

SIMULATED MOVING BED (SMB) SEPARATION OF PHARMACEUTICAL ENANTIOMERS AND CRYSTALLIZATION

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A promising solution for the separation of pharmaceutical enantiomers is the method of SMB liquid chromatography (SMB-LC). An optimised coupling of SMB-LC and crystallization processes can improve the efficiency of enantioseparation. In the first process both enantiomers can be produced in enriched concentrations in extract and raffinate streams of simulated moving bed chromatograph suitable for crystallization. Chromatographic investigations include the experimental determination of adsorption isotherms, bed voidage, NTP, HETP etc. on a suitable chiral stationary phase as well as the simulation and optimization of a the appropriate SMB-LC process. Mathematical models and joined computer programmes were published in our earlier papers had been developed and applied for the calculation of SMB-LC. We assumed isotherm, isochor equilibrium adsorption (competitive multicomponent Langmuir-adsorption equilibrium) in the mathematical model neglecting the effects of axial dispersion. The mathematical model was solved by finite differences, numerical mathematical method using PC. The SMB-LC separations were carried out on a laboratory scale (I.D. = 1 cm, L = 25 cm) four-column open loop eluent system equipment at 1:1:1:1 column configuration. The process variables of the SMB-LC (product purity, yield, productivity, specific solvent consumption) are favourable. The efficiency of the enantiomer separation can be increased by coupled crystallization of the enriched extract and raffinate fractions resulting >99% (w/w) pure enantiomer crystals. „Mother liquids” recirculation from crystallization to the SMB-LC process increases with great benefit the economical parameters of enantiomer separation.

Keywords: preparative liquid chromatography, simulated moving bed chromatography, pharmaceutical enantiomers, chiral chromatographic packing, crystallization

Introduction

Nowadays more than half of the registered medicals have got chiral structures owing special importance in pharmaceutical industry. From pharmaceutical points of view only one enantiomer has got good biological activity, meanwhile the other enantiomer is inactive or toxic. The conclusion is, that the enantiomer purity has determining effect. The classical resolution can be well applied for the production of optically active compounds. For example optically active compounds can be produced by stoichiometric catalytic asymmetric synthesis starting from achiral compounds. Enantiomers produced by synthesis can be separated by simulating moving bed liquid chromatography (SMB-LC) process. This last process has growing importance in pharmaceutical industry as it is well applicable in case of the high purity separation of wide scale of racemic mixtures. The advantages of SMB-LC compared to the traditional batch chromatography are: continuous process, constant product purity for both enantiomers, high yields, high productivities, low specific solvent consumptions. The chromatographic packing (CSP) is used in full columns length, so the productivity and yield is high in case of

SMB-LC compared to batch chromatography. Disadvantages of SMB-LC are: high investment costs and process parameter sensitivity.

In analytical, preparative and SMB liquid chromatography the polysaccharide derivatives proved to be versatile among industrial chiral stationary phases (CSP). The productivity of SMB-LC in case of these CSP packings are as high as 0.2-2 kg racemic mixture/kg packing/day. The coupled crystallization to SMB-LC provides possibility to impurity removal, thus the valuable enantiomers can be achieved with more than 99% (w/w) purity [1-5]. The next crystallization methods can be applied: resolution by entrainment, separation via formation of diastereomer salts and crystallization from optically active solvents. The triangle solubility diagram of two enantiomers/one solvent system presents the possibility of enantiomer enrichment. The yield of crystallization process depends on the above mentioned triangle solubility diagram data and the inlet liquid concentration. This publication is on the SMB-LC and the coupled crystallization of enantiomers. In the first step SMB-LC process is used for enantiomer enrichment, then in the second step pure enantiomer is produced by crystallization. Valuable

enantiomer crystallization mother liquid after evaporation was recirculated to the feed of SMB-LC. Determination of optimal concentration of the valuable enantiomer coming from SMB-LC is an important task during the planning of hybrid process (SMB-LC, liquid evaporation, crystallization with cooling, mother liquid recirculation, etc.).

Enantiomer separation by chiral liquid chromatography

The first and significant step in planning of chiral chromatographic enantiomer separation is to determine the proper moving and stationary phases can be made by screening experiments in analytical scale. These data are used for SMB-LC mathematical models, calculations helping previous planning and size increase of SMB-LC process.

Fundamentals of SMB-LC chromatography

Nowadays the SMB-LC process is widely used and well applied in various separation problems. The large scale SMB-LC process was developed by UOP in 1960 and since this time it has gone over tremendous development. Since 1980 it has been used especially in fine chemical industry and for the last decade in the field of pharmaceutical industry.

On Fig. 1. the classical four column open eluent loop SMB-LC process is presented. The SMB-LC chromatographic process is a multi column system with two inlet (fresh eluent and feed of enantiomers) and three outlet (extract, raffinate and outlet liquid) streams where the liquid and solid phases are moving in counter current direction. The counter current stream of liquid and solid phases is not real, but simulated as the stationary phase is moving by columns at each switching time. Simulated solid phase movement is carried out at the switching time by the proper periodical change of inlet and outlet points of the equipment. These points divide the SMB-LC equipment for four zones (I, II, III, IV). The enantiomer solution (A, B) for separation is fed (F = feed) into the equipment amidst II and III zones. As the component A adsorbs stronger on CSP than component B, the previous is moving rather with the solid phase while the other one is moving with the liquid phase in the SMB-LC equipment. Components A and B in zones II and III separate from each because of their different adsorption affinity and the resulted pure A and B components can be got in extract and raffinate streams respectively (Fig. 1) Solid phase regeneration happens in zone I with fresh eluent, while in zone IV the eluent regeneration is carried out by retaining the less adsorbing component B.

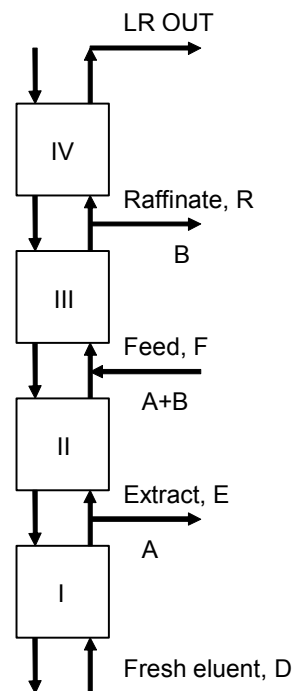


Fig. 1: Simulated moving bed (SMB) adsorber: I, II, III, IV-zones, respectively HPLC columns; D-desorbent (solvent, eluent); E-extract stream with the stronger adsorbed component A; F-feed stream with the components A and B; R-raffinate stream with the less adsorbed component B; LR OUT- outlet liquid.

Working points and the separation range of SMB-LC processes [6, 7]

The main task in SMB-LC planning is the proper choice of working conditions. Obviously it means the determination of relative volumetric streams of liquid and solid phases in each zone. Five parameters must be determined in a two component mixture separation task using SMB-LC equipment. These are the liquid volumetric streams in four zones and the so called switching time. Morbidelli and co-workers significantly contributed to the handling of this non trivial planning problem. According to the above Morbidelli theory or triangle was used for the initial parameters calculation of SMB-LC.

Assume the next component balance equation for equilibrium adsorption without component indexes.

$$B_f \left(\frac{\partial c}{\partial z} \right)_t + (1 - \varepsilon) A_f \left(\frac{\partial q}{\partial t} \right)_z + \varepsilon A_f \left(\frac{\partial c}{\partial t} \right)_z = 0$$

Where B_f = volumetric velocity (cm^3/min), c = concentration in liquid phase (mg/cm^3), z = axial distance (cm), t = time (min), ε = bed voidage (cm^3 liquid/ cm^3 column), A_f = column cross section (cm^2), q = concentration in solid phase (mg/cm^3).

Assuming linear adsorption equilibrium isotherm:

$$q = K \cdot c$$

The linear velocity of liquid element with c concentration, u_c = (cm/min) after the de Vault equation:

$$\left(\frac{\partial z}{\partial t}\right)_c = u_c = \frac{\frac{B_f}{A_f}}{\varepsilon + (1-\varepsilon)K}$$

The column is packed with granular solid adsorbent, which does not fill the full volume. The column can be characterised by the bed voidage (ε).

In our case “A” and “B” two component mixture was investigated with K_A and K_B adsorption equilibrium distribution coefficient.

In our case $K_A > K_B$, K measure unit is (g component/ cm^3 solid)/(g component/ cm^3 liquid).

Determination of the Morbidelli parameters [6,7]

Let examine the III zone of the SMB-LC equipment. Presume, that the adsorbent have neither “A” nor “B” components and during “T” switching time “A” and “B” components are fed into the III zone. As the volumetric velocity in the given III zone is $D-E+F$, so the velocities of “A” and “B” components in III zone are as follows (see Fig. 1):

$$u_A = \frac{\frac{D-E+F}{A_f}}{\varepsilon + (1-\varepsilon)K_A} \quad u_B = \frac{\frac{D-E+F}{A_f}}{\varepsilon + (1-\varepsilon)K_B}$$

The length of the III zone is L (cm), the cross section of the column is A_f .

“A” is not allowed to run out of the III zone, but “B” must leave it.

$$\varepsilon + (1-\varepsilon)K_B < \frac{\frac{D-E+F}{A_f}}{\frac{L}{T}} < \varepsilon + (1-\varepsilon)K_A$$

$$K_B < \frac{\frac{D-E+F}{A_f} T - L \varepsilon}{L(1-\varepsilon)} < K_A$$

$$K_B < m_{III} < K_A$$

m_{III} – Morbidelli parameter, relative velocity for the III zone

The next can be written for the II zone of the SMB-LC equipment:

“B” component must leave the II zone and “A” is not allowed to get through:

$$\varepsilon + (1-\varepsilon)K_B < \frac{\frac{D-E}{A_f}}{\frac{L}{T}} < \varepsilon + (1-\varepsilon)K_A$$

$$K_B < \frac{\frac{D-E}{A_f} T - L \varepsilon}{L(1-\varepsilon)} < K_A$$

$$K_B < m_{II} < K_A$$

m_{II} – Morbidelli parameter, relative velocity for the II zone

In the I zone of the SMB-LC equipment no “A” can be remained, so regeneration must be perfect.

$$m_I = \frac{\frac{D}{A_f} T - L \varepsilon}{L(1-\varepsilon)} > K_A$$

m_I – Morbidelli parameter, relative velocity for the I zone

“B” component is not allowed to leave the IV zone of the SMB-LC equipment.

$$m_{IV} = \frac{\frac{(D-E+F-R)}{A_f} T - L \varepsilon}{L(1-\varepsilon)} < K_B$$

m_{IV} – Morbidelli parameter, relative velocity for the IV zone

These conditions are necessary for the separation of a two-component “A”, “B” mixture for pure “A” and “B” components.

Summary	$K_A < m_I$
	$K_B < m_{II} < K_A$
	$K_B < m_{III} < K_A$
	$m_{IV} < K_B$

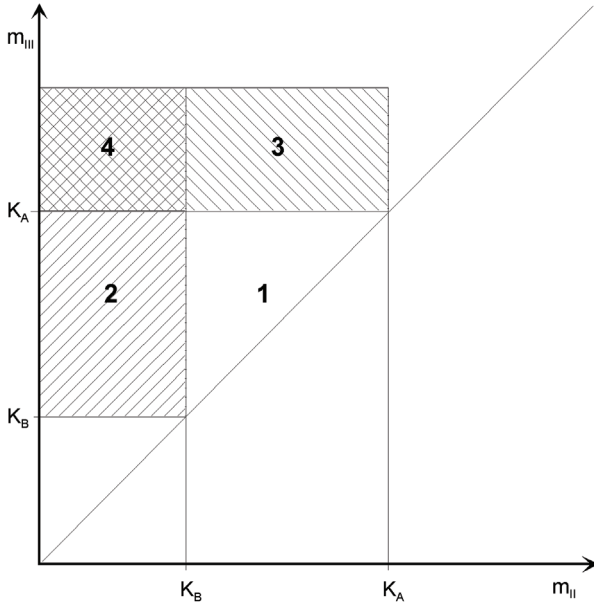


Fig. 2: Morbidelli triangle in case of linear adsorption isotherms assuming independent adsorption: Region 1: pure "A" and pure "B", Region 2: pure "B" in raffinate, impure "A" in extract, Region 3: pure "A" in extract, impure "B" in raffinate, Region 4: impure "A" in extract, impure "B" in raffinate.

Theoretical analysis in case of non linear adsorption isotherms [6,7]

If linear adsorption equilibrium isotherms and independent adsorption conditions are not existing during the theoretical description of equilibrium adsorption described in the previous chapter, than the Morbidelli triangle modifies.

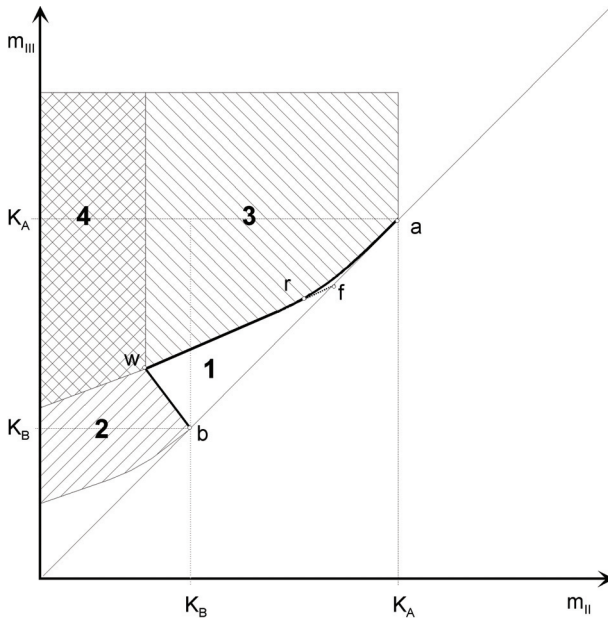


Fig. 3: Modification of Morbidelli triangle in case of competitive Langmuir isotherms

It can be seen from the *Figure 3*, that the modified Morbidelli triangle is also divided into four regions: Region 1: pure "A" and pure "B"; Region 2: pure "B" in raffinate, impure "A" in extract; Region 3: pure "A" in extract, impure "B" in raffinate; Region 4: impure "A" in extract, impure "B" in raffinate

Let the two component competitive Langmuir adsorption isotherm be valid:

$$q_i = \frac{a_i c_i}{1 + b_1 c_1 + b_2 c_2} \quad i = 1, 2$$

Where:

- q_i – solid phase concentration (mg/cm³ adsorbent)
- c_i – liquid phase concentration (mg/cm³)
- a_i – Langmuir constant (cm³ liquid/cm³ adsorbent)
- b_i – Langmuir constant (cm³ liquid/mg "A" or "B" component).

The conditions of simultaneous production of pure "A" and "B" components are as follows:

$$m_I > K_A$$

$$m_{II, \min}(m_{II}, m_{III}) < m_{II} < m_{III} < m_{III, \max}(m_{II}, m_{III})$$

$$m_{IV} < m_{IV, Kr}$$

$$m_{IV, Kr} = \frac{1}{2} [K_B + m_{III} + b_B c_B^F (m_{III} - m_{II}) - \sqrt{[K_B + m_{III} + b_B c_B^F (m_{III} - m_{II})]^2 - 4a_B m_{III}}]$$

where F means the feed of SMB-LC equipment (cm³/min).

Explanation of *Fig 3*:

Point a $K_A = K_A$

Point b $K_B = K_B$

Point f $\omega_G = \omega_G$

Point r

$$\left[\frac{\omega_G^2}{K_A}, \frac{\omega_G [\omega_F (K_A - \omega_G) (K_A - K_B) + K_B \omega_G (K_A - \omega_F)]}{K_A K_B (K_A - \omega_F)} \right]$$

Point w

$$\left[\frac{K_B \omega_G}{K_A}, \frac{\omega_G [\omega_F (K_A - K_B) + K_B (K_B - \omega_F)]}{K_B (K_A - \omega_F)} \right]$$

Where ω_F and ω_G ($\omega_G > \omega_F > 0$) are the roots of equation given below

$$(1 + b_A c_A^F + b_B c_B^F) \omega^2 - [K_A (1 + b_B c_B^F) + K_B (1 + b_A c_A^F)] \omega + K_A K_B = 0$$

It can be seen on *Fig. 3* that the shape and area of Morbidelli triangle significantly changes caused by the competitive Langmuir isotherm affected by feeding total and component concentrations, real effects as adsorption kinetics (component transfer resistances), axial mixing phenomena, column packing efficiency (*Fig. 4*).

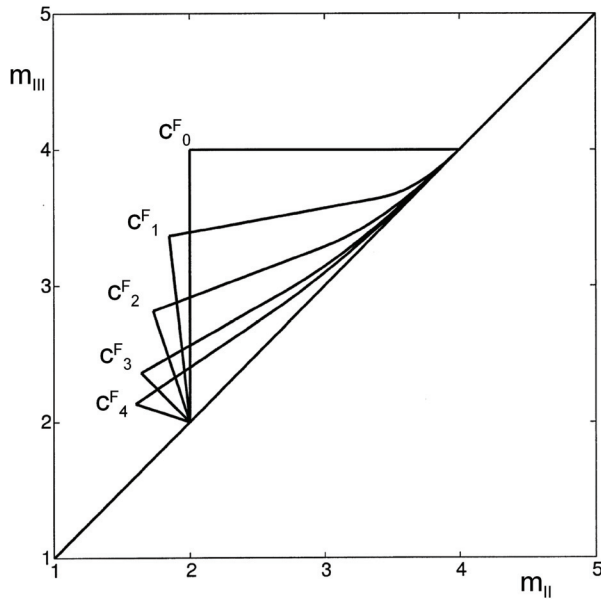


Fig. 4: Feeding concentration change on Morbidelli triangle in case of racemic mixture ($c_0^F < c_1^F < c_2^F < c_3^F < c_4^F$, $c_0^F \sim 0$ g/dm³)

Increasing the feeding concentration the triangle area decreases and deforms at constant Langmuir adsorption equilibrium parameters. It is unfavourable for enantiomer separation.

The mathematical model of SMB-LC

From the above equations, it can be seen that the pure extract-pure raffinate area is smaller by higher feed concentration than the rectangular triangle, consequently we have less possibility by parameter variation. The Morbidelli's area changes, when the following possibilities of intervention vary during the planning: temperature, changing adsorbent, and composition of the solvent (eluent).

When we have already selected the chromatographic packing for the separation, the next task is to choose operating variables: fresh eluent, recirculated eluent, feed, and extract, raffinate flow rates, switching time. During calculation, the influence of a given parameter is to be investigated, the others must be considered as constants.

Better productivity, product purity, yield, and eluent consumption can be achieved by optimizing operating conditions by computer with the exact mathematical model of the SMB. The basic equation of the mathematical model can be deduced from the component balance of the solid-liquid equilibrium system [8, 9]. The equal balance for the component "k" is:

$$v_0 \left(\frac{\partial c_k}{\partial z} \right)_t + (1 - \varepsilon) \left(\frac{\partial q_k}{\partial t} \right)_z + \varepsilon \left(\frac{\partial c_k}{\partial t} \right)_z = 0$$

Where v_0 = velocity of fluid phase (cm/min),
 dc_k/dz = place derivative of liquid concentration of component k,
 dc_k/dt = time derivative of liquid concentration of component k, and

dq_k/dt = time derivative of solid concentration of component k.

When we discuss a two-component equilibrium system the competitive multi-Langmuir type isotherm can be written as:

$$q_1 = \frac{a_1 c_1}{1 + b_1 c_1 + b_2 c_2} \quad q_2 = \frac{a_2 c_2}{1 + b_1 c_1 + b_2 c_2}$$

The denominator of this isotherm was replaced by "N":

$$N = 1 + b_1 c_1 + b_2 c_2$$

Considering the equal balance and concentration (c_1, c_2) derivatives of the above Eq. can be written:

$$-\frac{v_0}{1 - \varepsilon} N^2 \frac{\partial c_1}{\partial z} = \frac{\partial c_1}{\partial t} \left(\frac{a_1 N - a_1 b_1 c_1}{N^2} + \frac{\varepsilon}{1 - \varepsilon} \right) \frac{a_1 b_2 c_1}{N^2} \frac{\partial c_2}{\partial t}$$

The derivatives were substituted with difference quotients:

$$-\frac{v_0}{1 - \varepsilon} N^2 \frac{\Delta c_1}{\Delta z} = \frac{\Delta c_1}{\Delta t} \left(a_1 N + \frac{\varepsilon}{1 - \varepsilon} N^2 \right) - a_1 c_1 \left(b_1 \frac{\Delta c_1}{\Delta t} + b_2 \frac{\Delta c_2}{\Delta t} \right)$$

Difference Eq. above was rearranged and written for component "k":

$$-\frac{v_0}{1 - \varepsilon} N^2 \frac{\Delta c_k}{\Delta z} + a_k c_k \sum_{k=1}^n b_k \frac{\Delta c_k}{\Delta t} = \frac{\Delta c_k}{\Delta t} \left(a_k N + \frac{\varepsilon}{1 - \varepsilon} N^2 \right) - a_k b_k c_k$$

The above Eq. is the base of the numeric simulation software. The component transfer is calculated with this Eq. between the so-called equilibrium cascades. In our model, the number of equilibrium cascades are compliant with the number of theoretical plates. Based on the mathematical model, the KROM-N and SMB KROM-N computer programs were written in Delphi computer language [10]. Frontal adsorption experiments and SMB-LC measurements can be simulated by the computer programs. At 200 NTP/column the calculation time on a 3 GHz personal computer is 0,6% of the measurement time. The input data of the software: number of components, feed concentration, adsorption equilibrium properties (Langmuir parameters), column geometrical data (average) NTP/column, total porosity, bulk density, volumetric velocities (eluent, feed, extract, raffinate, recirculation).

The initial SMB operating conditions were calculated with the equilibrium triangle method. We considered the maximal flow rate of eluent pump, and minimal column switching time was chosen.

Simulated moving bed liquid chromatography (SMB-LC) coupling with crystallization [1-5]

Enantiomer purification by crystallization is based on their ternary solubility diagrams. According to the type of saturation curves in the phase diagram, three fundamental types (conglomerate, racemic compound, pseudo racemate) forming systems can be identified. Only 5-10% of racemates belong to the conglomerate forming group,

90-95% belong to the true racemates and the third group pseudo racemates are relatively few.

On Fig. 5 the solubility diagram of two, (S)- and (R)-enantiomers and solvent "H" can be seen on equilateral triangle at different temperatures.

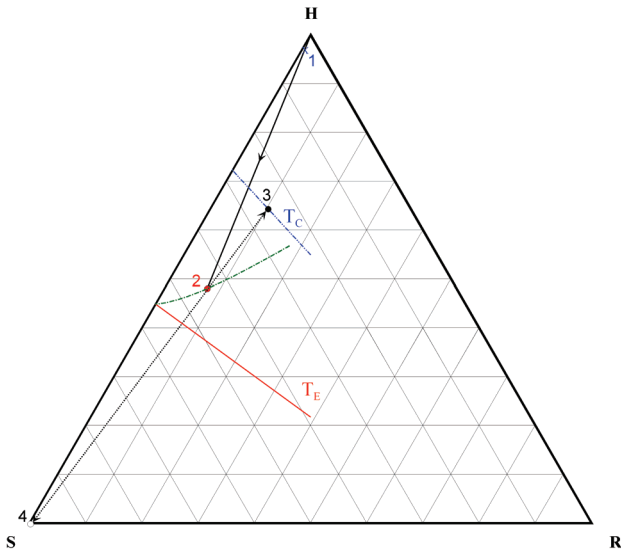


Fig. 5: The solubility diagram of two enantiomers, (S) and (R) in "H" solvent. (S): less retained enantiomer, (R): more retained enantiomer, H: n-hexane solvent, 1. Raffinate, 2. Evaporated raffinate, 3. Crystallization mother liquid, 4. (S) crystals, $T_C = -20\text{ }^\circ\text{C}$ cooling temperature, $T_E = 50\text{ }^\circ\text{C}$ evaporation temperature, -.-.- limiting evaporated liquid concentration curve of $>99\%$ (w/w) (S) crystals.

SMB-LC and joined crystallization processes are recommended for enantiomer separation according to Fig. 5. The (S)-enantiomer in required purity can be achieved, if crystallization started from an asymmetric component content.

Joined SMB-LC and crystallization process starts at Point 1 (Raffinate) with (S) enrichment. As the raffinate is a diluted unsaturated liquid (Point 1), it must be evaporated to put Point 2 between evaporation and cooling solubility curves. Cooling the liquid (Point 2) forms crystallization mother liquid (Point 3) and pure (S) crystal is resulted (Point 4).

SMB-LC, evaporation, cooling crystallization and crystallization mother liquid recycling

The joint process can be seen on the Fig. 6, where both enantiomers can be purified by crystallization. In our work only the (S)-enantiomer was crystallized and its crystallization mother liquid was recycled to the feed. In case of (R)-enantiomer only the eluent was recycled. The original (S) and (R) 50-50% (w/w) feed is to be changed because of the recirculation of (S) crystallization mother liquid and thus concentration of (S) increases over 50%. We have to work out an optimization method, which has got an objective function ((S) productivity maximalization, fresh solvent consumption minimalization) at higher than 99% (w/w) (S) purity, and higher than 99% (S) yield.

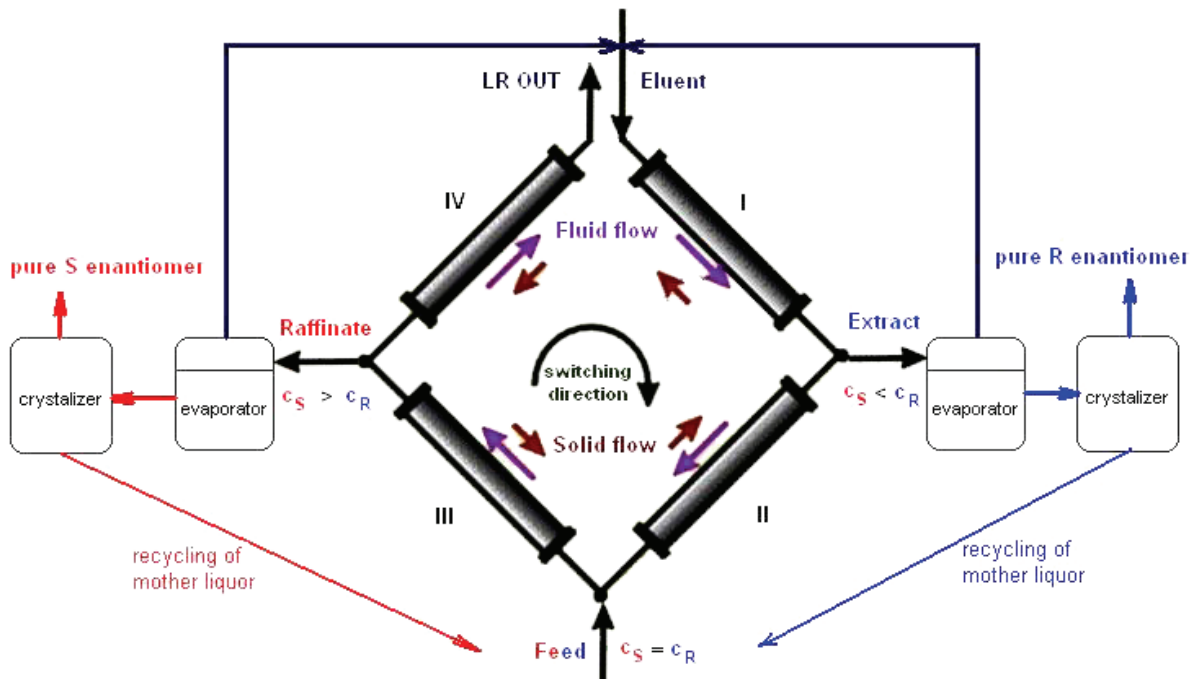


Fig. 6: SMB-LC, evaporator, cooling crystallization and crystallization mother liquor recirculation.

Purity and productivity are determined by the next equations in extract and in raffinate.

$$\text{Purity} = \frac{c_S}{c_S + c_R} \cdot 100$$

$$P_S = \frac{R \cdot c_S^R}{4V_C \rho_B} \quad P_R = \frac{E \cdot c_R^E}{4V_C \rho_B}$$

c_S – (S) concentration in extract or raffinate streams (g/dm³)

c_R – (R) concentration in extract or raffinate streams (g/dm³)

P – productivity (mg component/g packing/day)

R, E – raffinate and extract volumetric streams (cm³/min)

V_C – column volume (cm³)

ρ_B – bulk density (g/cm³)

In case of the high purity production of (S)-enantiomer only by SMB-LC the (S) productivity is low. Joining SMB-LC and crystallization the (S) productivity can be significantly increased.

Experiments

Chiral racemic ester mixture separation was investigated as a model system. The two pure (S)- and (R)-enantiomers can not be reached in the market and no data can be found in special literature on them. During our research work both chromatographic and crystallization measurements were carried out. By previous analytical and preparative scale HPLC measurements adsorption isotherms were estimated for chiral stationary phase-eluent systems. The ternary system solubility (S, R, solvent) diagrams were determined at different temperatures in laboratory scale experiments. Several laboratory scale SMB-LC and joined crystallization experiments were carried out based on previous chromatographic and solubility data.

Further we describe in details the applied experimental methods.

Chiral chromatographic packing selection

Chiral racemic ester mixture separation was investigated by analytical HPLC columns (I.D. = 0.46 cm, L = 25 cm, d_p = 5-10 μ m) at 20 °C. Previous selection of CSP was done by DAICEL handbook. Accordingly the next CSP of DAICEL were chosen: Chiralcel OD-H, Chiralcel OJ, Chiralpak AD, Chiralpak IA, Chiralpak AS. The applied eluents are as follows: n-hexane, IPA, Et-OH, AcN, Me-OH, MTBE, DKM in different volumetric ratio with 1 cm³/min volumetric velocity. The 20 μ l, 5 g racemic mixture/dm³ eluent concentration sample was separated by analytical HPLC column. The enantiomers were detected by GILSON type UV spectrophotometer at 254 nm wavelength.

Investigation of CSP packing properties

The SUPELCO (I.D. = 1 cm, L = 25 cm) column was packed with the 20 μ m Chiralcel OD by dry vibration method applying 60 min vibration time. Air was removed by n-hexane:IPA = 95:5% (v/v) eluent. After that the NTP was determined by injection method. Applied eluents were: n-hexane:IPA = 80:20, 90:10, 95:5% (v/v). Reaching equilibrium condition with eluent, a 100 μ l 50 g racemic mixture/dm³ eluent sample was injected to the column by a Rheodyne type injector at 20°C temperature. Detection of enantiomers happened by WATERS UV detector at 254 nm wavelength or CHIRALYSER type chiral detector. Applied eluent volumetric velocities: 2.5, 5, 10, 15, 20, 30 cm³/min. Evaluation of residence time distribution curves were done by triangle method (determination of σ , t_R , NTP, α values). The packing total porosity (ϵ) was determined by TTBB (Tri-tert-butyl-benzene) method. Bulk density of CSP was calculated from weight measurements of dry packing. Values of Langmuir a* constants were calculated from k' values. Langmuir b constants were calculated from frontal adsorption-elution measurement data [11].

Frontal adsorption-elution measurements

Measurements were carried out by SUPELCO column (I.D.=1 cm, L=25 cm) packed with 20 μ m Chiralcel OD at 20 °C temperature using n-hexane:IPA = 95:5, 95:7% (v/v) eluent.

2, 5, 10 g racemic mixture/dm³ eluent sample in 10 cm³ volume was injected to the column with 2.5, 5 cm³/min volumetric velocities. Detection happened by GILSON type UV spectrophotometer at 254 nm wavelength. Based on the above data the Langmuir adsorption isotherm a* and b values were calculated by Felinger et al. [10] method.

Description of laboratory scale SMB-LC equipment

The SMB-LC equipment with four columns (I.D. = 1 cm, L = 25 cm SUPELCO type stainless steel, mounted with liquid distributor and porous stainless steel plates), four zones and open eluent loop was constructed in the Central Mechanical Workshop of the Pannon University. The joint pipes were made of 1/16" stainless steel material, the liquid stream controlled by VALCO 5 four-ways and 4 two-ways cocks, and by 4 GILSON type HPLC pumps with maximum volumetric velocity: one of them is 5 cm³/min, two are 10 cm³/min and one is 25 cm³/min. Volumetric velocities (D, E, F, R) were measured by EMX 100/211 type digital balance connected to a computer. Pressure gauge was connected after the eluent pump. Extract and raffinate outlet streams were detected on-line by UV or chiral detectors.

There is no liquid recirculation in open loop SMB-LC equipment, but the liquid from IV zone is collected (LR OUT) separately. The feed composition is 2.5 g (S)/dm³ eluent and 2.5 g (R)/dm³ eluent. The eluent composition is: n-hexane:IPA = 95:5% (v/v), 20 °C temperature, column configuration 1:1:1:1. Volumetric velocities adjusted on different pumps: fresh eluent D = 20 cm³/min, feed F = 0.5-1.5-2.9 cm³/min, extract E = 4 cm³/min, raffinate R = 6 cm³/min. Switching time is 5 min. The SMB-LC equipment was working by the usual four-zone process, in each 5 minutes inlet and outlet points were changed.

Examination of SMB-LC product streams

Extracts, raffinates and LR OUT liquid streams were collected in the 1st, 2nd, 3rd and 4th cycle separately through 20-20 minutes. During the first switching time of the 5th cycle extract, raffinate and LR OUT streams were fractionated in each 75 seconds for studying concentration transients. The above samples were analysed by GILSON HPLC equipment, on Chiralcel OD-H packing at 20 °C temperature, at UV 254 nm wavelength using n-hexane:IPA = 95:5% (v/v) eluent.

Solubility investigations

20 mg 80:20, 85:15, 90:10, 95:5, 100:0% (w/w) (S):(R) samples were solved in 4 cm³ n-hexane at 20 °C temperature, later cooled to -22 °C and kept staying for 8 hours. Crystals and crystallization mother liquid were separated from each other and analyzed by GILSON analytical HPLC equipment. Crystal purity in each case was higher than 99% (w/w) (S). The solubility of pure (S)-enantiomer in n-hexane at -22 °C was 1.2 g/dm³, at 20 °C it was 28.5 g/dm³. Solubility of pure (R) in n-hexane at -22 °C was also 1.2 g/dm³. Solubility of "SR" racemate at -27 °C was 0.735 g/dm³, at 20 °C was 25.45 g/dm³. Solubility of (S) and (R) was examined in n-hexane-IPA mixtures at 0, 5, 10, 20% (v/v) IPA content too.

Extract and raffinate fractions of the SMB-LC measurements were separately evaporated in vacuum at 40-60 °C temperature in Rotadest equipment according to Fig. 5. Because of the n-hexane-IPA vapour liquid equilibrium data at 95:5% (v/v) n-hexane:IPA concentration during evaporation only n-hexane remained in liquid phase. Evaporation was done on the way, that enantiomer concentration was about 5 g/dm³ in the evaporated liquid. The evaporated liquid was kept in a -20...-27 °C freezer through 8 hours. Crystals and crystallization mother liquid were separated from each other and analyzed by GILSON analytical HPLC equipment.

Results

CSP packing selection

Capacity relations and selectivity coefficients were calculated from analytical HPLC measurements.

$$k' = \frac{t_R - t_0}{t_0} \quad \alpha_S^R = \frac{k'_R}{k'_S} \quad \varepsilon = \frac{d^2 \pi \cdot L}{F \cdot t_0}$$

Where t_R – retention time of the given enantiomer (min)

t_0 – dead time (min),

d = 0.46 cm, inner diameter of column,

L = 25 cm, length of column,

F = 1 cm³/min, eluent volumetric velocity

ε – bed voidage, total porosity (determined by TTBB method)

On *Table 1* are summarized the experimental data. The best α_S^R selectivity was given in case of Chiralcel OD-H packing and n-hexane-IPA eluent. Thus this system was used in further experiments.

Table 1: Screening measurement results by analytical HPLC equipment

Eluent	Concentration (% v/v)	Packing				
		Chiralcel OD-H	Chiralcel OJ	Chiralpak AS	Chiralpak AD	Chiralpak IA
n-hexane:IPA	70:30		$\alpha^R_s=1$			
n-hexane:IPA	80:20	$\alpha^R_s=1.17$	$\alpha^R_s=1.196$			
n-hexane:IPA	90:10	$\alpha^R_s=1.173$	$\alpha^R_s=1$		$\alpha^R_s=1$	
n-hexane:IPA	97.5:2.5					$\alpha^R_s=1.06$
n-hexane:IPA	95:5	$\alpha^R_s=1.19$	$\alpha^R_s=1$			$\alpha^R_s=1.03$
n-hexane:Et-OH	95:5	$\alpha^R_s=1.122$				
n-hexane:IPA:AcN	80:10:10		$\alpha^R_s=1$			
AcN	100			$k'_s=0.24$	$\alpha^R_s=1$	
AcN-Me-OH	80:20			$\alpha^R_s=1$	$\alpha^R_s=1$	
Ethanol	100				$\alpha^R_s=1$	
n-hexane:MTBE	80:20	$k'_s>20$				
n-hexane:MTBE	60:40	$k'_s=6.10$				
n-hexane:MTBE:Et-OH	(60:40)+5% Et-OH	$k'_s=1.086$				
n-hexane:IPA						$\alpha^R_s=1.08$
n-hexane:Met-OH	99:1					$\alpha^R_s=1.12$
n-hexane:Et-OH	99:1					$\alpha^R_s=1.11$
n-hexane:DKM	75:25					$\alpha^R_s=1.03$
n-hexane:IPA:EtOH	95:2.5:2.5	$\alpha^R_s=1.15$				
n-hexane:IPA:MetOH	95:2.5:2.5	$\alpha^R_s=1.05$				
n-hexane:EtOH:MetOH	95:2.5:2.5	$\alpha^R_s=1.05$				

Investigation of CSP packing properties

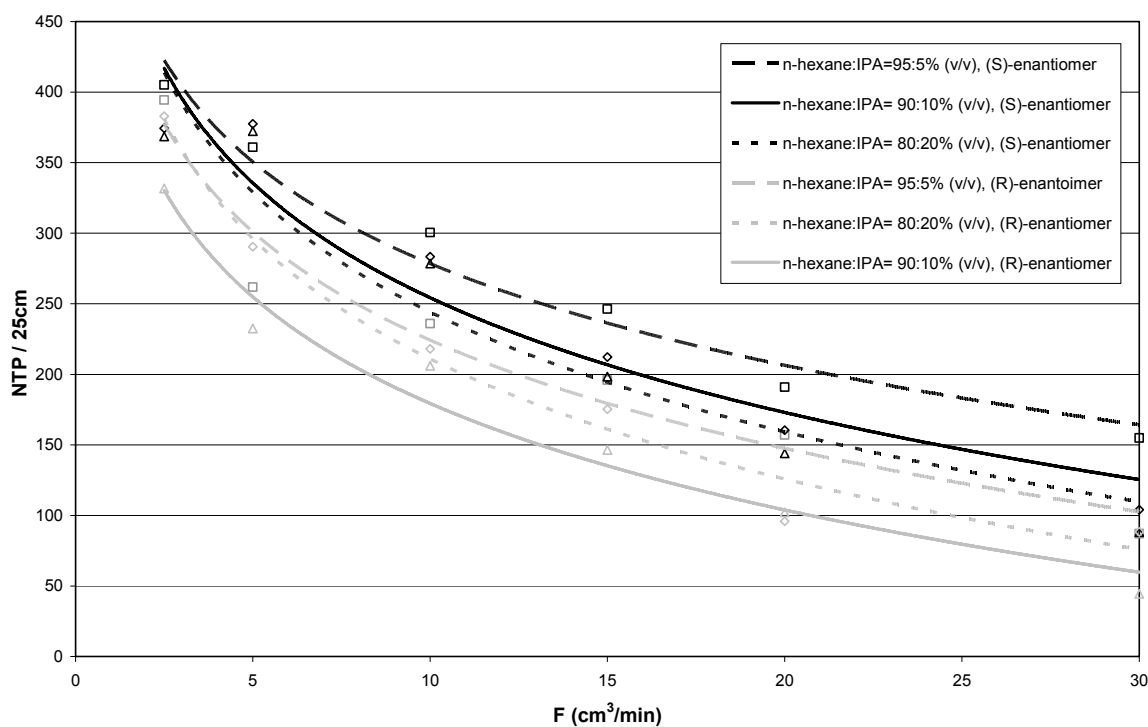


Fig. 7: Results of elution chromatographic experiments, SUPELCO HPLC column (I.D. = 1 cm, L = 25cm), Chiralcel OD (particle size: 20 μm) packing, T = 20 $^{\circ}\text{C}$, sample: 50 g chiral racemic mixture/ cm^3 eluent, 100 μl injection, eluent: n-hexane:IPA.

For example the n-hexane:IPA = 95:5% (v/v), (S) isomer experimental curve can be described by the next fitting equation.

$$NTP = 643.36F^{-0,3876}$$

Where F – volumetric velocity (cm^3/min),

NTP – Number of theoretical plates/25 cm column,

ε = 0.67 bed voidage, total porosity

ρ_B = 0.6 g dry CSP/ cm^3 column volume, bulk density

Frontal adsorption-elution measurement and simulation

On the basis of k' capacity relation values determined by elution measurements (Chiralcel OD-H, 95:5% (v/v) = n-hexane:IPA) K Morbidelli parameters and Langmuir adsorption a^* values were determined at 20 °C temperature, b values of Langmuir adsorption isotherm were calculated by Felinger et al. [11] method using the KROM-N simulation program (Table 2).

Table 2: Input data of KROM-N software

Data input to the KROM-N software	
Number of components:	2
Column inner diameter:	I.D. = 1 cm
Column length:	L = 25 cm
Free volume coefficient:	$\varepsilon = 0.67 \text{ cm}^3 \text{ liquid free volume/cm}^3 \text{ column}$
Bulk density:	$\rho_B = 0.6 \text{ g packing/cm}^3 \text{ column}$
Langmuir constants:	as given
Volumetric velocity:	2-2.5-5 cm^3/min
Sample feeding time:	2-4-5-6 min (10 cm^3)
Sample concentration:	$c_B^F = 1 - 2.5 - 5 \text{ g (S) component/dm}^3 \text{ liquid}$ $c_A^F = 1 - 2.5 - 5 \text{ g (R) component/dm}^3 \text{ liquid}$
Number of Theoretical Plates:	NTP = 200/25 cm column
End of calculation time:	400 min

$$K_{A(R)} = k'_{A(R)} \frac{\varepsilon}{1 - \varepsilon} = 12.998 \frac{\text{cm}^3 \text{ liquid}}{\text{cm}^3 \text{ adsorbent}}$$

$$K_{B(S)} = k'_{B(S)} \frac{\varepsilon}{1 - \varepsilon} = 10.058 \frac{\text{cm}^3 \text{ liquid}}{\text{cm}^3 \text{ adsorbent}}$$

$$\rho_{BULK} = 0.6 \frac{\text{g packing}}{\text{cm}^3 \text{ column}} \quad \alpha_S^R = 1.29$$

$$\varepsilon = 0.67 \frac{\text{cm}^3 \text{ liquid}}{\text{cm}^3 \text{ column}} \text{ (Tri - tert - butil - benzol method)}$$

$$a_{A(R)}^* = K_{A(R)} \frac{1 - \varepsilon}{\rho_{BULK}} = 7.1489 \frac{\text{cm}^3 \text{ liquid freevolume}}{\text{g packing}}$$

$$b_{A(R)} = 0.016 \frac{\text{cm}^3 \text{ liquid freevolume}}{\text{mg A component}}$$

$$a_{B(S)}^* = K_{B(S)} \frac{1 - \varepsilon}{\rho_{BULK}} = 5.5320 \frac{\text{cm}^3 \text{ liquid freevolume}}{\text{g packing}}$$

$$b_{B(S)} = 0.012 \frac{\text{cm}^3 \text{ liquid freevolume}}{\text{mg B component}}$$

KROM-N simulation program was used for simulation and compared to the measurement results. The conclusion is, that small concentration (2 g/dm^3 sample) measurements provide the best separation.

Results of SMB-LC simulations

We examined the inlet concentration effect on Morbidelli triangle. Enantiomers in feed are in equal ratio, $(R)=(S)$ as a racemic mixture. By data given in previous chapter.

$$K_R = 12.998 \frac{\text{cm}^3 \text{ liquid}}{\text{cm}^3 \text{ adsorbent}}$$

$$K_S = 10.058 \frac{\text{cm}^3 \text{ liquid}}{\text{cm}^3 \text{ adsorbent}}$$

Fig. 8 shows, that increase of inlet concentration decreases deforms the pure components separation area of Morbidelli triangle.

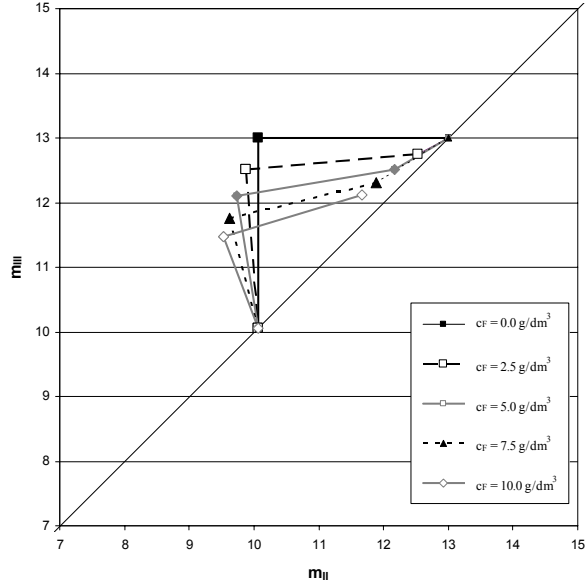


Fig. 8: Changes in Morbidelli triangle in function of feed concentration, $c_F = c_S + c_R$, $c_S = c_R$ in feed.

The SMB-LC process volumetric velocity by zones and the switching time were determined in linear adsorption isotherm region using the Morbidelli theory. On the basis of that the next relations must be true to produce pure (S)- and pure (R)-enantiomers respectively in product streams:

$$\begin{aligned} 12.998 &= K_R < m_{I} \\ 10.058 &= K_S < m_{II} < K_R = 12.998 \\ 10.058 &= K_S < m_{III} < K_R = 12.998 \\ m_{IV} &< K_S = 10.058 \end{aligned}$$

Assuming 5 min switching time by the above relations the fresh eluent (D) must be over 19.47 cm^3/min , extract stream between 0.53 and 4.34 cm^3/min , the feed stream between 0 and 3.47 cm^3/min values. The minimal raffinate stream was 3.24 cm^3/min value. During simulation the aim was to achieve 80, 85, 90, 95, 99% (w/w) (S) pure raffinate at >99% (S) yield. The fresh (D) volumetric velocity can not be higher than 20 cm^3/min because of the allowed highest 50 bar pressure (CSP packing specification). The working points of the simulations can be found in Morbidelli triangle on Fig. 9, the parameters in Table 3 and the results in Table 4.

Table 3: Input data of SMB KROM-N software

Data input to the SMB-KROM-N software								
Number of components:	2							
Column inner diameter:	I.D. = 1 cm							
Column length:	L = 25 cm							
Number of columns:	N = 4							
Free volume coefficient:	$\epsilon = 0.67 \text{ cm}^3 \text{ liquid free volume/cm}^3 \text{ column}$							
Bulk density:	$\rho_B = 0.6 \text{ g packing/cm}^3 \text{ column}$	SIM 1.	SIM 2.	SIM 3.	SIM 4.	SIM 5.	SIM 6.	SIM 7.
Feed:	cm^3/min	3.47	3.2	2.9	2.6	2.35	1.5	0.5
Fresh eluent:	$D = 20 \text{ cm}^3/\text{min}$							
Extract:	$E = 4 \text{ cm}^3/\text{min}$							
Raffinate:	$R = 6 \text{ cm}^3/\text{min}$							
Recycling:	$\text{REC} = 0 \text{ cm}^3/\text{min}$							
Langmuir constants:	as given							
Feed concentration:	$c_B^F = 2.5 \text{ g (S) component/cm}^3 \text{ liquid}$ $c_A^F = 2.5 \text{ g (R) component/cm}^3 \text{ liquid}$							
Number of Theoretical Plates:	$\text{NTP} = 200/25 \text{ cm column}$							
Switching time:	5 min							
Calculation time:	400 min							

Table 4: Results of simulations

Simulation	Purity		Yield		Productivity		Eluent consumption	
	S% (A/A)	R% (A/A)	S (%)	R (%)	(mg S / g packing/day)	(mg R / g packing/day)	($\text{cm}^3 \text{ eluent/mg S}$)	($\text{cm}^3 \text{ eluent/mg R}$)
SIM 1.	76.44	99.61	99.97	68.84	268.31	184.16	2.27	3.32
SIM 2.	80.43	99.63	99.97	75.29	245.40	184.15	2.49	3.32
SIM 3.	85.88	99.65	99.98	83.04	222.50	184.06	2.74	3.33
SIM 4.	93.18	99.66	99.98	91.77	199.63	182.37	3.06	3.35
SIM 5.	97.43	99.64	99.97	96.10	180.54	173.61	3.38	3.54
SIM 6.	99.73	99.35	99.96	98.06	115.32	112.43	5.3	5.44
SIM 7.	99.98	98.38	99.96	97.98	38.55	37.41	15.86	16.34

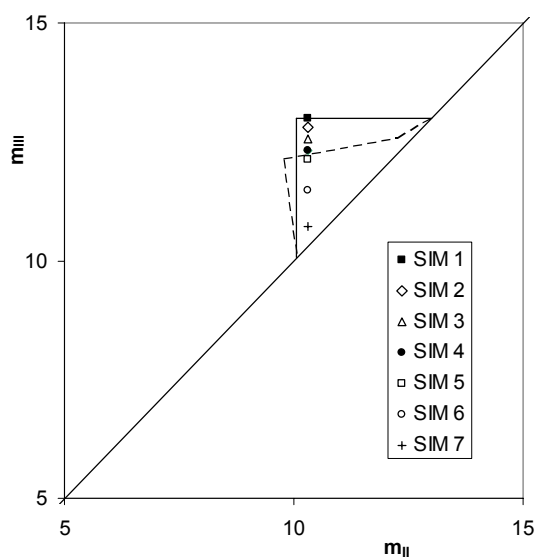


Fig. 9: The working points of the SMB simulations, simulated with SMB KROM-N program, dash line: non-linear Morbidelli triangle at $c_F = 5 \text{ g/dm}^3$.

Summarized results for (S)-enantiomer (purity, yield, productivity, eluent consumption) can be seen on Fig. 10.

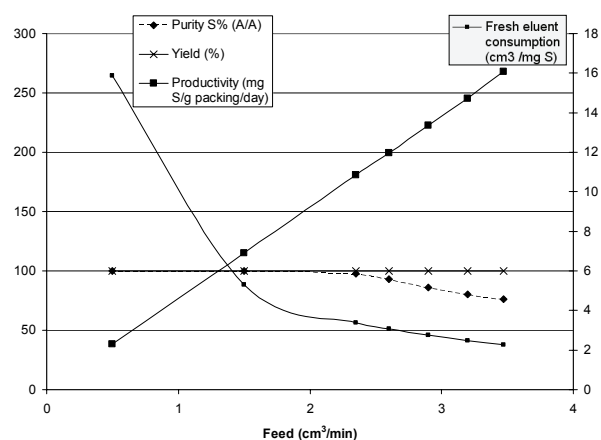


Fig. 10: Summarized results for (S)-enantiomer in Raffinate stream.

Evaporation of raffinate streams, crystallization, crystallization mother liquid recirculation

Simulation calculation were done. Solubility of both (S)- and (R)-enantiomers at $-20 \text{ }^\circ\text{C}$ temperature were 1.2 g/dm^3 . Raffinates of SMB-LC were evaporated until $5 \text{ g (S)+(R)-enantiomers/dm}^3$ concentration followed by cooling to $-20 \text{ }^\circ\text{C}$ temperature. Purity of crystal (S) was assumed 99.9% (w/w). On Table 5 are summarized data for the calculation of crystallization.

Table 5: Summarized data for the calculation of crystallization

Solution to cooling +20°C, 5 g/dm ³ total concentration			Solution at -20°C		Crystal		η
<i>S</i> (g/dm ³)	<i>R</i> (g/dm ³)	<i>S</i> : <i>R</i>	<i>S</i> (g/dm ³)	<i>SR</i> (g/dm ³)	<i>S</i> (g)	<i>S</i> (%)	
4.00	1.00	80:20	1.2	2.0	1.8	45.0	
4.25	0.75	85:15	1.2	1.5	2.3	54.1	
4.50	0.50	90:10	1.2	1.0	2.8	62.2	
4.75	0.25	95:5	1.2	0.5	3.3	69.5	
4.90	0.10	98:2	1.2	0.2	3.6	73.5	
5.00	0.00	100:0	1.2	0.0	3.8	76.0	

Crystallization mother liquid was evaporated to 5 g (*S*)+(*R*)-enantiomers/dm³ concentration, which was mixed to the SMB-LC feed. In the recycled evaporated mother liquid IPA concentration was adjusted at 5% (v/v)

Table 7: Calculated data after the third full calculation

Simulation, Crystallization code	Feed (cm ³ /min)		Concentration (g/dm ³)		Raffinate purity <i>S</i> % (w/w)	Fresh eluent consumption (cm ³ eluent/mg <i>S</i>)	Productivity (mg <i>S</i> /g packing/day)
	Rec.	Fresh	<i>c</i> ^F _{<i>S</i>}	<i>c</i> ^F _{<i>R</i>}			
SIM. III.01. C III.01.	1.37	2.10	2.85	2.15	81.71	3.80	160.96
SIM. III.02. C III.02.	1.08	2.12	2.83	2.17	84.67	3.77	162.01
SZIM. III.03. C III.03.	0.77	2.13	2.81	2.19	89.07	3.74	163.50
SZIM. III.04. C III.04.	0.47	2.13	2.79	2.21	94.70	3.73	163.43
SZIM. III.05. C III.05.	0.33	2.02	2.78	2.22	97.82	3.94	155.33
SZIM. III.06. C III.06.	0.17	1.33	2.77	2.23	99.76	6.00	102.05
SZIM. III.07. C III.07.	0.00	0.50	2.50	2.50	99.98	15.86	38.55

On the basis of the above data productivity maximum was at values 2.13 cm³/min fresh feed and 0.77 cm³/min recirculation feed, when raffinate purity was 89.07% (w/w) (*S*). Fresh eluent consumption (calculated for the total SMB+crystallization system) was 3.74 cm³ fresh eluent/mg (*S*).

Considering the pure (*S*)-enantiomer 28.5 g (*S*)/dm³ solubility in n-hexane at 20 °C temperature and (*R*) and (*S*) 1.2 g/dm³ solubility in n-hexane at -20 °C temperature, crystallization process was recalculated (See Table 8). During calculation purity of (*S*) 99.9% (w/w) was assumed in crystals. The so called limiting curve belonging to the 99.9% (w/w) (*S*) crystal value can be drawn (Fig. 11).

Table 8: Summarized data for the calculation of crystallization of 99.9% (w/w) pure (*S*)

Solution to cooling +20°C			Solution at -20°C		Crystal		η
<i>S</i> (g/dm ³)	<i>R</i> (g/dm ³)	<i>S</i> : <i>R</i>	<i>S</i> (g/dm ³)	<i>SR</i> (g/dm ³)	<i>S</i> (g)	<i>S</i> (%)	
4.80	1.20	80:20	1.2	2.40	2.40	50.0	
6.80	1.20	85:15	1.2	2.40	4.40	64.7	
10.80	1.20	90:10	1.2	2.40	8.40	77.7	
22.80	1.20	95:5	1.2	2.40	20.40	89.4	
27.93	0.57	98:2	1.2	1.14	26.16	93.7	
28.50	0.00	100:0	1.2	0.00	27.30	95.7	

value. Evaporation of raffinate streams, crystallization, crystallization mother liquid recirculation was repeated by this modified feed concentration twice and three times. After the third full calculation quasi-stationary condition was reached. Calculated data can be found in Table 6 and 7.

Table 6: First step crystallization calculated data

Crystallization code	99.9% purity crystal (mg/min)	Recirculation (cm ³ /min)	Fresh Feed (cm ³ /min)	<i>c</i> ^F _{<i>S</i>} (g/dm ³)	<i>c</i> ^F _{<i>R</i>} (g/dm ³)
C 1.01	3.80	1.50	1.97	2.82	2.18
C 1.02	4.07	1.18	2.02	2.81	2.19
C 1.03	4.38	0.81	2.09	2.79	2.21
C 1.04	4.65	0.47	2.13	2.77	2.23
C 1.05	4.59	0.32	2.03	2.76	2.24
C 1.06	3.00	0.15	1.35	2.75	2.25
C 1.07	1.00	0.00	0.50	2.50	2.50

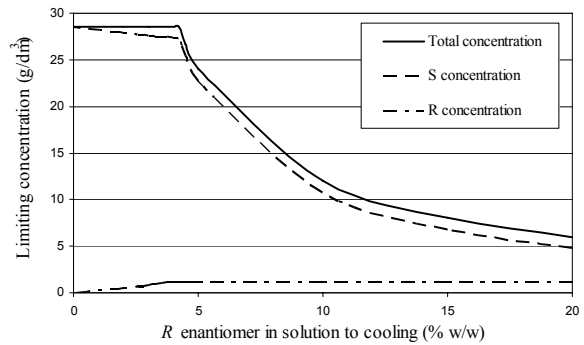


Fig. 11: Concentration limiting curve of 99.9% (w/w) (*S*) crystals, calculated.

Using the above so called limiting curve data belonging to the 99.9% (w/w) (*S*) crystal product this hybrid process (evaporation of raffinate streams, crystallization, crystallization mother liquid recirculation) were recalculated three times. Data can be seen on Table 9 and Fig. 12. By these data the optimum of hybrid system was at 2.32 cm³/min fresh feed, 0.18 cm³/min recirculated feed and 95.6% (w/w) (*S*) raffinate purity. At optimum value productivity was 177.83 mg (*S*)/g packing/day, fresh eluent consumption (calculated for the total SMB+crystallization system) was 3.44 cm³ fresh eluent/mg (*S*).

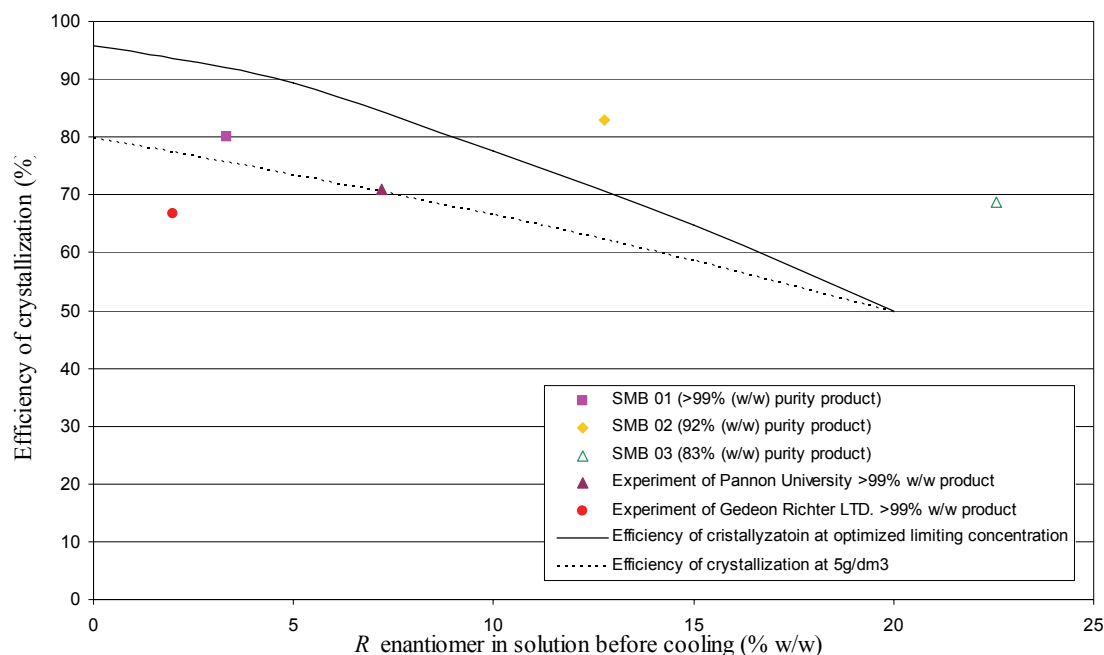


Fig. 14: Measurement results and the earlier measurement results done at Richter Gedeon Ltd. and Pannon University, together with the optimized limiting curve belonging to the 99.9% (w/w) (*S*) crystal value.

Summary

In this work separation of pharmaceutical enantiomers coupling with crystallization using SMB-LC joined crystallization hybrid system were studied. The aim of this work was the production of valuable (*S*)-enantiomer of racemic ester mixture from raffinate with higher than 99% (w/w) (*S*) purity and higher than 99% (*S*) yield for getting maximal (*S*) productivity and minimal fresh solvent consumption.

The main parts of hybrid system and processes are: enrichment of (*S*) in SMB-LC raffinate stream, evaporation of raffinate, cooling, separation of (*S*) crystals and crystallization mother liquid, recirculation of crystallization mother liquid to the feed after (*S*)+(*R*) and eluent concentration adjustment. Evaporated eluent was recirculated from raffinate and extract streams to the SMB-LC equipment inlet. After total evaporation of extract stream (*R*) component was produced could be transformed to (*S*)-enantiomer by chemical or other processes outside the SMB-LC crystallization hybrid system.

By previous laboratory scale measurements fundamental data of SMB-LC and joined crystallization necessary for computer simulation were determined. We concluded, that in SMB-LC crystallization hybrid system productivity could significantly be increased from 115.32-177.83 mg (*S*)/g packing/day compared to the separation method using only SMB-LC equipment at higher than 99% (w/w) (*S*) purity and higher than 99% (*S*) yield.

The above hybrid system applicability was proven by laboratory and industrial scale SMB-LC, crystallization experiments.

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