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EXAMINATION OF CRYSTALLIZATION COUPLED TO THE SMB-LC PROCESS

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The simulated moving bed preparative liquid chromatography (SMB-LC) technique is a highly promising method for optical isomer separation. Optimal coupling of SMB-LC to crystallization respectfully increases the effect of enantioseparation. Extract and raffinate products enriched by SMB-LC are suitable for further process by evaporation, cooling crystallization. Chromatographic packing and eluent system selection being the best for the separation and determination of optimal working parameters of SMB-LC coupled with crystallization can be found in our previous publications. The goal of this paper was the detailed study of crystallization process, investigation of crystallization kinetics, such as sample concentration, composition, cooling speed and end-cooling temperature, application of pure seeding crystals with particular influence on the mass and composition of crystals.

Keywords: enantioseparation, crystallization, SMB-LC, conglomerates, racemic compounds.

Introduction

There are several cases in pharmaceutical industry, when one of the two enantiomers has got good biological activity, while the other is inefficient or human health destroying, toxic. Therefore there is high interest in the production of pure enantiomers [1]. The chemical synthesis in most cases results racemic mixture, thus efficient separation methods are required. Chromatography is one of the most effective separation technique in this field. In the last years were invented the applications of simulated moving bed liquid chromatography (SMB-LC) and high performance chiral stationary phases to increase separation productivity [2]. This type of separation has got growing importance in chemical engineering practice in the field of industrial chromatography. Unfortunately the method is expensive, thus there is a growing interest in coupling the cheapest traditional separation technology with chromatography [3-7], for example coupling crystallization with SMB-LC chromatography seems to be a cheap alternative.

Since Pasteur's famous separation [8] invention (sodium-ammonium-tartarate enantiomer direct crystallization) numerous researchers have dealt with crystallization process to produce pure enantiomers. Selective crystallization can be realized on the bases of solid-liquid equilibrium [9].

Our model racemic ethyl-ester mixture in n-hexane liquid belongs to the conglomerate type racemates, when enantiomers crystallize separately [7, 10, 11]. In this case enantioseparation with crystallization can be done directly from the liquid, if composition is significantly different from that of the racemic. This method is efficient if there are only few amounts from one of the enantiomers in the mixture. Enrichment of an enantiomer can be realized by SMB-LC equipment followed by enantiomer purification with evaporation, cooling crystallization.

Selection of the best chromatographic packing and eluent system for SMB-LC separation and determination of the optimal working parameters of SMB-LC coupled with crystallization can be found in our previous publications [7, 10, 11].

Crystallization

The goal of crystallization is the recovery from auxiliary materials, separation from other compounds, purification, formation to get new crystal morphology, etc. Crystallization can be realized from gas, liquid or melting phase. Further we focus on the crystallization from liquid phase.

In case of crystallization from liquid the driving force is the over saturated condition of the liquid can be achieved by cooling or evaporation. *Fig. 1* provides equilibrium saturation curve, over saturated curve and three ranges (stable, metastable, unstable).

While planning and optimizing of crystallization process the area of metastable range is important can be determined experimentally [12, 13].

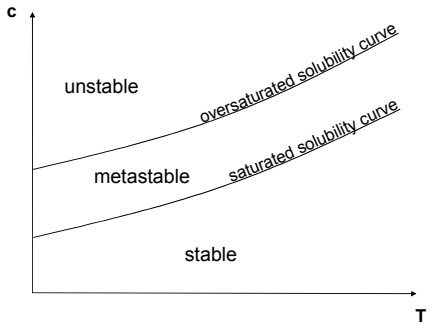

Figure 1

On Fig. 1 liquid concentration versus temperature diagram shows, that the working point of the liquid to be crystallized can be placed in three areas, namely stable, metastable, unstable areas. Stable area (unsaturated liquid area) is under the equilibrium saturation curve, where no crystal forming and increase exist. In the metastable area (limited by the saturated and over saturated solubility curves) there is low crystal seed formation, but the existing crystal particle can increase. There is spontaneous crystal forming in the unstable area and the speed of crystal seed forming quickly rises. If over saturation is high enough, the crystal seed forming is extremely rapid. Over-saturation curve and saturation curve seems nearly parallel bordering the metastable area. Wideness of metastable area depends on different parameters (cooling speed, mixing conditions, concentration, etc.) and can definitely be determined experimentally.

The kinetics of crystallization [12, 13]

Theoretically the kinetics of crystallization is simplified as a two-step process. In the first step crystal seeds are forming, in the second step they are growing. These two steps go on simultaneously in practice. Crystal seed forming are homogenous or heterogeneous.

Generally crystal seed forming is started by crystal seed injection into the over saturated liquid. The injected crystal seed may be the fine grinded crystal powder product itself.

Crystal growth starts around the crystal seeds. The kinetics of crystallization is influenced mainly by the degree of over saturation. In case of high over saturation degree crystal products are small in size, while at low over saturation crystals are big in size.

Kinetic resolution is a novel chemical engineering process in the field of crystallization which is detailed in Chapter Kinetic resolution with crystal seed injection in case of conglomerate type racemic system.

Phase diagrams [14]

Solid-liquid equilibrium phase data are presented on the so called phase diagrams. It is important to have detailed phase diagram, if enantiomer separation or purification with crystallization is planned. Biner melting point diagrams can be seen on *Fig. 2*, showing the melting behaviour of the two enantiomers. By Roozeboom [9] there are three basic types of enantiomer systems, as follows: conglomerate (a), racemic (b), pseudoracemate (c). 5–10 % of racemates belong to most easily separable conglomerates, 90–95 % belong to the true racemate. The rest is called pseudoracemate.

Solubility data in a solvent are figured in triangle diagram. *Fig. 3* shows theoretically our conglomerate type triangle diagram. [7, 10, 11].

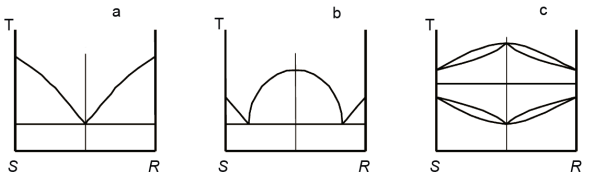

Figure 2

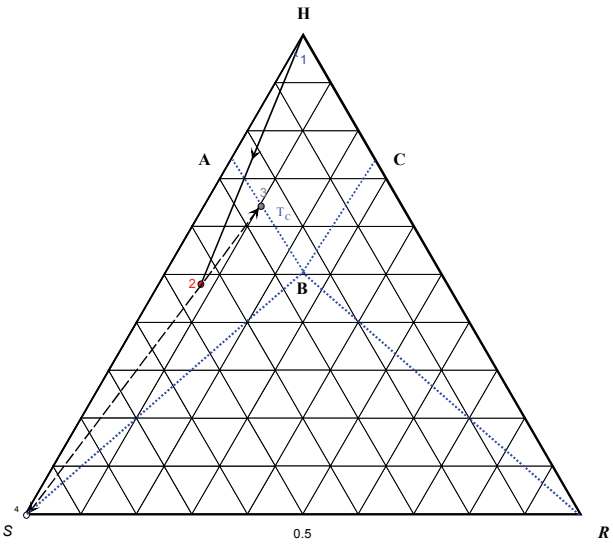

Figure 3: (S) and (R) enantiomer,
H solvent ternary solubility diagram at T = const.

Pure components are placed at the triangle peaks: S and R enantiomers are on the lower left and right peaks, H solvent is at the top. The triangle sidelines represent binary system in mol fraction or mass fraction units as S enantiomer/solvent, R enantiomer/solvent, S/R enantiomer systems. 0.5 is the point, where both enantiomers are present in 50-50 %. Each inner point of the triangle represents a ternary mixture containing all the three (S, R, H) components. A-B-C-H borders the unsaturated one-phase area, A-B-S and C-B-R are two-phase areas, which contain pure enantiomer solid phase and a saturated liquid phase.

Pure enantiomer solubility at a given T temperature in solvent is represented by A and C points, while racemic mixture is signed by B point. The solubility curve of the terner system is represented by the A-B-C lines. Unsaturated liquid area is above this line, while the multi-phase areas are under it. Within the area of triangles A-B-S or C-B-R the solid phase containing one of the enantiomers is in equilibrium with the saturated liquid above. S-B-R is a three phase area, where the

liquid concentration is racemic in case of equilibrium while the solid phase is enriched only in one of the enantiomers.

Let see Points 1, 2, 3 and 4 on Fig. 3 In point 1 there is a solvent enriched in S enantiomer, which is evaporated (TE temperature) thus getting to point 2 Liquid in point 2 is cooled down to TC temperature, when saturated liquid in point 3 and pure S crystal in point 4 is received. It means that pure S enantiomer can be produced with crystallization coupled to SMB-LC, when point 1 is the concentration of raffinate coming out of the SMB-LC unit. Point 2 is the evaporated liquid concentration inside the A-B-S triangle. Point 3 is the concentration of crystallization mother liquor. Point 4 is the concentration of pure crystal S.

Kinetic resolution with crystal seed injection in case of conglomerate type racemic system [1, 15-17]

Crystal seed injection method can be applied in case of conglomerate type racemic system to produce pure enantiomers. *(Fig. 4)*.

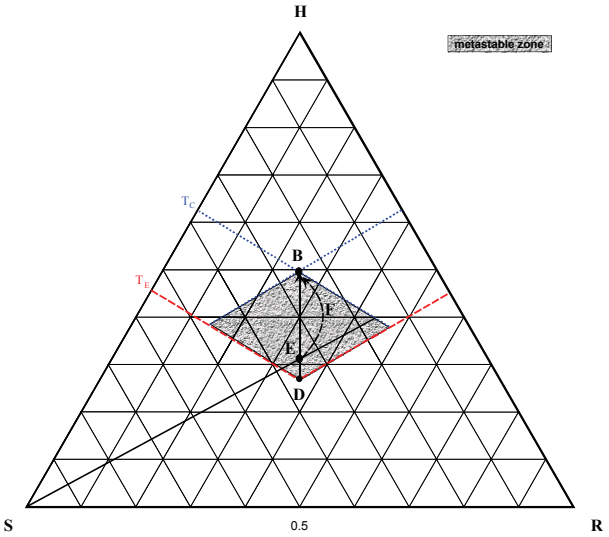

Figure 4: Theory of kinetic resolution with crystallizaton

Let the enantiomer containing racemic mixture saturated at TE temperature (point D) and cool it down to point E resulting over saturation in metastable area. Then add small amount of pure S crystal seed and continue cooling solution to TC temperature. Then concentration of liquid does not move directly to point B, but follows the E-F-B trajectory because of the homo chiral crystal surface phenomena. The consequence is, that we receive S enriched crystal fraction on E-F section and naturally the liquid gets concentrated in R component. On F-B section R concentration of crystal mother liquor decreases to the racemic concentration B. In F-B section crystal is richer in R enantiomer. Looking at the full E-F-B trajectory the resulted integral crystal concentration is racemic.

According to the above mentioned theory the so called "butterfly crystallization" was invented being able to be used for pure enantiomer production from conglomerate type racemic mixtures with kinetic crystal seed injection method. The method is well described in publication of H. Lorenz and A. Seidel-Morgenstern [1]. The main point of the method is that non racemic mixture is cooled while R enantiomer crystal seed is injected. R crystals are filtered out of the liquid. Racemic mixture is added to the crystallization mother liquid while heating it. Later on S enantiomer crystal seed is added to the liquid while cooling it. Crystal S is filtered, and liquid is heated while racemic mixture is added. Then we get back to the starting point, the first cycle of the cyclic process is finished and can be repeated according to the above described method.

Crystallization coupled to the SMB-LC process

There are several cases in pharmaceutical industry, when one of the two enantiomers has got good biological activity, while the other is inefficient or human health destroying, toxic. Therefore there is high interest in the production of pure enantiomers [1]. The chemical synthesis in most cases results racemic mixture, thus efficient separation methods are required. Chromatography is the most effective separation technique in this field. In the last years were invented the applications of simulated moving bed liquid chromatography (SMB-LC) and high performance chiral stationary phases to increase separation productivity [2]. This type of separation has got growing importance in chemical engineering practice in the field of industrial chromatography. Unfortunately the method is expensive, thus there is a growing interest in coupling the cheapest traditional separation technology with chromatography [3-7], for example coupling crystallization with SMB-LC chromatography seems to be a cheap alternative.

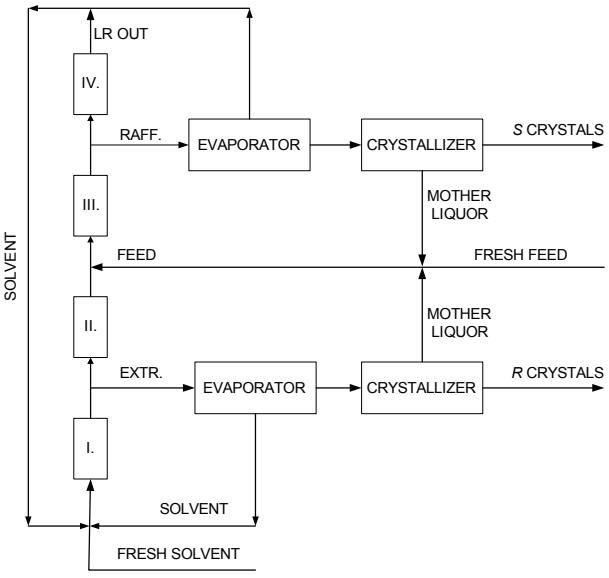

Figure 5: SMB with crystallizaton

System examined by Gál et al. [7] can be seen on *Fig. 5*, which is evaporation, cooling crystallization with crystallization mother liquor and solvent recirculation coupled to SMB-LC equipment. The laboratory scale SMB-LC equipment can be seen on *Fig. 6*.

The end product, the S enantiomer enriched in raffinate is planned to be produced in 99% w/w purity in crystal form in at least 99% yield while minimizing solvent consumption. Raffinate from SMB-LC is evaporated, then we crystallized with cooling it, crystal S is filtered and dried. Crystallization mother liquor is recirculated to the SMB-LC feed. Extract from SMB-LC after full evaporation results >99% w/w crystal. Solvents after evaporation are recirculated to the SMB-LC when IPA concentration is adjusted in n-hexane. The productivity of SMB-LC with coupled crystallization can significantly be increased. Details can be found in 7, 10, 11 publications.

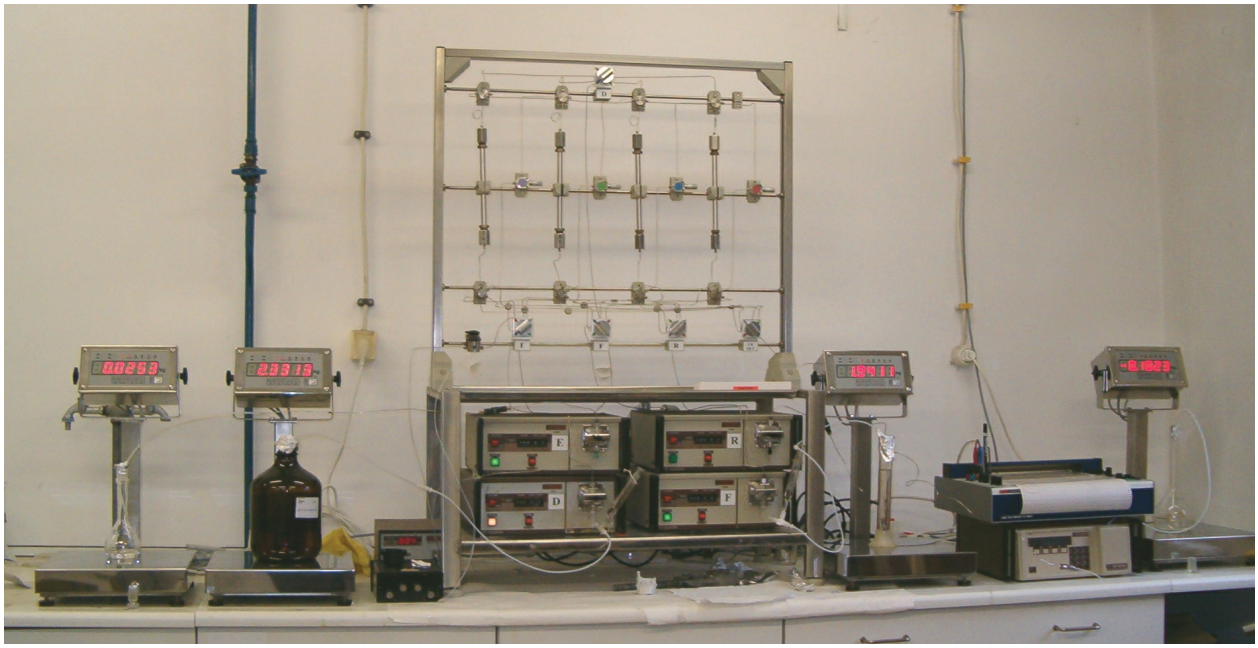

Figure 6: The laboratory scale SMB-LC

Experiments

Determination of solubility data

During the measurement pure S enantiomer or SR racemic mixture (produced by Richter Gedeon Ltd.) was solved in n-hexane (Merck extra pure) at 20 °C till solid phase appeared in the solvent. The saturated liquid was kept during 8 hours at given temperature. After that liquid sample was filtered by MILLEX GN type 0.2 µm filter and analyzed by MERCK-HITACHI La Chrom type HPLC equipment using Chiralcel OD-H packing and n-hexane:IPA = 95:5% v/v eluent.

Solubility-temperature function is given by Profir et al. [15] equation.

$$\ln x = a + \frac{b}{T}$$

where:

x - is the mol fraction

T - is the absolute temperature [K]

a, b - constants

Measurements and calculated data can be seen in *Table 1, 2* and *Figs* 5 and *7*.

Table 1: Solubility of S enantiomer in n-hexane

T [°C]	1000/T [1/K]	c [g/dm³]	x [mol/Σmol]	lnx
20	3.413	28.5	$1.538 \cdot 10^{-2}$	-4.175
-17.5	3.914	1.7515	$9.589 \cdot 10^{-4}$	-6.95
-19	3.937	1.4948	$8.185 \cdot 10^{-4}$	-7.108
-20	3.953	1.20	$9.417 \cdot 10^{-4}$	-6.968
-27	4.065	0.920	$5.039 \cdot 10^{-4}$	-7.593

Table 2: Solubility of SR racemic mixture in n-hexane

T [°C]	1000/T [1/K]	c [g/dm³]	x [mol/Σmol]	lnx -
20	3.413	50.9	$2.714 \cdot 10^{-2}$	-3.607
-20	3.953	2.71	$1.483 \cdot 10^{-3}$	-6.514
-27	4.065	1.47	$8.049 \cdot 10^{-4}$	-7.125

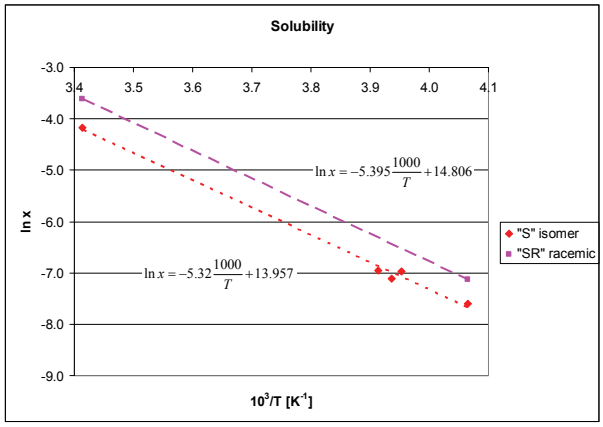

Figure 7: Solubility of S and SR, ln mol fraction versus 1000/T

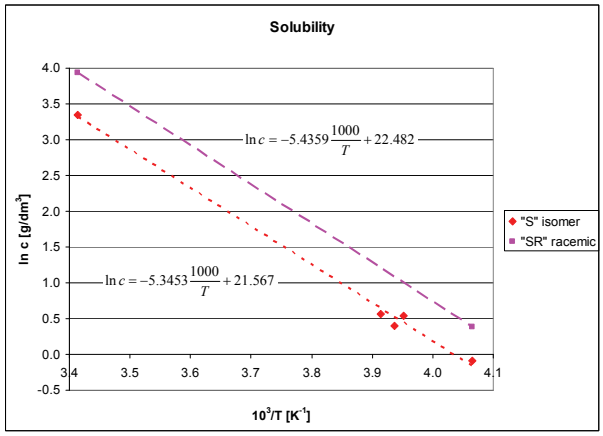

Figure 8: Solubility of S and SR, ln concentration versus 1000/T

Solubility data presentation in a triangle diagram

Solubility measurements were also carried out, where different S:R mixtures (77.4:22.6, 80:20, 85:15, 87.2:12.8, 90:10, 92.8:7.2, 95:5, 96.7:3.3, 98:2) were prepared from pure S enantiomer and pure R enantiomer (S+R about 5 g/dm³) at 20 °C in n-hexane similarly to the previous chapter. These mixtures were cooled down and kept through 8 hours at -10 °C, -15 °C, -20 °C and -27 °C temperatures. Liquid and crystal phase was analyzed. Results of solubility measurements in mass fraction measure unit are described in *Fig. 10* assuming 660 mg/cm³ n-hexane density at 20 °C. The photos of crystals can be seen on *Fig. 9*.

By Fig. 10 the S-R-H system is a conglomerate type one.

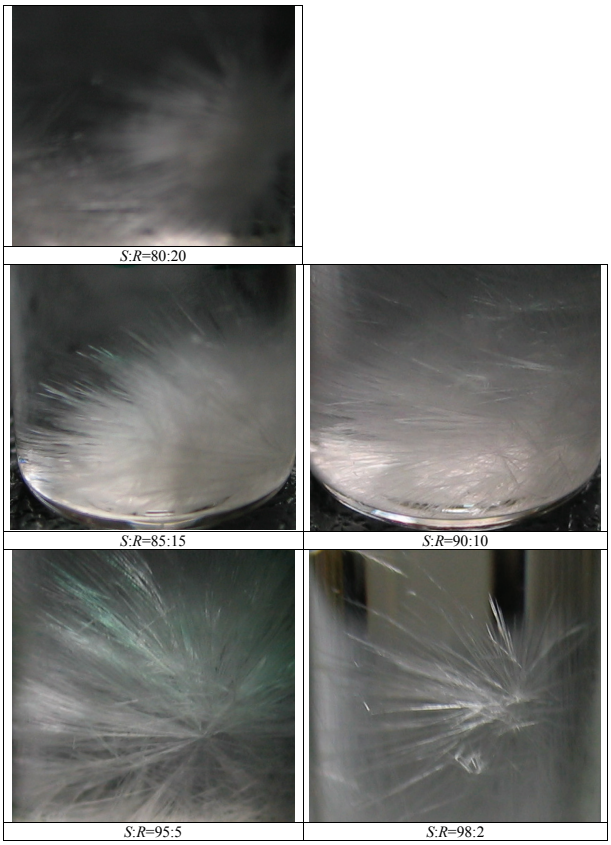

Figure 9: The crystals

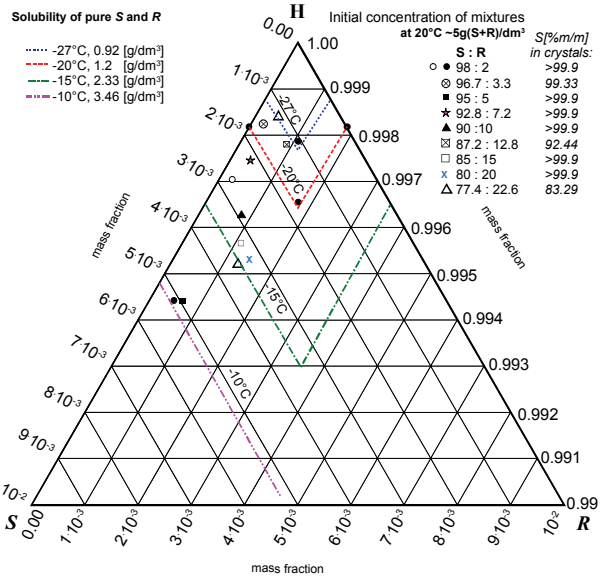

Figure 10: Results of solubility measurements

Solubility model calculation results

Our calculation was based on calculations of Profir et al. [15] being an ideal model for conglomerate systems. By this model SR solubility is twice as much as S or R solubility. For example solubility of S at -20 °C is 1.2 g/dm³, solubility of R at -20 °C is 1.2 g/dm³. Thus solubility of SR is 2.4 g/dm³.

Be the total concentration of solution for cooling 5 g/dm³ for S+R enantiomers. Cool down to -20 °C different S:R (80:20, 85:15, 90:10, 95:5, 98:2, 100:0) solutions. 1.2 g/dm³ S remains in the liquid and the originally present max. 1.2 g/dm³ R. The rest precipitates as pure S crystal from the yield (η_S) of S can be calculated.

Results can be seen in *Table 3* and *Fig. 11*.

Table 3: Solubility model calculation results

Solute +20 °C, 5 g/dm³ concentration			Solute -20 °C		Crystal	η_S
S [g/dm³]	*R* [g/dm³]	*S*:*R*	*S* [g/dm³]	*R* [g/dm³]	*S* [g]	[%]
4	1	80:20	1.2	1	2.8	70
4.25	0.75	85:15	1.2	0.75	3.05	71.8
4.5	0.5	90:10	1.2	0.5	3.3	73.3
4.75	0.25	95:5	1.2	0.25	3.55	74.7
4.9	0.1	98.2	1.2	0.1	3.7	75.5
5	0	100	1.2	0	3.8	76

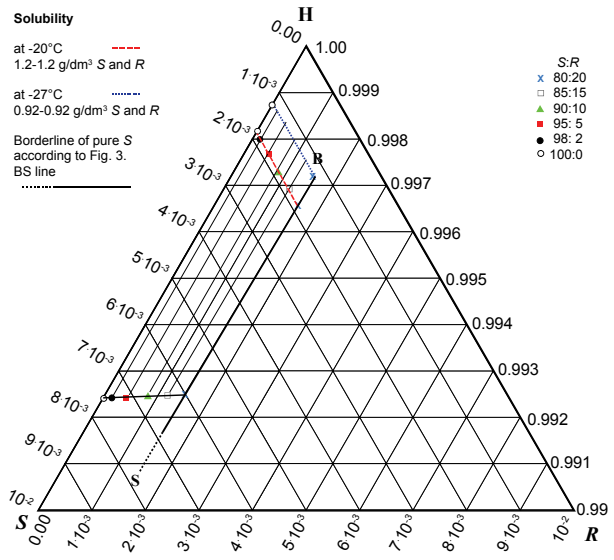

Figure 11: Solubility model calculation results

Results of calculations after the Profir et al. [15] model can be seen on the previous Fig. 10 using different signs as differently coloured continuous and dotted lines.

Determination of metastable areas in n-hexane with cooling crystallization in case of SR racemic mixture solution concentrated in S enantiomer

Experimental equipment

The cooling system was MLW MK70 type cryostate containing 16 dm³ antifreeze ethylene-glycol, diethylene-glycol solution circulated. 100 cm³ volume jacketed glassware was used for the four experiments and antifreeze solution was circulated in the jacket. The sample to be cooled and the mixture of crystal mother liquor were mixed by METROHM-E 349 type magnetic mixer. The system was thermo-isolated.

Starting the experiment 50 cm³ solution was prepared with 5 g(S+R)/dm³ n-hexane concentration. After starting the cooling and mixing the experiment lasted for 150–180 minutes while solution temperature was changed from +20 °C to -28 °C. Temperature was measured by mercury-in-glass thermometer fixed in crystallization glassware. During the experiment 1 cm³ liquid sample taken out of the crystallization glassware at given times was filtered by MILLEX GN type 0.2 µm nylon filter (ID = 13 mm). The filtered liquid was collected separately while the crystals remained on the filter was washed off with 1 cm³ 20 °C n-hexane into separate sample holders. Both the filtered liquid and crystal containing liquid were analyzed by MERCK-HITACHI La Chrom type HPLC equipment using Chiralcel OD-H packing and n-hexane:IPA = 95:5% v/v eluent.

According to Fig. 3 the KR4 measurement was done with initial concentration 85.84% w/w S and 14.16% w/w R to carry out crystallization in A-B-S area. During KR1, KR2 and KR3 measurements crystallization happened in S-B-R area. In case of KR1 the measurement started with racemic mixture, of KR2 S crystal seeds were additionally used, of KR3 solution slightly differing from racemic mixture was cooled (52.7% w/w S and 47.3%w/w R). The cooling speed initially was 50 °C/h decreasing to 10 °C/h after 60 minutes. After 2 hours it was 5 °C/h.

Measuring results are summarized in *Table 4*.

Table 4: Measuring results

KR2		SR racemic								S isomer							
		Mother liquor				Crystals				Mother liquor				Crystals			
time	T	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
[min]	[°C]	c [g/dm³]	c [g/dm³]	[%w/w]	[%w/w]	c [g/dm³]	c [g/dm³]	[%w/w]	[%w/w]	c [g/dm³]	c [g/dm³]	[%w/w]	[%w/w]	c [g/dm³]	c [g/dm³]	[%w/w]	[%w/w]
0	22																
10	12																
15	8.7	2.85	2.86	49.89	50.11					3.25	0.18	94.84	5.16				
30	0	2.77	2.76	50.12	49.88	0.13	0.28	32.55	67.45								
45	-7.85	2.72	2.72	50.00	50.00	0.17	0.29	36.58	63.42								
55	-11.5	2.67	2.68	49.94	50.06	0.17	0.28	38.43	61.57								
65	-13.75	2.54	2.54	49.99	50.01	0.05	0.14	26.17	73.83								
75	-16.25	1.92	1.73	52.67	47.33	0.22	0.28	44.54	55.46								
80	-17.5									1.75	0.00	100.00	0.00	0.59	0.00	100.00	0.00
85	-18.25	1.29	1.13	53.25	46.75	0.13	0.19	40.22	59.78								
90	-19									1.49	0.00	99.80	0.20	0.77	0.00	100.00	0.00
95	-20	1.10	0.94	53.74	46.26	0.25	0.30	45.65	54.35								
105	-21.4	0.98	0.83	54.19	45.81	0.21	0.25	45.54	54.46								
115	-22.5	0.94	0.78	54.59	45.41	0.10	0.09	52.93	47.07								
125	-23.5	0.79	0.69	53.36	46.64	0.11	0.15	42.21	57.79								
135	-24.2	0.22	0.18	55.47	44.53	0.03	0.04	41.90	58.10								
145	-25	0.26	0.21	55.84	44.16	0.05	0.05	48.62	51.38								
155	-25.5	0.61	0.47	56.54	43.46	0.04	0.08	31.34	68.66								
165	-26	0.58	0.44	56.88	43.12	0.03	0.08	31.02	68.98								

KR4		SR racemic							
		Mother liquor				Crystals			
time	T	S	R	S	R	S	R	S	R
[min]	[°C]	c [g/dm³]	c [g/dm³]	[%w/w]	[%w/w]	c [g/dm³]	c [g/dm³]	[%w/w]	[%w/w]
0	20	4.43	0.73	85.84	14.16				
30	0	4.40	0.70	86.23	13.77	0.38	0.06	87.09	12.91
45	-6	4.44	0.73	85.90	14.10	0.37	0.06	86.23	13.77
60	-10.5	4.37	0.71	86.10	13.90	0.39	0.06	87.30	12.70
85	-15.5	2.58	0.68	79.08	20.92	3.08	0.12	96.28	3.72
100	-17.5	2.64	0.99	72.69	27.31	2.36	0.10	96.05	3.95
115	-19	2.15	0.83	72.08	27.92	2.73	0.09	96.80	3.20
130	-20	1.87	0.74	71.59	28.41	2.77	0.10	96.49	3.51
145	-21	1.68	0.64	72.36	27.64	2.98	0.12	96.11	3.89
170	-22	1.35	0.47	74.18	25.82	2.59	0.12	95.55	4.45
200	-23	1.22	0.34	78.38	21.62	3.61	0.14	96.31	3.69

KR3		SR racemic							
		Mother liquor				Crystals			
time	T	S	R	S	R	S	R	S	R
[min]	[°C]	c [g/dm³]	c [g/dm³]	[%w/w]	[%w/w]	c [g/dm³]	c [g/dm³]	[%w/w]	[%w/w]
0	19.5	2.67	2.40	52.70	47.30				
30	-1.6	2.65	2.37	52.78	47.22	0.19	0.17	52.97	47.03
45	-8.2	2.65	2.37	52.79	47.21	0.27	0.28	49.23	50.77
60	-13.6	2.58	2.31	52.80	47.20	0.27	0.20	57.30	42.70
75	-17.2	2.53	2.26	52.77	47.23	0.27	0.15	64.22	35.78
90	-20	1.39	1.19	53.86	46.14	0.93	0.78	54.35	45.65
105	-22	1.10	0.90	55.00	45.00	1.28	1.12	53.37	46.63
120	-23.5	1.00	0.80	55.56	45.89	1.50	1.33	53.00	47.00

KR1		SR racemic							
		Mother liquor				Crystals			
time	T	S	R	S	R	S	R	S	R
[min]	[°C]	c [g/dm³]	c [g/dm³]	[%w/w]	[%w/w]	c [g/dm³]	c [g/dm³]	[%w/w]	[%w/w]
0	17	2.83	2.82	50.10	49.90	0.00	0.00	0.00	0.00
30	-3	2.87	2.72	51.35	48.65	0.27	0.26	50.93	49.07
45	-10	2.64	2.63	50.09	49.91	0.29	0.28	50.10	49.90
60	-15	2.47	2.46	50.06	49.94	0.21	0.22	48.88	51.12
75	-17					0.15	0.15	50.05	49.95
90	-19.3	2.39	2.38	50.10	49.90	0.18	0.16	53.81	46.19
105	-22.2	0.99	0.97	50.52	49.48	0.58	0.53	51.89	48.11
120	-24.6	0.79	0.75	51.03	48.97	0.42	0.39	51.99	48.01
135	-26	0.66	0.64	50.91	49.09	0.36	0.34	51.25	48.75
150	-27.4	0.58	0.56	50.83	49.17	0.23	0.26	47.28	52.72
155	-28	0.48	0.17	74.39	25.61	0.94	0.97	49.36	50.64
						0.62	0.64	49.15	50.85

Results of KR4 measurements

Starting the experiment 50 cm³ solution was prepared with 4.4263 g S/dm³ n-hexane and 0.7301 g R/ dm³ n-hexane concentration (S 85.84% w/w and R 14.16% w/w). The solution was cooled down during 170 minutes from 20 °C to -23 °C beside 300 1/min mixing speed. The metastable area is expanding from -7 °C to -23 °C or from 30 min to 160 min. Examining the concentration data slight concentration wave was found indicating kinetic resolution in function of temperature and cooling time (*Fig. 12* and *Fig. 13*).

About 97% w/w S concentration crystal precipitates from the initially 86% w/w S concentration liquid (S yield 70.65%).

It should be noted, that on the basis of the previously described experimental data (see Chapter Solubility data presentation in a triangle diagram) with slow cooling without mixing >99% w/w S pure crystal could be prepared with better than 70% S yield.

Results of KR3 measurements

50 cm³ n-hexane solution was cooled. Its initial concentrations were as follows: 2.6419 g S/dm³, 52.7% w/w S and 2.3986 g R/ dm³, 47.3% w/w R.

Solution was cooled down within 165 minutes from 19.6 °C to -26.4 °C with 300 1/min mixing speed. On *Fig. 14* can be seen S+R concentration of liquid phase in function of temperature and time. SR solubility curve was reached at about -17 °C temperature and at 55 min cooling time. Metastable area ranges to -20 °C, and 90 minutes.

S concentrations in liquid phase varied between 52.7 and 54.23% w/w S values. Concentration of S crystal was between 49.23 and 64.22% w/w S. Considering the concentration data slight concentration wave was found indicating kinetic resolution in function of temperature and cooling time (Table 4).

Results of KR1 measurements

SR racemic mixture was solved in 50 cm³ n-hexane (2.8311 g S/dm³ and 2.8199 g R/dm³, then the liquid was cooled from 17 °C to -28 °C temperature during 160 min with slow mixing (60 1/min). Results of measurement can be seen on *Fig. 15* and Table 4)

Metastable area ranges from -10 °C to -22 °C temperature or from 45 to 105 min. Observation of kinetic resolution phenomena is expected within this area. S concentration of liquid changes by data in Table 4 between 50.06–51.35% w/w S values, while S concentration of crystal changes between 50.1–53.81% w/w S. Concentration data show slight kinetic resolution effect (Table 4).

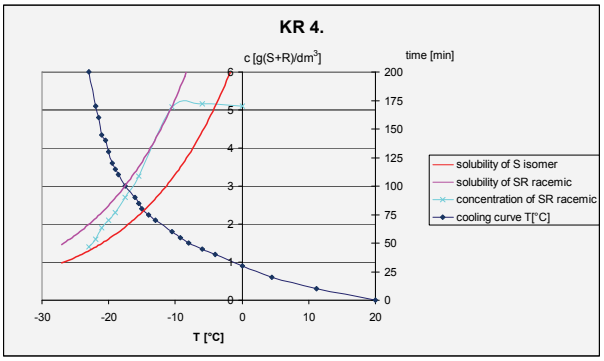

Figure 12: KR4 measurement liquid (S+R) concentration, temperature, time diagram with S and SR solubility curves

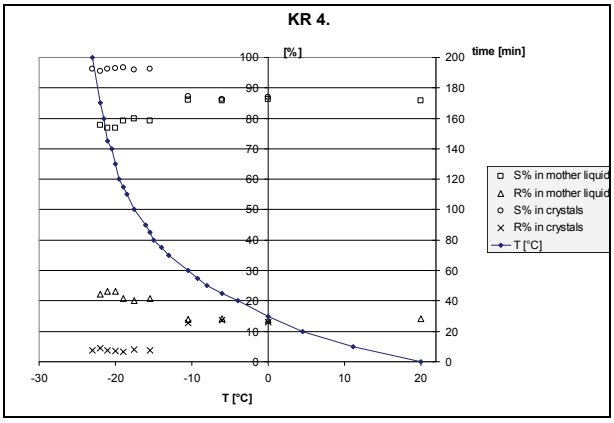

Figure 13: KR4 measurement: crystallization, mother liquid and crystal concentration (S and R % w/w) temperature-time diagram

Results of KR2 measurements

KR1 measurement was repeated using crystal seed injection. SR racemic mixture was solved in one of crystallization glassware in 50 cm³ n-hexane (2.8467 g S/dm³ and 2.8594 g R/dm³), in the other S enantiomer was solved in 12.5 cm³ n-hexane (3.2539 g S/dm³, 40.67 mg S and 0.1771 g R/dm³, 2.21 mg R). Then both crystallization glasswares were cooled down to -26 °C during 150 min beside slow mixing speeds (60 1/min).

During measurement 5 cm³ liquid (2.1 mg S/cm³, 10.5 mg S and 0.1771 mg R/cm³, 0.8855 mg R) and crystal (5.76 mg S) was taken out at -15 °C from the S enantiomer containing glassware and was injected as crystal seed to the SR racemic mixture containing crystallization glassware. Measurement data can be found in *Fig. 16* and Table 4). Metastable area ranges from -10 °C to -16 °C and 50–75 min. S concentration of liquid changes initially between 49.89-50.00 % w/w S, then after crystal seed injection 52.67-56.88 % w/w S.

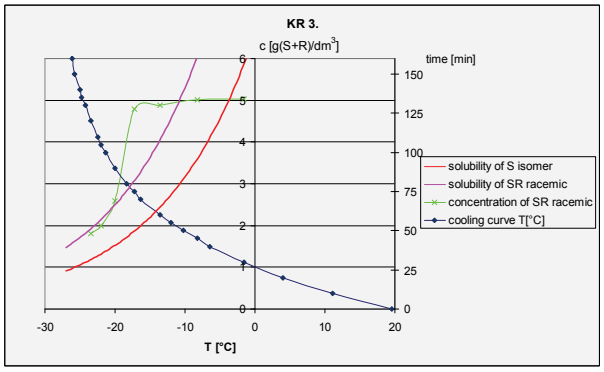

Figure 14: KR3 measurement liquid (S+R) concentration, temperature, time diagram with S and SR solubility curves

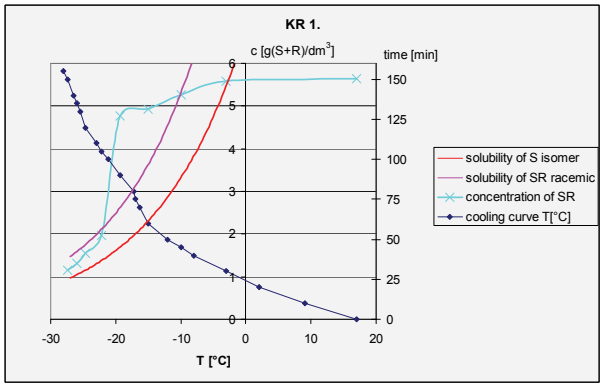

Figure 15: KR1 measurement liquid (S+R) concentration, temperature, time diagram with S and SR solubility curves

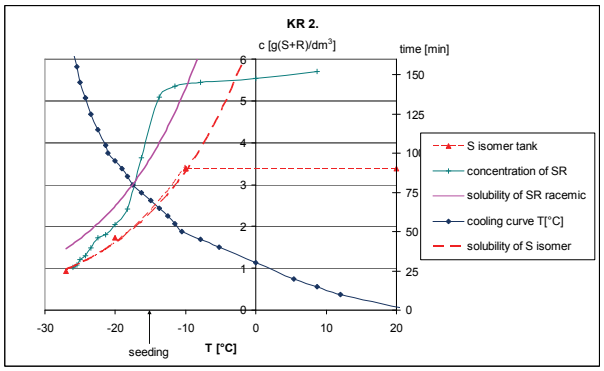

Figure 16: KR2 measurement liquid (S+R) concentration, temperature, time diagram with S and SR solubility curves

Results

Within the frame of this work we examined the crystallization coupled to the SMB-LC process. For this purpose we determined the solubility data of a chiral ethyl ester, as S and R, and SR racemic mixture in

n-hexane (H) at different temperatures. Solubility data were represented on ternary S-R-H diagram. Profir et al. [15] solubility equation for ideal crystallization mixtures was used for describing solubility data and concluded, that S-R-H system is conglomerate type from crystallization point of view.

During crystallization kinetic investigation we examined cooling of mixtures enriched in S enantiomer and racemic SR mixture in n-hexane between +20 °C and -28 °C temperatures with and without S crystal seed injection. During 0–200 minutes cooling time significant metastable areas were observed (see KR1, KR2, KR3, KR4 measurements, Fig. 12, Fig. 14, Fig. 15, Fig. 16, *Fig. 17*). The observation of kinetic resolution phenomena is expected in the above mentioned metastable areas.

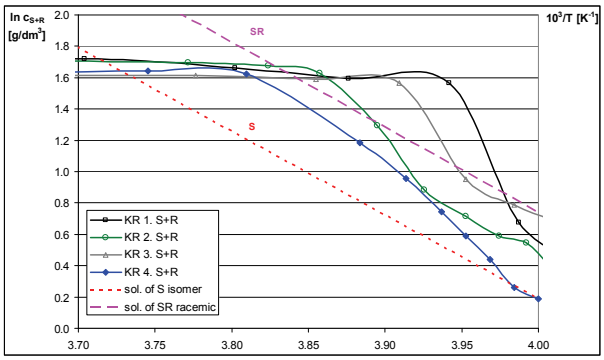

Figure 17

Cooling speed data can be seen on *Fig. 18*. The cooling speed initially was 50 °C/h and decreased to 10 °C/h after 60 minutes. After 2 hours it was 5 °C/h.

According to Fig. 3 the KR4 measurement was done with initial concentration 85.84% w/w S and 14.16% w/w R to carry out crystallization in A-B-S area. During KR1, KR2 and KR3 measurements crystallization happened in S-B-R area. In case of KR1 the measurement started with racemic mixture, of KR2 S crystal seeds were additionally used, of KR3 solution slightly differing from racemic mixture was cooled (52.7 % w/w S and 47.3 w/w R). The above measurement liquid concentration data are described in *Fig. 19*, Table 4 liquid concentration and crystal concentration data show slight kinetic resolution. This liquid concentration change based on concentration data of Table 4 can be seen on Fig. 19 show small concentration fluctuation.

Looking at the composition of crystals from measurements KR1…KR4 it can be concluded, that in case of KR4 measurement 97% w/w S crystal was given in about 70.65% yield.

It should be noted, that on the basis of the data written in Chapter Solubility data presentation in a triangle diagram, namely with slow cooling during 8 hours, from 20 °C to -20 °C without mixing >99% w/w S pure crystal could be prepared with better than 70–76% S yield. See solubility measurement data carried out with different S:R mixtures (80:20, 85:15, 90:10, 95:5, 98:2, S+R about 5 g/dm³) and cooled down the solution from 20 °C in n-hexane through 8 hours to -20 °C temperatures (Table 3).

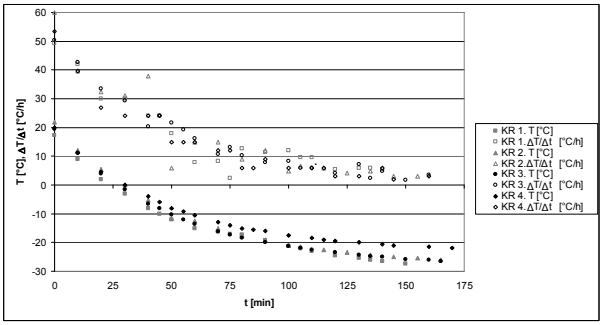

Figure 18

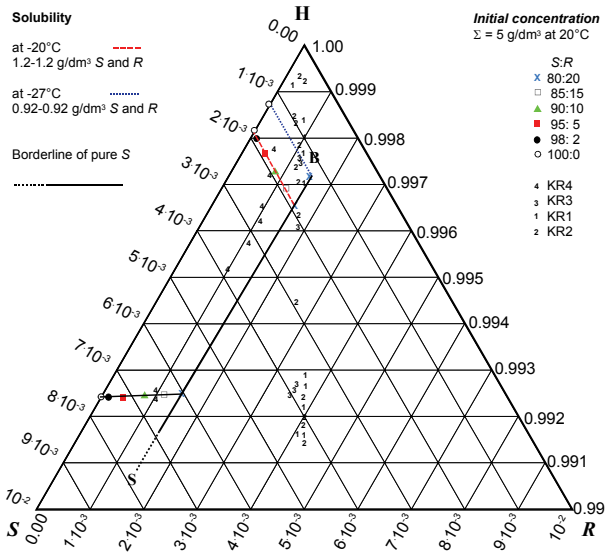

Figure 19

ACKNOWLEDGEMENTS

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