University of Szeged
Doctoral School of Pharmaceutical Sciences

Pharmaceutical Technology PhD program

Program manager: Prof. Dr. Piroska Szabó-Révész D.Sc.

Department of Pharmaceutical Technology

Supervisor: **Dr. Zoltán Aigner PhD.**

Ágnes Szabados-Nacsa Dr.

Improvement of the solubility and bioavailability of loratadine by pharmaceutical technological methods

Committee of final exam:

Head: *Prof. Dr. István Erős*, Univ. of Szeged, Dept. of Pharmaceutical Technology
Members: *Dr. Gábor Blazsó Ph.D.*, Univ. of Szeged, Dept. of Pharmacodynamics and Biopharmacy
Dr. Éva Hunyadvári, Phoenix Zrt.

Committee of reviewers:

Head: *Prof. Dr. Judit Hohmann*, Univ. of Szeged, Dept. Of Pharmacognosy
Reviewers: *Dr. György Stampf Ph.D.*, Semmelweis Univ., Dept. Of Pharmaceutics
Dr. Miklós Vecsernyés Ph.D., Univ. of Debrecen, Dept. of Pharmaceutical Technology
Members: *Dr. Attila Dévay Ph.D.*, Univ. of Pécs, Inst. of Pharmaceutical Technology and Biopharmacy
Dr. Róbert Gáspár Ph.D., Univ. of Szeged, Dept. of Pharmacodynamics and Biopharmacy

Szeged
2011

INTRODUCTION

Oral administration is the most common route for drug administration. It is estimated that 40% or more of APIs identified through combinatorial screening programs are poorly soluble in water. However, after oral administration, the absorption may be erratic and incomplete. Therefore it is a great challenge for the pharmaceutical technologists to formulate suitable therapeutic effect disposed products from these materials. Recently it would be advantageous if the pharmacokinetic properties of drug candidates could be predicted before clinical phases.

Per os administered APIs should possess the following properties: they should be stable in gastrointestinal (GI) fluids, they should have suitable solubility in water in order to be able to dissolve in the GI fluids, however they should be somewhat lipophilic to be able to get across the membranes.

One of the prerequisites for successful oral drug therapy is sufficient intestinal absorption. The rate and extent of intestinal absorption are mainly dependent on the dissolution rate of the drug in the gastrointestinal fluids and the rate of transport across the intestinal membrane. These two factors were the base of the BCS. There are several possibilities to modify the APIs to reach better physico–chemical parameters, thereby better BA (Fig. 1). The rate and extent of absorption of Class II compounds is highly dependent on the performance of the formulated product. These drugs can be successfully formulated for oral administration, but care needs to be taken with formulation design to ensure consistent BA. In this thesis I investigated the possibilities of solubility enhancement of a BCS II compound, loratadine (LOR) with cyclodextrins (CDs) and polyvinylpyrrolidones (PVP). The solubility of LOR highly depends on the pH, therefore it is advisable to make the solubility independent of the pH for suitable absorption, i.e. bioavailability (BA).

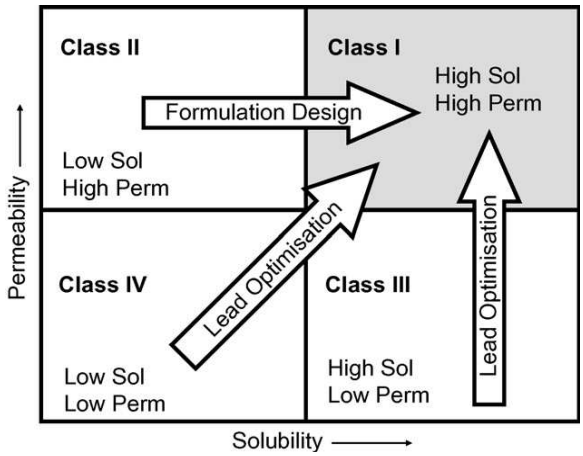

Fig. 1 Formulation strategies of APIs belonging different classes of BCS to improve their BA

AIMS

During my work the following targets have been set:

- to investigate the effect of different CD derivatives on solubility;
- to prepare products with the above chosen CD derivative by different methods;
- to study the dissolution rate of the products; to find out relationship between the preparation methods and the dissolution rate (which preparation method and composition is the best);
- to examine the pH-dependence solubility of the products;
- to study the interaction between the API and CD in the products with direct (mass spectrometry – ESI-MS) and indirect (thermoanalytical measurement – DSC, TG; Fourier transform inrfrared spectroscopy – FT-IR, diffusion ordered ^{1}H-NMR – DOSY) instrumental methods;
- to test the products in *in vitro* permeability model in order to predict the absorption;
- to investigate the absorption in *in vivo* experiments and
- to evaluate the results.

MATERIALS AND METHODS

Active ingredient: Loratadine (LOR)

Manufacturer: TEVA Hungary Zrt.

Chemical structure: see on Fig. 2.

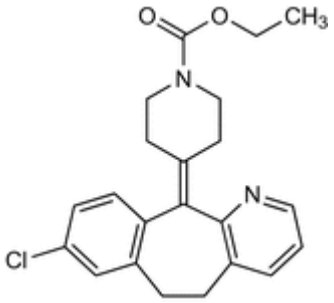

Fig. 2: Chemical structure of LOR

Molecular formula: $C_{22}H_{23}ClN_2O_2$

M_w: 382.89

Melting range: 132 – 135 ˚C

Trade names: Clarinase®, Claritine®, Claritine akut®, Erolin®, Flonidan®, Lorano®, Loratadin Hexal®, Loratadin–ratiopharm®, Roletra®

LOR is a tricyclic, piperidine derivative of antihistamines. It belongs to the second generation antihistamines, so it has non–sedating properties. H_1 antihistamines are applied in the treatment of allergies: they prevent symptoms such as itching, congestion, rhinorrhoea, tearing and sneezing.

LOR belongs to Class II of the BCS. Chemically LOR is a weak base, therefore its solubility increases with decreasing pH. At lower pH values, LOR – which is a nitrogen base – is protonated, and therefore becomes more soluble in water. However according to the modified Hendersson–Hasselbach equation, at ~ pH 7 and higher LOR is totally unionized, which is the form able to absorb, so LOR will probably absorb from the intestines, in which it has poor solubility. Accordingly there is a need to improve the solubility of LOR in order to achieve acceptable biological effect.

Excipients:

- α–CD, β–CD, γ–CD, random methyl–β–CD (RAMEB), 2–hydroxypropyl–β–CD (HPBCD), methyl–β–CD, hydroxy–butyl–β–CD and heptakis–(2,6–di–O–methyl)–β–cyclodextrin (DIMEB); Cyclolab Ltd. (Budapest)
- β–CD–sulfobutyl–ether (Captisol); CyDex Pharmaceuticals Inc. (Lenexa, USA)
- compound 48/80 (N-methyl-4-methoxy-phenethylamine); Sigma-Aldrich Logistic GmbH (Germany)
- other chemicals, e.g. methanol, ethanol were of analytical grade purity (Spektrum 3D Kft., Debrecen)

Methods

During the preliminary experiments the effects of the various CD derivatives on the solubility of LOR were investigated. The best solubility enhancement was achieved with DIMEB, which resulted in a ~ 300–fold increase in solubility, and accordingly this derivative was used in the further examinations.

Phase-solubility studies

The phase–solubility diagrams were recorded by the Higuchi–Connors method. For this purpose aqueous solutions of DIMEB of various concentrations were prepared at a specific pH value (7.5) An excess amount of LOR was added to these solutions, and they were then shaken at room temperature. After 72 h, the suspensions were filtered through 0.45 μm membrane filters. After dilution, their absorption was measured by UV spectrophotometry.

Preparation of products

The DIMEB-containing products were prepared in three molar ratios (LOR:DIMEB = 1:1, 1:2 and 1:3), while PVP-containing solid dispersions were produced in four weight ratios (LOR: PVP K25 = 1:1, 1:2, 1:4, 1:6).

Physical mixtures (PM): LOR was mixed carefully in a mortar with the calculated amount of DIMEB. ***Kneaded products (KP):*** the physical mixtures were suspended with the same mass of 50% ethanol, and the solvent was evaporated off at room temperature. After drying, the products were ground. ***Microwave products (MWs):*** the preparation is the same with kneaded products to the end of suspension step. Then the evaporation of the solvent was carried out by microwave power (150 W, 90 s, 60 °C) and vacuum drying. ***Spray–dried products (SDs):*** the physical mixtures were dissolved in 50% ethanol, and SDs were obtained by using a Büchi Mini Spray Dryer B–191 (inlet temperature: 105 °C, compressed air flow: 800 L/h, nozzle diameter: mm). All of the samples were sieved (100 μm) and stored at room temperature under normal conditions.

The **solid dispersions** of LOR in PVP K-25were prepared by the solvent evaporation method (**SEs**). LOR and PVP K-25 were dissolved in methanol and the solvent was removed by vacuum dryer during 6 hours. All of the products were pulverized in a mortar and sieved through a 100 μm sieve.

***In vitro* dissolution studies**

200 mg samples of pure LOR or products containing 200 mg of LOR was examined in 100 mL of dissolution media (simulated gastric medium or simulated intestinal medium). The paddle was rotated at 100 rpm and sampling was performed up to 120 min (sample volume 5.0 mL). Aliquots were withdrawn at 5, 10, 15, 30, 60, 90 and 120 min and immediately filtered. After filtration and dilution, the LOR contents of the samples were determined spectrophotometrically.

Study the effect of pH on the solubility

Seven buffer solutions were prepared with different pH values between 1.2 and 7.5. The defined daily dose of LOR is 10 mg, so 10 mg of LOR or product containing 10 mg of LOR was examined in 900 mL of dissolution media at 37 °C. The paddle was rotated at 100 rpm. After 2 h the removed samples were filtered and the LOR concentrations were measured spectrophotometrically.

Investigation of complexation by instrumental methods

Thermoanalytical (DSC and TG), Fourier-transform infrared spectroscopy (FT-IR), mass-spectrometry (ESI-MS) and diffusion ordered ^{1}H-NMR (DOSY) measurements were performed in order to characterize the products and to prove the complexation and to determine the stoichiometry of the complexation.

***In vitro* membrane permeability assay (PAMPA)**

PAMPA "sandwiches" were formed from an acceptor 96-well microtitre plate and a matching filter plate with apparent porosity of 0.45 µm, coated with 5 µL of 1 w/v% *n*-dodecane solution of lecithin. The plate sandwich was allowed to incubate at 25±1 °C for 16 hours without stirring, in an atmosphere saturated in humidity. Afterwards, sample concentrations in both the acceptor and donor wells were determined by HPLC method. Effective permeability coefficients, P_e, were determined by taking into account the apparent filter porosity and sample mass balance. The donor (V_D=150 µL) and acceptor (V_A=300 µL) compartments were both constituted of pH 7.4 buffer solutions.

The permeability rates were calculated using by the equation below:

$$\log P_e = \log\{C \bullet -\ln(1-\frac{[drug]_{acceptor}}{[drug]_{equilibrium}})\}$$

$$C = (\frac{V_D \bullet V_A)}{(V_D + V_A) Area \bullet time}$$

$[drug]_{acceptor}$: drug concentration in the acceptor phase

$[drug]_{equilibrium}$: equilibrium concentration of the drug

Area: area of the PVDF membrane

Time: incubation time

***In vivo* experiments**

150 ± 5 g of male Wistar rats were used to test the following materials: LOR, DIMEB, KP 1:1 and KP 1:2 (LOR:DIMEB). The test substances were given orally in suspension in 0.25% methylcellulose in a dose of 10 mg/kg. 1 h later the histamine liberator compound 48/80 in physiological solution (10 µg/0.1 mL) was administered subplantarly to elicit the inflammatory reaction. The intensity of the arising inflammatory reaction was measured after 30 min with the use of a plethysmometer. Then blood samples were taken for plasma concentration analysis (HPLC). Statistical analyses were performed with Prism 4.0 software by ANOVA method.

RESULTS AND DISCUSSION

The type of the phase solubility diagram is AL, which means, that the solubility of the drug raises with the increase of CD concentration in linear relationship.

According to that LOR has good solubility in acidic medium, the dissolution of products was evaluated in alkaline medium. We have found out that all DIMEB-containing products improved the rate of dissolution, however the extent was largely influenced by the composition (molecular ratio) and the preparation method. The lowest enhancement of dissolution rate was experienced by the 1:1 products; within these products the following order has been arisen: PM<KP~MW<SD. For the 1:2 (Fig. 3) and 1:3 compositions the whole of the investigated samples dissolved in the first 15 min (except the PMs), which means that the same good dissolution would be obtained at the extreme pH values of the gastrointestinal tract on the use of these DIMEB products. Accordingly, if the rate–limiting step of absorption was not the dissolution, the permeability would regulate the passage through the membrane. As LOR has good permeability, the application of LOR complexed with DIMEB would lead to a greater quantity of drug being absorbed, consequently better BA would be obtained.

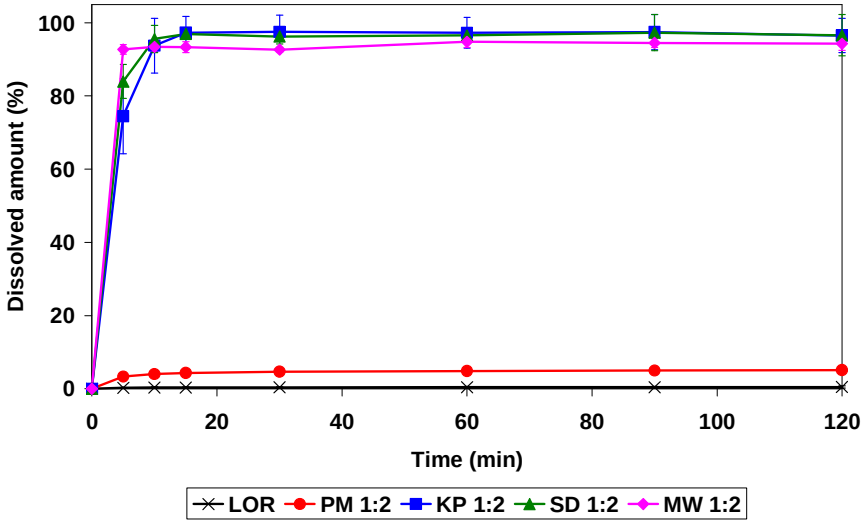

Fig. 3: Dissolution of LOR and 1:2 products in SIM

However LOR SEs did not give much better result in simulated intestinal medium. The dissolution did not change in SIM, where 1:1 and 1:2 ratios showed worse dissolution compared to raw drug. In the case of 1:4 and 1:6 product the initial appearance of increased dissolution rate can be explained that amorphous drug dissolves faster than the crystalline LOR, and after the rapid dissolution the API recrystallizes. As the SEs did not improve the solubility and dissolution rate of LOR adequately, they were not tested in the further studies.

The solubility of LOR has been reported to decrease with increasing pH. As can be seen in Fig. 4 and 5, the applied dose of pure LOR did not dissolve at the pH of intestines, from where it is absorbed. In the acidic range (up to pH ~3) both the 1:1 and 1:2 compositions can provide that the applied dose is dissolved. However in the case of 1:1 products with the increasing pH, DIMEB is not able to dissolve the whole quantity, therefore the BA will not be good enough. According to the dissolution results, PM shows worse dissolution, than KP and SD. By the PM 1:2 product the result is similar to the previous one. In contrast, virtually the whole quantity of LOR dissolved from the KP and SD 1:2 products both in the acidic and alkaline media, subsequently the solubility of LOR became independent of the pH. This clearly suggests an opportunity to ensure smooth dissolution for LOR, thereby achieving better and more uniform BA. In case of the 1:3 products it can be stated the same as by the 1:2 products.

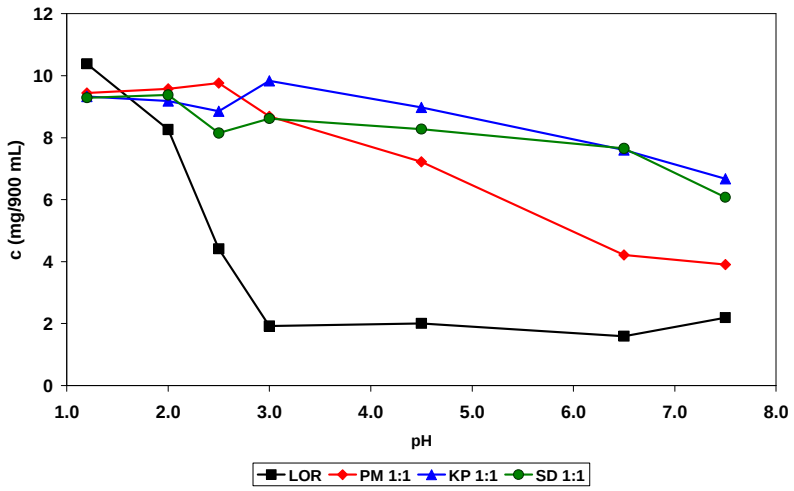

Fig. 4: pH–dependence of the solubility of LOR and 1:1 products

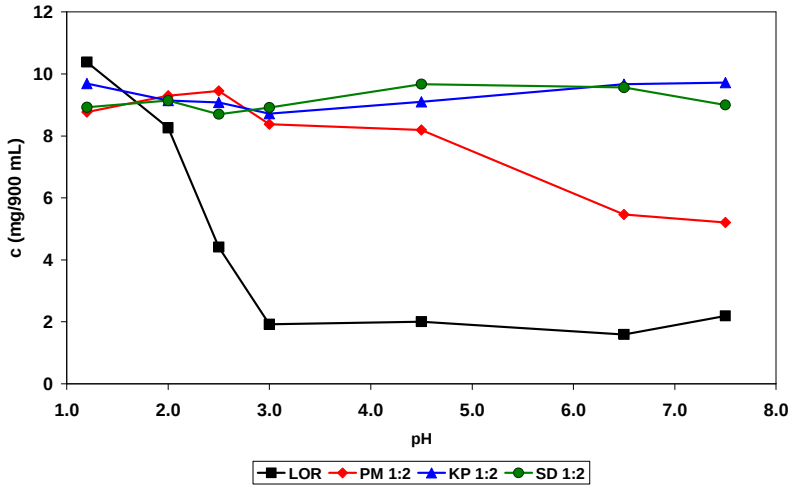

Fig. 5: pH–dependence of the solubility of LOR and 1:2 products

Based on the thermoanalytical studies the PMs did not result in total complexation, which was also proved by FT-IR (data not shown).

For the KP, MW and SD samples total complexation occurred, which is confirmed by the absence of the endotherm reflected the melting point of LOR (Fig. 6) and the shift of the characteristic carbonyl stretching in LOR on the FT-IR spectra (Fig. 7). These results lead us to assume that the –COO group provides the complex–forming bonds to the outer surface of DIMEB and that complex formation alters the hydrogen–bonded cyclic dimeric structure involving the carboxyl group. A lipophilic part of LOR will probably be attached to the inner surface of DIMEB, like the aromatic rings, however in the FT–IR spectrum of LOR, the characteristic stretching frequencies of these aromatic parts are masked by DIMEB, accordingly these interactions can not be detected with this method.

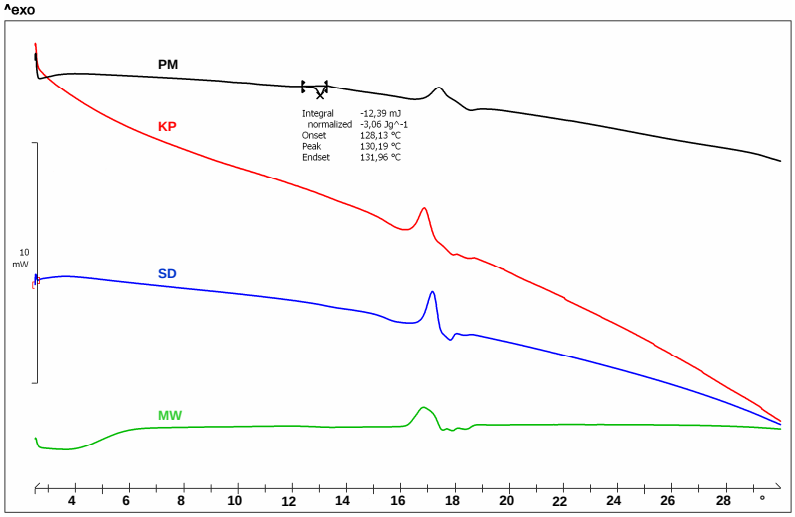

Fig. 6: DSC curves of 1:2 products

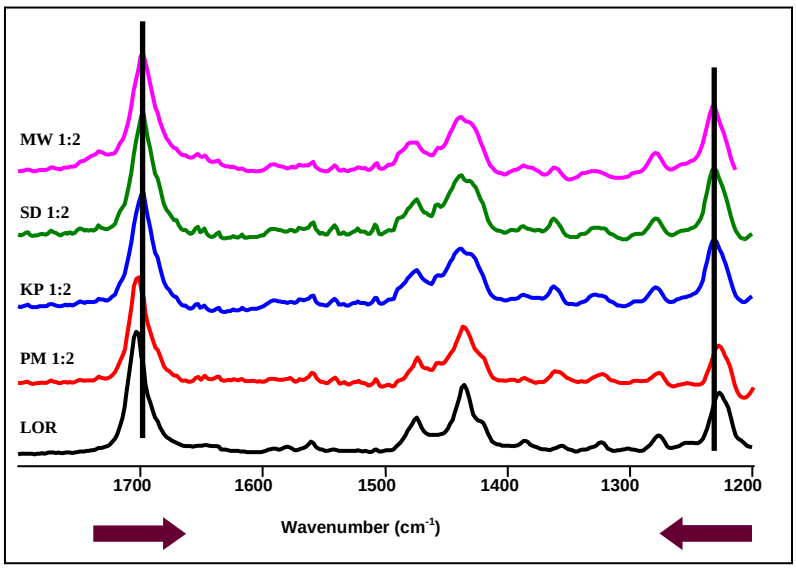

Fig. 7: FT-IR difference spectra of LOR and 1:2 products

For determining the stoichiometry of the complex mass spectrometry (ESI-MS) and diffusion ordered ^{1}H-NMR were applied. As expected, the ESI-MS speactra (Fig. 8 and 9) essentially involved peaks due to singly charged ions of pure LOR [at m/z 383 as $(LOR)H^{+}$] and pure DIMEB [at m/z 1331 as $(DIMEB)H^{+}$]. A new signal corresponding to the inclusion complex as a singly charged ion $(DIMEB+LOR)H^{+}$ is observed at m/z 1730 independent from the molar ratios (1:1 and 1:2), which suggests that the DIMEB inclusion complex in the gas phase has a certain stoichiometry (1:1).

In the DOSY spectra (Fig. 10) of KP 1:1 and 1:2, log D is the same for every chemical shift, which is possible only when LOR is complexed in DIMEB, when they compose a unit. If LOR is not complexed, it should diffuse more quickly due to its small molecular weight; it should have a smaller D value. The D value measured for the LOR:DIMEB complexes indicates that it is best formulated as the 1:1 complex.

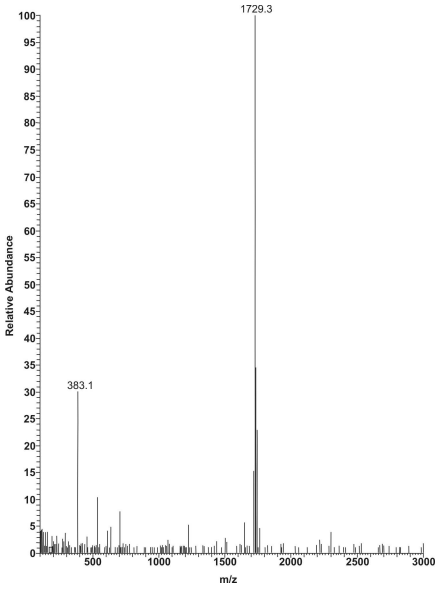

Fig. 8: ESI-MS spectra of KP 1:1

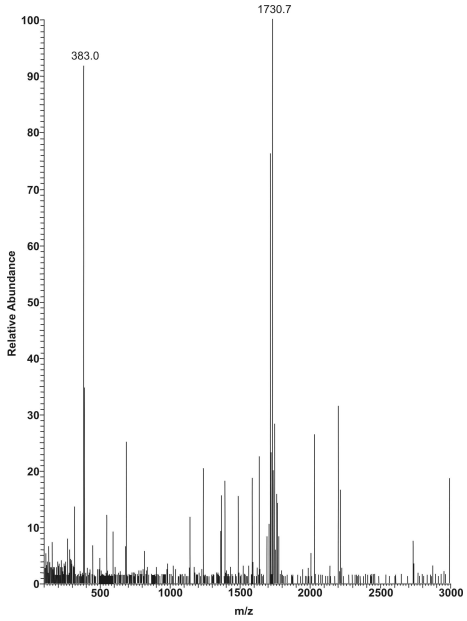

Fig. 9: ESI-MS spectra of KP 1:2

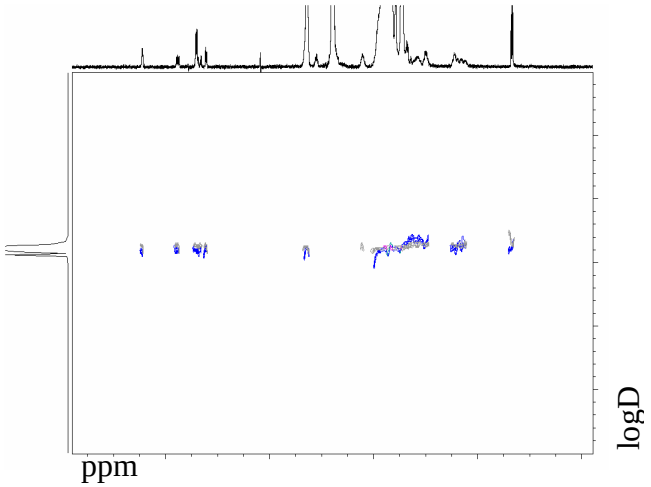

Fig. 10: Representative DOSY spectra of KP 1:1 (grey) and KP 1:2 (blue)

The permeability results originated from PAMPA model can be summarized as followings: There is an observable relationship between the amount of DIMEB and the calculated permeability. The higher the concentration of DIMEB, the lower the permeability is. This confirms the theory that the association-dissociation balance is shifted to association, and the back-diffused LOR is recomplexed by free DIMEB.

It was found that in the *in vivo* experiments DIMEB did not influence the extent of oedema relative to the control group, and thus the effects of the DIMEB-containing products can only be due to the LOR in the products. Rats pretreated with LOR, KP 1:1 and KP 1:2 significantly decreased the compound 48/80-induced oedema (Fig. 11). It is evident that the abilities of LOR, KP 1:1 and KP 1:2 to decrease oedema differ significantly; the complexation of LOR with DIMEB resulted in better BA.

The results confirm that in the absorption process of LOR active transport mechanisms play also an important role in addition to passive diffusion (in the PAMPA method only passive diffusion part of the absorption can be investigated). As DIMEB has surface activity, therefore it can inhibit the P-glycoprotein (P-gp), which inhibits the absorption of LOR. Hence, one part of the DIMEB will affect P-gp, and the other part remains for the recomplexation. This can be another explanation why there is a need for excess DIMEB to achieve much better BA. These two mechanisms probably play a role in the enhanced absorption, and therefore the greater BA.

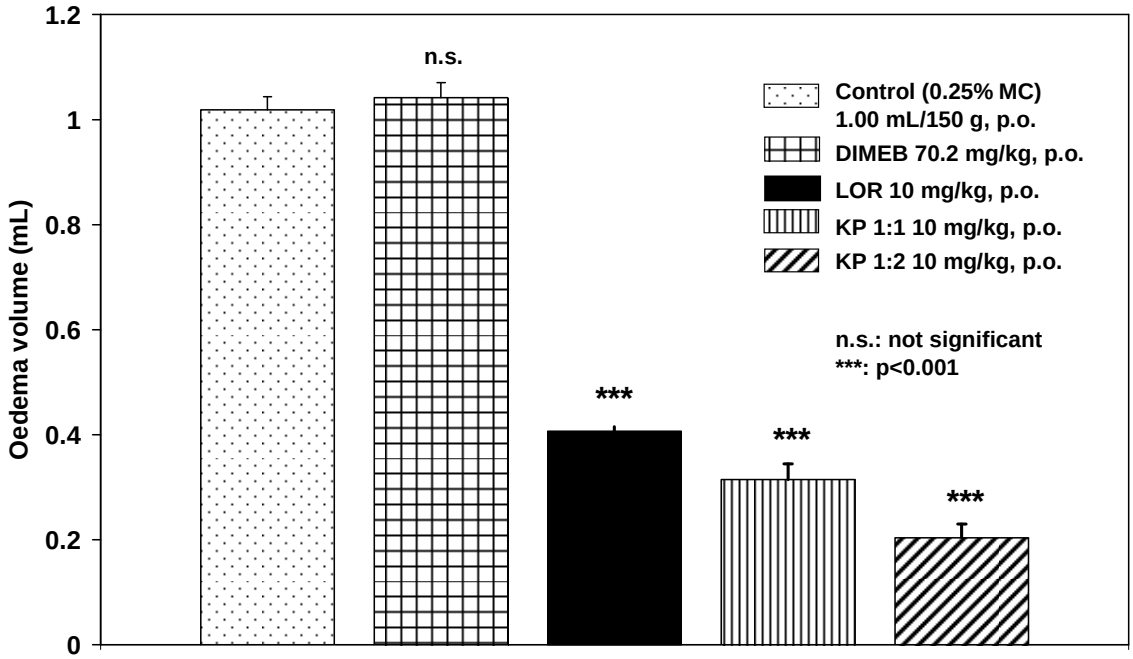

Fig. 11: Oedema decreasing effect of LOR and selected KPs in rats

SUMMARY

1. From the available 9 CD derivatives DIMEB was chose based on preliminary experiments, because it increased the solubility of LOR to the largest extent, to about 300–fold.
2. Different mole ratios (LOR:DIMEB = 1:1, 1:2 and 1:3) and four methods (PMs – physical mixtures, KPs – kneaded products, MWs – microwave products and SDs – spray dried products) were applied to form complexes. Besides solid dispersions with PVP K25 were also made in four different mass ratios (LOR:PVP K25 = 1:1, 1:2, 1:4, 1:6).
3. The greatest dissolution was obtained by the higher amount of DIMEB containing products (1:2 and 1:3 compositions). From the preparation methods only the physical mixing and the solid dispersions with PVP K25 did not show much better results than LOR only.
4. With the application of DIMEB we achieved pH-independent solubility of LOR by some of the compositions (KP, MW, SD: 1:2 and 1:3), therefore better and smoother bioavailability can be expected.
5. The extent of inclusion complexation has been proved by indirect (DSC, FT-IR, DOSY) and direct (ESI-MS) methods. For PMs there is only particular complexation, while in case of KPs, MWs and SDs full complexation occurred. The stoichiometry of these complexes is 1:1 based on the ESI-MS and DOSY results, which can be seen on the phase solubility diagram, as well.
6. The physical and chemical characteristic of KP and MW are quite similar, the thermoanalytical and FT-IR studies proved that the microwave power did not cause any physical or chemical change in the molecule of LOR. Therefore microwave power can be safely applied as a drying method.
7. By the in vitro membrane modelling (PAMPA) it has been proven that the absorption of LOR is not only affected by passive diffusion, but other mechanisms also play role in this process.
8. With *in vivo* experiments the KP 1:1 and 1:2 decreased the extent of the induced oedema, i.e. improved the BA of LOR, because of some factors: products has better dissolution, the dissolution of the products is independent of the gastrointestinal pH and DIMEB inhibits the P-gp (which effluxes the absorbed LOR, therefore the BA is low and very variable). It has been proven that there is a need for excess amount of DIMEB

(1:2 composition) to achieve the best pharmacological effect although the stoichiometry of the complex is 1:1.

9. The obtained results are very useful in early drug discovery. Most of the new APIs are very hydrophobic and some of them have an ionizable function group, i.e. pH-dependent solubility and in these cases DIMEB can be a good choice to improve their solubility, so they could become suitable for other important investigations, like toxicological studies, effectiveness assays and so on.

PUBLICATIONS

I. **Nacsa Á.**, Aigner Z. és Szabóné Révész P.:
Loratadine oldékonyságának és biohasznosíthatóságának növelése gyógyszer–technológiai módszerekkel.
Orvostudományi Értesítő, **79 (2)**, 257–260 (2006)

II. **Á. Nacsa**, R. Ambrus, O. Berkesi, P. Szabó–Révész and Z. Aigner:
Water–soluble loratadine inclusion complex: Analytical control of the preparation by microwave irradiation.
J. Pharm. Biomed. Anal., **48**, 1020–1023 (2008)

III. R. Ambrus, **Á. Nacsa**, P. Szabó–Révész, Z. Aigner and S. Cinta–Panzaru:
Polyvinylpyrrolidone as carrier to prepare solid dispersions – Pros and cons.
Rev. Chim., **60**, 539–543 (2009)

IV. **Á. Nacsa**, O. Berkesi, P. Szabó–Révész and Z. Aigner:
Achievement of pH–independence of poorly–soluble, ionizable loratadine by inclusion complex formation with dimethyl–β–cyclodextrin.
J. Incl. Phenom. Macrocycl. Chem., **64**, 249–254 (2009)

V. **Á. Szabados-Nacsa**, P. Sipos, T. Martinek, I. Mándity, G. Blazsó, Á. Balogh, P. Szabó-Révész and Z. Aigner:
Physico-chemical characterization and in vitro/in vivo evaluation of loratadine:dimethyl-β-cyclodextrin inclusion complexes
J. Pharm. Biomed. Anal., **55**, 294–300 (2011)

ABSTRACTS

I. **Nacsa Á.:**
Loratadine oldékonyságának és biohasznosíthatóságának növelése gyógyszer–technológiai módszerekkel.
Tudományos Diákköri Konferencia, Szeged, 2006. április 5–7.
Abstract/Verbal

II. **Nacsa Á.**, Aigner Z. és Szabóné Révész P.:
Loratadine oldékonyságának és biohasznosíthatóságának növelése gyógyszer–technológiai módszerekkel.
Erdélyi Múzeum Egyesület, Orvos– és Gyógyszerésztudományi Szakosztály, XVI. Tudományos Ülésszak, Csíkszereda, Románia, 2006. április 27–29.
Abstract/Verbal

III. **Nacsa Á.**, Aigner Z. és Szabóné Révész P.:
Loratadine oldékonyságának és biohasznosíthatóságának növelése gyógyszer–technológiai módszerekkel.
Congressus Pharmaceuticus Hungaricus XIII., Budapest, 2006. május 25–27.
Poster/Abstract

IV. **Nacsa Á.:**
Loratadine oldékonyságának és biohasznosíthatóságának növelése gyógyszer–technológiai módszerekkel.
II. Szent–Györgyi Albert Konferencia, Budapest, 2008. március 7–8.
Abstract/Verbal

V. Z. Aigner, **Á. Nacsa**, R. Ambrus, O. Berkesi and P. Szabó–Révész:
Preparation of cyclodextrin inclusion complexes by microwave treatment.
6th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Barcelona, Spain, April 7–10, 2008.
Poster/Abstract

VI. **Nacsa Á.:**
Loratadine ciklodextrines termékeinek összehasonlító vizsgálata.
Magyar Tudomány Ünnepe
Szeged, 2008. november 27.
Verbal

VII. **Nacsa Á.:**
Loratadine és ciklodextrines komplexeinek állatkísérletes vizsgálata.
IX. Clauder Ottó Emlékverseny, Budapest, 2009. április 23–24.
Abstract/Verbal

VIII. **Á. Nacsa**, Z. Aigner, P. Sipos, T. Martinek, G. Blazsó, Á. Balogh and P. Szabó–Révész:
Enhanced oral bioavailability of loratadine via a pH–independent inclusion complex.
3rd BBBB International Conference on Pharmaceutical Sciences, Antalya, Turkey, October 26–28, 2009.
Poster/Abstract

IX. **Nacsa Á.**, Sipos P., Martinek T., Blazsó G., Balogh Á., Szabóné Révész P., Aigner Z.:
Loratadin biohasznosíthatóságának növelése pH–független zárványkomplex–képzéssel.
Congressus Pharmaceuticus Hungaricus XIV., Budapest, 2009. november 13–15.
Poster/Abstract

This work was supported by the Sanofi-Aventis Fellowship.