OPTICAL RESOLUTION TRIALS AND A PROCESS-CONCEPT ASSESSMENT OF THE INDUSTRIAL SCALE UP

Theses of Ph. D. dissertation

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1. Introduction

During chemical reactions the two optical isomers of the asymmetric molecules is produced in the same ratio as a rule. Since the different pharmacological effects of the enantiomers being the mirror images of one another came to light, the demands to produce the chiral compounds in the pure enantiomer form rose exponentially and at the present developments it is already an official requirement to incorporate in the preparations only the enantiomer form carrying the useful effect.

According to the observations the physico-chemical characteristics of the two antipodes are similar to a degree that to find a resolution method suitable to separate them, can only be expected following a lengthy and troublesome work if it succeeds at all. Parallel with the increase of demands diversified research has been started, developing a broad scale of processes aiming at producing optically active compounds. Resulting from its economic efficiency however, the chemical resolution resting on the diastereomeric salt formation and selective crystallization is the most widely used procedure in the industry at present times too.

The goal of my work for Doctor degree was the resolution of pipecolic acid xylidides having local anaesthetic effect, the detailed clearing up of the resolution process and rendering it more governable. I studied the possibilities of scaling up the processes applied and validation of the production operations too.

2. Reference background

The reference bases of the resolutions resting on the diastereomeric salt formation and selective crystallization go back to the middle of the XIX. Century. Fogassy and his research team extended our knowledge about the resolution considerably too. Currently the most up to date source cited the most frequently too is the handbook edited by Dávid Kozma, containing and evaluating the data of several hundreds of resolutions.[1]

Depending on the interactions between the antipodes conglomerate and racemate types of enantiomer mixtures can be distinguished. In the case when one of the enantiomers reacts first of all with the one similar to it, there the crystallization of the conglomerate is the favoured one. In the case of conglomerates the spontaneous resolution can be carried out too by direct crystallization, inoculating the mixture with a crystal of one of the enantiomers.[2]

There are two sections of the melting point-composition diagram of the enantiomer mixtures known. In the case of conglomerates or racemic molecular compounds the composition-temperature relationship over the enantiomer section can be described with the simplified form of the Schröder-van Laar equation.[3,4]

$$\ln x = \frac{H_E}{R}\left[\frac{1}{T_E} - \frac{1}{T}\right]$$

x – composition (mole fraction)
H_E – melting enthalpy of the pure enantiomer (nearly identical with melting enthalpy of the almost pure substance) (J/mole)
T_E – melting point of the pure enantiomer (ºK)
T – melting end temperature of the composition x (ºK)

In the case of racemic molecular compounds the section between the two eutectic points can be described with the Prigogine-Defay equation.[5]

$$\ln 4x(1-x) = \frac{2H_R}{R}\left[\frac{1}{T_R} - \frac{1}{T}\right]$$

H_R – melting enthalpy of the racemate (J/mole)
T_R – melting point of the racemate (ºK)
T – melting end temperature of the composition x (ºK)
x – composition (mole fraction)
R – universal gas constant

[1] Kozma, D., *Handbook of Optical Resolution via Diastereomeric salt Formation*, CRC Press, Boca Raton 2002
[2] Jacques, J.; Leclercq, M.; Brienne, M.J., *Tetrahedron* **37**, 1727-33 (1981)
[3] Schröder, I., *Zeitschrift für Phys. Chem., Stoechiometrie und Verwandtschaftslehre* **11**, 449-65 (1893)
[4] Van Laar, J., .J., *Archives Nederland. sc. exact. et nat*. **8**, 264-84 (1903)
[5] Prigogine, I., Defay, R., *Chemische Thermodynamik,* Deutscher Verlag für Grundstoffindustrie, Leipzig 1962

I used the idea of efficiency of the resolution ('S') to typify the resolution.[6]

$$'S' = \frac{Y\%^{X}}{100} \quad \frac{OP\%}{100}$$

Y – yield of the diastereomeric salt settled out
OP – optical purity of the enantiomer released from it

The equation describing the relationship between the efficiency of the resolution, the chemical structural and the physicochemical parameters contains the hydrophobic constant (π), the Taft constant (the σ^* calculable from the inductive and mesomeric parameters), the steric parameter (M_R), and the empirical polarity of the solvent applied (E_T) as independent variables. [6] The steric factor can frequently be omitted.

$$'S' = 0{,}517 \cdot \pi + 2{,}280 \cdot \sigma^* - 0{,}015 \cdot E_T - 0{,}001$$

π – hydrophobic constant,
σ^* – Taft constant,
E_T – empirical polarity of the solvent

The characteristics of the solvent frequently influence decisively the efficiency of the resolution. Lately it became known upon the publications of Sakai that the resolution process can be managed in a controlled way by varying the dielectric constant of the solvent.[7]

While producing and resolving the model compounds I controlled the progress of the procedure carrying out TLC tests. This method supplied other useful data being characteristic for features of the substance to be resolved, too. The retention capacity (R_m) of the applied chromatographic system concerning the individual components can be described with help of a logarithmic equation containing the principal members of the dissociation exponent of the given component (pKa) and its lipophility, that is the distribution ratio (log P or lg k).[8]

$$R_m = \lg k + pK_a + \lg(A_s/A_m) + pH$$

R_m – retention capacity of the applied chromatographic system concerning the component given
K - chromatographic distribution ratio of the sample substance between the mobile and the stationary phases
pK_a - dissociation exponent of the component
A_s/A_m – ratio of the contacting surfaces at the stationary and mobile phases
pH pH of the eluent mixture applied

The efficiency of the resolution depends decisively on the chiral recognition ability of the resolution agents. The Cambridge Structure Database contains an ever increasing number of data resulting from the X-ray diffraction tests of monocrystals, and the mathematical and graphic software suitable to processing those allows assessments from several aspects.[9]

3. Experimental and calculation methods

Melting point- The Büchi 535 type apparatus has been used to the determination.
DSC: The plots were recorded and integrated using a Perkin-Elmer DSC C-2 type apparatus. Approximately 2 mg of the substance was heated at 2.5°C/min rate in a sealed container.
Optical rotation: The individually prepared solutions were measured using a Perkin-Elmer 241 type polarimeter.
TLC: 5 µg of the sample solution (0.1 g/5 ml chloroform) inject at the start line onto a 20 cm x 20 cm size glass plate coated with Kieselgel GF_{254} silica in 0.25 mm thickness,. The eluent was a mixture of *n*-butanol : glacial acetic acid : water in 60 : 15 : 25 volume ratio. Saturation of the chromatographic tank to the equilibrium was attained after 2 hours. The development were stopped at reaching the 15 cm frontline. The spots are visualized by using Draggendorf reagent and permanganate solution. In order to clear up the forces acting behind the phenomena observed during the TLC tests, the observed and measured data ('S', R_f, pK_a) were

[6] Fogassy, E.; Lopata, A.; Faigl, F.; Darvas, F.; Ács, M.; Tőke, L., *Tetrahedron Letters* **21**, 647-650 (1980)
[7] Sakai, K., Sakurai, R., Hirayama, N., *Tetrahedron Assymmetry* **15**, 1073-1076 (2004)
[8] Golumbic, C.; Orchin, M.; Weller, S., *J.Am.Chem.Soc.* **71**, 2624 27 (1949)
[9] Allen, F., H., Motherwell, W., D., S., *Acta Cryst. B* **38**, 407-422 (2002)

evaluated together with the values calculated on basis of the molecule structures (dipole moment, proton affinity, log D), the empirical polarity of the solvents available from the literature E_T) and the dielectric constant of those (ϵ). To calculation of the dipole moment (d) and the proton affinity (Σβ) the Gaussian 03, B3LYP/6-31G(D) software, the logP data the version 9.0 of the ACD/Labs' Chromatography Application database software has been used. The pK_a values were derived from the data of potentiometric titration.

The SAS system 8.2 statistical software was used to the statistical evaluations.

FT-IR: in solid phase, measured in KBr disc, using FT-IR Bruker IFS-113 v, resp. Perkin-Elmer Spectrum One type spectrophotometer.

NMR: The NMR spectra have been recorded using Bruker AVANCE DRX 400 and WM-250 spectrometers. The samples dissolved in DMSO-d_6 or $CDCl_3$ solvent were examined at a temperature of T=295°K, applying the resonance frequencies at 400.13, and 250 MHz ^{1}H and 100,6 and 62.7 MHz ^{13}C. The values of the chemical shifts were given in ppm, related to the TMS (δ=0.00 ppm) and DMSO (δ=39.50) internal standards, and indicated on the δ scale.

MS: Mass spectra were recorded using a Kratos 80 type apparatus, at 150°C, in a 70eV field.

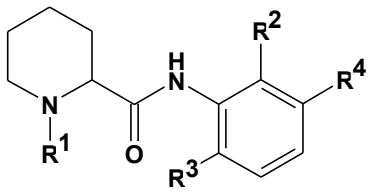
CHN: The elementary analysis was carried out using a Perkin-Elmer 2400 CHN type elementary analyzer apparatus.

XRD: The single crystal of *2S*-(-)- mepivacaine-*O,O*-dibenzoyl-2*R*,3*R*-tartrate (mepivacaine-DBTA) has been developed in ethanol-ethyl acetate mixture, by standing at room temperature. The X-ray diffraction scans were taken using an Enraf-Nonius MACH3 type diffractometer, at 293°K (Mo Kα radiation λ=0.71073Å, ω motion). Raw data were evaluated using the XCAD4 software. The structure was solved using direct methods by SIR-92 and refined on F^2 using the SHELX-97 program. For crystallographic calculations the PLATON program, and for the illustration of the structure the WINGX-97 suite software was applied.

We retrieved the crystallographic data from the "Cambridge Structure Database System 2008 release" database with help of the "ConQuest v.1.10." software. According to those data the structures were demonstrated using the Mercury 1.4.2. graphic program.

4. Results

1. ***I synthesized 13 pcs. N-alkyl-pipecolic acid-anilide derivatives*** **(1-13)**. I used the pipecolic acid-xylidides with local anaesthetic effect (**1-5**) as model compounds to studying the resolution processes (***2**-mepivacaine, ****4**-ropivacaine, *****5**-bupivacaine).

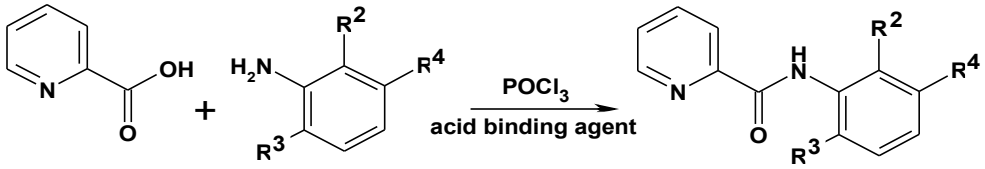


1-13

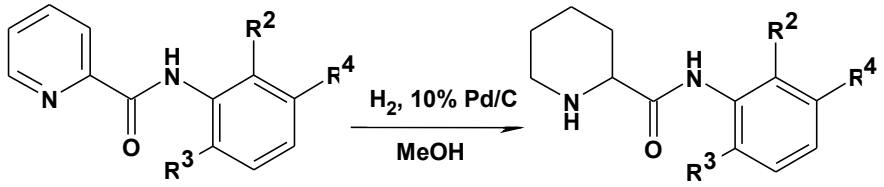
	R^1	R^2	R^3	R^4	Group
1.	H	CH_3	CH_3	H	Pipecolic acid xylidides
* **2.**	Me	CH_3	CH_3	H	
3.	Et	CH_3	CH_3	H	
** **4.**	*n*-Pr	CH_3	CH_3	H	
*** **5.**	*n*-Bu	CH_3	CH_3	H	
6.	H	H	H	H	Pipecolic acid anilides
7.	Et	H	H	H	
8.	*n*-Pr	H	H	H	
9.	*n*-Bu	H	H	H	
10.	H	H	H	CF_3	Pipecolic acid trifluoromethyl anilides
11.	Et	H	H	CF_3	
12.	*n*-Pr	H	H	CF_3	
13.	*n*-Bu	H	H	CF_3	

The synthesis path consisted of three steps:

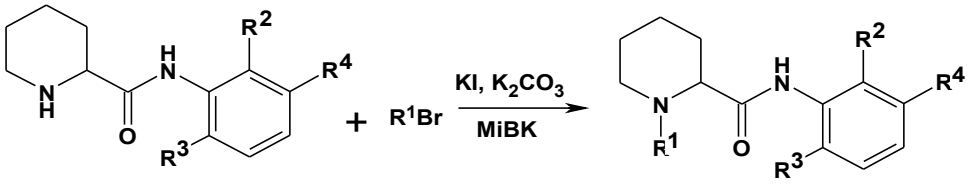
a.) The aniline derivatives were acilated with picolinic acid in the presence of phosphorus oxychloride and *N,N*-dimethyl aniline (Y_{av}: 85 %).



b.) The pyridine ring was saturated to piperidine by catalytic hydrogenation, under 4-5 bar pressure hydrogen atmosphere, applying 10% Pd/C catalyst at 45-50°C (Y_{av}: 91%).



c.) The *N*-alkyl derivatives were synthesized by reacting the pipecolic acid anilides with alkyl halogenides, in presence of potassium iodide and potassium carbonate . (Y_{av}: 63-90%).



The pipecolic acid xylidides were resolved in alcoholic solvents, with *O,O'*-dibenzoyl-2*R*,3*R*-tartaric acid (DBTA) and 2*R*,3*R*-tartaric acid (TA). These processes were optimized.

	R^1	Resolv. agent	Solvent	Mole ratio (settled out)	Y-Yield %	OP-Optical purity %	'S'-Efficiency of resolution
1	H	DBTA	*i*-PrOH	2:1	75.4	94.0	**0.71**
2	Me	DBTA	EtOH	1:1	93.3	99.7	**0.93**
3	Et	DBTA	*i*-PrOH	2:1	63.4	73.2	**0.46**
4	*n*-Pr	TA	96% EtOH	1:1	93.4	95.1	**0.89**
5	*n*-Bu	TA	*i*-PrOH	2:1	68.4	99.9	**0.68**

Two general processes have been applied to resolution of the pipecolic acid xylidides applying DBTA and TA. In the case where the resolution with DBTA was functioning, there it cannot be resolved when applying TA and *vice versa*.

2. When applying the eluent mixture *n*-butanol:glacial acetic acid:water for the TLC investigations of the diastereomers produced during the resolution the spots of the acidic and basic parts ran according to the free base or acid. ***I found a relationship between the differences of the R_f values of the acids and bases and the efficiency of the resolution.*** In the case where the difference was small there the resolution was unsuccessful, and where it was great there the resolution functioned well.

During the TLC tests the bases and the resolution agents were developed in other alcohol-glacial acetic-acid-water mixtures in place of the *n*-butanol too. The resolution was carried out successfully in the alcohol, where the difference between the R_f values of the acids and bases showed maximum value.

I tried to find the theoretical principles of those observed, too. The Golumbić rule describing the chromatographic retention (R_m) and the equation serving to predict the efficiency of the resolutions ('S') contain the parameters characteristic for the hydrophobic character and the proton affinities of the molecules as the main factors alike.

Alcohol component of the eluent: *n*-butanol (BuOH) 2-propanol (*i*-PrOH) ethanol (EtOH) methanol (MeOH)

DBTA

Pipecolic acid-xylidides (1-5)

TA

TA **1 2 3 4 5** DBTA TA **1 2 3 4 5** DBTA TA **1 2 3 4 5** DBTA TA **1 2 3 4 5** DBTA

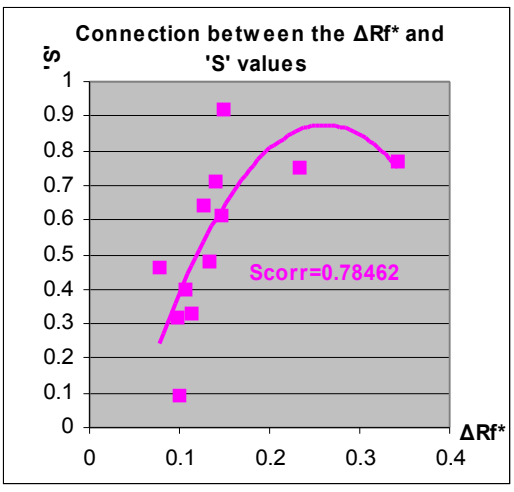
$R_m = \lg (A_S / A_m) + \lg k + pH + pK_a$

$'S' = 0{,}517\,\pi + 2{,}280\,\sigma^* - 0{,}015 \bullet E_T - 0{,}001$

I marked the factors varying at standard TLC conditions and the main parameters affecting the efficiency of the resolutions in circles.

Having the TLC data we were able to ***design the resolutions*** – to select the suitable resolution agent and solvent. During resolution of the pipecolic acid anilides and –trifluoromethyl anilides in the first trial-series we were able to produce bases with an optical purity exceeding 77% in four cases from the altogether eight ones. In frame of the second trial all of the eight compounds were resolved. The number of the trials decreased considerably.

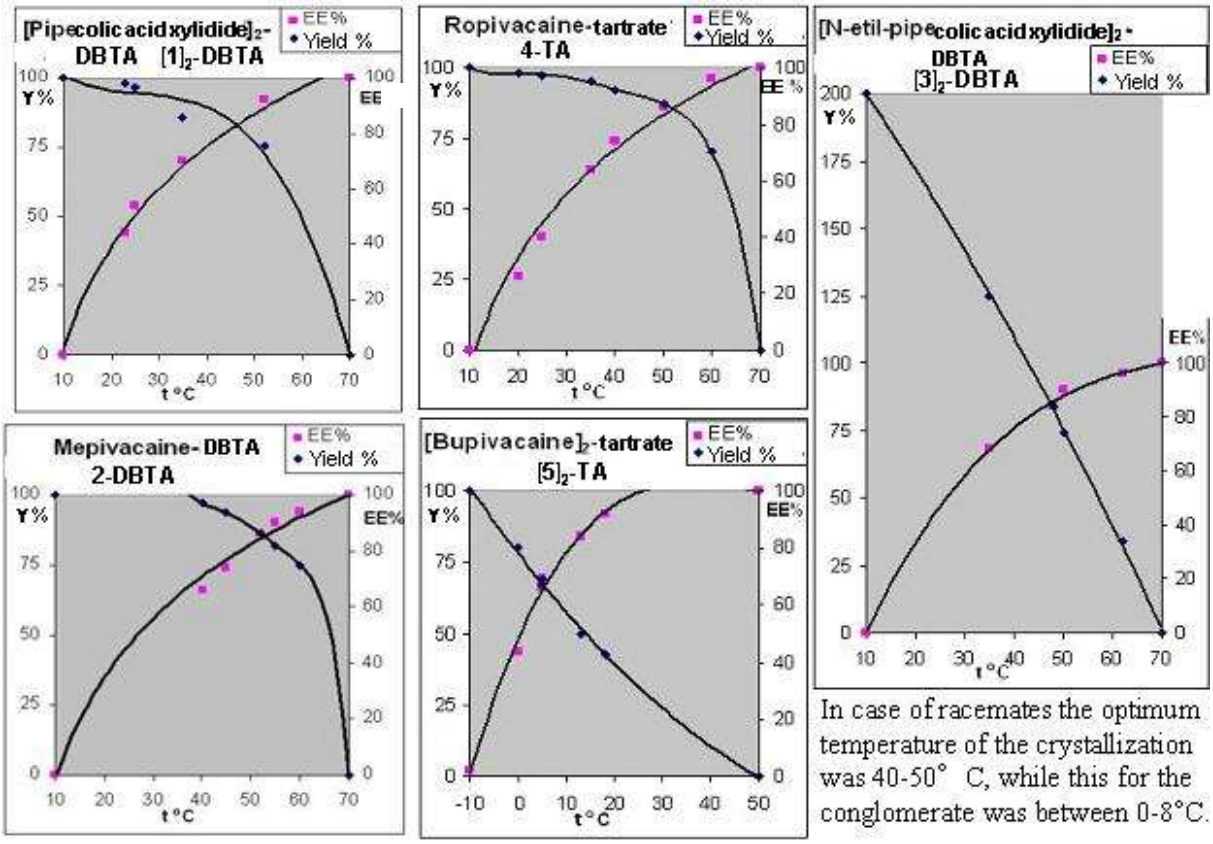
	With tartaric acid						**With dibenzoyl tartaric acid**				
R^1	**Sol-vent**	**ΔR_f***	**Yield % /trial**	**O.P. %**	**$[\alpha]_D^{20}$**	**R^1**	**Sol-vent**	**ΔR_f***	**Yield % /trial**	**O.P. %**	**$[\alpha]_D^{20}$**
$R_2 = R_3 = H$, $R_4 = H$, Pipecolic acid anilides (**6-9**)											
H (**6**)	$EtOH_{aq}$	0,570	67,5/**1**	10,0	+1,5	Et (**7**)	*i*-PrOH	0,133	50,0/**1**	95	-120,4
n-Bu (**9**)	$EtOH_{aq}$	0,563	54,8/**2**	?	-26,0	*n*-Pr (**8**)	*i*-PrOH	0,097	42,0/**1**	77	-102,6
$R_2 = R_3 = H$, $R_4 = CF_3$ Pipecolic acid trifluoromethy anilides (**10-13**)											
H (**10**)	EtOH	0,587	65,2/**2**	15,0	+1,7	Et (**11**)	*i*-PrOH	0,147	66,8/**1**	91,4	-56,6
n-Bu (**13**)	EtOH	0,594	58,5/**2**	71,8	-64,0	*n*-Pr (**12**)	$EtOH_{aq}$	0,113	41,6/**1**	80,0	-62,4



I extended the test to different structure compounds too. The own results were completed with the data of 6 additional resolutions known from the literature, the Rf values of the substances chosen were measured. The connection between difference of the retention factors: ΔR_f* and the efficiency of resolution: 'S' is significant, the value of the correlation coefficient according to Spearman at the 95% confidence level: Scorr=0.7846. Considering the data of the most important industrial resolutions the connection is even stronger, Scorr=0.8286. The data of tisercin, the menthol and the asparagin are located exactly on the line of the plot**.**

3. We examined the effects of the substituents exerted on yield of the diastereomeric salts. The yield data show a decreasing trend with the increase of the weight of the *N*-alkyl substituents: R^1. The substituents of the anilide skeleton: R^2, R^3 and R^4 influence yield of the salts precipitated in crystalline form only slightly in case of the DBTA, but more considerably in case of the TA.

4. I studied the optimum temperature of the resolutions too.

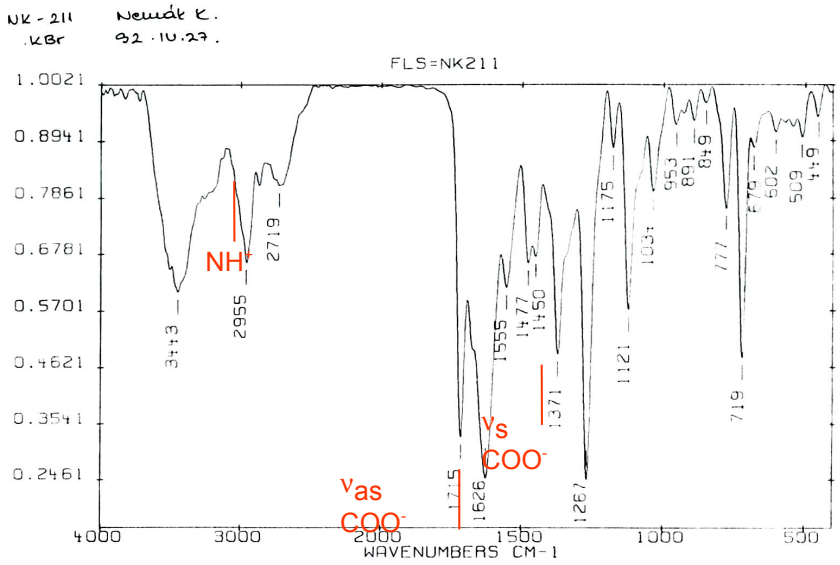


In case of racemates the optimum temperature of the crystallization was 40-50° C, while this for the conglomerate was between 0-8°C.

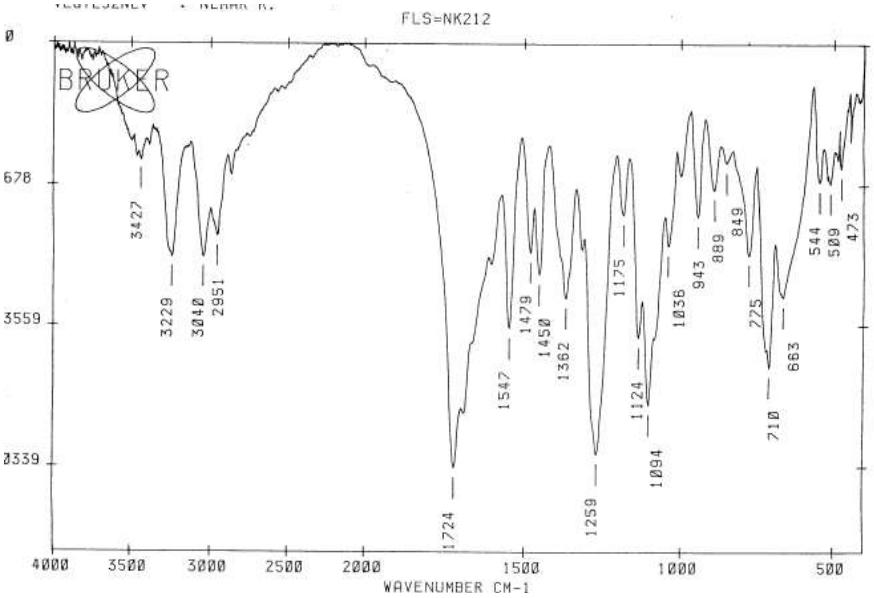
5. We examined the thermodynamic characteristics of the enantiomer mixtures. The melting point-composition diagram of the pipecolic acid xylidides was plotted according to data from DSC measurements. The complete diagram can be plotted having only a few data and it provides substantial help during purification of the enantiomers. The shape of the diagrams reveals the character of the interaction between the enantiomers too.

6. We evaluated the IR- and NMR-spectra of the diastereomers. During this procedure we found two basic types. From the 13 diastereomers examined 6 showed "not salt-like" behaviour during the spectroscopic tests and we could see the expected, classical "salt-like" signals only in 5 of the cases.

Group	***N*-alkyl substituents (R^1)**				
	H	**Me**	**Et**	***n*-Pr**	***n*-Bu**
Pipecolic acid xylidides (**1-5**)	DBTA 2:1 salt	DBTA 1:1 complex	DBTA 2:1 salt	TA 1:1 complex	TA 2:1 salt
Pipecolic acid anilides (**6-9**)	TA 2:1 salt		DBTA 1:1 complex	DBTA 1:1 complex	TA 1:1 complex
Pipecolic acid trifluoromethyl-anilides (**10-13**)	TA 2:1 salt	XRD	DBTA 1:1 complex	DBTA 1:1 complex	TA 1:1 complex



"salt-like" IR-spectrum of [*N*-ethyl-pipecolic acid-xylidide]$_2$ – DBTA with 2719 t-amine NH, 1626 ν_{as} COO^-, and 1371 ν_s COO^- signals



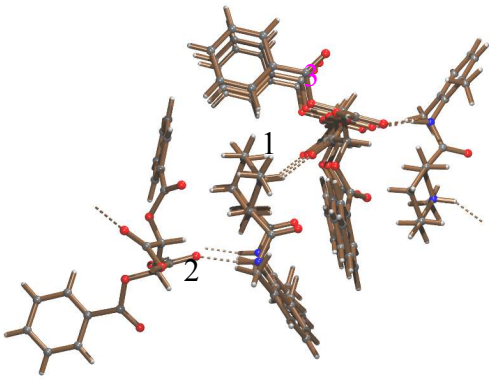
In the **"not salt-like",** IR spectrum of the mepivacaine – DBTA no signals similar to the previous ones appear.

The behaviour of protons located next to the ionic centre of mepivacaine (**2**) was examined in a magnetic field.

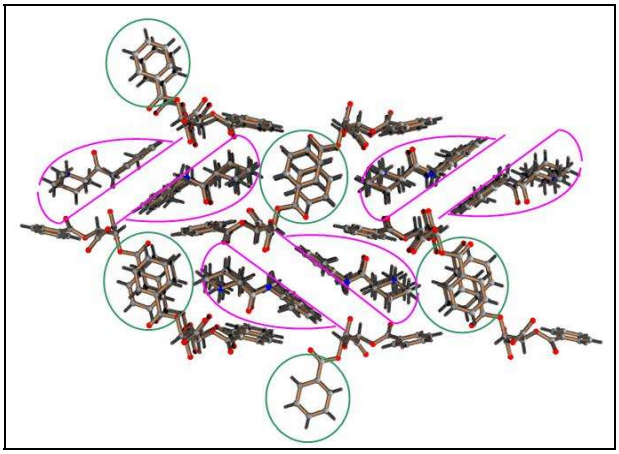
Chemical shift (ppm)	mepivacaine base	mepivacaine - DBTA	mepivacaine HCl
6-H_{ax}	2.00	2.68	3.13
6-H_{eq}	2.91	3.27	3.39
2-H_{ax}	2.57	3.51	4.22
7-H_3	2.26	2.59	2.80

In case of the dibenzoyl tartrate the chemical shift of the 6-H_{ax}, 6-H_{eq}, the 2-H_{ax}, and the 7-H_3 protons is situated between the similar signals of the mepivacaine base and the mepivacaine hydrochloride, somewhat nearer to the signal of the hydrochloride.

7. We examined the supramolecular interactions on the by monocrystal X-ray diffraction method. The following stabilizing hydrogen bridge bonds were identified in the structure of the diastereomer:



	D-H..A	Symmetry code	D..A (Å)
1	N1-H1…O20		2.717(5)
2	N2-H21…O24	1-x, -1/2+y, 1/2-z	2.830(5)
3	**O25-H25...O21**	1+x, y, z	2.482(4)



We observed, that in the structure of the (S)-mepivacaine-dibenzoyl tartrate the homofil interactions between the tartrate parts were significantly stronger, than the heterofil attractions between the mepivacaine and the tartrate parts.

The structure of the crystal is fixed by strong interactions:

- bitartrate chain structures bounded by hydrogen bridges between the carboxylates,
- π-π interactions between the phenyl rings
- repelling powers between aliphatic groups,
- strong interactions within the mepivacaine fixing also the xylil ring, as a result of which some 13°deformation of the torsion angle between the amide bond and the planar ring has been observed.

We would like to clear-up the supramolecular principles of the "not salt-like" behaviour observed during the spectroscopic tests, too. From the Cambridge Crystallographic Database we selected the data series of the tartrates and dibenzoyl tartrates, altogether 631pcs. Narrowing the scope of our examination we set apart 28 twice-basic, neutral salts and 25 acidic salts furthermore 31 pcs of non-ionic structures. Finally we selected 10 representative compounds for which we analyzed the data in details. On the average the hydrogen-mediated bonds were 2.7 Å long, and these were shorter (O…H…O 2.5 Å) for the non-ionic connection types: in case of the clathrats and the acidic salts, and longer (N..H...O 2.8 Å) in case of neutral salts formed with more basic amines. (In the mepivacaine hydrochloride the N..H...Cl distance is 3.1 Å). The shortest are the "head-foot" bonds within the tartrate chains.

8. During my work as process engineer I developed a method suitable to the ***planning of process validations based on risk-assessment***. To further the industrial application of the resolution processes described here I carried out the risk-assessment and marking out the measurement points of the process validation concerning the procedures of diastereomer formation and the selective crystallization too. I presented the procedures suitable to the specific technical assessment of effects of the scale ups and technology transfers.

9. Together with my colleagues at the Chinoin Co,. Ltd. ***we developed a complex quality assurance system*** for supervision of developments functioning with widespread teamwork. In the preparing phase the more detailed designs, predictions, while after the manufacture the multidirectional evaluation of the results renders the development of products smooth, therefore we suggest employing it to the industrial realisation of the resolutions described.

5. *Theses*

1. The binary phase diagram of the enantiomers can be plotted from a few data and it indicates the crystallization type of the enantiomer mixtures (conglomerate, racemate). Size of the racemate area shows the intensity of the interaction between the enantiomers and thus it is suitable to the pre-evaluation of the ability to separate those. The enantiomer ratio achieved by the recrystallization can be red from the diagram according to the melting point. S1

2. The chromatographic retention and the resolution alike are processes depending decisively on secondary interactions. The difference between the R_f values of the acids and bases can be calculated from the data of TLC-s developed under standard conditions with different alcoholic eluent mixtures. We can select the solvent and the resolving agent suitable to the resolution at the greatest difference. S2

3. When changing the temperature at the crystallization of diastereomers the optical purity and the yield change according to opposite trends. When illustrating the optical purity and the yield data as functions of the crystallization temperature in a common diagram, then the optimum of the crystallization temperature can be plotted from a few data. In case of the racemates this was between 40-50°C, while for the conglomerate between 0 and 10°C. S3

4. When studying the structure of the diastereomers we observed in several cases that the IR-spectrum did not contain neither the protonated amine signal of bases nor the carboxilate signal of the acids. According to NMR results in case of the diastereomers showing "not salt-like" behaviour the base : acid molar ratio was always 1:1, while with 2:1 molar ratio the diastereomers had classical "salt-like" IR-spectrum. Based on these data the idea arose that perhaps a complex formation happens here and over the ionic connections the resolution can be achieved with structures developed only by secondary interactions too. S4

5. According to the X-ray diffraction test the crystal structure of the *S*-(-)-mepivacaine-DBTA is determined basically by three strong hydrogen bridges. The homofil interactions between the tartrate parts were stronger than the heterofil ones connecting the mepivacaine. We investigated 84 structures out of 631 tartrates we found in crystallographic database. We determined that in the complexes and the acidic salts of DBTA the shortest hydrogen bridge is of the O…H...O type. In the structure of the *S*-(-)-mepivacaine-DBTA the torsion angle of the amide bond and the xylil ring was deformed. It can be supposed that in the compact structure the power demand of the vibration excitation is modified because of the overlapping electromagnetic fields, and the signals shift to an other area of the IR-spectrum. S5

6. We developed a method resting on risk-evaluation and suitable to the estimation of effects of the scale up and planning the process validations. The arrangement before and the evaluation later on of the manufactures require a careful preparatory, analytical work covering everything alike. We recommended the introduction of the SPECTRAL method as a guide to the analysises. The introduction of the complex quality assurance system provides good frame to the widespread team work. S6

6. *Application or possibilities for applying the results*

From the above theses paragraphs 1., 3., and 6. describe generally applicable techniques.

From my activities described here the presentation of the risk-evaluation methods (6) set off international response. During the quality assurance of the developments the employment of this process can bring very significant results – by increasing the security. A half year after its presentation at the Dublin conference (E1) the methodology described here was included in

the collection of examples attached to the ICH Q9 guideline issued upon the three-parties agreement issued by the International Conference on Harmonization (the supervising board of the joint harmonization activities for the pharmaceutical authorities of America, Europe and Japan). http://www.ich.org/LOB/media/MEDIA3199.pdf (Development, 34-35 pp)

The paper about the examination of structures of the diastereomers (S4) has raised significant attention. There can be found 18 citation for this article in the Scopus database. i.e. Dávid Kozma also cited this paper acknowledging that the idea for applying the dibenzoyl tartaric acid to the resolution of racemates not containing any basic groups came from it.

Preparing the phase diagrams, searching the optimum temperature of the crystallization can be used generally and it is expedient to carry out too.

According to my opinion the application of the resolution planning method could facilitate considerably the laboratory work, relieving the staff from a lot of unnecessary trials. The paper about this (S2) appeared only a few months ago, and is not generally known as yet.

The methods developed to the resolution of the pipecolic acid xylidides are suitable to industrial realisation and optimized.

7. Publications

Publications covering the theme of the dissertation:

S1. **Nemák, K.**, Ács, M., Kozma, D., Fogassy, E.: Racemic compound formation-conglomerate formation Part 4.: Optical resolution and determination of the melting phase diagrams of 2',6'-pipecoloxylidide and four 1-alkyl- 2',6'-pipecoloxylidides **J. Thermal Anal. Vol. 48, pp.691-696 (1997),** *(IF: 1,483, CI: 3)*

S2. **Nemák, K.**, Fogassy, E., Bényei, A., Hermecz, I.,: The Role of TLC in Investigation of Diastereomeric Salt formation by a Gruop of Pipecoloxylidides, **Journal of Planar Chromatography 21(2), 125-128 (2008),** *(IF: 1,531, CI: 0)*

S3. **Nemák, K.**, Kozma, D., Fogassy, E.: Study of the mechanism of optical resolutions via diastereomeric salt formation. Part 4. The role of the crystallization temperature in optical resolution of pipecolic acid xylidides, **Mol. Cryst. Liquid Cryst. 276, 31-36 (1996),** *(IF: 0,554, CI: 4)*

S4. **Nemák, K.**, Ács, M., Jászay, M., Zs., Kozma, D, Fogassy, E.: Study of tthe Diastereisomers formed between (*N*-alkyl)-pipecolic acid-anilides and 2R,3R-tartaric acid. Do the Tartaric Acid Form molecular-Complexes, instead of Salts during Optical resolutions? **Tetrahedron Vol. 52, 1637-1642 (1996),** *(IF: 2,879, CI: 16)*

S5. **Nemák, K.**, Bényei, A., Halász, J., Simon K. and Hermecz, I.,: Optical Resolution of Mepivacainee and Supramolecular Characterization of the Less Soluble Diastereomer – **draft of paper** under opinioning – **Journal of Pharmaceutical and Biomedical Analysis** *(IF: 2,761, CI: 0)*

S6. **Nemák, K.**, Vajdai, M., Kiss, T., Halász, J., Hermecz, I.,: Adapting to New Regulatory Requirements: FDA's Risk-Based Approach to Compliance - How Industry Can Comply with New Concepts in Quality Management, - Accepted to publication: **American Pharmaceutical Outsourcing**

Lectures disclosing results presented in the dissertation:

E1. **Nemák, K.**, Vajdai, M., Kiss, T., Halász, J., Hermecz, I.,: Adapting to New Regulatory Requirements: FDA's Risk-Based Approach to Compliance - How Industry Can Comply with New Concepts in Quality Management, General Session 2. (Main Conference 90 minute Sessions), Active Pharmaceutical Ingredient Conference, June 21-24, 2005, Dublin, Ireland

E2. **Nemák, K.**: Establishing Acceptance Criteria, Interactive Session 2. (Main Conference 90 minute Sessions) Mastering the Science of Process Validation Symposium, December 5., 2006, San Diego, CA, brochure on net: http://www.ivthome.com/pdf/1206_pv_sandiego.pdf

E3. **Nemák, K.**,: Minimizing/Analyzing Process Validation Risks – Planning, Organization and Execution, Advanced General Session 11. (Main Conference 90 minute Sessions) Mastering the Science of Process Validation Symposium, December 7., 2006, San Diego, CA

E4. **Nemák, K.**,: Quality by Design: Scale Up and Process Validation, Specialities of Tech Transfers, Advanced Workshop C Part II.(Half-Day Workshops) Mastering the Science of Process Validation Symposium, December 6., 2006, San Diego, CA

E5. **Nemák, K.**,: New guidelines of the authorities: Application of the approach resting on risk-evaluation, Pharmaceutical Research Symposium, 4. November, 2005., Pécs

Poster lecture presenting some results described in the dissertation:

P1. **Nemák, K.**, Fogassy, E., Bényei, A., Hermecz, I.,: The Role of TLC Methods in the Investigation of Diastereomeric Salt Forming Behaviour Within a Group of Pipecolo-xylidides, poster: P-86, 7th Balaton Symposium on High-Performance Separation Methods, September 5-7, 2007, Siófok