runs B, E and F. Operating point B resulted in complete separation, which is a confirmation of this approach. Therefore it can be assumed that the region of complete separation assumes another shape than predicted. Going away further from the diagonal (run C) leads as predicted by the theory to a loss in purity.

A further confirmation of this theoretical analysis is provided by runs E (pure raffinate only) and run F (pure extract only) and by their relative position with respect to runs A and B. It should be noted that the experiments E and F have been performed with a fourfold switch time ($t^* = 4.96$ min) as compared to runs A to C and with a set of flow-rates to fulfill Eq. (6).

The most interesting experiment is run D, which has been performed with a threefold switch time ($t^* = 3.72$ min) and with a set of drastically different internal flow-rates, but keeping the m_j values as in run B, and in fact both product streams were essentially pure.

These findings are illustrated in Fig. 6, where the purity of the outlet streams is reported as a function of m_2 for the experimental runs corresponding to the operating points along the same straight line in the m_2,m_3 plane as mentioned above.

The typical behavior of the purities in extract and

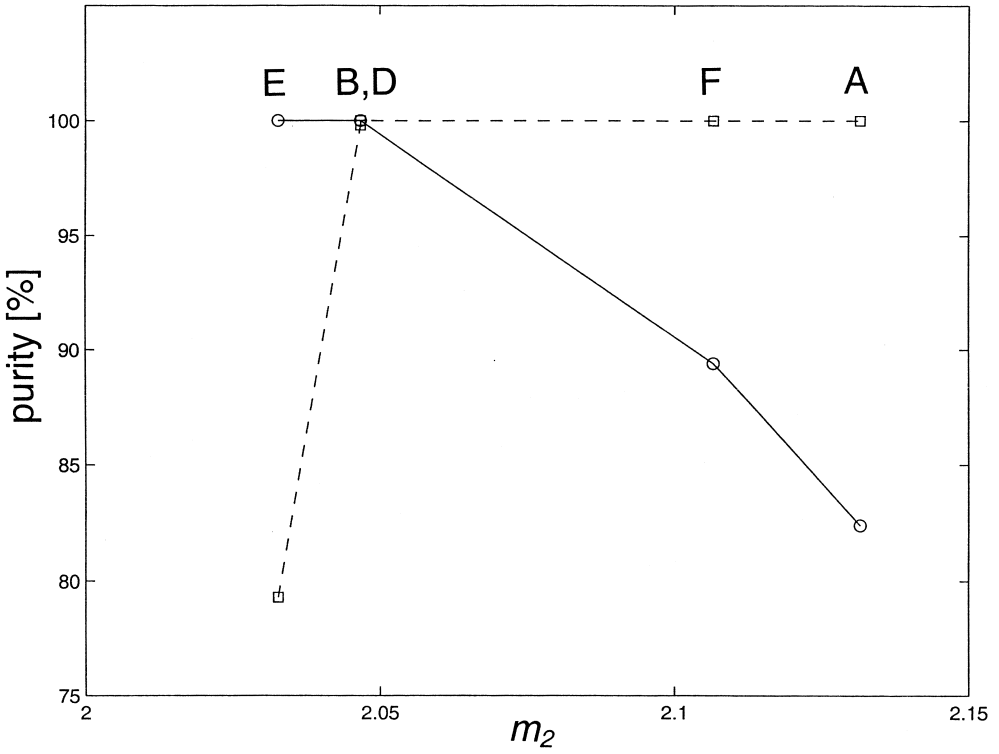

Fig. 6. Effect of the operating points on a parallel line to the diagonal in the m_2,m_3 plane on the purities of extract (- - -) and raffinate (—).

raffinate stream follows what is predicted by the ''triangle theory'' and has in this form already been presented by several researchers [24,25]. It should be noted that the performance in the operating point C is not inconsistent to the other experiments.

Thus it can be concluded that the triangle corresponding to the real isotherm should have the vertex close to point B; the left hand boundary should be located between the points E and B, the right hand boundary between points B and F. In order to obtain the correct isotherm and the precise triangle a deeper investigation of the adsorption equilibria would be necessary.

Since the operating point of run B is very close to the vertex of the triangle of complete separation it can be considered as the achievable optimum operating point of this test separation. However, this optimal operating point cannot be considered as robust; already small perturbations in the operating conditions, such as small flow-rate changes, variations of the feed composition or temperature changes will result in a shift from the expected location in the complete separation region, to another position which is likely to be outside the complete separation region itself, e.g., as in the case of experiment E. It was demonstrated recently that this vertex represents optimal operating conditions with respect to productivity and desorbent consumption [7].

The productivity PR is defined as the amount of components separated per unit of time and mass of stationary phase (g product obtained per day for 1 kg of stationary phase). PR is determined through the feed flow-rate, the concentration of the feed solution and the amount of stationary phase employed in the columns. For run B (complete separation) a PR of 735 g day^{-1} kg^{-1} is found. For the small scale separation mentioned above an optimized PR of ~820 g day^{-1} kg^{-1} has been reported [10].

The desorbent requirement DR is defined as the amount of mobile phase used to recover a given amount of pure product (l solvent used for the purification of 1 kg target compound). As the mobile phase is fed to the SMB unit through both, the eluent and the feed pump, their flows have to be added for the determination of the desorbent requirement. For run B the desorbent requirement is in the range of 400 l/kg. This value is considerably higher than the DR reported for the small scale separation of the test system, where DR was in the range of 50 l/kg [10]. It should be noted that the latter favorable situation is most probably the result of the high feed concentration of 120 g/l used for the cited separation, as compared to a feed concentration of 20 g/l in run B.

5. Conclusion

The test separation of a binary mixture of cycloheptanone and cyclopentanone allows to perform a quick and easy performance test for simulated moving bed units. The Licosep 10x50 demonstrated its ability to produce over 250 g/day of each test substance with a purity of greater than 99.9%. Setting up the system to achieve this throughput took only two days, which is of great importance in an industrial environment.

Following a standard procedure for the determination of adsorption isotherms the Novasep software provides a good first approximation of a feasible operating point based on a small number of simple experiments. However, if the proposed adsorption isotherm is incorrect, the access to stable and robust conditions and complete separation as well as the understanding of the system's behavior is simplified through the recently introduced ''triangle theory''. The theory provides a clear approach using the available data without a lengthy trial and error procedure for the determination of the region of complete separation with few experiments.

In view of these test results the separation of up to 1 kg of a racemate, e.g., a pharmaceutically active substance for phase I studies, will be feasible per day. The efficiency of the system compared to conventional isomer separations has a favorable impact on the resources required for drug development by allowing correspondingly smaller amounts or precursors to be synthesized.

6. Notation

a	coefficient, defined by Eq. (14)
A	more retained compound
b	coefficient, defined by Eq. (14)
B	less retained compound
c	mobile phase concentration
DR	desorbent requirement
HETP	height equivalent to a theoretical plate
H	Henry constant
K	adsorption equilibrium constant
m	flow-rate ratio, defined by Eq. (6)
$\underline{n}$	adsorbed phase concentration
$\underline{N}$	saturation capacity
N_p	number of plates
PR	productivity
Q	volumetric flow-rate
Q_S	solid flow-rate in an hypothetical TMB
S	selectivity
t^*	switch time in a SMB unit
V	volume of a single column of a SMB
V_S	solid volume in an hypothetical TMB

6.1. Greek letters

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

6.2. Subscripts

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

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