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Ph.D. Thesis

IMPROVEMENT OF THE SOLUBILITY AND BIOAVAILABILITY OF LORATADINE BY PHARMACEUTICAL TECHNOLOGICAL METHODS

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CONTENTS

Publications

I. **Nacsa Á.**, Aigner Z. és Szabóné Révész P.:
Loratadine oldékonyságának és biohasznosíthatóságának növelése gyógyszer–technológiai módszerekkel.
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S-Y. Lin et al.: J. Pharm. Biomed. Anal., 53, 799-803 (2010)

III. R. Ambrus, **Á. Nacsa**, P. Szabó–Révész, Z. Aigner and S. Cinta–Panzaru:
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ABBREVIATIONS

API	active pharmaceutical ingredient
BCS	Biopharmaceutical Classification System
BA	bioavailability
LOR	loratadine
pK_a	dissociation constant
DMSO	dimethyl-sulfoxide
SCF	supercritical fluid
CD	cyclodextrin
GI	gastro-intestinal
logP	logarithm of effective permeability
PAMPA	parallel artificial membrane permeability assay
M_w	molecular weight
DIMEB	dimethyl-β-CD
P-gp	P-glycoprotein
PM	physical mixture
KP	kneaded product
SD	spray dried product
MW	microwave
SGM	simulated gastric medium
SIM	simulated intestinal medium
DSC	differential scanning calorimetry
FT–IR	Fourier–transform infrared spectroscopy
ESI–MS	electrospray ionization mass spectrometry
DOSY	diffusion ordered NMR spectroscopy
EDTA	ethlyenediamine-tetraacetate

1. 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 [1]. 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 [2].

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. [3]. These two factors were the base of the BCS [4]. Poorly water–soluble drugs (BCS II and IV class) are associated with slow drug absorption leading eventually to inadequate and variable BA [4, 5]. 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 [6].

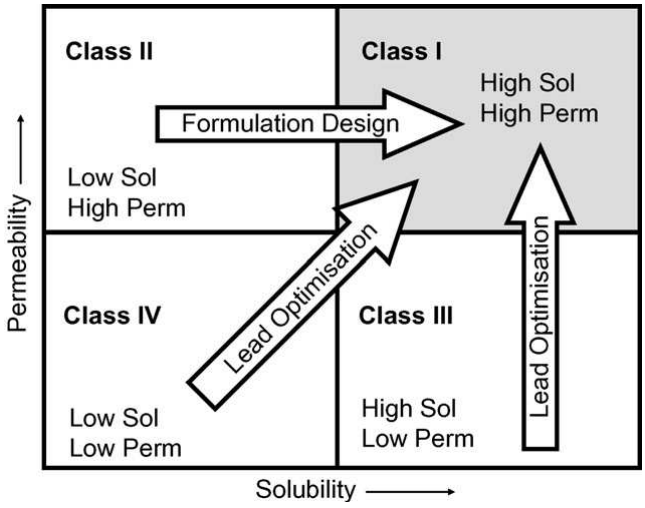

Fig. 1 Formulation strategies of APIs belonging different classes of BCS to improve their BA [6]

This thesis is based on investigations of an antihistamine drug, LOR belonging to the Class II of BCS to increase its solubility, dissolution rate and BA.

2. Bioavailability

The BA of pharmacons is basically determined by their solubility and permeability. All drugs must possess some degree of aqueous solubility in order to be pharmacologically active, and they need to be lipophilic to be able to permeate biological membranes [7]. The rate–limiting step of oral absorption is the dissolution by APIs with solubility < 0.1 mg/ml, e.g. drugs belonging in classes II and IV of the BCS [5, 8]. The solubility of ionizable compounds varies with the pH of the gastrointestinal juices, depending on their pK_a [9]. The pH of the gastrointestinal fluids is therefore one of the most important factors influencing on the saturation solubility of ionizable drugs [10]. As the pharmacon proceeds along the gastrointestinal tract, it passes into a medium with somewhat higher pH. During 90% of a fasting state, the gastric pH is <3 [11]. The presence of food in the stomach can influence the release, dissolution and gastro–duodenal transport of a drug [12]. As the gastrointestinal pH can vary widely, the rate of dissolution of an ionizable pharmacon will also vary considerably. Only the dissolved drug is capable of absorption, so its BA, and hence the pharmacokinetic parameters will be very variable [13].

2.1. Solubility and dissolution rate

First we need to distinguish between “kinetic” and “thermodynamic solubility”. By the kinetic solubility tests the compound is dissolved in an organic solvent (generally in DMSO), then added to the aqueous buffer until precipitation. In the case of thermodynamic solubility the aqueous solvent is added directly to solid crystalline material (the solid is in excess) and long mixing time is applied to ensure equilibrium (e.g. 24 – 72 hours). The kinetic solubility in general tends to be higher, than the thermodynamic, because the presence of DMSO helps to enhance solubility, there is no need to overcome the crystal lattice energy by water molecules [14, 15].

There is a difference between solubility and dissolution rate, too. Solubility is how much of a compound can dissolve in a solution. Dissolution rate is how fast a compound can dissolve into solution [14].

Intrinsic dissolution rate has been defined as ”the rate of dissolution of a pure pharmaceutical active ingredient when conditions such as surface area, temperature, agitation

or stirring speed, pH and ionic strength of the dissolution medium are kept constant" [16]. It is specific for the substance. The apparent dissolution rate reflects the liberation of drug from a dosage form [17].

Noyes and Whitney noticed that the rate of dissolution is proportional to the difference between the instantaneous concentration, C at time t, and the saturation solubility, C_s. This relationship was modified by Brunner, Tolloczko and Nernst (see Equation 1), who considered the exposed surface (S), the thickness of the diffusion layer (h), the volume of the dissolution medium (V) and the diffusion coefficient (D) [18].

$$\frac{d[C]}{dt} = \frac{D \cdot S}{V \cdot h}(C_s - C) \qquad \text{Equation 1.}$$

Based on this equation, there are several possibilities for increasing the solubility and dissolution rate:

- increasing the surface area;
- decreasing the thickness of diffusion layer;
- better wettability;
- increasing the apparent solubility by suitable physiological conditions.

There are several pharmaceutical technological methods to enhance the solubility and the dissolution rate, thereby the BA (Table 1).

Table 1 Possibilities for the solubility enhancement

Particle size reduction	**Dispersion in a carrier**	**Amorphisation**	**Molecular complexation**	**Other methods**
• micronization • nanotechnology • interactive mixtures • SCF technology	• solid solutions • solid dispersions • eutectic mixtures	• with excipients • without excipients	• CDs • terner systems (organic acids, macromolecules)	• polymorphism • crystal habit • salt formation • cosolvents • oily solutions • emulsions, microemulsions

2.1.1. Particle size reduction

The reduction of the particle size results in the rise of specific surface area, which effects favourably on the dissolution rate.

Micronization is a term used to describe size reduction where the resulting particle size distribution is less than 10 μm. Micronization involves acceleration of particles so that grinding occurs by particle–to–particle impact or impact against a solid surface [19]. N. Resenack *et al.* enhanced markedly the dissolution rate of ibuprofen, itraconazole and ketoconazole by *in situ* micronization method [20].

Nanosuspensions are sub–micron colloidal dispersion of pure particles of drug, which are stabilized by surfactants. Nanosuspensions can be formed by bottom up techniques (building particles as in precipitation) or by top down methods (desintegration as in milling). In both case new surface area is formed. Therefore it has more free energy and system tends to agglomerate which is prevented by addition of surfactants. Surfactants cause high energy barrier and prevents particles coming together [21]. P. Kocbek *et al.* improved the dissolution rate of ibuprofen by formulation of nanosuspension. The dissolution rate proved to be higher for smaller drug particles, thus influencing the BA of the drug and improving safety for the patient by decreasing gastric irritancy [22].

If a free–flowing powder is mixed with a cohesive powder an interactive mixture can be obtained. The cohesive particles adhere to the free–flowing particles (now called carrier particles) to form interactive units [23]. An interactive mixture may not contain free aggregates of the cohesive powder, which means that all small particles must be adhered to the larger ones. The difference from an ordered mixture is instead that all carrier particles do not need to be of the same size and a different number of small particles attached to each one. A narrow size range of the carrier particles is preferred to avoid segregation of the interactive units [24]. Interactive mixtures can be used enhancing the dissolution of poorly soluble drugs. [25] B. Alway *et al.* improved the dissolution of diazepam in lactose interactive mixtures [26].

The SCF technology has many advantages. A pure component enters the supercritical status when both temperature and pressure are above its critical p and T values. In this region, the SCF exists in an intermediate phase between liquid and gas phases. The SCF is dense but highly compressible, particularly near the supercritical region. Thus, any change of pressure alters its density and consequently the solvent power [27]. The compound is dissolved in an

SCF solvent (usually CO_2), then the solution gets into a special nozzle, where the pressure is rapidly decreased. So the solution becomes supersaturated, which resulted in precipitation of small particles [28]. V. Majerik *et al.* enhanced the solubility and dissolution properties of oxeglitazar by SAS technology. SAS is a type of SCF technology, where the fluid is used not for solvent, but antisolvent [29].

2.1.2. Dispersion in a carrier

Many hydrophilic carrier can be applied for this purpose e.g. polyethylene glycol, polyvinylpyrrolidone (PVP), polyvinylalcohol (PVA), crospovidone, PVP–PVA copolymer, cellulose derivatives, polyacrylates and polymetacrylates, urea, sugar, polyols and their polymers, emulsifiers, organic acids and their derivatives [5].

A simple eutectic mixture consists of two compounds which are completely miscible in the liquid state but only to a very limited extent in the solid state. When a mixture of A and B with composition of C is cooled, A and B crystallize out simultaneously; whereas when other compositions are cooled, one of the components starts to crystallize out before the other. When a mixture with composition C, consisting of a poorly soluble drug and an inert, highly water soluble carrier, is dissolved in an aqueous medium, the carrier will dissolve rapidly, releasing very fine crystals of the drug. The large surface area of the resulting suspension should result in an enhanced dissolution rate and thereby improved BA.

Solid solutions are comparable to liquid solutions, consisting of just one phase irrespective of the number of components. In the case of solid solutions, the drug's particle size has been reduced to its absolute minimum viz. the molecular dimensions and the dissolution rate are determined by the dissolution rate of the carrier [5].

Solid dispersion differs from the solid solution in that the particle size is greater, non–molecular.

S.G. Kapsi *et al.* prepared solid solutions of itraconazole with different carriers and they observed better BA compared to marketed product [30]. C.S. Yong *et al.* improved the solubility of ibuprofen with menthol due to formation of an eutectic mixture. So ibuprofen will be in molecular distribution during the preparation of the suppository, therefore a solution–format of suppository can be prepared, a faster therapeutic effect can be achieved [31]. R. Ambrus *et al.* improved the solubility and permeability of poorly water–soluble niflumic acid by application of PVP [32].

Polyvinylpyrrolidone

PVP (Fig. 2) is an amorphous carrier and it is suitable for formulating solid solutions, glass solutions and amorphous precipitations with a crystalline API. The glass transition temperature (T_g) is high and for this reason PVPs have only limited applications for the preparation of solid dispersions by the hot-melt method. Due to their good solubility in a wide range of organic solvents, they are particularly suitable for the preparation of solid dispersions by the solvent method [33]. The aqueous solubility of the PVPs becomes poorer with increasing chain length, and a further disadvantage of the high M_w PVPs is their much higher viscosity at a given concentration, however they can be used for controlled release. [34]. The aim of the preparation of PVP solid dispersions is generally to transform the drug into the amorphous form and thus to achieve faster dissolution. Dispersions containing crystalline areas exhibit biphasic release profiles, with the amorphous areas dissolving quickly and the crystalline areas more slowly. For example, for a crystalline drug with a very poor aqueous solubility, the formation of an amorphous mixture with a water-soluble additive can provide an opportunity to enhance the dissolution and perhaps bioavailability [35].

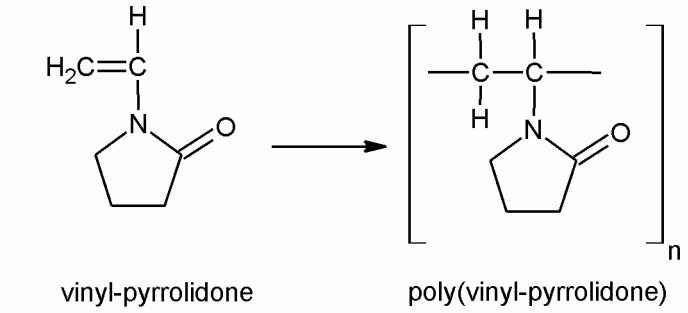

Fig. 2 Structural formula of PVP

2.1.3. Amorphization

The amorphous state is lacking distinct crystalline structure or organization. A rigid material, whose structure lacks crystalline periodicity. The amorphization process can be processed by three ways: solvent method, hot–melt technology and milling technology. By the solvent method the API is dissolved in a suitable solvent then the solvent is evaporated rapidly. In case of hot–melt technology the API is melt, and then it is suddenly cooled or the

excipient is melt and in this melt the API can be dissolved and then it is cooled down. The theoretical base of the milling technology is that the cohesion in the crystal can be disrupted by appropriate external forces, and the crystalline state is left off. Since the amorphous state has higher energy than the crystalline form, the amorphous materials due to thermodynamic principles are going to recrystallize beyond a certain date. To stabilize the amorphous form, we can use crystallization–inhibitors (PVP, sugars, sugar–alcohols etc.) [36]. The amorphous state is a good technique to improve solubility of poorly soluble drugs [37].

2.1.4. Complexation

A complex implied a reversible association of molecules, atoms or ions through weak chemical bounds. From viewpoint of drug formulation complexation can be sorted as follows [17]:

- metal complexes

  Also known as coordination compounds. The cohesion is valence force or intermolecular force. Such compounds are e.g. citric acid, tartaric acid or EDTA (ethylenediamine-tetraacetate) which complexes the decomposition catalysing hard metals.
- molecular complexes

  The bond is given by hydrogen–bonding or dipole–dipole interaction. Such complexes are e.g. caffeine–sodium benzoate or sodium–salicylate.
- inclusion complexes

  The cohesion is intermolecular forces. The most frequently applied complex forming agents are cyclodextrins and latterly the calixarenes. A calixarene is a macrocycle or cyclic oligomer based on a hydroxyalkylation product of a phenol and an aldehyde. The word calixarene is derived from calix or chalice because this type of molecule resembles a vase and from the word arene that refers to the aromatic building block. Calixarenes have hydrophobic cavities that can hold smaller molecules or ions [38].

2.1.5. Changing the crystal habit

Crystals can be rated into needle–like, isometric, tabular, lamellar and columnar morphological classes based on their habit. The different crystal habit can cause substantial differences in the dissolution rate [39]. The different crystal habits are chemically the same, but they differ in many physicochemical properties e.g. melting point, density, flow properties, solubility properties. R. Adhiyaman *et al.* got the conclusion that because the *in vitro* dissolution data are good predictor of in vivo performance therefore it can safely concluded that the improved dissolution rate of the modified crystals will give better BA and better therapeutic activity clinically [40].

2.1.6. Polymorphism, pseudopolymorphism

Polymorphism is the ability of a solid material to exist in more than one form or crystal structure by the same chemical structure. The different crystal forms of the same compound have different free energy, therefore their thermodynamic activity is variable. Polymorphs can differ in melting point, melting enthalpy, solubility, dissolution rate, bioavailability, permeability, chemical reactivity, habit, stability, density, refraction index, electrical conductivity, heat of solution and from other utilization and processing aspect important properties [39, 41, 42]. These different physical properties may affect the therapeutic efficacy, toxicity, BA, pharmaceutical processing and stability of the drug product [43]. The relative stability of polymorphs depends on their free energies, such that a more stable polymorph has a lower free energy. Under a defined set of experimental conditions only one polymorph has the lowest free energy. This polymorph is the thermodynamically stable form and the other polymorph is termed a metastable form. The metastable form is sometimes desirable on account of its special properties, such as higher BA, better behaviour during grinding and compression, or lower hygroscopicity. However a metastable form has a thermodynamic tendency to reduce its free energy by transforming into stable form [44].

Pseudo–polymorphism is a kind of polymorphism that is characterized by solvate compounds, including water solvates (hydrates) [42].

2.1.7. Prodrugs

A prodrug is a bioreversible chemical derivative of an active parent drug. Chemical modification of the poorly water–soluble parent drug can result in a water–soluble prodrug.

The synthesis of N–alkoxycarbonyl prodrugs of anthelminthic mebendazole has been shown to increase its aqueous solubility by two orders of magnitude. Amino acid ester prodrugs have been synthesized both to improve intestinal membrane permeability and increase aqueous solubility of acyclovir. The poorly water–soluble etoposid has shown some evidence for improved oral BA in preliminary studies utilizing phosphate ester prodrug. The addition of the phosphate ester functionality on phenytoin resulted in a prodrug stable in aqueous solution with over a 7000–fold increase in aqueous solubility [45–49].

2.1.8. Salt formation

Salt formation is the most common and effective method of increasing solubility and dissolution rates of acidic and basic drugs [50]. The process is a simple way to modify the properties of a drug with ionizable functional groups to overcome undesirable features of the parent drug. Salts are formed when a compound that is ionized in solution forms a strong ionic interaction with an oppositely charged counterion, leading to crystallization of the salt form [51].

H.S. Gwak *et al.* improved the dissolution rate of a BCS II drug, piroxicam by salt formation with mono–, di– and triethanolamin. In the three salts the highest dissolution rate belonged to the monoethanolamin–salt at pH = 6.8, however there was no difference at pH = 1.2 [52].

2.2. Permeability

The absorption of an orally administered drug involves the extent and rate of transfer of the drug from the GI–tract to the portal blood supply. Absorption is a complex process that is influenced not only by physicochemical properties (e.g. solubility, charge, lipophilicity, permeability, etc.) of the drug but also by physiological GI properties (e.g. intestinal transit times, active transporters, gut wall metabolism etc.) and formulation differences. Because a

significant number of small molecules that are synthesized in drug discovery laboratories are transported across the GI epithelial barrier by a passive transcellular mechanism, the solubility and permeability of the drug are key surrogate absorption markers [53].

There are several methods to model the absorption and to screen the permeability. The Fig. 3 shows these possibilities in the order of predictability, throughput and cost–effectiveness. Recently there are many computational programs (*in silico*) which have a great database and can predict some parameters such as pK_a, logP, logD, solubility etc. from the formula of a molecule. Unfortunately the reliability of the obtained data is very limited. The measured physico–chemical parameters predict primarily the passive transport. The PAMPA is one of the most often applied methods in the early stage of drug discovery because it is easy to carry out, about 100 compounds can be studied in the same time and requires small amount of samples. Its primal disadvantage is that it can predict only the passive transport. If the active transport plays also a role, the suitable method is the Caco–2 model, but it is costly and slow. The in vivo animal tests approach considerably well the human absorption, but there are minor differences between the absorption parameters. Certainly the most effective and the most expensive way is the human assay [54].

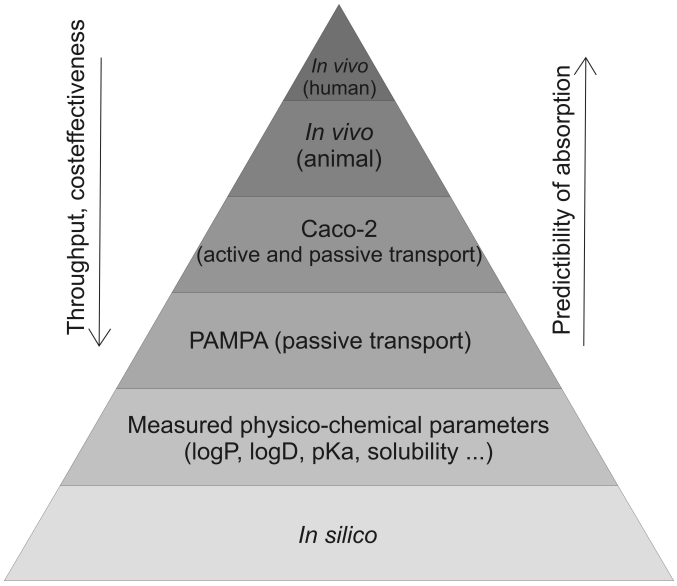

Fig. 3 Measuring and predicting possibilities of absorption

3. Cyclodextrins

CDs were discovered approximately 100 years ago. They are cyclic, non–reducing oligosaccharides built up from six, seven or eight glucopyranose units [55]. CDs are obtained by starch bioconversion using cyclodextrin glucosyltransferase. This enzyme is produced by a variety of bacteria, mainly by several species of Bacillus, Klebsiella or Thermoanaerobacter. Different types of starch can be used as substrate, but potato starch is the most commonly used for CD production. Maize starch and wheat starch are also used but they contain a higher percentage of amylase, which gives lower yields of CD than amylopectin [56].

Owing to their apolar cavity, they are able to form complexes with molecules, or part of them, changing the physicochemical properties, such as solubility and stability of the guest compounds [55]. Guest molecules can be solid, liquid or even gaseous.

CDs are capable of forming inclusion complexes with compounds having a size compatible with the dimensions of the cavity. The extent of the complex formation depends, however, also on the polarity of the guest molecule.

The characteristic feature of the host molecule is that the binding sites are oriented to the same spatial direction, whereas in the guest molecule the binding sites are diverging in the complex.

CD monographs can be found in several Pharmacopoeias [57–59].

Table 2 CDs in Pharmacopoeias

General name	Name in the Pharmacopoeia	In which part	Pharmacopoeia
α-CD	Alfadex	In Monographs	Ph. Eur., USP 34 – NF 29, BP 2012
	α -Cyclodextrin	In Reagents	Ph. Eur., USP 34 – NF 29, BP 2012
β-CD	Betadex	In Monographs	Ph. Eur., USP 34 – NF 29, BP 2012
	β-cyclodextrin	In Reagents	Ph. Eur., BP 2012
γ-CD	Gamma Cyclodextrin	In Monographs	USP 34 – NF 29
HPBCD	Hydroxypropylbetadex	In Monographs	Ph. Eur., USP 34 – NF 29, BP 2012
	Hydroxypropyl-betacyclodextrin	In Reagents	Ph. Eur., USP 34 – NF 29, BP 2012
DIMEB	Dimethyl-beta-cyclodextrin	In Reagents	Ph. Eur., USP 34 – NF 29, BP 2012

3.1. Derivatives

It is well known that derivatisation changes the physical and chemical properties of the CDs, modifying their solubility, complex–forming capacity, thermal properties and chemical stability. Most common substituents used in CD derivatives: methylated, acetylated, carboxymethylated, hydroxypropylated, naphtylethyl carbamate, 3,5–dimethylphenyl carbamate, p–toluoyl, α–methylbenzamine, naphthylethylamine, pyridylethylene diamine, nitropyridylethylene diamine, phenyl carbamate, cationic β–CDs [60].

More than 1500 different CD derivatives have been described in the literature. One can distinguish hydrophilic, lipophilic and ionizable derivatives. Chemical substitution elongates the CD torus in an axial direction by introducing larger substituents. Modification of the 2– or 3–hydroxyl group results in disruption of the hydrogen bonding occurring around the ring of the CD molecule. The disruption allows more interactions of these hydroxyl groups with water molecules, resulting in altered solubility [61].

From the viewpoint of the optimization of pharmacotherapy, drug release should be controlled in accordance with the therapeutic purpose and the pharmacological properties of active substances. Various CD derivatives have been used in order to modify drug release in oral preparations. The hydrophilic and hydrophobic CDs are useful for the immediate release and the prolonged release type formulations, respectively. Recently, highly hydrophilic CD derivatives, such as HPBCD, 6–O–maltosyl–β–CD and sulfobutyl–β–CD have been used to obtain an immediate release formulation, which is readily dissolved in the GI tracts, promising an enhancement of oral BA of poorly water–soluble drugs. Hydrophobic CDs having a weak acidic group (e.g. 6–O–(carboxymethyl)–O–ethyl–β–CD) are less soluble in water at low pH but soluble in neutral and alkaline regions. Under the control of this pH–dependence, the delayed release dosage form which passes from the stomach into the higher pH environment would experience increased drug release. Most of the slow–release preparations have been aimed to provide a constant blood level for a long period of time. For this purpose, hydrophobic CDs such as alkylated and acylated derivatives are useful as slow–release carriers for water–soluble drugs. When the stomach is one of the important absorption sites, there should be no lag time in drug release from the dosage form. To attain an efficient BA for such acidic drugs, a double–layer tableting was useful, consisting of hydrophilic CDs as the fast–release portion and hydrophobic cellulose derivatives as the slow–release portion [62].

3.2. Physico–chemical properties of natural CDs and CD derivatives

The natural CDs consist of 6, 7 or 8 D–glucopyranose units – by α–1,4–glycoside linkage – and are referred to as α–, β– and γ–CD, respectively. Because of the $^4C_1$ chair conformation of each glucopyranose units, the whole molecule has the shape of a hollow truncated cone. The interior of the cavity is composed of hydrogen atoms of C_3, C_5 and oxygen atoms of the glycoside linkage, which makes the intracavity hydrophobic while the exterior of the cavity is hydrophilic due to assembling large numbers of alcoholic hydroxyl groups [63]. As results CDs can form host–guest inclusion complexes by weak intermolecular interaction.

Unsubstituted, native CDs are crystalline, non–hygroscopic, homogenous substances.

Table 3 Physical and chemical characteristics of CDs [60]

	α–CD	β–CD	γ–CD	DIMEB
Glucose units	6	7	8	7
Internal diameter (nm)	0.47 – 0.53	0.60 – 0.65	0.75 – 0.83	6 A
Depth of cavity (nm)	0.79	0.79	0.79	>0.79
pK_a value	12.3	12.2	12.1	*
M_w	972	1135	1297	1331
water solubility (mg/ml 25 ˚C)	145	18.5	232	Op.: 295-300
Crystal water (%, w/w)	10.2	13.2 – 14.5	8.13 – 17.7	*

* no data available

The C_2 hydroxyl group of one glucopyranose unit can form a hydrogen bond with the C_3 hydroxyl group of the neighbouring glucopyranose unit. In the β–CD intermolecular hydrogen bonding occurs between the secondary hydroxyl groups which reduces the number of hydroxyl groups capable of forming hydrogen bonds with the surrounding water molecules, so it can explain the observation that β–CD has the lowest solubility of all native CDs [60, 64].

Substituted β–CD derivatives are characterized by much higher solubility. Random substitution of the hydroxyl groups, even by hydrophobic moieties such as methoxy functions, will result in dramatic improvements in their solubility. The main reason for the solubility

enhancement is that the random substitution transforms the crystalline CDs into amorphous mixtures of isomeric derivatives [7]. DIMEB is prepared from β–CD by the selective methylation of all C_2 secondary hydroxyl groups and all C_6 primary hydroxyl groups (C_3 secondary hydroxyl groups remain unsubstituted) [65]. DIMEB is soluble in organic solvents, and very soluble in cold water – because it is strongly hydrated in the sense of Pauling's clathrate model of liquid water – 20–25% solutions of increased viscosity can readily be prepared. An uncommon property of DIMEB is that the homogenous and clear solution will suddenly crystallize on heating (above 50 ˚C). The feasible explanation of the negative solubility coefficient of methylated CDs in water that the hydration structure breaks down at elevated temperature. Hence, the hydrophobic DIMEB molecules become less soluble and aggregate, as could be expected from the hydrophobic effect, leading finally to crystallization In contrast to the native CDs, DIMEB has surfactant activity [55, 66].

The thermoanalytical measurements indicated that CDs have no well–defined melting point, but from about 250 to 400 ˚C they begin to decompose. It has been frequently shown that the thermal stability of CD derivatives depends on the type, location and number of any substituents. As previously mentioned CDs are not hygroscopic, but form various stable hydrates [60].

CDs are insoluble in most organic solvents; but they are soluble in some polar, aprotic solvents. CDs glass transition occurs at about 225 to 250 ˚C. The glass transition temperature varies with the degree of substitution. Strong acids such as hydrochloric acid and sulphuric acid hydrolyze CDs. CDs are very stable against bases [61].

In forming inclusion complexes, the physical and chemical properties of both the drug molecule and the CD molecule can be altered. Co–solvents will increase the solubility of a poorly water soluble drug in a nonlinear fashion with respect to co–solvent concentration. The linear relationship between solubility and CD concentration has a number of advantages, one of which is the lack of precipitation of the formulation on dilution in contrast to the co–solvent method. It is important to realize that the kinetics of inclusion complex formation and dissociation between a CD and a drug molecule is fast. The half–lives for formation/dissociation are much less than one second and occur at rates very close to diffusion controlled limits [67].

3.3. Complexation mechanisms

Complexation of molecules to CDs occurs through a non–covalent interaction between the molecule and the CD cavity. This is a dynamic process whereby the guest molecule continuously associates and dissociates from the host CD. CD inclusion is a stoichiometric molecular phenomenon in which usually only one molecule interacts with the cavity of the CD molecule to become entrapped. Inclusion complex formation can be regarded as "encapsulation" of the drug molecule, or at least the labile part of the molecule. Many techniques are used to form CD complexes, like co–precipitation, slurry complexation, paste complexation, damp mixing, heating method, extrusion and dry mixing [61].

The driving forces for inclusion complexation are both enthalpic and enthropic in nature and not fully understood [67]. The main driving force for complex formation, at least in the case of β–CD and its derivatives, appears to be release of the enthalpy–rich water molecules from the CD cavity which lowers the energy of the system. These water molecules located inside the central cavity are replaced by either the whole drug molecule, or more frequently, by some lipophilic structure of the molecule. However, other forces, such as van der Waals interactions, hydrogen bonding, hydrophobic interactions, release of structural strains and changes in surface tension, may also be involved in the complex formation. No covalent bonds are involved in the complex formation and drug molecules located in the cavity are in a very dynamic equilibrium with free drug molecules out in the solution. In aqueous solutions drug/CD complexes are constantly being formed and broken at rates very close to the diffusion controlled limit [64].

3.4. Cyclodextrins and drug delivery

In general, formulation techniques that increase the apparent aqueous solubility of Class II and Class IV drugs without decreasing their lipophilicity will enhance their absorption through biological membranes. These techniques include particle size reduction, salt formation, solid dispersion, melt extrusion, spray–drying, and complexation, as well as drug solutions in microemulsions, liposomes and non–aqueous solvents. The chemical structures of CDs, their molecular weight and their very low octanol/water partition coefficient are all characteristics of compounds that do not readily permeate biological membranes. Only the free form of the drug, which is in equilibrium with the drug/CD

complex, is capable of penetrating lipophilic membranes. The physicochemical properties of the drug, the composition of the drug formulation and physiological composition of the membrane barrier will determine whether CDs will enhance or hamper drug delivery through biological membranes. Most biological membrane barriers are lipophilic with an aqueous exterior, which forms a structured water layer at the membrane surface frequently referred to as unstirred diffusion layer. If drug permeation through the aqueous diffusion layer is the rate–limiting step of drug permeation through the barrier, CDs can frequently enhance the permeation [7].

CD lowers permeability and raises solubility, but the two effects are most often not equal in magnitude [68]. CDs can enhance the aqueous solubility of lipophilic drugs without changing their intrinsic ability to permeate biological membranes. Thus, through CD complexation it is possible to move BCS Class II drugs, and sometimes even Class IV drugs, into Class I. However, CDs can decrease BA of Class I drugs and will in most cases not improve BA of Class III drugs. In general CDs enhance drug delivery through biomembranes by increasing the drug availability at the membrane surface. At the surface the drug molecules partition from the CD cavity into the lipophilic membrane. Thus, properly designed CD formulation will increase the drug concentration gradient over the membrane, which will increase the drug flux through the membrane. Since drug/CD complexes do not readily permeate biomembranes, excess CD in pharmaceutical formulations can reduce drug BA. Including CDs in pharmaceutical formulations will however increase the formulation bulk of solid dosage forms. Even under best conditions, CD complexation will result in 4 – 10–fold increase in the formulation bulk. This limits the use of CDs in solid oral dosage forms to potent drugs that possess good complexing properties. [69].

3.4. Application of CDs in pharmaceutics

In Fig. 4 there are introduced the diversified availabilities of CDs with some examples.

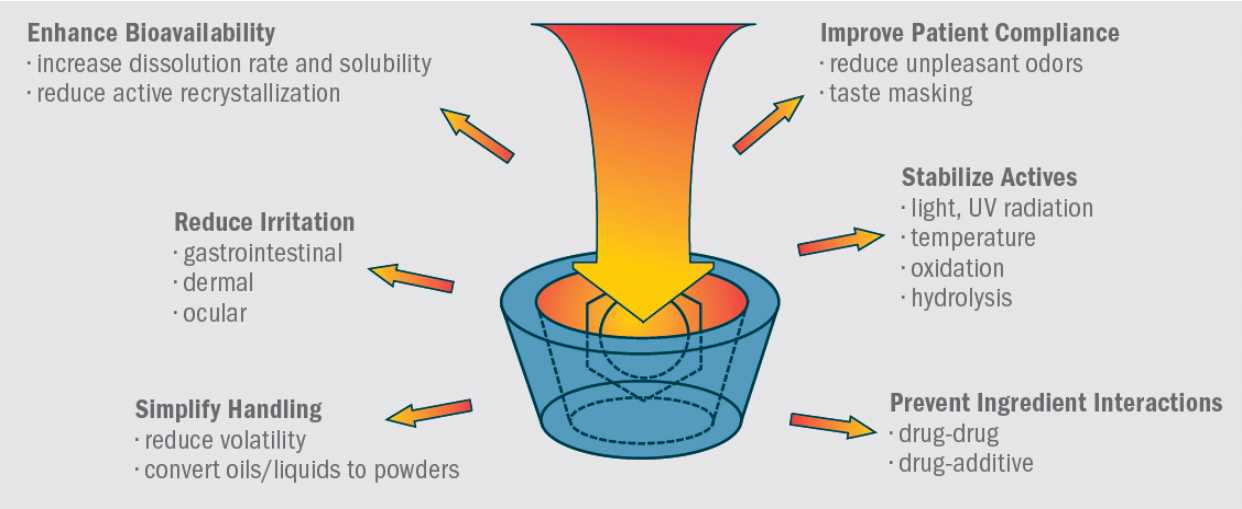

Fig. 4 Application fields of CDs [70]

CDs applications in drug delivery are the followings: oral, parenteral, ocular, nasal, rectal, controlled, colon–specific, peptide and protein, gene and oligonucleotide, dermal and transdermal, brain targeting, liposomes, microspheres, microcapsules, nanoparticles [71]. Worldwide about 40 different pharmaceutical products containing CDs are on the market, which are summarized in Table 4.

If a poly–α–amino acid chain is combined with β–CD, a new amphiphilic completely biodegradable polymer can be afforded. Both biodegradability and amphiphilicity are extremely significant properties for some applications of biomedical polymers. The amphiphilic biodegradable polymers may be used as controlled release of drugs [72].

HPBCD and Captisol are typically used in formulation for preclinical studies [73]. A.G. Ellis *et al.* used Captisol for making an aqueous formulation with appropriately high concentration for the assessment of tissue distribution and pharmacokinetic studies of the active ingredient [74].

C.E. de Matos Jensen *et al.* showed that β-CD improved the solubility and BA of valsartan, because the complex reduced the arterial blood pressure much better than valsartan alone in *in vivo* rat experiments [75]. Besides hundreds of article can be found in the literature in that solubility was improved by CDs.

Table 4 CD containing pharmaceutical products [76, 77]

Drug	Trade name	Formulation	Company
	α–CD		
Alprostadil (PGE_1)	**Prostavasin** Caverject Dual Edex	**iv. solution and infusion**	**Ono, Schwarz Pfizer**
Limaprost (OP-1206)	Opalmon, Prorenal	tablet	Ono
Cefotiam–hexetil HCl	Pansporin T	tablet	Takeda
	β–CD		
Benexate HCl	Ulgut Lonmiel	capsule	Teikoku Shionogi
Cephalosporin	Meiact	tablet	Meiji Seika
Cetirizine	Cetrizin	chewing tablet	Losan Pharma
Chlordiazepoxide	Transillium	tablet	Gador
Dexamethasone	Glymesason	ointment, tablet	Fujinaga
Dextromethorphan	Rynathisol	tablet	Synthelabo
Diphenhydramine HCl, chlortheophylline	Stada–Travel	chewing tablet	Stada
Iodine	Mena–Gargle	solution	Kyushin
Meloxicam	Mobitil	tablet and suppository	Medical Union Pharmaceuticals
Nicotine	Nicorette	sublingual tablet	Pfizer
Nimesulide	Nimedex	tablet	Novartis
Nitroglycerin	Nitropen	sublingual tablet	Nihon Kayaku
Omeprazole	Omebeta	tablet	Betafarm
PGE_2	Prostarmon E	sublingual tablet	Ono
Piroxicam	Brexin Flogene Cicladol	tablet, suppository, liquid	Chiesi Aché
Tiaprofenic acid	Surgamyl	tablet	Roussel-Maestrelli
	2–hydroxy–propyl–β–CD (HP–β–CD)		
Cisapride	Propulsid	suppository	Janssen
Hydrocortisone	Dexocort	solution	Actavis
Indomethacin	Indocid	eye drop solution	Chauvin
Itraconazole	Sporanox	oral and iv. solutions	Janssen
Mitomycin	Mitozytrex, MitoExtra	iv. infusion	Novartis
	Random methylated β–CD (RMBCD)		
Chloramphenicol	Clorocil	eye drop solution	Oftalder
17–β–estradiol	Aerodiol	nasal spray	Servier
	Sulphobuthylether–β–CD (Captisol)		
Aripiprazole	**Abilify**	**im. solution**	**Bristol Myers Squibb, Otsuka Pharm.**
Maropitant	**Cerenia**	**sc. solution**	**Pfizer Animal Health**
Voriconazole	**Vfend**	**iv. solution**	**Pfizer**
Ziprasidone mesylate	**Zeldox** Geodon	**im. solution**	**Pfizer**
	2–hydroxy–propyl–γ–CD (HP–γ–CD)		
Diclofenac Na	**Voltaren ophtha**	**eye drop solution**	**Novartis**
Tc–99 Teoboroxime	CardioTec	iv. solution	Bracco

The products with bold Roman type are marketed in Hungary, too.

3.5. Application of CDs beyond pharmaceutics

β–CD is used in food industry for removing cholesterol from cream [78], reduction of undesired taste, extension of food products shelf life [79]. Y.Q. Tian *et al.* demonstrated that β–CD has significant impact on the staling of crust and cumb. The retarding effect of β–CD was strongly supported by the less changes of hardness, cohesiveness and springiness [80].

CDs play role in enzyme mimicking, chiral chromatographic separations and a new applicability is that β–CD is a template in a biomimetic synthesis of spherical hydroxyapatite crystals [81].

CD complexation represents a unique and effective strategy for improving the protein therapy by stabilizing them against aggregation, thermal denaturation and degradation [82].

CDs improve the steroids biotransformation. M. Wang *et al.* represented that HP–β–CD enhances the 1–en–dehydrogenation of cortisone–acetate catalyzed by a microorganism. It not only increases the reaction rate but also improves the final substrate conversion rate [83].

It was possible to form inclusion complexes between the CDs and metal ions like Pb^{2+}. The β–CD and its derivatives have been used for the removal of polluting species from wastewater. The use of water–insoluble β–CD immobilized on polymeric matrices or solid supports has led to the development of novel decontaminating agents [84].

The self–assembles monolayers of thiolated CD derivatives has constructed on the surface of Au electrodes, producing a modified electrode selective towards electrochemical reactions characterized by cyclic voltammetry. This modified electrode is an environmentally friendly alternative to substitute the hanging mercury drop electrode [84].

The separation of structural isomers can be achieved by using β–CD thanks to inclusion complex formation which depends on size and polarity of the host molecule and its shape. β–CD has been used as a mobile phase component in reversed–phase HPLC and also in stationary phases, both in liquid and gas chromatography [85].

Some researchers have shown the efficiency of CDs for PAH (polycyclic aromatic hydrocarbons) removal from soils by aqueous washing [86, 87].

4. Loratadine (LOR)

Chemical name: ethyl–4–(8–chloro–5,6–dihydro–11H–benzo[5,6]cyclohepta[1,2–b]pyridin–11–ylidene)–1–piperidinecarboxylate

Chemical structure (see Fig. 5):

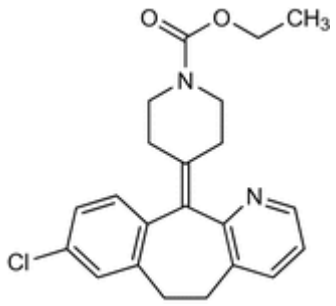

Fig. 5 Chemical structure of LOR

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

M_w: 382.89

Melting point: 132 – 135 ˚C

Description: white or off–white crystals or powder [88]

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 [89].

LOR belongs to Class II of the BCS [90]. LOR is a weak base; its pK_a value at 25 °C has been reported as 4.85 – 6.00 [89–92]. The solubility of bases increases with decreasing pH at pH values less than the pK_a [9]. At lower pH values, LOR – which is a nitrogen base – is protonated, and therefore becomes more soluble in water [91]. However according to the modified Hendersson–Hasselbach equation [93], 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.

It is metabolized by the cytochrom P450 to an active metabolite, desLOR, which is 153 times more potent than LOR. The presumed reason for this notable affinity difference is that LOR's amine group cannot interact with the carboxylate part of the receptor (Asp 107) since it is not protonated in physiological pH. DesLOR does not have the electrophilic ethyl–ester group which reduces the basicity of the molecule, so desLOR has pK_a value at 8.65, the amine group can be protonated at physiological pH [94]. It has a very big affinity to the plasma proteins (~98%). The defined daily dose of LOR is 10 mg [95]. LOR is a substrate for the P-gp. P-gp is an ATP–dependent efflux transporter that affects the absorption, distribution and excretion of compounds [96]. Increased intestinal expression of P-gp can reduce the absorption of drugs that are substrates for P-gp. Thus, there is a reduced BA, therapeutic plasma concentrations are not attained [97].

LOR is official in the undermentioned pharmacopoeias: British Pharmacopoeia 2012 [57], The United States Pharmacopoeia 34 – National Formulary 29 [58], European Pharmacopoeia 7.4 [59].

5. Materials and methods

5.1. Materials

α–CD, β–CD, γ–CD, randomly methylated–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) were purchased from Cyclolab Ltd. (Budapest, Hungary), β–CD–sulfobutyl–ether (Captisol) originated from CyDex Pharmaceuticals Inc. (Lenexa, USA). LOR (ethyl 4–(8–chloro–5,6–dihydro–11H–benzo–[5,6]–cyclohepta[1,2–b]pyridin–11–ylidine)–1–piperidine–carbo–xylate) was kindly provided by TEVA Pharmaceutical Industries Ltd. (Hungary). Compound 48/80 (N-methyl-4-methoxy-phenethylamine) was supplied by Sigma-Aldrich Logistic GmbH, Germany. Other chemicals were of analytical reagent grade purity.

5.2. Methods

5.2.1. Preliminary experiments

The effects of the various CD derivatives on the solubility of LOR were investigated at 25 ºC. 20 mg of LOR and 200 mg of the CD derivatives were suspended in 20 mL of distilled water. The mixture was stirred at 10 min with a magnetic stirrer and then filtered, and after suitable dilution the UV spectrum was recorded in the range 220–300 nm.

5.2.2. Phase solubility studies

The phase–solubility diagrams were recorded by the Higuchi–Connors method [8]. For this purpose aqueous solutions of CDs of various concentrations were prepared at a specific pH value (7.5) (250 mL of 0.2M KH_2PO_4, 204 mL of 0.1 M NaOH made up to 1000 mL with distilled water). 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 (λ = 248 nm). The presence of the CD did not disturb the spectrophotometric assay. Each experiment was performed in triplicate.

5.2.3. Preparation of products

DIMEB proved to demonstrate the best enhancement of the solubility (see section 6.1). The products were prepared in three molar ratios (LOR:DIMEB = 1:1, 1:2 and 1:3) by four methods: physical mixing, kneading (two different methods for removing the solvent) and spray–drying. ***Physical mixtures (PMs):*** LOR was mixed carefully in a mortar with the calculated amount of CD. ***Kneaded products (KPs):*** 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 in a MW oven (Milestone Ethos TC MW apparatus, Advanced Microwave Labstation, Italy). The method of MW treatment was as follows: 150 W, 90 s, 60 °C, and the samples were then dried under vacuum [98]. ***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 (BÜCHI Labortechnik AG, Flawil, Switzerland), at an inlet

temperature of 105 ^{o}C, with a compressed air flow of 800 L/h and a nozzle diameter of 0.5 mm. The aspirator rate was 75–80%, and the pump rate was 5–10%. All of the samples were sieved (100 μm) and stored at room temperature under normal conditions.

The **solid dispersions** of LOR in PVP K-25 containing four different ratios (1:1, 1:2, 1:4 and 1:6 w/w) were 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.

5.2.4. In vitro dissolution studies

The modified paddle method with the USP dissolution apparatus (Erweka Type DT, Germany) was used to examine 200 mg samples of pure LOR or products containing 200 mg of LOR in 100 mL of simulated gastric medium (SGM) (pH = 1.1 ± 0.1; 94.00 g of 1 M HCl, 0.35 g of NaCl, and 0.50 g of glycine made up to 1000 mL with distilled water) or simulated intestinal medium (SIM) (pH= 7.0 ± 0.1; 14.4 g of $Na_2HPO_4 \cdot 2H_2O$ and 7.1 g of KH_2PO_4 made up to 1000 mL with distilled water). 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. At each sampling time, an equal volume of fresh media was added, and the correction for the cumulative dilution was calculated. After filtration and dilution, the LOR contents of the samples were determined spectrophotometrically (λ_{SGM} = 276 nm, λ_{SIM} = 248 nm).

5.2.5. Study the effect of pH on the solubility

Seven buffer solutions were prepared with different pH values between 1.2 and 7.5 [11]. 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.

5.2.6. Thermoanalytical measurements

5.2.6.1. DSC

The DSC records were obtained with a Mettler Toledo DSC 821^{e} (Mettler Inc., Schwerzenbach, Switzerland) apparatus. Between 2 and 5 mg of sample was measured in a standard aluminium pan (40 μL) and heated from 25 to 300 °C at a heating rate of 5 °C/min under a constant purge of argon at 10 L/h.

5.2.6.2. Thermogravimetric measurements

The TG, DTG and DTA curves were recorded in parallel in platinum crucibles with the same thermal program (heating range 25–300 °C, heating rate 5 °C/min), using a MOM Derivatograph–C (MOM Co., Hungary). The reference was a crucible containing aluminium oxide.

5.2.7. FT–IR

Samples with a LOR content of 0.5 mg were ground and mixed with 150 mg of dry KBr in an agate mortar, and the mixture was then compressed into a disc at 10 t. Each disc was scanned 64 times at a resolution of 4 cm^{-1} over the wave number region 4000–400 cm^{-1} with an FT–IR spectrometer (Thermo Nicolet AVATAR 330, USA). The evaluation was carried out with the GRAMS/AI Ver. 7 program.

5.2.8. ESI–MS

The compounds were characterized by MS, using a Finnigan MAT 95S sector field mass spectrometer equipped with an electrospray ion source. Positive-ion ESI-MS spectra were obtained. The solutions were prepared in a 1:1 mixture of acetonitrile/water. The solutions were infused directly into the mass spectrometer at a rate of 200 μL/min. Data were collected for approximately 100 scans. The scan range was 100-3000 m/z. The spectrometer was used at a resolution of ~ 1000–1500.

5.2.9. DOSY

Diffusion coefficients (D) were estimated via DOSY NMR experiments. In the DOSY spectra, chemical shifts were located along the F2 axis and D values along the F1 axis. From

the D values, it is possible to infer the size of the species and therefore the absolute stoichiometry of the supramolecular complexes.

The NMR spectra were recorded at 25 ºC on a Bruker Avance DRX 400 MHz spectrometer. For 2D DOSY ^{1}H NMR, pulsed field-gradient spin-echo NMR measurements were performed by using the stimulated echo and longitudinal eddy current delay (LED) sequence [99]. A time of 1.5 ms was used for the dephasing/refocusing gradient pulse length (δ), and 100 ms for the diffusion delay (Δ). The gradient strength was changed quadratically from 5% to 95% of the maximum value (B-AFPA 10 A gradient amplifier), and the number of steps was 16. Each measurement was run with 32 scans and 16K time domain points. For the processing, an exponential window function and single zero filling were applied. During the diffusion measurements, the fluctuation of the temperature was less than 0.1 K. Prior to the NMR scans, all the samples were equilibrated for 30 min. The data were analysed by using XWINNMR 2.5 software.

5.2.10. PAMPA

PAMPA "sandwiches" (Fig. 6) were formed from an acceptor 96-well microtitre plate (Millipore MATRNPS 50) and a matching filter plate (Millipore Multiscreen®-IP, MAIPNTR 10) with apparent porosity of 0.45 μm, coated with 5 μL of 1 w/v% *n*-dodecane solution of lecithin. The initial donor sample concentrations were about 150 μM. 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 (Equation 2.) below:

$$\log P_e = \log\{C \bullet -\ln(1-\frac{[drug]_{acceptor}}{[drug]_{equilibrium}})\}, \text{ where} \qquad \text{Equation 2.}$$

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

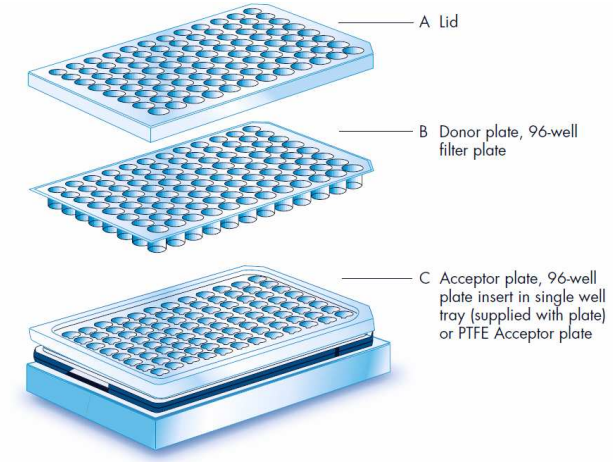

Fig. 6 Schematic structure of the PAMPA model [100]

5.2.11. In vivo experiments

This study was approved by the Committee on Animal Research at the University of Szeged (IV./01758-6/2008). A colony of inbred 150 ± 5 g male Wistar rats (Charles River Laboratories, Germany) was used, fed commercial rodent pellets and tap water. The animals were housed in groups of 5 at a controlled room temperature (22 ± 1 ºC) and maintained under an alternating 12 h light/12 h dark cycle (light on at 6:00 am).

The following materials were tested in this study: 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 (1.00 mL/kg). The animals were divided into five groups, with 6 rats per group. Group I received the vehicle and served as a control. To study the influence of DIMEB on the oedema, the Group II animals received DIMEB. LOR, KP 1:1 and KP 1:2 were administered to the other three groups, respectively.

In these experiments, at first the animals were treated orally by the above mentioned suspensions. 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 [101, 102]. In light isoflurane narcosis the intensity of the arising inflammatory reaction was measured after 30 min with the use of a plethysmometer (Ugo Basile, Harvard Apparatus, Germany) on the basis of the volume difference between the right hind leg treated with compound 48/80 and the left hind leg treated with vehicle (physiological saline). Immediately after measuring the extent of the oedema, blood samples were taken from rats by cardiac puncture and collected in tubes containing sodium-EDTA. Then these samples were centrifuged and the obtained plasmas were frozen until the measurement of LOR-content (see below).

A high-performance liquid chromatographic (HPLC) method has been developed for quantitative analyses of LOR in *in vitro* (PAMPA model) and *in vivo* samples. HPLC measurements were performed with a JASCO PU-1580 binary pump (JASCO Inc., Japan) and a programmable variable UV-visible detector. LOR was chromatographed on a 100 mm × 4.6 mm i.d., 3 μm particle, Phenomenex Luna C8(2), 100 Å analytical column under reversed-phase conditions at 30 ºC, protected with a SecurityGuard Cartridge (4.0 mm × 2.0mm) pre-column. The degassed mobile phase was a 47:42:11 (v/v) mixture of acetonitrile, purified water and a phosphate buffer solution (0.5 M, pH 3.0 ± 0.1, adjusted by the addition of 85% orthophosphoric acid). The flow rate was 1.0 ml/min and the analyte was monitored at 250 nm.

Calibration plots were constructed by analysis of working solutions (concentrations of 5, 10, 25, 50 and 75 μg/mL (*in vitro)* and 5, 10, 50, 75 and 100 ng/mL (*in vivo*)) of LOR in the mobile phase and plotting concentration against peak-area response for each injection. The calibration curves were linear throughout the whole range tested and described by the equations $y = 20.318 \cdot x - 23{,}775$ (R2 = 0.9912) and $y = 1083.4 \cdot x + 31.777$ ($R^2 = 0.9957$) for the *in vitro* and *in vivo* measurements, respectively. Unknown samples were quantified by reference to these calibration plots. Inter-day precision was calculated from results on the calibration sample of 5 μg/mL analysed on 20 consecutive days ($n = 5$). The mean amount found was 4.98 μg/mL and the RSD value was 2.47%. The limits of detection (LOD) and quantification (LOQ) were determined on the basis of the S.D. of the response (y-intercept) and the slope of the calibration plot. LOD and LOQ for LOR were 0.004 and 0.013 μg/mL, respectively.

500 μL of mobile phase was added to 500 μL of plasma, and this mixture was then centrifuged at 17 000 rpm for 15 min. The clear supernatant was next collected and filtered through a 0.22 μm membrane filter (Millipore). From this solution, a 100 μL aliquot was injected for HPLC analysis.

Statistical analyses were performed with Prism 4.0 software (GraphPad, San Diego, CA, USA). Differences in paw oedema between the treatment and control groups were determined by one-way analysis of variance (ANOVA) with the Newman-Keuls *post hoc* test. The criterion for statistical significance was taken as $p < 0.05$. An experimental group contained 6 rats. All values are expressed as mean ± S.E.

6. Results

6.1. Preliminary studies

The results were compared with the data of the CD–free system (see Table 5). The best solubility enhancement was achieved with DIMEB, which resulted in an approximately 300–fold increase in solubility, and accordingly this derivative was used in the further examinations.

Table 5 Effect of CD derivatives on solubility enhancement (25 ºC)

	c (μg/mL)	solubility enhancement (–fold)
LOR	2.43	1.00
+ α–CD	23.69	9.75
+ γ–CD	34.73	14.29
+ Captisol[a]	80.05	32.94
+ HP–β–CD[b]	128.14	52.73
+ H–Bu–β–CD[c]	181.69	74.77
+ β–CD	212.02	87.25
+ RAMEB[d]	496.89	204.48
+ Me–β–CD[e]	592.62	243.88
+ DIMEB	**730.87**	**300.77**

[a] sulphobuthylether–β–CD
[b] 2–hydroxypropyl–β–CD
[c] hydroxybuthyl–β–CD
[d] random methylated–β–CD
[e] methyl–β–CD

6.2. Phase–solubility studies

A relevant diagram is shown in Fig. 7, the solid line indicates the best linear regression fit of the experimental data. Higuchi and Connors [8, 55] defined 2 main types of diagrams. In general, type A diagram describes the characteristics of the water–soluble CD derivatives, while type B illustrates the properties of the less–soluble natural CDs. In the case of type A, the solubility of the drug raises with the increase of CD concentration. B–type phase–

solubility profiles reflect the formation of complexes with limited solubility in aqueous medium. Type A has 3 subtypes (A_N, A_L and A_P). The subtype of the present diagram is A_L. The most common type of CD complexes is the 1:1 drug:CD complex. The most common assumption is that a slope of less than 1 for a type A_L diagram indicates the formation of a 1:1 complex. The stability constant ($K_{1:1}$) of the complex can be calculated from the slope and the intrinsic solubility of the drug in the aqueous medium (Equation 3). In the absence of DIMEB, the equilibrium water solubility of LOR (S_0) was determined to be 0.81 ± 0.14 mg/L. The $K_{1:1}$ value of LOR:CD complex is very large: 1.48 x 10^6 M^{-1}. The linear regression coefficient (R^2) is 0.9991.

$$K_{1:1} = \frac{slope}{S_0(1-slope)}$$ Equation 3.

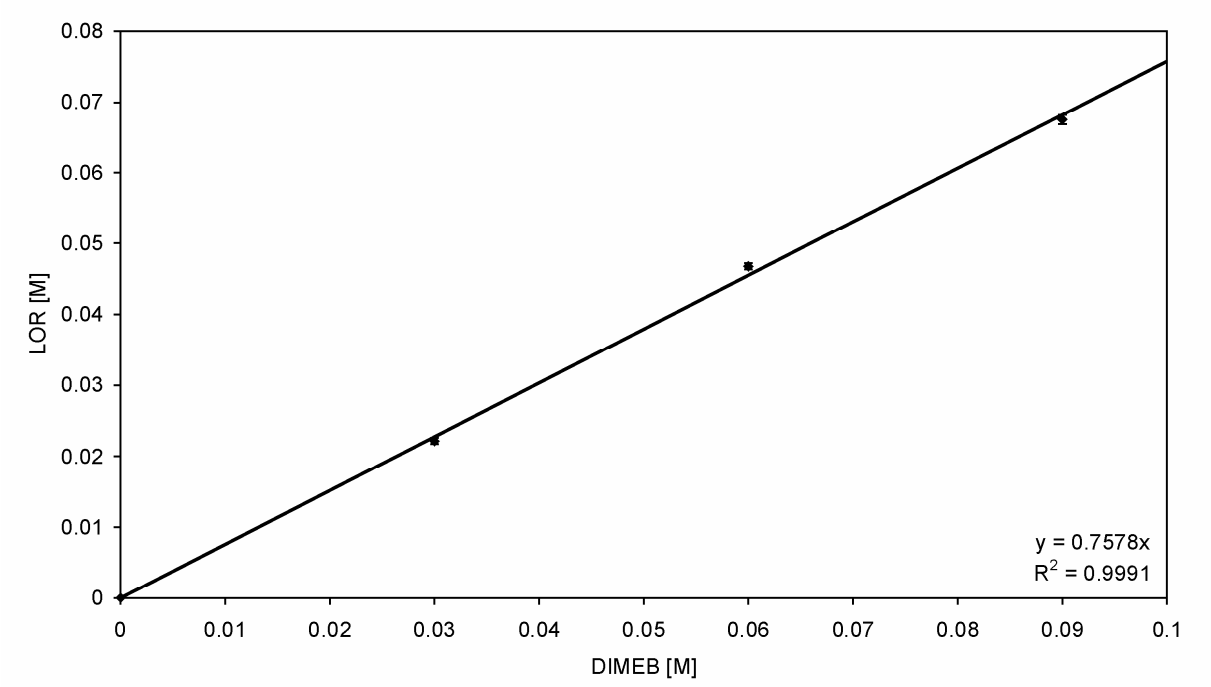

Fig. 7 Phase–solubility diagram of LOR and DIMEB

6.3. *In vitro* dissolution studies

According to the pK_a value the solubility of LOR depends on the pH: LOR can undergo protonation on the N of the pyridine ring in acidic media, forming salts with good solubility, so it exhibits good dissolution in acidic medium (e.g. SGM), but dissolves poorly in alkaline medium (e.g. SIM). The presence of DIMEB did not alter the solubility of LOR in SGM: the total investigated amount of the sample was dissolved in the first 5–10 min, independently from the preparation method and the composition. Concerning to the dissolution of DIMEB products in SIM, the rate of dissolution was improved for all of the products, but the extent of this increase depended on the preparation method and the molar ratio. For the 1:1 compositions (Fig. 8), none of the preparation methods resulted in 100% dissolution. The lowest solubility enhancement was observed for the PM (8.7–fold), as expected, as this mode of preparation did not result in a complex generally. The KP and MW furnished similar solubility increase (67.6– and 71.5–fold). However the best enhancement was achieved with the SD (142.9–fold). For the 1:2 and 1:3 preparations (Fig. 9 and Fig. 10) the PM displayed similar results as for the 1:1 product, with only a slight further improvement in solubility (10.26– and 13.7–fold, respectively). For the KP, MW, SD 1:2 and 1:3 products, the whole of the investigated samples dissolved in the first 15 min, i.e. the dissolution in SIM was as good as in SGM. This means that the same good dissolution can be obtained at the extreme pH values of the gastrointestinal tract with 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 a CD such as DIMEB would lead to a greater quantity of drug being absorbed, so that better BA would be obtained.

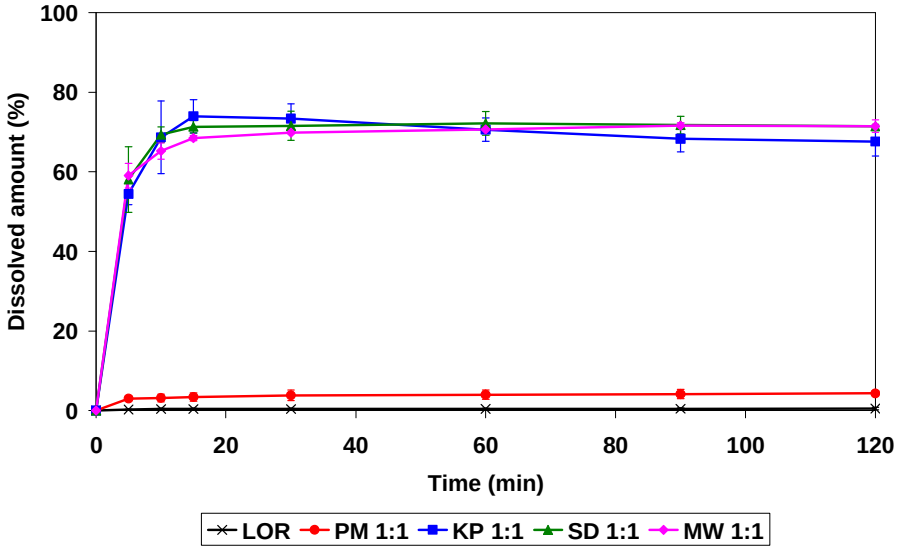

Fig. 8 Dissolution of LOR and 1:1 products in SIM

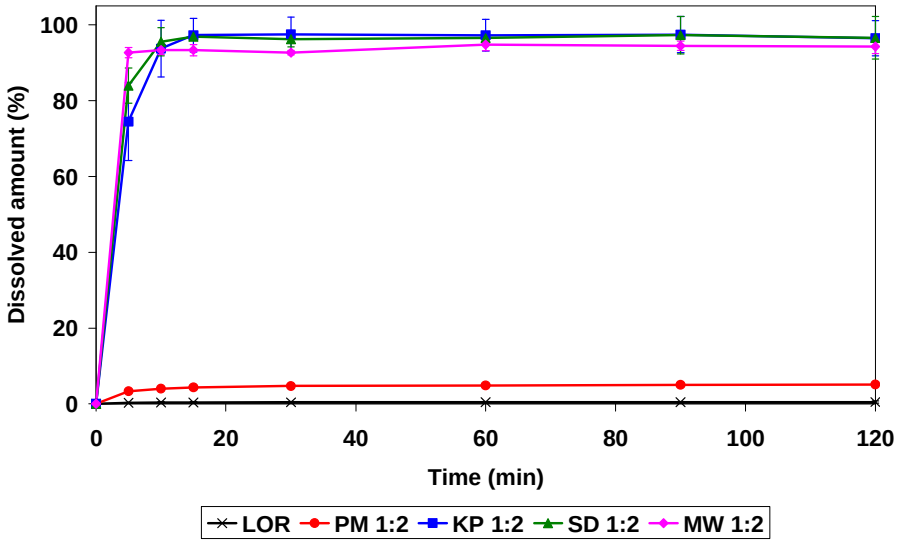

Fig. 9 Dissolution of LOR and 1:2 products in SIM

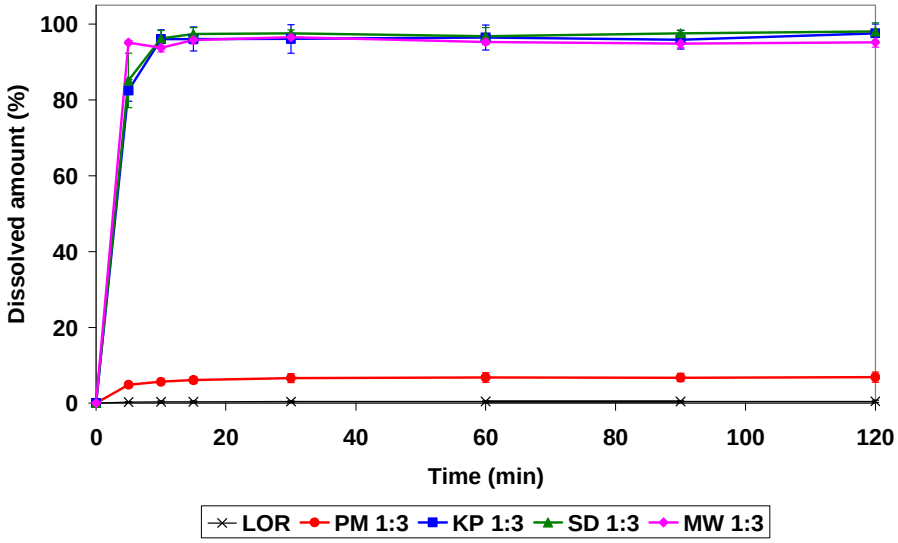

Fig. 10 Dissolution of LOR and 1:3 products in SIM

However LOR SEs did not give much better result in SIM (Fig. 11). The dissolution did not change in SIM, where 1:1 and 1:2 ratios showed worse dissolution compared to the raw drug. In the case of 1:4 and 1:6 product the initial appearance of increased dissolution rate can be explained by the faster dissolution of the amorphous drug 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.

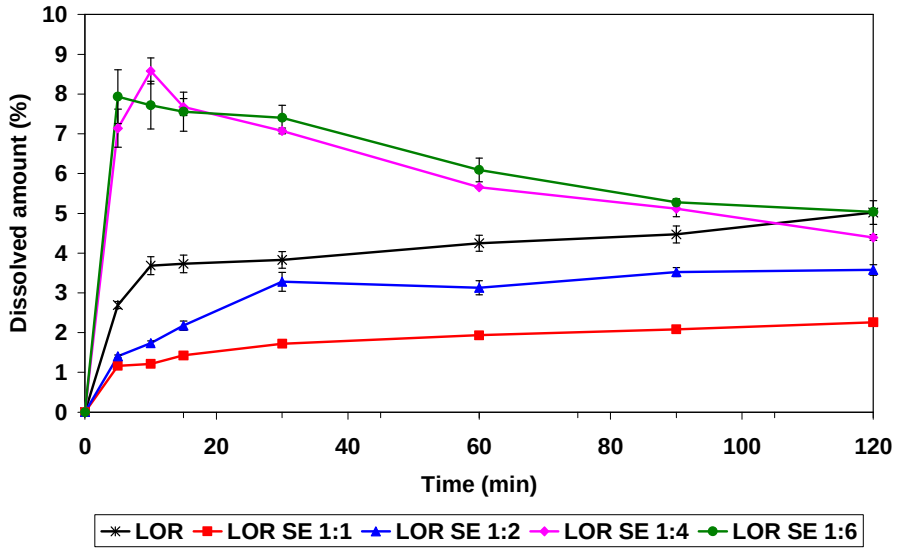

Fig. 11 Dissolution of LOR and SE products in SIM

6.4. Study the effect of the pH on the solubility

The defined daily dose of LOR is 10 mg. The solubility of LOR has been reported to decrease with increasing pH [90]. As can be seen in Fig 12 and 13, 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 with the increasing pH, DIMEB is not able to dissolve the whole quantity, so 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, so 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.

As the SEs did not improve the solubility and dissolution rate of LOR adequately, they were not tested in this study.

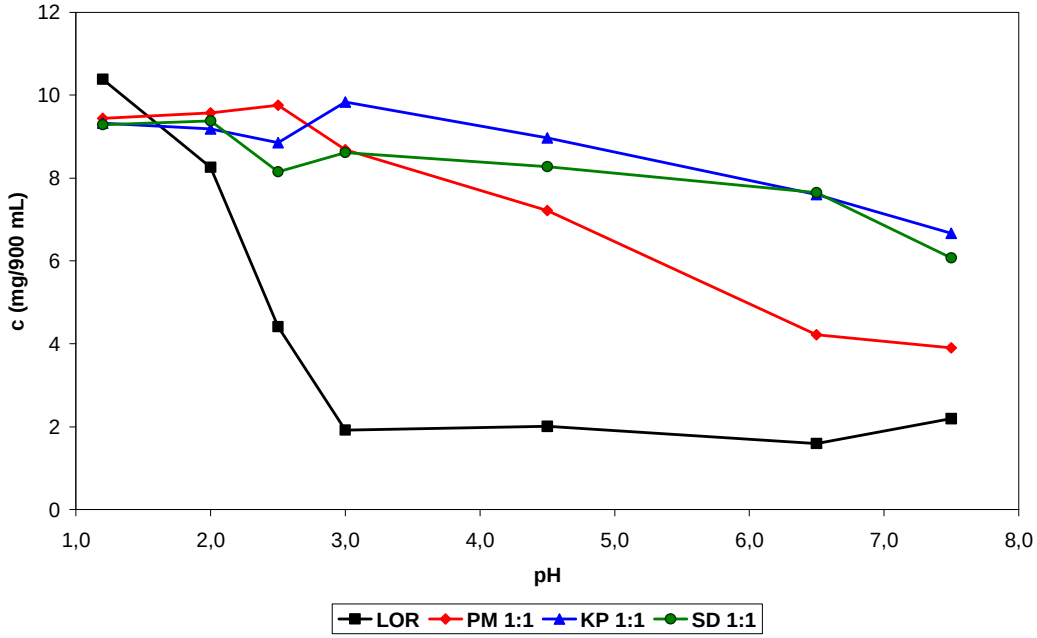

Fig. 12 pH–dependence of the solubility of LOR and 1:1 products

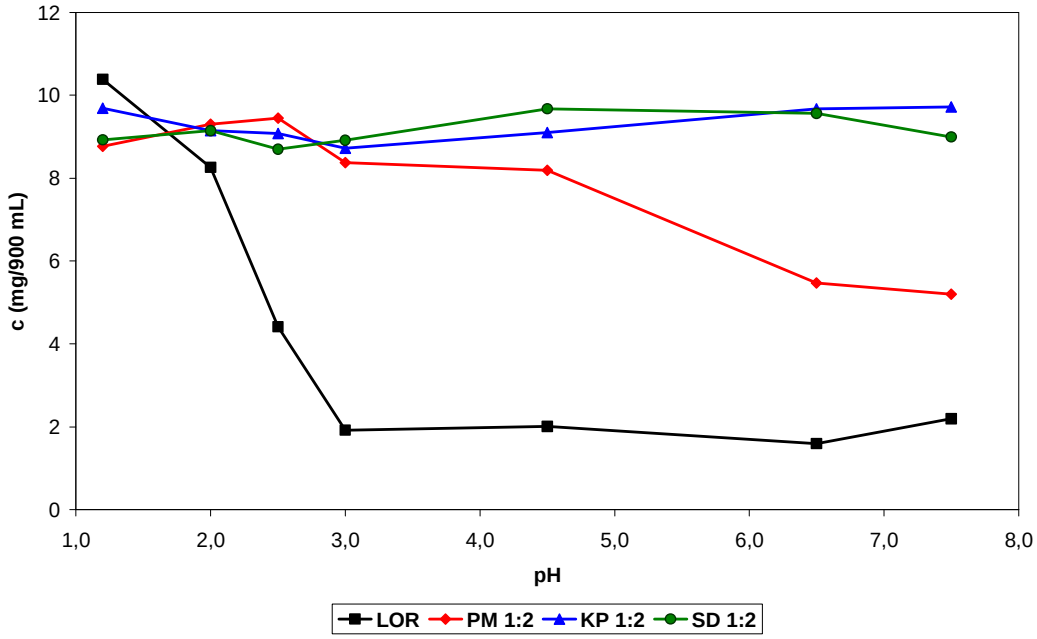

Fig. 13 pH–dependence of the solubility of LOR and 1:2 products

6.5. Results of thermal analysis

DSC thermograms of LOR and its products are shown in Fig. 14-17. The sharp, narrow endothermic peak in the DSC spectrum of LOR (peak 133.16 ^{o}C, normalized melting enthalpy 89.48 J/g) denotes the melting point of the material. The stability of LOR was not affected (no degradation was observed) up to 300 ^{o}C. Our DIMEB was amorphous, and there was no thermoanalytical indication at the melting point of LOR, however there was an exothermic peak reflecting the recrystallization of DIMEB at 181 ^{o}C (loss in mass was not detected in the TG curve). Above 320 ^{o}C, a broad endothermic peak was observed, associated with decomposition of the material.

LOR has no moisture content, as concluded from the TG curve. The moisture content of DIMEB is less than 0.5%, as determined from the TG curve and traditional gravimetry after drying in a drying chamber. The moisture contents of the products were also very low, there was no broad endothermic peak under 100 ^{o}C (representing the water content).

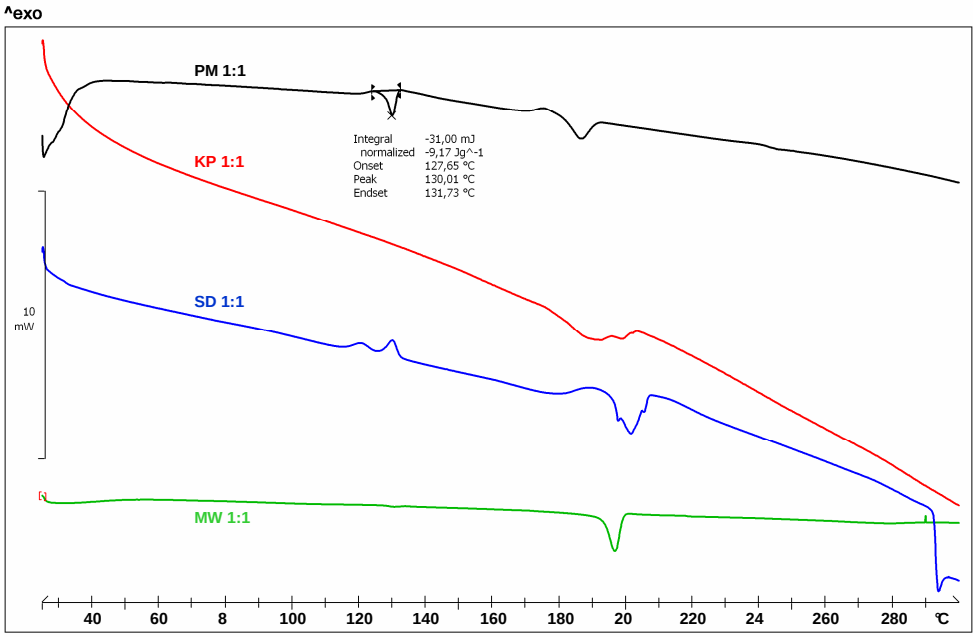

Fig. 14 DSC curves of 1:1 products

The results of the 1:1 compositions are presented in Fig. 14. For the PM, the melting point of LOR was seen several degrees lower than of pure LOR (this is characteristic for CD complexes) and the area under the peak was quite proportional to the amount of LOR in the sample. Hence, total inclusion complexes were not formed in the PM product. For the KP and SD samples, no endotherm reflected the melting point of LOR. For the MW and SD samples it is possible that the product becomes amorphous during the preparation method or that total complexation is occurred. For the KPs, similar phenomena could be observed, although there is no possibility for amorphization during the slow drying, accordingly total complexes were formed.

In case of all the 1:2 and 1:3 products similar conclusion can be drawn (as shown in Fig. 15 and 16). The PM method did not result in total inclusion complexation. KPs effected total inclusion complexes, MWs and SDs can be amorphous or inclusion complexes. To answer this question, further investigations were performed (FT-IR).

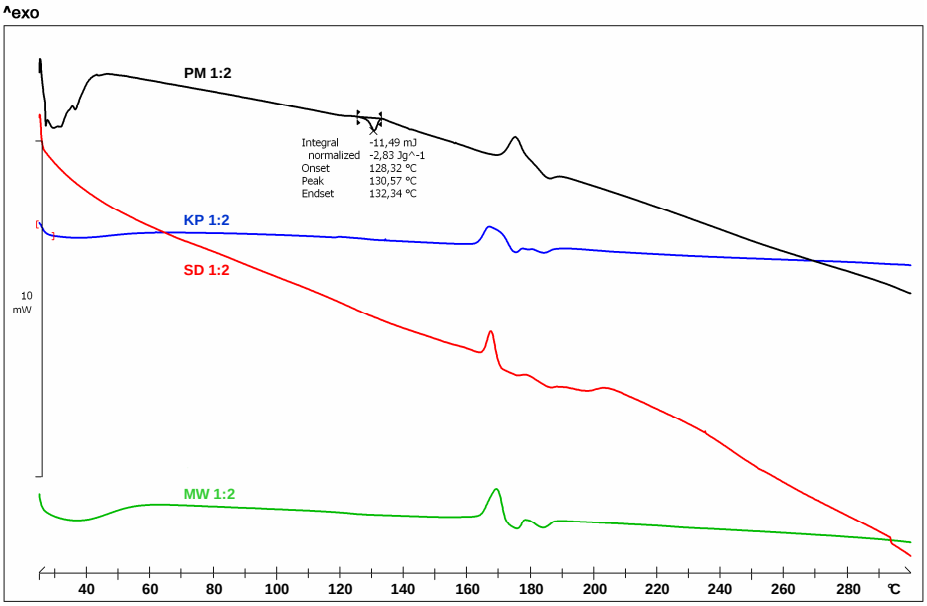

Fig. 15 DSC curves of 1:2 products

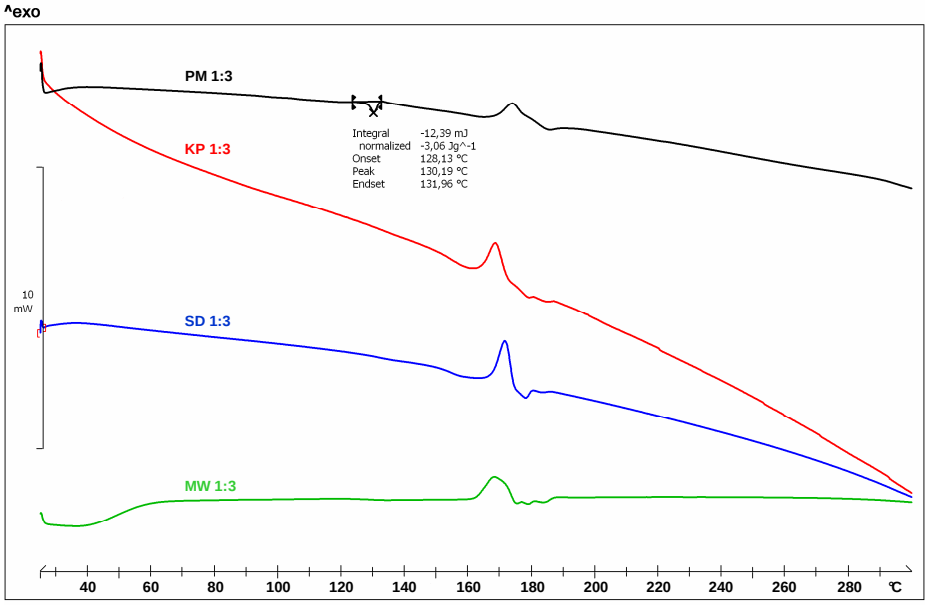

Fig. 16 DSC curves of 1:3 products

For these products the exotherm due to DIMEB was observed to have moved to lower temperature (about 170 ^{o}C). This phenomenon was seen for both KPs and MWs, and it can be stated consequently that applying the MW power did not cause changes in the chemical structure of the LOR molecule.

For the PMs the presumed uncomplexed guest (active material) percentages were estimated semiquantitatively from the DSC curves by using the following equation (Equation 4.):

$$c_{un} = \frac{\Delta H_i}{\Delta H_0 \times c} \times 10^4 \qquad \text{Equation 4.}$$

where c_{un} is the uncomplexed guest %; ΔH_i is the normalized integral value for the product; ΔH_o is the normalized integral value for the active ingredient; and c is the percentage of active ingredient in the product. Table 6 presents the results for the different compositions.

Table 6 LOR + DIMEB PMs complex ratio

	Uncomplexed LOR %
1:1	40,8
1:2	22,9
1:3	27,7

Based on these results it can be stated that the PM products are partial complexes.

In the case of SEs the DSC curves (Fig. 17) represented no melting point of the drug, except the 1:1 product, where a little shifted endotherm peak appeared at around 133 ^{o}C. Due to the preparation method it is expectable to forming an amorphous product which was proved by X-ray powder diffractometry (data not shown). However the 1:1 composition contained a small crystalline phase, as well.

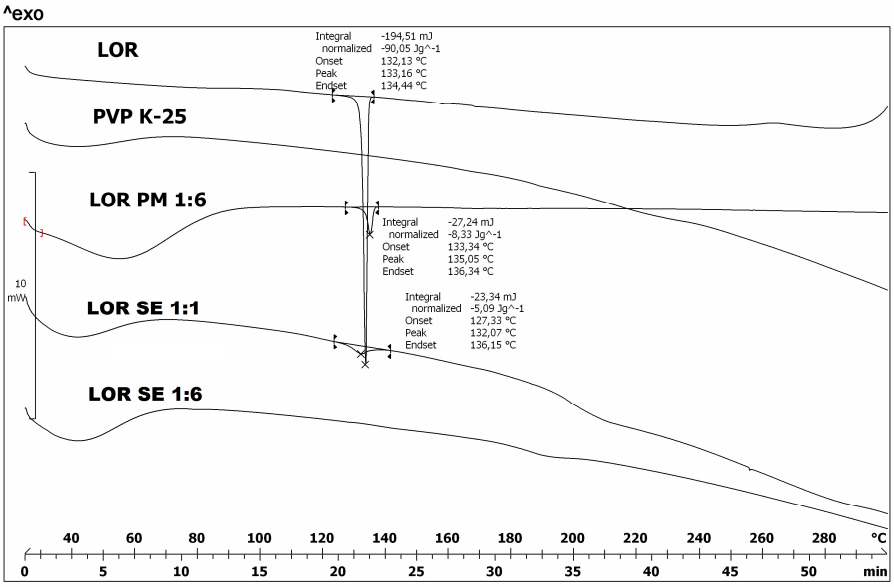

Fig. 17 DSC curves of LOR, PVP K-25 and SE products

6.6. FT–IR examinations

The spectral changes were evaluated by subtraction of the spectrum of DIMEB from the spectra of the samples. The spectra of the products involving different molar ratios and preparation methods did not differ appreciably.

The FT–IR spectra of LOR show the presence of the following peaks: 2983 cm^{-1} (aromatic C–H stretching), 2883 cm^{-1} (aliphatic C–H stretching), 1703 cm^{-1} (C=O stretching), 1580 and 1560 cm^{-1} (C=C stretching), 1474 and 1323 cm^{-1} (aliphatic C–H blending), 1435 cm^{-1} (C=N stretching), 1227 cm^{-1} (C–O stretching), 1117 cm^{-1} (C–Cl stretching), 831 and 764 cm^{-1} (aromatic C–H blending) and the FT–IR spectra of DIMEB showed prominent absorption bands at 3424 cm^{-1} (for O–H stretching vibrations), 2930 cm^{-1} (for C–H stretching vibrations) and 1159 cm^{-1}, 1086 cm^{-1} (C–H, C–O stretching vibration).

The difference spectra of the PM products were practically identical to the spectrum of pure LOR, indicating negligible interaction between LOR and DIMEB. For the KP, MW and SD samples (see Fig. 18–20), the characteristic C=O stretching frequency (1702 cm^{-1}) was shifted to lower wave numbers, and the typical C–O stretching at 1227 cm^{-1} was shifted to higher range. 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. During the formation of the inclusion complex, hydrogen–bonds develop between LOR and DIMEB, and the

inclusion complex can therefore be regarded as co–crystals [103]. A lipophilic part of LOR will probably be attached to the inner surface of DIMEB, like the aromatic rings, but in the FT–IR spectrum of LOR, the characteristic stretching frequencies of these aromatic parts are masked by DIMEB, so these interactions can not be detected with this method.

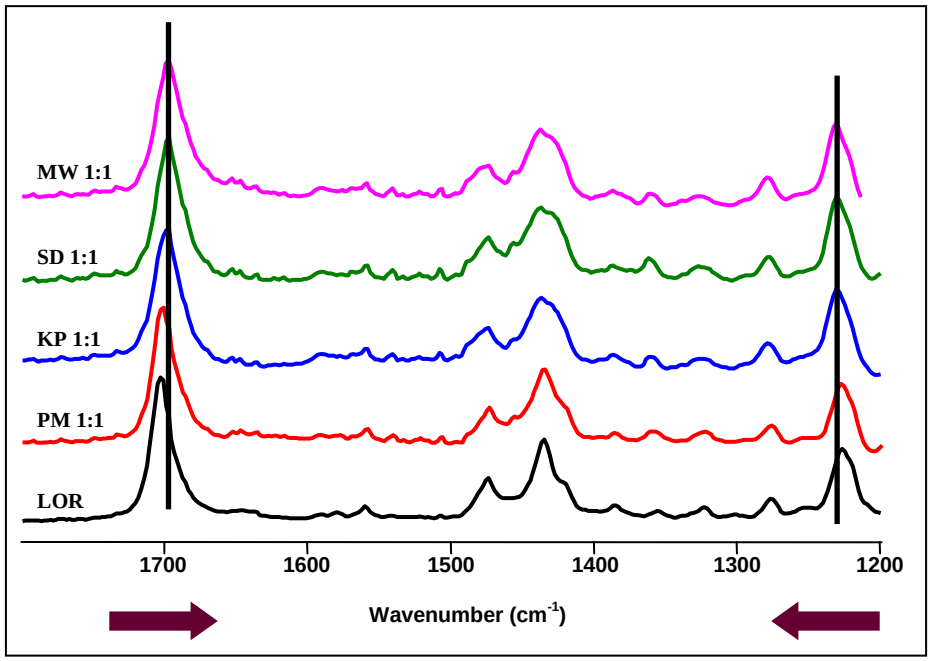

Fig. 18 FT-IR difference spectra of LOR and 1:1 products

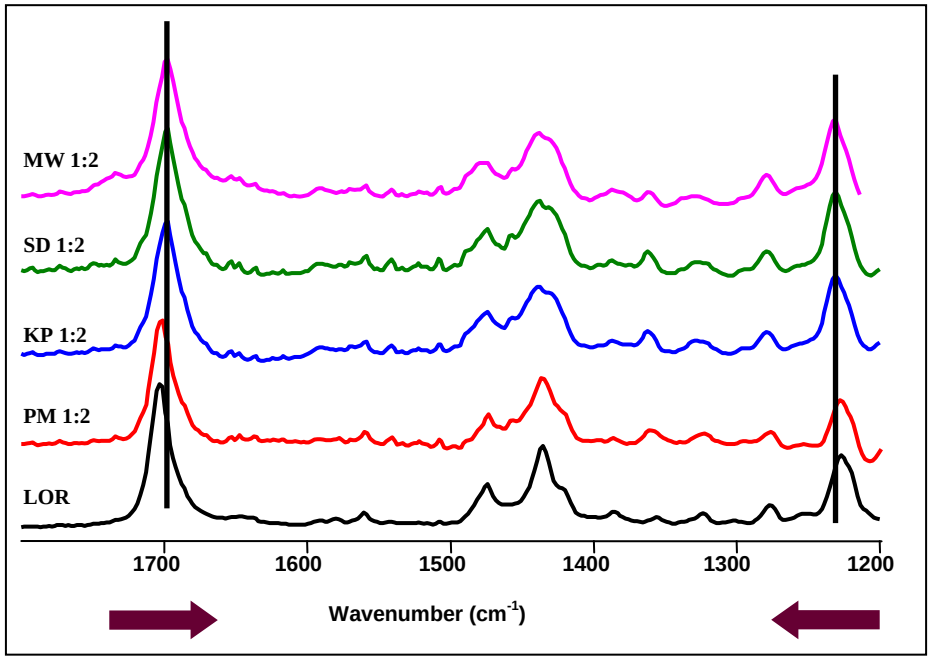

Fig. 19 FT-IR difference spectra of LOR and 1:2 products

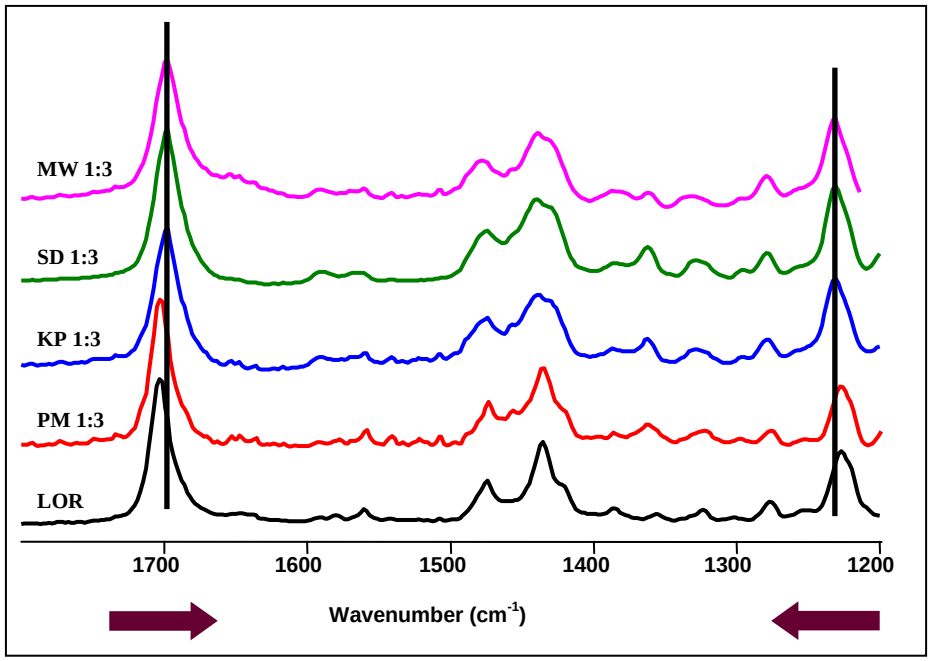

Fig. 20 FT-IR difference spectra of LOR and 1:3 products

In accordance with the DSC finding, in the KP products, total complexation occurred, and FT–IR also revealed total complexation for the MW and SD samples.

Based on the dissolution, the DSC and FT-IR results it was found that the applied microwave power did not cause any chemical changes in the molecule of LOR, the KP and MW products possess the same characteristic, therefore only one (the KP) of these preparations was investigated in the following studies.

On the FT-IR spectra of LOR SEs (data not shown) strong interaction can be seen between LOR and PVP. The PVP presents an apolar medium for LOR, and as LOR is a lipophilic drug, it rather likes to stay in an apolar medium, than to go to the hydrophilic, aqueous medium. As the SE products were not suitable of our aims (did not enhance the dissolution of LOR), other studies were not carried out with these products.

6.7. ESI–MS

ESI-MS is the most promising tool for the characterization of different kinds of host-guest complexes in the gas phase [104]. It can provide evidence of complexation and stoichiometry on the basis of the molecular weights of all vaporized species. The study of host-guest interactions in the gas phase allows the detection of specific interactions not

necessarily present in solution, thereby giving a complementary picture of the intrinsic phenomena responsible for molecular recognition. Also, there are interpretation ambiguities as concerns the ESI-MS spectra of supramolecular assemblies, e.g. deciding whether the species present in the mass spectra correspond to those present in solution, or they rather result from processes occurring under high-vacuum conditions. Moreover, it is not clear whether the molecular ions observed are real inclusion complexes or only ion-dipole external adducts, i.e. „false positives" [105]. In our particular case, however, a comparison can easily be made with the aid of results obtained from independent solution-phase techniques.

The hydroxyl groups of CDs are not easily protonated or deprotonated; ESI-MS analyses are usually carried out in the presence of salts in order to enhance detection.

The positive ESI spectra of KP 1:1 and KP 1:2 are reported in Fig. 21 and Fig. 22, respectively.

An interesting feature of the ESI-MS binding of the analytes with the CDs is the reproducible loss of water. This water loss is presumed to arise from displacement of water from the CD cavity.

Host-guest complexes formed in solution are also stable for characterization by ESI in the gas phase.

The ESI of DIMEB with positive ion detection leads to a series of protonated molecules, $[M+H]^+$, at *m*/*z* values depending on the number of methyl groups in each individual sugar unit of the CD derivative. The mass spectra reflect the average distribution of the methyl groups of DIMEB. It should be noted that the ion peak at *m*/*z* 1331 corresponds to 14 *O*-methyl groups (i.e. heptakis(2,6-di-*O*-methyl)cyclomaltoheptaose to a first approximation) [106].

As expected, the spectrum 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.

While the mixtures with different molar ratios (1:1 or 1:2) of LOR and DIMEB were analysed by ESI-MS, only the 1:1 complex was found in all the mass spectra, which suggests that the DIMEB inclusion complex in the gas phase has a certain stoichiometry.

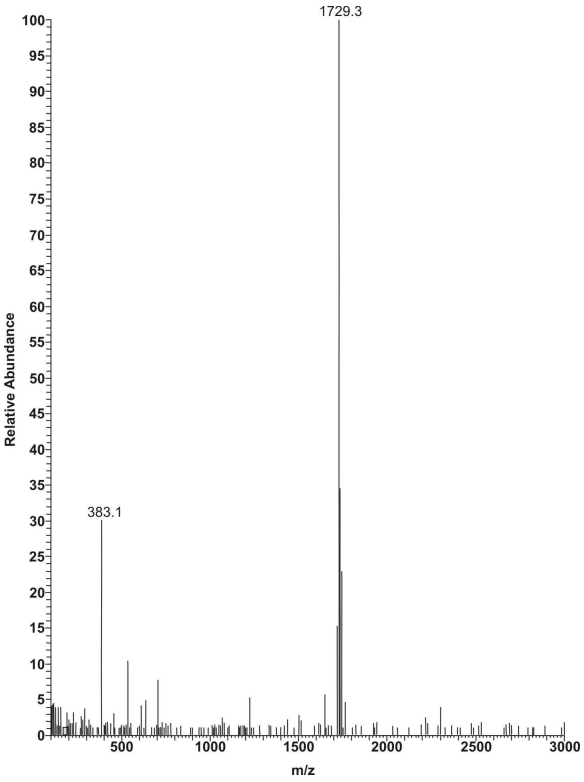

Fig. 21 ESI-MS spectra of KP 1:1

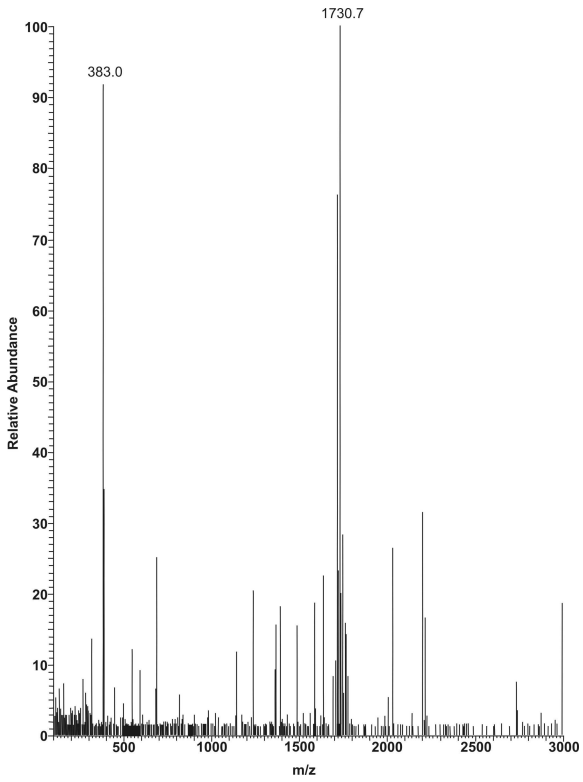

Fig. 22 ESI-MS spectra of KP 1:2

6.8. DOSY

The D value observed in the NMR experiment (fast-exchange condition) is the weighted average of those of the bound and the free guest. The rationale behind the extraction of the bound fraction from diffusion NMR measurements is simple. The host and guest have their own D values in the free state, reflecting their molecular weight and shape. The guest molecules are significantly smaller than CD, and the D values of the bound guests were taken to be equal to that of CD; it was assumed that, for the binding of a small guest molecule to a large host molecule, the D value of the host is not greatly perturbed and that of the host-guest complex can be assumed to be the same as that of the non-complexed host molecule. In the case of a weak or negligible association, the D values of the host and the guest will remain unchanged. For any other case, assuming fast exchange on the NMR time scale, the observed (measured) D values are weighted averages of the free and bound D values [107].

In the 2D DOSY spectra (Fig. 23), the F2 dimension shows the chemical shift and F1 stands for log D. Groups belonging to the same molecule will therefore appear in almost the same F1 row.

In the molecule of DIMEB, there are $=CH_2$ groups and $–O–CH_3$ groups, which have chemical shifts (δ) in the range 1–5 ppm. In the molecule of LOR, there are aromatic protons which have higher δ values, at about 7–8 ppm. In the DOSY spectrum of KP 1:1, 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 would diffuse more quickly due to its small molecular weight; it would have a smaller D value.

The D value measured for the complex indicates that it is best formulated as the 1:1 complex. For perfect spheres, theory predicts that increasing the molecular weight n-fold should lead to a D value decreased by a factor of n-1/3. Unfortunately, in the absence of well-defined ^1H NMR spectra, X-ray crystallographic results or ESI-MS data, assignment of absolute stoichiometry to these aggregates is speculative [108].

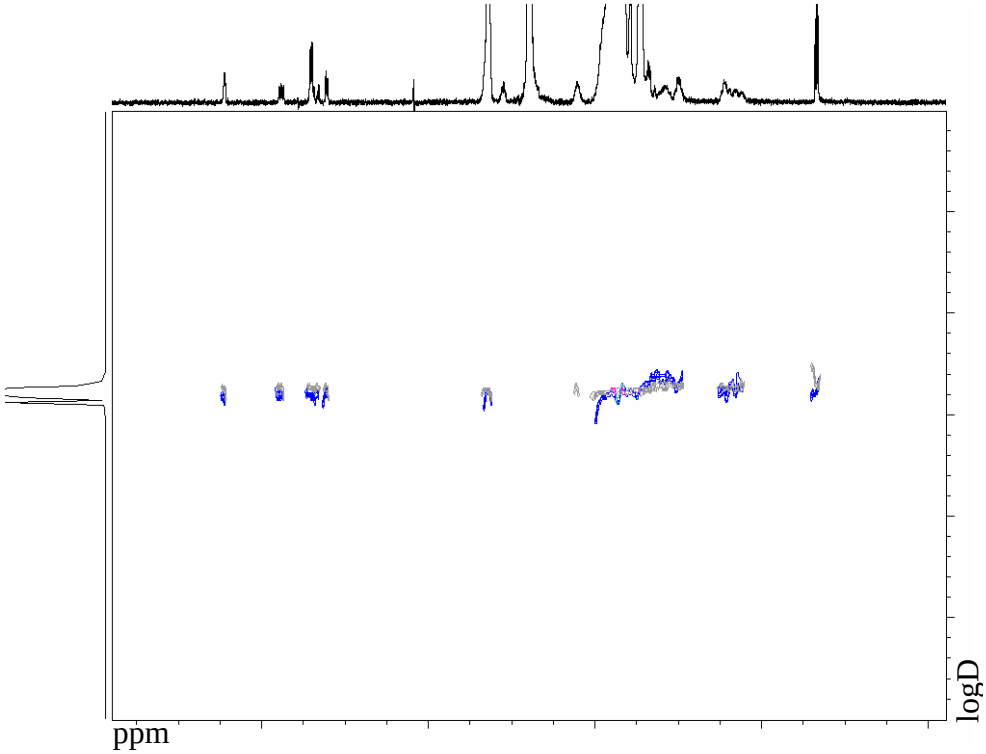

Fig. 23 Representative DOSY spectra of KP 1:1 (grey) and KP 1:2 (blue)

6.9 PAMPA

LOR is a highly variable drug from the aspect of BA: its absorption depends greatly on the content of the GI tract (fasted or fed state). In the fasted state, different enzymes and natural solubilizers (e.g. bile acids) are released, which help to dissolve the drug. All forms of

food have their own acidic (e.g. meats, cheese, egg, alcohol, mustard, sweeties, etc.), basic (e.g. potato, carrot, onion, mushroom or coconut) or natural characteristics, which influence the pH in the GI tract, and hence the solubility of this ionizable API. The pH of the stomach is known to differ significantly among individuals and is within the range of 1 to 3 or even higher (1 to 5) under fasting conditions. If undissolved LOR is emptied from the stomach, the absorption rate will be dramatically lower as compared with that in individuals where complete dissolution occurs in the stomach [90].

As LOR is a BCS class II drug, it readily passes through the intestinal wall, with a permeability value of $2.7 \cdot 10^{-3}$ – $4.8 \cdot 10^{-5}$ cm/min [90]. The PAMPA model represents only passive diffusion, and deceptive results can be observed if other mechanisms also play a role in the absorption of the API. LOR is a substrate of P-gp, which hinders its absorption, and thus the BA of this molecule. Figure 24 indicates that LOR has higher permeability than the DIMEB-containing products.

The results demonstrate that, if the main force of absorption was passive diffusion, DIMEB would hinder this process. It is possible that the diffusion through this artificial membrane is bidirectional, and therefore the absorbed LOR can back-diffuse to the donor side and become recomplexed by DIMEB. Another explanation is that the stability of the complex is too high, and the association-dissociation balance is shifted to association. There is an observable relationship between the amount of DIMEB and the calculated permeability. The higher the concentration of DIMEB is, 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. The statistical analysis reveals that the differences between the P_e values of LOR and KP 1:1, LOR and KP 1:2, and KP 1:1 and KP 1:2 are significant ($p<0.05$).

The permeability of LOR rises with increasing pH: at acidic pH, LOR is in a protonated form, which cannot pass through the membrane, but at basic pH it is not protonated (nonionized), and can be absorbed. This tendency also holds for KP 1:2, but the permeability is always lower at each pH value, which corresponds to the above discussion.

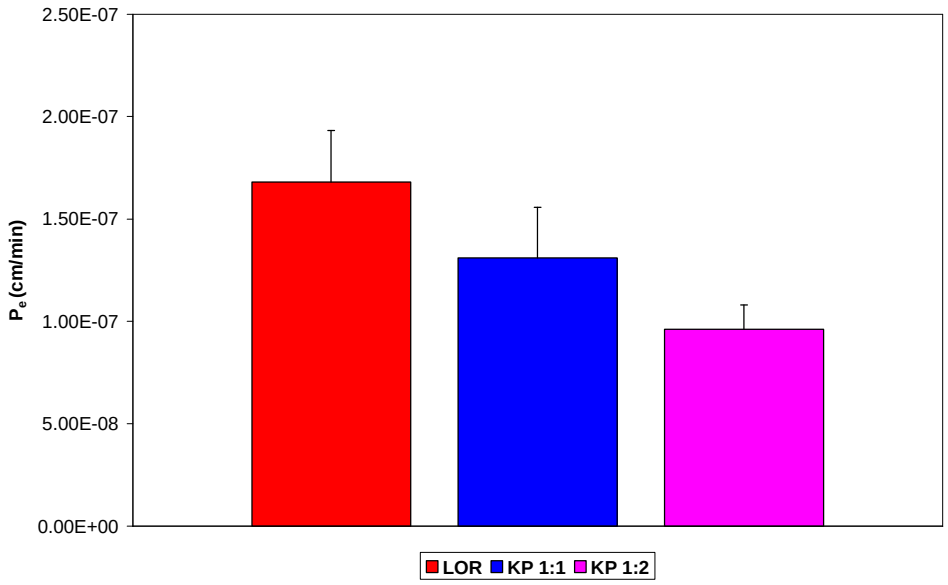

Fig. 24 Calculated effective permeability values of LOR, KP 1:1 and KP 1:2

6.10. In vivo experiments

The main effect of the oedema inducer compound 48/80 is the release of histamine from the mastocytes, and hence the effect of the antihistamine LOR can be well examined with this model [101, 102].

LOR, DIMEB and the inclusion complexes were investigated to establish to what extent they can reduce induced oedema. The volumes of the oedema induced in the right back paws are presented in Fig. 25. It was found that 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. The compound 48/80-induced oedema has been significantly decreased by rats pretreated with LOR, KP 1:1 and KP 1:2. The results of the Newman-Keuls multiple comparison test are presented in Table 7. 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.

We also measured the blood concentrations with HPLC. The data shown in Fig. 26 relate to the effects on oedema, except that the blood concentrations of LOR and KP 1:1 do not differ significantly, which demonstrates that KP 1:1 does not reach the desired BA. Excess DIMEB (as in KP 1:2) is needed to obtain pH-dependent solubility and more consistent and greater BA.

LOR was well separated from the biological background under the described chromatographic conditions, with mean RT=7.50 min. No interference with plasma matrix constituents was observed. The mobile phase used guaranteed good repeatability of the retention times. The LOR concentration in samples from the animals is about a few ng/mL, and thus the analytical wavelength was selected according to the maximum LOR absorbance with respect to a stable baseline.

P-gp recognizes many compounds as substrates, and tends to have high affinity for hydrophobic and positively charged compounds at physiological pH. DIMEB appears not to be a substrate of P-gp because it is a hydrophilic and electrically neutral cyclic oligosaccharide with a relatively high molecular weight. Furthermore, DIMEB should not compete with P-gp substrates due to its lack of cell permeability. Thus, DIMEB must have an alternative inhibitory effect on P-gp activity, differing from the P-gp inhibitors that suppress the efflux by their surface activity. Some results suggested that DIMEB has a direct inhibitory effect on the P-gp level on the cell surface. DIMEB did not exhibit cytotoxicity on Caco-2 cells and had no effect on the paracellular and transcellular transport [109].

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 in the greater BA. Consequently these observations also suggested that the increase in the AUC value of LOR by DIMEB may perhaps be caused not only by the solubilizing activity of DIMEB, but also by inhibition of the efflux in the GI tract.

Table 7 Results of the Newman–Keuls Multiple Comparison Test

Comparisons	Mean difference	Q	P value
Control vs. DIMEB	–2.333	1.097	>0.05
Control vs. LOR	–60.83	30.08	<0.001
Control vs. KP 1:1	–70.33	33.08	<0.001
Control vs. KP 1:2	–81.43	35.98	<0.001
LOR vs. KP 1:1	–9.50	4.015	<0.01
LOR vs. KP 1:2	–20.60	8.273	<0.001
KP 1:1 vs. KP 1:2	–11.10	4.311	<0.01

P>0.05 = non–significant (ns.), P<0.05 = *, P<0.01 = **, P<0.001 = ***

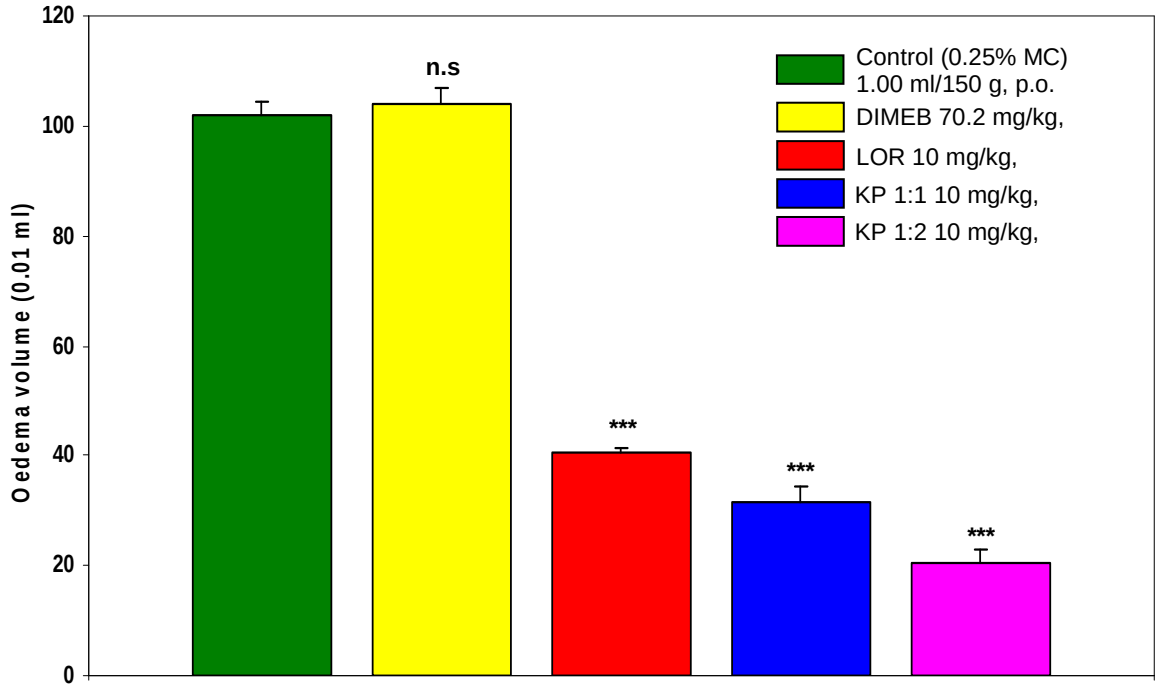

Fig. 25 Oedema decreasing effect of LOR and selected KPs in rats

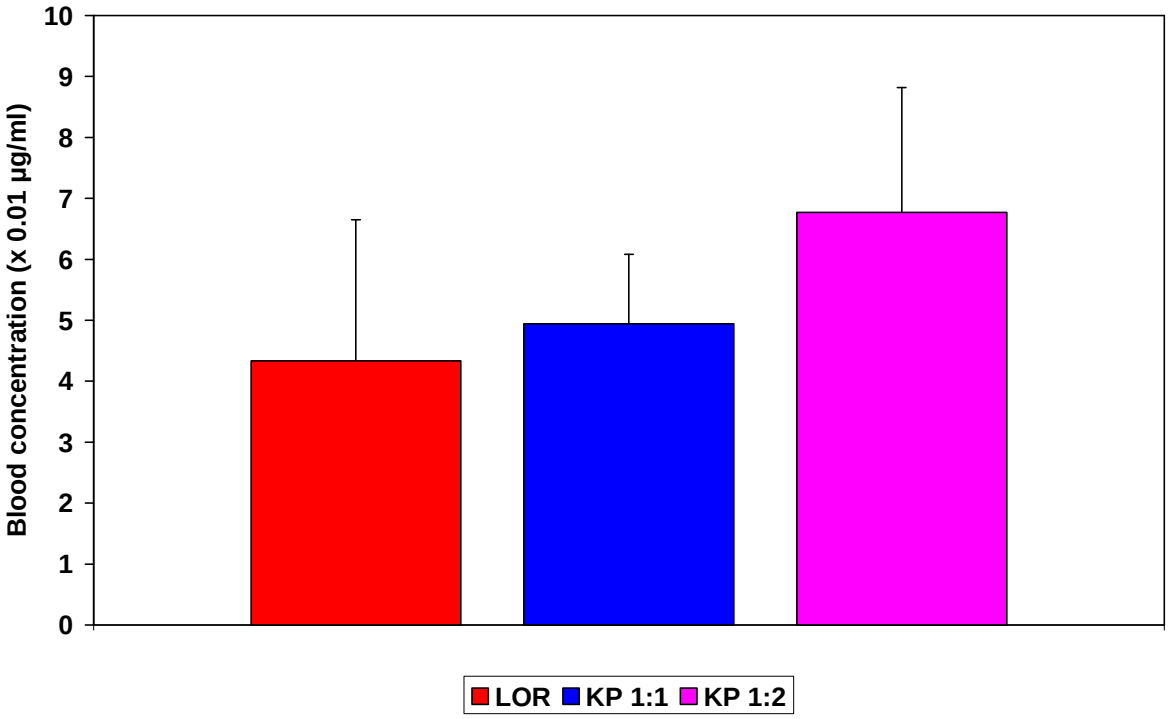

Fig. 26 Blood concentrations of LOR and selected KPs

7. Summary

The aim was to increase the solubility, dissolution rate and BA of the investigated API (LOR) by means of several pharmaceutical technological methods. This work involved a preformulation study to introduce the technological possibilities of a generic formulation.

The research work can be summarized as follows:

1. From the available 9 CD derivatives DIMEB was chose based on preliminary experiments, since it increased the solubility of LOR to the largest extent, to about 300–fold.
2. Different molar 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 utmost dissolution was obtained by the higher amount of DIMEB containing products (1:2 and 1:3 compositions). From the preparation methods, no more than the physical mixing and the solid dispersions with PVP K25 did not show much better results than LOR on its own.
4. With the application of DIMEB pH-independent solubility of LOR was achieved 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.

7. By the in vitro membrane modelling (PAMPA) it has been proven that the absorption of LOR is not only affected by passive diffusion, nevertheless 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, as a result of some factors: products has better dissolution, the dissolution of the products has become 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 the 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 forth.

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ANNEX

I.

Orvostudományi Értesítő, 2006, 79 (2): 257-260

Loratadine oldékonyságának és biohasznosíthatóságának növelése gyógyszertechnológiai módszerekkel

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Creşterea solubilităţii şi a biodisponibilităţii loratadinei prin procedee tehnologice
Loratadina este un agent antihistaminic H1 din generaţia a II-a. Aparţinând grupei a II-a a sistemului de clasificare biofarmaceutică are solubilitate mică şi permeabilitatea bună. Solubilitatea ei depinde de pH-ul lichidului gastrointestinal. Dimetil-β-ciclodextrina este capabilă să-i mărească solubilitatea, dependenţa de pH a produşilor rezultaţi cu CD fiind mai mică faţă de substanţa pură. Aceşti produşi s-au obţinut prin: amestecare mecanică, malaxare şi uscare prin pulverizare. În lucrare sunt prezentate rezultatele obţinute la investigarea acestor produşi. Interacţiunile, conducând la formare de complecşi, au fost examinate prin metoda DSC, FTIR şi RMN-^{1}H

Increasing the solubility and the bioavailability of loratadine with methods of pharmaceutical technology.
Loratadine is a II. generation of antihistaminic active substance. It belongs to the II. group of the Biopharmaceutics Classification System, with low solubility and good permeability. The solubility depends on the pH of the gastrointestinal liquid. Dimethyl-β-cyclodextrin was able to increase the solubility, so the pH-dependence of CD-products was smaller than the pure substance. The products were prepared from this sparingly soluble pharmacon by means of methods such as physical mixing, kneading and spray drying. This publication details the investigation results of these products. The interactions leading to complex formation between the components of the products were examined by DSC, FTIR, and ^{1}H-NMR methods.

Gyakori probléma a hatóanyagok rossz vízoldékonysága, és emiatt az ilyen farmakonokból lényegesen többet kell alkalmazni egy adott gyógyszerformában, ami megnöveli a mellékhatások kialakulásának valószínűségét. A hatóanyag vízoldékonyságának növelése javíthatja a biohasznosíthatóságát, ezáltal a dózist csökkenteni lehet és a mellékhatások is mérsékelhetők [3].

A biológiai hatáskifejlődés módosítására többféle gyógyszertechnológiai módszer alkalmazható. Ilyen a kristályforma módosítása, szemcseméret csökkentése (aprítás, mikronizálás, SCF technológia), a hatóanyag diszpergálása hordozóban (szilárd diszperzió képzése) és a ciklodextrines komplexképzés.

Munkánk során egy 2. generációs antihisztamin, a loratadine (LOR) oldékonyságának és biohasznosíthatóságának növelését vizsgáltuk. A loratadine a BCS (Biopharmaceutics Classification System) besorolása szerint a II. osztályba tartozik, ami azt jelenti hogy kicsi az oldékonysága és nagy a permeabilitása. A hatóanyagunk oldékonysága erősen pH-függő, ilyenkor jól használhatók a ciklodextrinek (CD), melyek alkalmazásával az oldékonyság függetleníthető a kémhatástól [1, 2].

Az előkísérletek során többféle CD-t kipróbáltunk, ezek közül a dimetil-b-CD-nek (DIMEB) volt a legnagyobb oldékonyságnövelő hatása (300x), ezért ezt a segédanyagot választottuk a 12 féle termék előállításához. A termékek különböző molekulaarányokban (hatóanyag:segédanyag = 2:1, 1:1, 1:2 és 1:3) készültek az alábbi módszerekkel: fizikai keverék (PM), gyúrás (KP) és porlasztásos szárítás (SD).

A termékek vizsgálatait 2 csoportba sorolhatjuk. Az oldékonyság változásának tanulmányozására meghatároztuk az oldódási sebességet, az *n*-oktanol / víz megoszlási hányados és a nedvesedési peremszög értékeket. A hatóanyag és a CD-molekula között kialakuló interakciót műszeres vizsgálatokkal igazoltuk.

Anyagok és vizsgálati módszerek

Loratadine (TEVA Magyarország Rt.); heptakis-2,6-di-O-metil-β-CD (DIMEB) (Cyclolab R&D Laboratory Ltd., Magyarország). A vizsgálatok során felhasznált anyagokat a Reanal (Budapest, Magyarország) gyártotta.

Hatóanyagtartalom kioldódása – forgólapátos kioldó készülék (20–200 mg hatóanyagtartalmú minta, 50–100 ml térfogat, 37 °C hőmérséklet, mesterséges gyomornedv vagy bélnedv, spektrofotometriás hatóanyagmeghatározás); *nedvesedési peremszög* – Dataphysics OCA 20 Contact Angle System (0,15 g termék, 13 mm átmérőjű préselmény, 1 tonna préserő, 4,3 ml desztillált víz cseppentés); *termoanalitika* – Mettler Toledo DSC 821^e készülék (2–5 mg minta, 25–300 °C, 5 °C/perc lineáris fűtés, 167 ml/perc argon); *FT-IR* – Thermo Nicolet AVATAR 330 FT-IR készülék; *^{1}H-NMR* – Bruker Avance DRX 400.

Eredmények

Az elővizsgálatok során az alkalmazott CD-származékok közül a DIMEB növelte legjobban a hatóanyag oldékonyságát, így a termékek készítéséhez ezt a származékot használtuk (1. táblázat).

1. táblázat. Ciklodextrin származékok oldékonyságnövelő hatása (25 °C-on)

	c (μg / ml)	*oldékonyságnövelés*
LOR	2,43	1,00
+ α-CD	23,69	9,75
+ γ-CD	34,73	14,29
+ Captisol	80,05	32,94
+ HP-β-CD	128,14	52,73
+ H-Bu-β-CD	181,69	74,77
+ β-CD	212,02	87,25
+ RAMEB	496,89	204,48
+ Me-β-CD	592,62	243,88
+ DIMEB	730,87	300,77

Mind az *n*-oktanol / víz megoszlási hányados, mind a nedvesedési peremszög értékek a termékeknél egyaránt kisebbek voltak, mint a hatóanyag esetében, ami azt jelezte, hogy a CD javította a hatóanyag nedvesedését, így várhatóan a termékekből nagyobb lesz a hatóanyag kioldódása a tiszta hatóanyaghoz viszonyítva (2. táblázat).

2. táblázat. LOR és termékeinek nedvesedési peremszöge és n-oktanol / víz megoszlási hányadosa

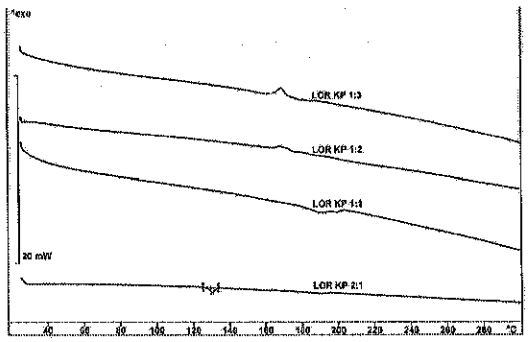
	Teta (°) ± SD					*c_o/c_v*			
LOR	*73,13 ± 7,01*				*LOR*	*6833,33*			
Termékek	2:1	1:1	1:2	1:3	Termékek	2:1	1:1	1:2	1:3
PM	34,00 ± 3,46	18,90 ± 2,26	19,35 ± 1,77	20,45 ± 1,14	PM	0,30	0,15	0,74	1,00
KP	33,20 ± 1,25	29,10 ± 1,23	36,05 ± 2,34	34,37 ± 1,42	KP	8,80	–	0,17	0,18
SD	–	36,00 ± 0,00	31,50 ± 0,28	25,00 ± 0,00	SD	–	8,70	0,02	–

A LOR oldékonysága 2 óra után mesterséges gyomornedvben 202,59 mg/100 ml, mesterséges bélnedvben 1,00 mg/100 ml. Jól látszik, hogy a hatóanyag sokkal jobban oldódik savas, mint lúgos pH-n. A CD-tartalmú termékek hatóanyag-kioldódása gyomornedvben ugyanakkora volt, mint a tiszta hatóanyagé, viszont bélnedvben a készítési módtól és az összetételtől függően akár 200-szorosára is tudtuk növelni az oldékonyságot. A CD-ek segítségével a hatóanyag oldékonyságát pH-tól függetlenné tudtuk tenni (3. táblázat).

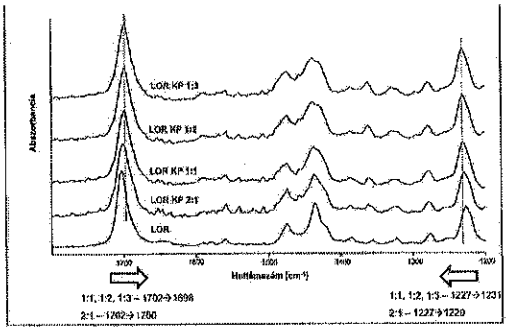
3. táblázat. LOR és termékeinek kioldódási eredménye (mg /100 ml)

		Mesterséges gyomornedv				*Mesterséges bélnedv*			
Termék		*10. perc*	*SD*	*120. perc*	*SD*	*10. perc*	*SD*	*120. perc*	*SD*
LOR		204,07	2,63	202,59	1,66	0,74	0,23	1,00	0,30
PM	2:1	51,36	0,22	54,11	5,36	5,21	0,35	5,48	0,04
	1:1	11,90	0,22	11,63	0,15	6,32	0,96	8,69	0,66
	1:2	28,41	0,66	28,59	0,24	8,05	0,46	10,26	0,14
	1:3	35,39	0,96	36,90	2,25	11,27	0,70	13,68	1,34
KP	2:1	200,50	9,09	199,89	10,68	30,60	0,45	26,79	0,23
	1:1	197,20	15,82	195,79	18,32	68,71	9,14	67,61	3,58
	1:2	197,81	1,12	201,12	3,00	187,53	7,50	193,03	4,66
	1:3	188,20	6,60	192,36	3,20	192,14	2,50	195,27	2,45
SD	2:1	99,22	–	97,90	–	5,61	2,04	20,11	3,61
	1:1	99,19	2,74	100,03	1,66	138,67	1,98	142,89	0,51
	1:2	96,68	2,60	97,22	2,76	191,16	3,73	193,17	5,62
	1:3	97,55	1,38	97,69	0,77	192,54	2,12	196,14	2,34

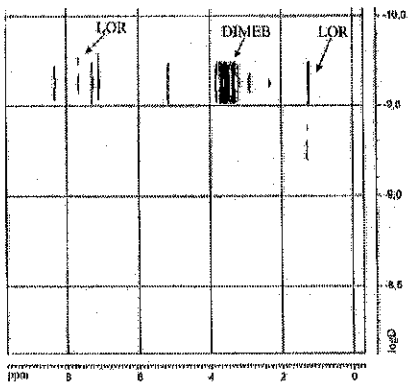
A molekuláris komplex kialakulását többféle műszeres vizsgálat is igazolta. A DSC és a FT-IR vizsgálatok minden esetben összhangban voltak. PM termékeknél nem alakult ki zárványkomplex, KP termékeknél a legkisebb CD-tartalmú termék (2:1) esetében részleges, míg a többi összetételnél teljes molekula-komplexek alakultak ki (1–2. ábra). SD termékeknél összetételtől függetlenül teljes komplexek alakultak ki. Az ^{1}H-NMR vizsgálatot csak a KP 1:1 összetételnél végeztük el, ahol a DOSY spektrummal kimutatható volt, hogy a hatóanyag és a CD együtt diffundálnak (azonos a diffúziós állandó), vagyis a komplex létrejött (3. ábra).



1. ábra. Gyúrt termékek DSC vizsgálata



2. ábra. LOR és gyúrt termékek FT-IR felvételei és értékelése



3. ábra. LOR gyúrt 1:1 összetételű termék DOSY spektruma

Következtetések

Az elővizsgálatok során legjobban a DIMEB növelte a hatóanyag oldékonyságát. A nedvesedési peremszög és az *n*-oktanol / víz megoszlási hányados értékeknek megfelelően növekedett a termékek vízoldékonysága a hatóanyagéhoz képest. A CD-származék növeli a hatóanyag oldódási sebességét és oldékonyságát, melynek értéke függ a kioldó közeg kémhatásától, a termékek összetételétől és készítési módjától. A CD alkalmazásával lehetővé vált egyenletes hatóanyagkioldódás biztosítása a GI traktus kémhatásától függetlenül. A készítési módtól függően részleges vagy teljes molekulakomplex kialakulását bizonyítottuk a termoanalitikai (DSC), az FT-IR és az ^{1}H-NMR vizsgálatok segítségével.

Irodalom

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Short communication

Water-soluble loratadine inclusion complex: Analytical control of the preparation by microwave irradiation

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ABSTRACT

The majority of active pharmaceutical ingredients are poorly soluble in water. The rate-determining step of absorption is the dissolution of these drugs. Inclusion complexation with cyclodextrin derivatives can lead to improved aqueous solubility and bioavailability of pharmacons due to the formation of co-crystals through hydrogen-bonding between the components. Inclusion complexes of loratadine were prepared by a convenient new method involving microwave irradiation and the products were compared with those of a conventional preparation method. Dissolution studies demonstrated that the solubility and rate of dissolution of loratadine increased in both of the methods used. The interactions between the components were investigated by thermal analysis and Fourier Transform Infrared studies. The microwave treatment did not cause any chemical changes in the loratadine molecule.

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1. Introduction

It is estimated that 40% or more of active pharmaceutical ingredients identified through combinatorial screening programs are poorly soluble in water [1]. The bioavailability of drugs depends on their solubility and permeability. The drugs belonging in class II of the Biopharmaceutical Classification System have high membrane permeability, and the limiting factor of their absorption is therefore their solubility [2].

One of the methods applied to increase solubility is to prepare inclusion complexes with cyclodextrins (CDs) [3–5]. Such complexes have found extensive application in many fields, including pharmaceutical technology (to increase the aqueous solubility, dissolution rate, bioavailability and stability of drugs, to reduce bitterness and to decrease tissue irritation upon dosing) [6]. CDs are usually used for solubility enhancement from the preclinical stage during drug development. The methods widely utilized to prepare inclusion complexes are coprecipitation, kneading, freeze-drying and co-grinding [7]. They often involve time-consuming manufacturing processes and generally require large amounts of solvents. There is therefore a need for faster and more convenient processes for the preparation. Microwave (MW) irradiation, a method recently used to prepare CD inclusion complexes, has the major advantages of shorter reaction times and higher yields of products [8]. In pharmaceutical technology, MW has been used because of its thermal effect in drying processes (granules or crystals), and for the sterilization of injections and infusions [9–11].

Loratadine (LOR) (Fig. 1) is a second-generation tricyclic H_1 antihistamine, marketed for its non-sedating properties. H_1 antihistamines prevent and suppress the responses to histamine or allergen in the nose and conjunctivae, thereby eliminating such symptoms as itching, congestion, rhinorrhoea, tearing and sneezing [12]. The solubility of LOR depends on the pH: on increase of the pH, the solubility decreases exponentially. Because of this, its bioavailability exhibits high variability, which is a disadvantage for its oral administration [13] because the drug may suffer a reduced therapeutic efficacy.

In general kneading, which is a simple and scaleable preparation method, gives the best results [14], but in this case the removal of the solvent could be a critical parameter in the preparation. In the present work the applicability of MW irradiation to prepare inclusion complexes without chemical change was studied. The conventional (kneaded product; KP) and a convenient preparation technique using MW irradiation treatment were compared in the case of LOR + dimethyl-β-CD (DIMEB) inclusion complexes. Dissolution studies were performed with the paddle method, and the interactions of the components were investigated by

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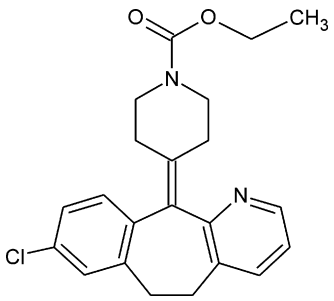

Fig. 1. Chemical structure of loratadine.

thermoanalytical methods [15] and Fourier Transform Infrared (FT-IR) spectrometry.

2. Materials and methods

2.1. Materials

Heptakis-2,6-di-*O*-methyl-β-cyclodextrin (DIMEB) was purchased from Cyclolab (Fig. 2); LOR (ethyl 4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidine)-1-piperidinecarboxylate) was kindly provided by TEVA Pharmaceutical Industries Ltd. (Hungary); other chemicals were of analytical reagent grade purity.

2.2. Preparation of products

Homogeneous powder mixtures of LOR and the appropriate amount of DIMEB were prepared in three molar ratios (LOR:DIMEB = 1:1, 1:2 and 1:3), the same mass of 50% alcohol was then added to the mixtures, and they were next homogenized, and treated in a MW oven (Milestone Ethos TC MW apparatus, Advanced Microwave Labstation, Italy). The method of MW treatment was as follows: 150 W, 90 s, 60 °C, and the samples were then dried under vacuum [7]. As a control KPs were made: the physical mixtures were suspended in the same mass of 50% ethanol, and the solvent was evaporated of at room temperature. The products were ground and sieved (100 μm), and stored at room temperature under normal conditions. The

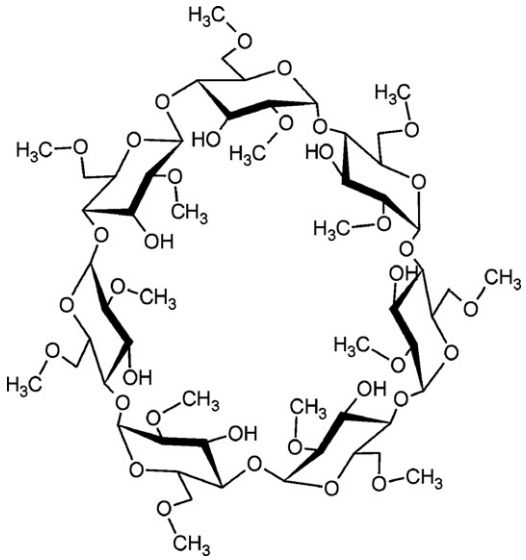

Fig. 2. Chemical structure of DIMEB.

actual LOR load of products was determined spectrophotometrically.

2.3. Dissolution study

In vitro dissolution studies (min. three parallel measurings) were performed in simulated intestinal medium (SIM) (phosphate buffer; pH 7.0 ± 0.1; 0.1 M) using the modified rotating paddle method. 50 mg of LOR, or product containing 50 mg of LOR (considering the LOR load of products and water content) was measured and added to 100 ml of SIM (100 rpm at 37 ± 0.1 °C). Aliquots were withdrawn at 5, 10, 15, 30, 60, 90 and 120 min and immediately filtered. At each sampling time, an equal volume of fresh SIM was added, and the correction for the cumulative dilution was calculated. The concentration of LOR was measured spectrophotometrically at 248 nm after the suitable dilution (Unicam UV/VIS spectrometer, Unicam, UK). The calibration curve was determined between 0 and 28 μg/ml, the slope was 0.0366, the linearity (r^2) was 0.9997. There was no absorption of DIMEB at the absorption maximum of the LOR. The limit of quantification (LOQ) value was 500 μg/l.

2.4. Thermoanalytical studies

2.4.1. Differential scanning calorimetry (DSC)

The DSC records were obtained with a Mettler Toledo DSC 821^e apparatus. Between 2 and 5 mg of sample was crimped in a standard aluminum pan (40 μl) and heated from 25 to 300 °C at a heating rate of 5 °C/min under a constant purge of argon at 10 l/h.

2.4.2. Thermogravimetric measurements

The TG, DTG and DTA curves were recorded in parallel in platinum crucibles with the same thermal program (heating range 25–300 °C, heating rate 5 °C/min), using a MOM Derivatograph-C (MOM Co., Hungary). The reference was a crucible containing aluminum oxide.

2.5. FT-IR analysis

The sample with a LOR content of 0.5 mg was ground and mixed with 150 mg of dry KBr in an agate mortar, and the mixture was then compressed into a disc at 10 t. Each disc was scanned 64 times at a resolution of 4 cm^{-1} over the wavenumber region 4000–400 cm^{-1} with an FT-IR spectrometer (Thermo Nicolet AVATAR 330, USA). The evaluation was carried out with the GRAMS/AI Ver. 7 program.

3. Results and discussion

3.1. Dissolution study

The investigated active pharmaceutical ingredient LOR has different solubility properties at the various pH levels in the gastrointestinal tract. LOR can undergo protonation on the N of the pyridine ring in acidic media, forming salts with good solubility; in contrast, in SIM it is practically insoluble (0.38 mg/100 ml). Since LOR is a weak base, it is absorbed from the intestine. Accordingly, the dissolution properties were studied in SIM. The dissolution curves are demonstrated in Fig. 3. For both the KP and MW products, the dissolution rate and the solubility of LOR depended on the molar ratio. At 1:1, the quantity dissolved was about 60%, whereas for the 1:2 and 1:3 products close to 100% release was observed. These investigations revealed that the KP and MW products yield similar dissolution results.

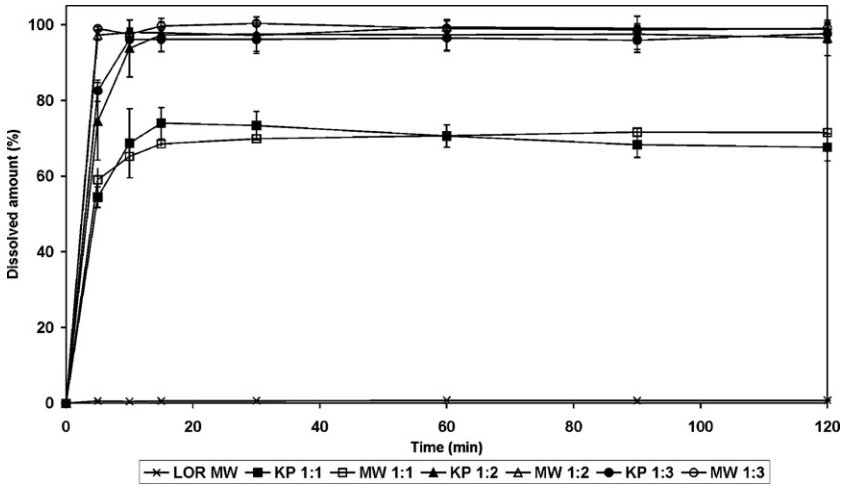

Fig. 3. Dissolution of loratadine in simulated intestinal medium.

3.2. Thermal analysis

The sharp, narrow endothermic peak in the DSC spectra of LOR (peak 133.16 °C, normalized melting enthalpy 89.48 J g^{-1}) and LOR MW (peak 135.48 °C, normalized melting enthalpy 65.94 J g^{-1}) denotes the melting point of the material. There was no significant difference between the treated and the untreated LOR. According to the TG curves, practically none of them contains residual water. The stability of the LOR was not affected (no degradation was observed) up to 300 °C. DIMEB is amorphous, and there is no thermoanalytical indication of the temperature of melting point of the LOR, but it has an exothermic peak which reflects the recrystallization of DIMEB at 181 °C (loss in mass was not detected in the TG curve). The water content of DIMEB is about 0.9%. The results of DSC investigations are presented in Fig. 4. No endotherm was detected for the LOR:DIMEB products at around the temperature of the melting point. Thus there are two possible explanations for the MW products: the samples were amorphized during the preparation method, or total complexation occurred. For the KPs, similar phenomena could be observed, but there is no possibility for amorphization during the slow drying. For the products the exotherm due to DIMEB was observed to have moved to lower temperature (about 170 °C). This phenomenon was seen for both KPs and MWs, and it can therefore be stated that the MW did not cause changes in the chemical structure of the LOR molecule. The water content of the KPs and MWs was similar, their quantity was about 0.9–2.7% and increased with the rising of CD ratio.

3.3. FT-IR spectra

The spectral changes were evaluated by subtraction of the spectrum of DIMEB from the spectra of the samples. The spectra of treated and untreated LOR and the calculated subtraction spectra are presented in Fig. 5. The spectra of the products involving different molar ratios and preparation methods did not differ appreciably. For all of the products, the characteristic C=O stretching frequencies (1702 cm^{-1}) were shifted to lower wavenumbers, and the typical C–O stretching at 1226 cm^{-1} was shifted to the higher range. These results lead us to

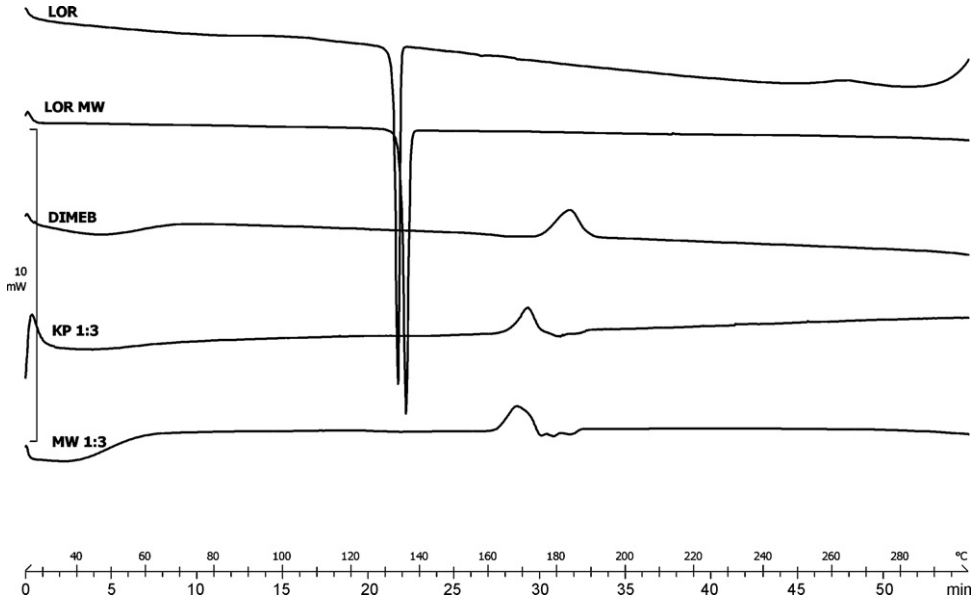

Fig. 4. DSC curves of loratadine and products.

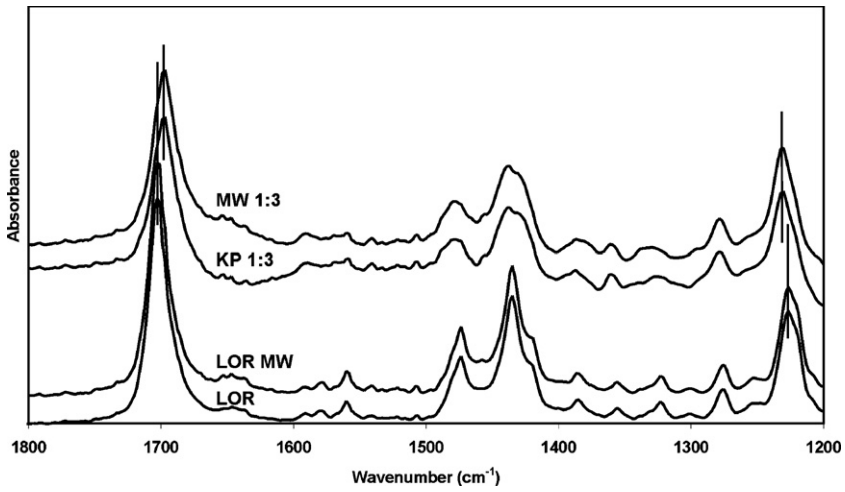

Fig. 5. FT-IR spectra of loratadine products.

surmise, that the –COO group provides the complex-forming bonds and that complex formation alters the hydrogen-bonded cyclic dimer structure of the carboxyl group. In accordance with the DSC finding, DIMEB forms an inclusion complex with LOR.

4. Conclusions

The inclusion complexes of LOR prepared with the application of MW irradiation were compared with those prepared by a conventional method. During the formation of the inclusion complex, hydrogen bonds develop between LOR and DIMEB, and the inclusion complex can therefore be regarded as co-crystals [16]. On inclusion complex formation, the solubility and rate of dissolution of LOR were increased. The interactions between the components were demonstrated by thermal analysis and FT-IR studies. The results were similar for the materials made by the two different preparation methods, but MW irradiation has several advantages. The drying time is substantially shorter, and on an industrial scale up it is therefore simpler to handle the greater quantities, and the MW irradiation method can accelerate the preparation of preclinical samples from poorly water-soluble drugs and CDs. MW irradiation treatment has proved to be a suitable technique for the preparation of CD inclusion complexes, yielding products with good bioavailability.

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III.

Polyvinylpyrolidone as Carrier to Prepare Solid Disperssion - pros and Cons

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Enhancement of the solubility of poorly-soluble drug substances is one of the most important tasks in pharmaceutical technology. It is possible to solve this problem with new drug carrier systems and/or with new technological processes. The solid dispersion technology is generally accepted technique which is able to enhance the dissolution characteristics of drugs with poor water solubility. For this purpose, the drug substance is dispersed in a water-soluble inert polymer matrix; at the higher surface area, due to the presence of the polymer, sometimes the drug solubility and dissolution rate may increase. This work summarizes the theoretical basics of solid dispersion technology and introduces pros and cons of studies using polyvinylpyrrolidone (PVP) as a carrier in the cases of two chosen active pharmaceutical ingredients (APIs), niflumic acid (NIF) and loratadine (LOR). Wettability properties and dissolution were characterized. The drug-polymer interaction was investigated by using differential scanning calorimetry, and powder X-ray diffraction. Even though the instrumental examinations show similar results for both drugs, the dissolution rate improved only by NIF significantly. The explanation for this phenomena could be sought in the different chemical structure and the strength of the connection between the PVP and the drugs

Keywords: polyvinylpyrrolidone, niflumic acid, loratadine, solid dispersion, DSC, X-ray, dissolution

Drug absorption from a solid dosage form after oral administration depends on the release of the drug product, the dissolution or solubilization of the drug under physiological conditions, and the permeability across the gastrointestinal tract. On the basis of their solubility and permeability, drugs can be divided into high/low solubility-permeability classes (biopharmaceutical classification system, BCS). If a drug candidate has reasonable membrane permeability then often the rate-limiting process of absorption is the drug dissolution step [1, 2]. To focus on formulation of classes II API, can also be used to develop a more optimised dosage form based on fundamental mechanistic features. Therefore there is a special importance of solid dispersion technology, too.

NIF (fig. 1.), an anthranilic acid derivative, is a frequently used anti-inflammatory drug, which also has a weak analgesic effect. It is primarily used to treat different forms of rheumatism, e.g. rheumatoid arthritis and arthrosis, and to decrease other inflammatory phenomena. It has some side-effects, such as nausea or vomiting [3]. According to the BCS, NIF can be considered a class II compound, i.e. a water-insoluble, lipophilic and highly permeable drug [4]. Since NIF is also widely prescribed for mild illnesses, the safety aspect becomes more and more important and efforts should be made to optimise the overall drug pharmacological profile.

LOR (fig. 1.) is a second generation of antihistaminic active substance. Loratadine is a long-acting antihistamine that blocks the actions of histamine that causes some of the symptoms of allergic reactions. It is one of a few antihistamines that doesn't cause sedation. Headache, fatigue, dry mouth, thirst, dry nose or hoarseness may occur as side effects. It belongs to the second group of the BCS, too. The solubility depends on the *p*H of the gastrointestinal liquid [5], so the bioavailability is very variable.

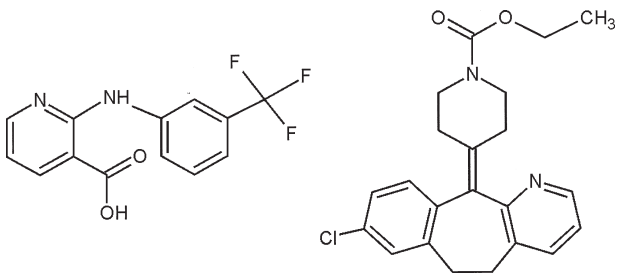

Fig. 1
Chemical structure of NIF and LOR

The concept of preparation of solid dispersions to improve the dissolution rate of sparingly soluble drugs has been widely explored since 1961 [6]. The drug-carrier interactions were classified into six representative structures: simple eutectic mixtures; solid solutions; glass solutions and glass suspensions; amorphous precipitations in a crystalline carrier; compound or complex formation; or combinations of these [7].

The frequently used procedures to prepare solid dispersions are the melting and solvent techniques. By the hot melt method the thermostability of the drug limits the application of this method. With the discovery of the solvent method, many of the problems associated with the melting method were solved. Those polymers that could not be utilized for the melting method because their high melting range (e.g. PVP) could now be considered as carrier possibilities [6, 8]. Finding the suitable drug carrier, the wettability of the product will be increased, and the better microenvironment conducives to dissolving of API [9, 10, 11, 12].

PVP (fig. 2.) is an amorphous carrier and it is suitable for formulate solid solutions, glass solutions and amorphous precipitations with a crystalline API. The glass transition

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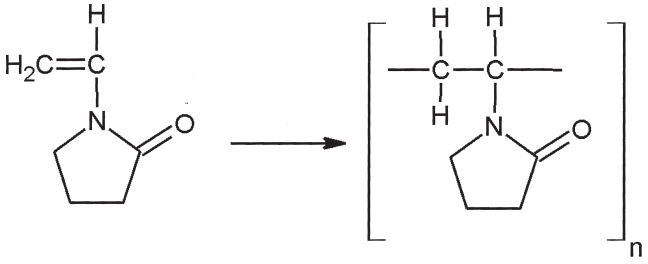

Fig. 2. Structure of PVP

temperature (T_g) is high and for this reason PVPs have only limited applications for the preparation of solid dispersions by the hot-melt method. Due to their good solubility in a wide variety of organic solvents, they are particularly suitable for the preparation of solid dispersions by the solvent method [13]. The aim of the preparation of PVP solid dispersions is generally to transform the drug into the amorphous form and thus to achieve faster dissolution. Dispersions containing crystalline areas exhibit biphasic release profiles, with the amorphous areas dissolving quickly and the crystalline areas more slowly. For example, for a crystalline drug with a very poor aqueous solubility, the formation of an amorphous mixture with a water-soluble additive can provide an opportunity to enhance the dissolution and perhaps bioavailability [14].

In the cases of both drugs cyclodextrin complexation was used to improve the solubility and dissolution rate [15, 16], which was good enough by LOR, but more less in case of NIF. Other possibility is the solid dispersion technology. However this paper will show the interactions between PVP and the APIs, which have different chemical properties and variant behaviour in the gastrointestinal liquids.

Experimental part

Materials

Niflumic acid: 2-[[3-(trifluoromethyl)phenyl]amino]-3-pyridine-carboxylic acid (G. Richter Pharmaceutical Factory, Hungary); Loratadine: ethyl-4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-1-piperidinecarboxylate (TEVA Hungary Ltd.); polyvinylpyrrolidone (PVP): Plasdone K-25 (M_w ~ 34000, T_g ~ 160 °C), (C/o ISP Customer Service GmbH, Germany). Other chemicals: methanol and acetone (Reanal Co., Budapest, Hungary).

Methods

Preparation of the samples

The solid dispersions of NIF in PVP K-25 containing four different ratios (1:1, 1:2, 1:4 and 1:6 w/w) were prepared by the solvent method (**NIF SD**). To a solution of NIF (1.0 g) in 30 mL of acetone, the appropriate amount of PVP K-25 was added. The minimum amount of methanol was added to solubilize the polymer. The solvents were then removed by evaporation under reduced pressure at 30°C and the residue was dried under vacuum at room temperature for 3 h [17]. The abbreviation SD refers the solid dispersion product by the solvent evaporation method.

The solid dispersions of LOR were prepared with the same ratios (**LOR SD**). LOR and PVP K-25 were solved in methanol and the solvent was removed by vacuum dryer during 6 h.

The physical mixtures (**PMs**) by both of the drugs as a control were mixed according to the ratios. All of the products were pulverized in a mortar and sieved through a 100μm sieve.

Method of DSC

The DSC measurements were performed by a Mettler Toledo DSC 821^e thermal analysis system with the STAR^e thermal analysis program V6.0 (Mettler Inc., Schwerzenbach, Switzerland). Approximately 2–5 mg of pure drug or product was examined in the temperature range between 25 °C and 300 °C. The heating rate was 5 °C min^{-1}. Argon was used as carrier gas, at a flow rate of 10 L/h.

Hot-stage microscopy (HSM)

Microscopic observations of morphological features and their changes during heating were carried out with a LEICA Thermomicroscope (LEICA MZ 6, Germany). The samples were observed under the microscope by using a scanning speed of 2°C/min. The magnification in the photographs was 59.7x.

X-ray powder diffraction (XRPD)

The physical state of the NIF and LOR in the different samples was evaluated by XRPD. Diffraction patterns were obtained on a Philips PW 1710 diffractometer, where the tube anode was Cu with Kα = 1.54242 Å. The pattern was collected with a tube voltage of 50 kV and 40 mA of tube current in step scan mode (step size 0.035, counting time 1 s per step).

Study of contact angles

The OCA Contact Angle System (Dataphysics OCA 20, Dataphysics Inc., GmbH, Germany) was uSDd for studies of the wettability of NIF, LOR and their products. 0.15 g of material was compressed under a pressure of 1 ton by a Specac hydraulic press (Specac Inc., USA). The wetting angles of the pressings were determined after 4.3 μL of distilled water was dropped onto the surface of the pressings. The change in the wetting angle was registered from 1 to 25 s (minimum of 5 parallel times), using the circle fitting method of the OCA System.

Methods of *in vitro* dissolution

The modified paddle method with the USP dissolution apparatus (Erweka Type DT, Germany) was used to examine 200 mg samples of pure NIF, LOR or products containing 200 mg of drugs in 100 mL of simulated gastric medium (SGM) (*p*H = 1.1 ± 0.1; 94.00 g of 1 M HCl, 0.35 g of NaCl, and 0.50 g of glycine to 1000 mL with distilled water), simulated intestinal medium (SIM) (*p*H= 7.0 ± 0.1; 14.4 g of $Na_2HPO_4 \cdot 2H_2O$, and 7.1 g of KH_2PO_4 to 1000 mL with distilled water). The paddle was rotated at 100 rpm and sampling was performed up to 120 min (sample volume 5.0 mL). After filtration and dilution, the drug content of the samples were determined spectrophotometrically (SGM_{NIF} = 256 nm, SIM_{NIF} = 288 nm and SGM_{LOR} = 276 nm, SIM_{LOR} = 248 nm). Previously the wavelengths of maximum absorption depending on *p*H were determined by calibration in the concentration-range between 0 and 28 μg/mL with 3 parallel measurements.

Results and discussion

Results of thermalanalysis

Figure 3. shows the DSC curves of NIF, LOR, PVP K-25 and their products. NIF gave a melting endotherm at 201.95 °C, which can be identified from the literature data as its melting point. The onset of melting was observed at 201.15 °C and the end at 203.81°C; the normalized enthalpy was 127.11 J g^{-1}. After the melting of NIF, the total mass of the investigated sample was progressively lost, with an

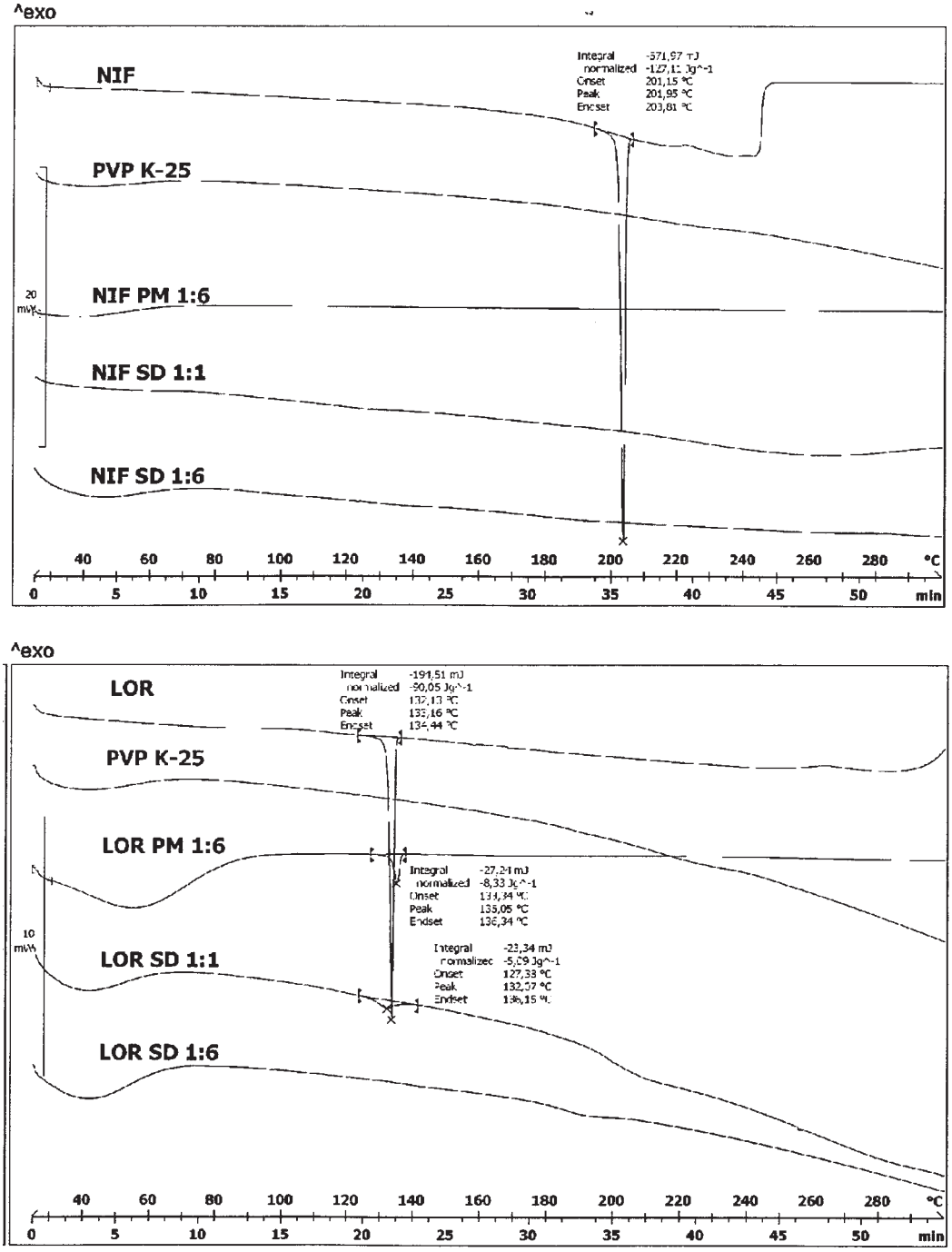

Fig. 3
DSC curves of APIs and products

endotherm at about 250°C relating to its decomposition. LOR has lower melting point at 133.16°C with 132.13°C onset and 134.44 °C endset and the normalized enthalpy was 90.05 J g^{-1}. The DSC curves did not reveal a melting peak for NIF in any of the solid dispersions and reference PMs. This may be due to the interaction between NIF and PVP in these systems. In contrast with the XRPD patterns of the NIF PMs (without any amorphous character of NIF), the melting point of the drug was not observed on the DSC curve. This indicated a NIF:PVP solid-state interaction induced by heating. However all curve of LOR PMs presented a little shifted endotherm peak at around 133°C. In the cases of LOR SDs this phenomena wasn't detected, so they referred amorphous character of the drug, except the 1:1 product.

To visualize the changes in the NIF and LOR PM samples during heating, HSM was used. This technique is complementary to DSC and may facilitate the interpretation of the DSC results. With increase of temperature, the PVP began to melt, and above 150 °C its melting was complete. It may be seen that the NIF particles were dispersed in the melt and subsequently dissolved with rising temperature. During heating, the drug particle size was steadily reduced, showing its dissolution in the PVP melt. The NIF was completely dissolved in the melt of PVP at close to 157°C, a temperature about 50 °C lower than the melting point of the pure drug. This finding can explain the absence of any sign of melting in the DSC thermograms of the PM. But this phenomena wasn't detected by LOR PMs, because LOR has melted before the T_g of PVP (~160°C), so it has not the possibility to dissolve in the melted PVP.

XRPD study

The XRPD patterns of APIs, PVP K-25, PMs and SDs in 1:6 ratio are shown in figure 4. PVP is an amorphous

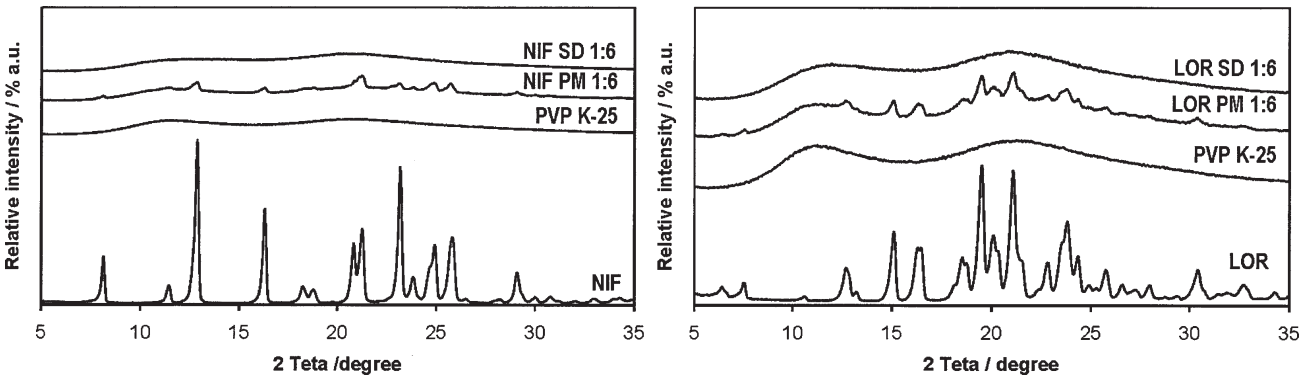

Fig. 4. XRPD patterns of APIs and 1:6 products

powder without crystalline structure. The presence of numerous distinct peaks in the XRPD spectrum indicates that NIF and LOR are crystalline materials. NIF has characteristic peaks at diffraction angles 2θ at 8.18, 12.92, 16.33, 21.30 and 23.21°. In the case of LOR we can see sharp peaks at 15.15, 19.45, 21.11, 23.84, 24.38 and 30.44°. The XRPD peaks of NIF in the PMs K-25 were similar to those for the pure drug, indicating that the crystallinity of NIF was not changed in these products. However, the crystalline structures of NIF in all the solid dispersions were different from that of the pure drug, as revealed by the differences in their XRPD patterns. These patterns were similar to those for the PVPs. The absence of diffraction peaks indicated the presence of NIF in amorphous form. By the LOR PMs the characteristics reflections can be identified well. As the DSC curve of LOR SDs shown the possibility of amorphous character, we saw this phenomenon on XRPD pattern too, except of LOR SD 1:1, where the PVP concentration was the smallest, so it was not enough to inhibit the crystallization of LOR. Figure 4 presents that PVP K-25 and LOR SD 1:6 are amorphous. The intensity of amorphous ring (by 22 degree) are the same by these samples, but the reflection (by 11 degree) of LOR SD 1:6 is half of the pure PVP.

Studies of wettability and dissolution tests

The contact angles at 5 s for NIF, LOR and their SDs were evaluated. The contact angle for NIF was 71.1° and for LOR was 73.1 °, i.e. they are very hydrophobic drugs. The contact angles of the investigated products were in all cases decreased. The investigation of the systems produced by NIF SDs revealed that, with increasing PVP content, the contact wetting angles increased. However in the cases of LOR SDs with increasing PVP content, the contact wetting angles were similar to each other. The NIF SD 1:1 (7.5°) and LOR SD 1:6 (38.04 °) gave the best results. Based on these results we can conclude that a better expected wettability may will resulted in better dissolution (table I.).

Solubility characteristics of NIF and LOR are different in the gastrointestinal media, so we investigated their dissolution in that media in which the API has worse solubility. Unfortunately both APIs are absorbed from that part of gastrointestinal tract, in which they don't dissolve

Table 1
THE CONTACT ANGLES OF NIF, LOR AND THEIR PRODUCTS

Table I: The wetting angles of NIF, LOR and their products				
	Teta (°) ± SD			
NIF	71,1 ± 0,2			
LOR	73,1 ± 7,0			
	1:1	**1:2**	**1:4**	**1:6**
NIF SD	7.5 ± 3.4	36.8 ± 6.4	44.6 ± 2.0	49.0 ± 2.3
LOR SD	– *	– *	43.0 ± 1.6	38.0 ± 1.5

* impossible to prepare pressings

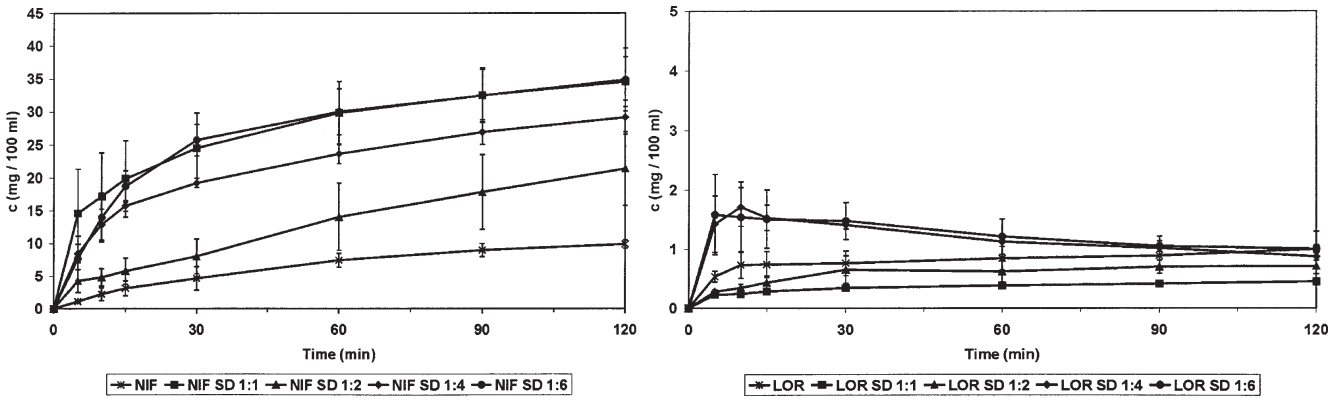

Fig. 5. Dissolution of NIF and its products in SGM and of LOR and its products in SIM

well. The dissolution from these media could improve by technological methods. Figure 5 presents the dissolution results of drugs and products from their adequate "absorption media". Nearly 3,5 times better dissolution was detected in the case of NIF SDs in SGM and in addition the dissolution of NIF SD in SIM was complete and it means that after the increased dissolution from gastric medium we could reach quantitative absorption from the intestine. But LOR SDs did not give much better result. The dissolution didn't change in SIM, where 1:1 and 1:2 ratios worsened the dissolution compared with raw drug. In the case of 1:4 and 1:6 product the initial appearance of increased dissolution rate can be explained by the fact the fact that amorphous drug dissolves faster than the crystalline LOR, and after the rapid dissolution the API recrystallizes.

This might be due to the surface tension-lowering effect of PVP, resulting in wetting of the hydrophobic crystalline surface [18]. This should retard any agglomeration or aggregation of the particles, which can slow the dissolution process. Differences were observed according to the investigated APIs. In the case of LOR significant dissolution increasing was detected in neither media.

Conclusion

In consequence of the poor water-solubility of the pharmaceutical ingredients, NIF and LOR, our aim was to increase their solubility and dissolution rate to form solid dispersions. PVP K-25 was used to reach amorphous drugs. It could be interpreted that the solvent evaporation method not always resulted in products with excellent dissolution. However the amorphisation was successful in the cases of both drugs, the increasing of dissolution was reached only by NIF. The PVP presents an apolar medium for LOR, and as LOR is a lipophil drug, it rather likes to stay in an apolar medium, than to go to the hydrophilic, aqueous medium. And in the case of LOR we need to optimise the amount of PVP too, because too much PVP inhibits the dissolution of API and too less isn't enough to increase the dissolution.

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IV.

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ORIGINAL ARTICLE

Achievement of pH-independence of poorly-soluble, ionizable loratadine by inclusion complex formation with dimethyl-β-cyclodextrin

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Abstract A tricyclic, piperidine derivative of antihistamines, loratadine, which belongs in class II of the Biopharmaceutical Classification System, was investigated. It is an ionizable drug, whose solubility depends on the gastrointestinal pH, and the bioavailability is therefore very variable. The aim of this work was to enhance the dissolution and make the solubility of loratadine independent of pH. Inclusion complexes were prepared between loratadine and dimethyl-β-cyclodextrin in two different molar ratios by three techniques (physical mixing, kneading and spray-drying). The formation and physicochemical properties of the inclusion complexes were investigated by means of dissolution tests, thermal analysis and Fourier Transform Infrared spectroscopy. The instrumental examinations proved the presence of partial or total complexes depending on the preparation method and molar ratio, which resulted in better dissolution. For some compositions and preparation methods, the application of this cyclodextrin made the solubility of loratadine independent of pH.

Keywords Loratadine · DIMEB · pH-independent solubility · Thermal analysis · FT-IR · Co-crystal

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Introduction

It is estimated that 40% or more of active pharmaceutical ingredients (APIs) identified through combinatorial screening programs are poorly soluble in water [1]. The bioavailability of pharmacons is determined by their solubility and permeability. All drugs must possess some degree of aqueous solubility in order to be pharmacologically active, and they need to be lipophilic to be able to permeate biological membranes [2]. The rate-limiting step of oral absorption is the dissolution by APIs with solubility <0.1 mg/mL, e.g. drugs belonging in classes II and IV of the Biopharmaceutical Classification System (BCS) [3, 4]. The solubility of ionizable compounds varies with the pH of the gastrointestinal juices, depending on their pK_a [5]. The pH of the gastrointestinal fluids is therefore one of the most important factors influencing on the saturation solubility of ionizable drugs [6]. As the pharmacon proceeds along the gastrointestinal tract, it passes into a medium with somewhat higher pH. During 90% of a fasting state, the gastric pH is <3 [7]. The presence of food in the stomach can influence the release, dissolution and gastroduodenal transport of a drug [8]. As the gastrointestinal pH can vary widely, the rate of dissolution of an ionizable pharmacon will also vary considerably. Only the dissolved drug is capable of absorption, so its bioavailability, and hence the pharmacokinetic parameters will be very variable [9].

The investigated API was loratadine (LOR), a second-generation antihistamine marketed for its non-sedating properties. H_1 antihistamines are applied in the treatment of allergies: they prevent symptoms such as itching, congestion, rhinorrhoea, tearing and sneezing [10]. LOR belongs in class II of the BCS [11]. LOR is a weak base; its pK_a value at 25 °C has been reported as 4.85–6.00 [10–14]. The

solubility of bases increases with decreasing pH at pH values less than the pK_a [5]. At lower pH values, LOR, which is a nitrogen base, is protonated, and therefore becomes more soluble in water [12]. According to the modified Hendersson-Hasselbach equation [15], bases are totally ionized at lower pH values, and at ~pH 7 and higher they are totally unionized, which is the form able to absorb, so LOR will probably absorb from the intestines, in which it has poor solubility.

There are several possibilities via which to increase the solubility of such APIs: the particle size reduction of fenofibrate resulted in a nanodispersion, and enhanced its bioavailability; and formation of an appropriate indinavir salt improved the stability and the aqueous solubility of the compound [16]. Complexation with cyclodextrins (CDs) is another method applied to improve the aqueous solubility of drugs [17–20]. The chemical structures of the CDs, their high molecular weights and their very low octanol/water partition coefficients are all characteristics of compounds that do not readily permeate biological membranes [21, 22]. However, the methylated β-CDs are able to permeate and enhance drug delivery through biological membranes [23]. The effects of CDs on oral drug absorption can be explained in the context of the BCS [24]. The permeation of BCS class II drugs through the aqueous diffusion layer is slow due to their low aqueous solubility. Water-soluble CD complexes of these drugs exhibit enhanced diffusion to the mucosal surface, leading to enhanced oral bioavailability [2].

The primary aim of the present work was to improve the solubility and dissolution rate of LOR, and to make the solubility independent of pH, thereby enhancing the bioavailability. We therefore prepared various CD inclusion complexes of LOR in different molar ratios by three preparation methods. The investigations of the inclusion complexes included thermal analysis, Fourier-Transform Infrared (FT-IR) spectrometry, solubility and dissolution tests.

Materials and methods

Materials

α-CD, β-CD, γ-CD, randomly methylated-β-CD (RAMEB), 2-hydroxypropyl-β-CD (HPBCD), methyl-β-CD, hydroxybutyl-β-CD and heptakis-(2,6-di-O-methyl)-β-cyclodextrin (DIMEB) were purchased from Cyclolab Ltd. (Budapest, Hungary), sulfobutyl-ether-β-CD (Captisol) originated from CyDex Pharmaceuticals Inc. (Lenexa, USA). LOR (ethyl 4-(8-chloro-5,6-dihydro-11H-benzo-[5, 6]-cyclohepta[1,2-b]pyridin-11-ylidine)-1-piperidine-carboxylate) was kindly provided by TEVA Pharmaceutical Industries Ltd. (Hungary). Other chemicals were of analytical reagent grade purity.

Methods

Preliminary experiments

The effects of the various CD derivatives on the solubility of LOR were investigated. 20 mg LOR and 200 mg of the CD derivatives were suspended in 20 mL of distilled water. The mixture was stirred at 10 min with a magnetic stirrer and then filtered, and after suitable dilution the UV spectrum was recorded in the range 220–300 nm.

Phase-solubility studies

The phase-solubility diagrams were recorded by the Higuchi-Connors method [3]. For this purpose aqueous solutions of CDs of various concentrations were prepared at a specific pH value (7.5) (250 mL of 0.2 M KH_2PO_4, 204 mL of 0.1 M NaOH made up to 1,000 mL with distilled water). 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 ($\lambda = 248$ nm). The presence of the CD did not disturb the spectrophotometric assay. Each experiment was performed in triplicate.

Preparation of the products

DIMEB proved to demonstrate the best enhancement of the solubility (see Sect. "Preliminary experiments"). The products were prepared in two molar ratios (LOR:DIMEB = 1:1 and 1:2) by three methods: physical mixing, kneading and spray-drying. *Physical mixtures* (*PMs*): LOR was mixed carefully in a mortar with the calculated amount of CD. *Kneaded products* (*KPs*): 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. *Spray-dried products* (*SDs*): the physical mixtures were dissolved in 50% ethanol, and SDs were obtained by using a Büchi Mini Dryer B-191 (BÜCHI Labortechnik AG, Flawil, Switzerland), at an inlet temperature of 105 °C, with a compressed air flow of 800 L/h and a nozzle diameter of 0.5 mm. The aspirator rate was 75–80%, and the pump rate was 5–10%. All of the samples were sieved (100 μm) and stored at room temperature under normal conditions.

In vitro dissolution studies

The modified paddle method with the USP dissolution apparatus (Erweka Type DT, Germany) was used to

examine 200 mg samples of pure LOR or products containing 200 mg of LOR in 100 mL of simulated gastric medium (SGM) (pH = 1.1 ± 0.1; 94.00 g of 1 M HCl, 0.35 g of NaCl, and 0.50 g of glycine made up to 1,000 mL with distilled water) or simulated intestinal medium (SIM) (pH = 7.0 ± 0.1; 14.4 g of $Na_2HPO_4 \cdot 2H_2O$ and 7.1 g of KH_2PO_4 made up to 1,000 mL with distilled water). The paddle was rotated at 100 rpm and sampling was performed up to 120 min (sample volume 5.0 mL). After filtration and dilution, the LOR contents of the samples were determined spectrophotometrically (λ_{SGM} = 276 nm, λ_{SIM} = 248 nm).

DSC

The DSC records were obtained with a Mettler Toledo DSC 821[e] (Mettler Inc., Schwerzenbach, Switzerland) apparatus. Between 2 and 5 mg of sample was measured in a standard aluminium pan (40 µL) and heated from 25 to 300 °C at a heating rate of 5 °C/min under a constant purge of argon at 10 L/h.

FT-IR

Samples with a LOR content of 0.5 mg were ground and mixed with 150 mg of dry KBr in an agate mortar, and the mixture was then compressed into a disc at 10 t. Each disc was scanned 64 times at a resolution of 4 cm^{-1} over the wave number region 4,000–400 cm^{-1} with an FT-IR spectrometer (Thermo Nicolet AVATAR 330, USA). The evaluation was carried out with the GRAMS/AI Ver. 7 program.

Study the effect of pH on the solubility

Seven buffer solutions were prepared with different pH values between 1.2 and 7.5 [11]. The defined daily dose of LOR is 10 mg, so 10 mg of LOR or the selected 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.

Results and discussion

Preliminary experiments

The results were compared with the data on the CD-free system (see Table 1). The best solubility enhancement was achieved with DIMEB, which resulted in an ~300-fold increase in solubility, and accordingly this derivative was used in the further examinations.

Table 1 Effect on solubility enhancement of CD derivatives

	c (µg/mL)	Solubility enhancement (-fold)
LOR	2.43	1.00
+ α-CD	23.69	9.75
+ γ-CD	34.73	14.29
+ Captisol[a]	80.05	32.94
+ HP-β-CD[b]	128.14	52.73
+ H-Bu-β-CD[c]	181.69	74.77
+ β-CD	212.02	87.25
+ RAMEB[d]	496.89	204.48
+ Me-β-CD[e]	592.62	243.88
+ DIMEB	**730.87**	**300.77**

[a] Sulfobutyl-ether-β-CD
[b] 2-Hydroxypropyl-β-CD
[c] Hydroxybutyl-β-CD
[d] Random methylated-β-CD
[e] Methyl-β-CD

Phase-solubility studies

A relevant diagram is shown in Fig. 1, the solid line indicating the best linear regression fit of the experimental data. Higuchi and Connors [3] defined two main types of diagrams. In general, type A is characteristic for the water-soluble CD derivatives, while type B is observed for the less-soluble natural CDs. In type A, the solubility of the drug increases with increasing CD concentration. B-type phase-solubility profiles reflect the formation of complexes with limited solubility in aqueous medium. Type A has three subtypes (A_N, A_L and A_P). The subtype of the present diagram is A_L. The most common type of CD complexes are the 1:1 drug:CD complexes. However, a slope of <1 for a type A_L diagram does not necessarily indicate that only a 1:1 complex is formed, though this is a common assumption. The stability constant ($K_{1:1}$) of the complex can be calculated from the slope and the intrinsic solubility of the drug in the aqueous medium (see Eq. 1). In the absence of

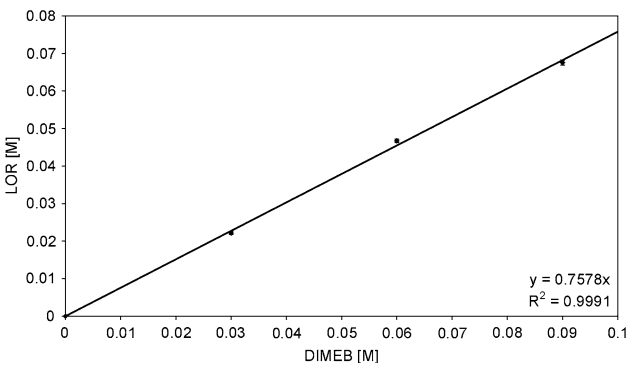

Fig. 1 Phase-solubility diagram of LOR at pH 7.5

DIMEB, the equilibrium water solubility of LOR (S_0) was determined to be 0.81 ± 0.14 mg/L. For LOR the $K_{1:1}$ value is very large: 1.48×10^6 M^{-1}. From linear regression, r^2 is 0.9991.

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

In vitro dissolution studies

According to the pK_a value the solubility of LOR depends on the pH: it exhibits good dissolution in acidic medium (e.g. SGM), but dissolves poorly in alkaline medium (e.g. SIM). The presence of DIMEB did not alter the good solubility in SGM: the total investigated amount of the sample dissolved in the first 5–10 min, independently of the preparation method and the composition. As concerns the dissolution in SIM, the rate of dissolution was improved for all of the products, but the extent of this increase depended on the preparation method and the molar ratio. For the 1:1 compositions (Fig. 2), none of the preparation methods resulted in 100% dissolution. The lowest solubility enhancement was observed for the PM (8.7-fold), as expected, because this mode of preparation does not generally result in a complex. The KP furnished a 67.6-fold solubility increase, but the best enhancement was achieved with the SD (142.9-fold). For the 1:2 preparations (Fig. 3) the PM displayed similar results as for the 1:1 product, with only a slight further improvement in solubility (10.26-fold). For the KP and SD 1:2 products, the whole of the investigated samples dissolved in the first 15 min, i.e. the dissolution in SIM was as good as in SGM, which means that the same good dissolution would be attained at the extreme pH values of the gastrointestinal tract on the use of these DIMEB products. Accordingly, the rate-limiting step of absorption would not be the dissolution: the permeability would regulate the passage through the membrane. As LOR has good permeability, the application of LOR complexed with a CD such as DIMEB would lead to a greater quantity of drug being absorbed, so that better bioavailability would be obtained.

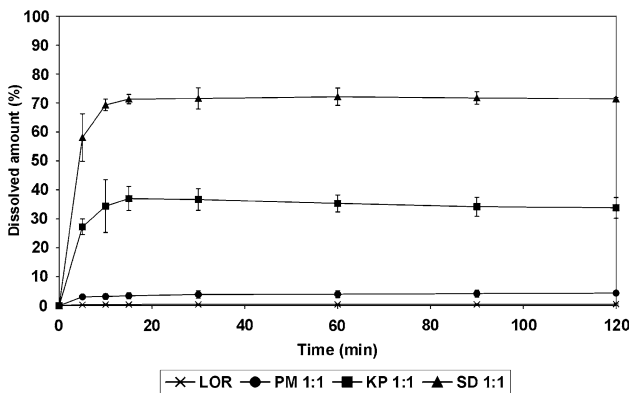

Fig. 2 Dissolution of 1:1 products in SIM

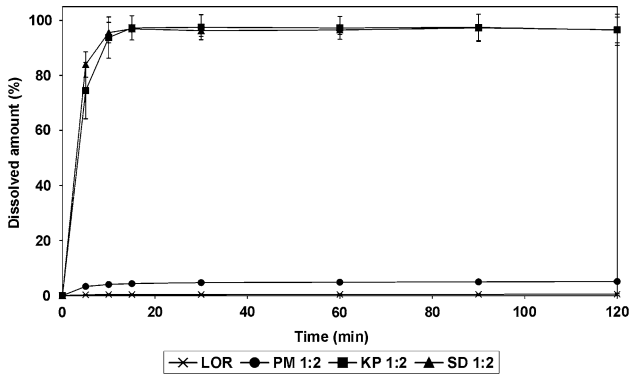

Fig. 3 Dissolution of 1:2 products in SIM

DSC results

The sharp, narrow endothermic peak in the DSC spectrum of LOR (peak 133.16 °C, normalized melting enthalpy 89.48 J g^{-1}) denotes the melting point of the material. The stability of LOR was not affected (no degradation was observed) up to 300 °C. Our DIMEB was amorphous, and there was no thermoanalytical indication at the melting point of LOR, but there was an exothermic peak reflecting the recrystallization of DIMEB at 181 °C. Above 320 °C, a broad endothermic peak was observed, associated with decomposition of the material. The results for the 1:2 compositions are presented in Fig. 4. For the PM, the melting point of LOR was seen several degrees lower than for pure LOR (this is characteristic for CD complexes) and the area under the peak was proportional to the amount of LOR in the sample. Hence, no inclusion complexes were formed in the PM product. For the KP and SD samples, no endotherm reflected the melting point of LOR. Accordingly, the KP sample exhibited total inclusion complexation, but

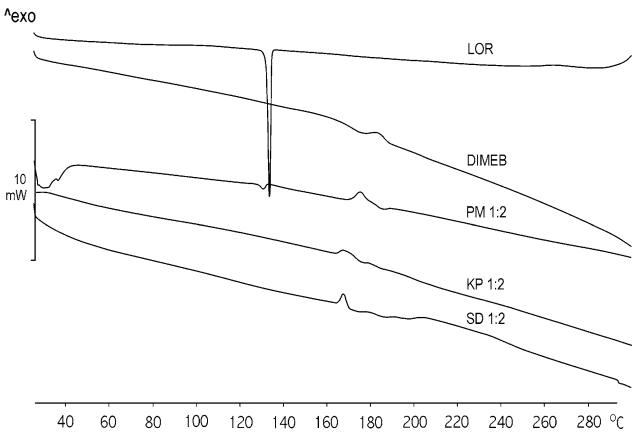

Fig. 4 DSC curves of LOR and 1:2 products

for the SD sample it is possible that the product was amorphized or that total complexation occurred during the preparation.

FT-IR studies

The spectral changes were evaluated by subtraction of the spectrum of DIMEB from the spectra of the samples. The spectrum of LOR and the calculated subtraction spectra of the 1:2 products are presented in Fig. 5. The spectra of the products involving different molar ratios and preparation methods did not differ appreciably. The difference spectrum of the PM product was practically identical to the spectrum of pure LOR, indicating negligible interaction between LOR and DIMEB. For the KP and SD samples, the characteristic C=O stretching frequency (1,702 cm^{-1}) was shifted to lower wave numbers, and the typical C–O stretching at 1,227 cm^{-1} was shifted to higher range. In accordance with the DSC finding, in the KP 1:2 product, total complexation occurred, and FT-IR also revealed total complexation for the SD sample. These results lead us to surmise 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. During the formation of the inclusion complex, hydrogen-bonds develop between LOR and DIMEB, and the inclusion complex can therefore be regarded as co-crystals [25]. A lipophilic part of LOR will probably be attached to the inner surface of DIMEB, like the aromatic rings, but in the FT-IR spectrum of LOR, the characteristic stretching frequencies of these aromatic parts are masked by DIMEB, so these connections can not be detected with this method.

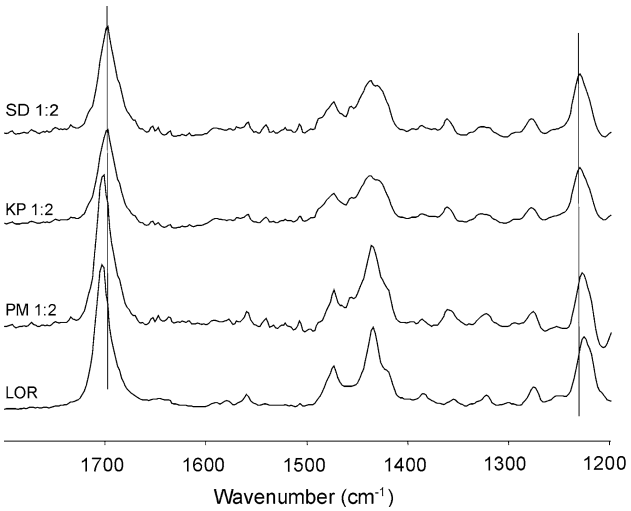

Fig. 5 FT-IR spectras of LOR and 1:2 products

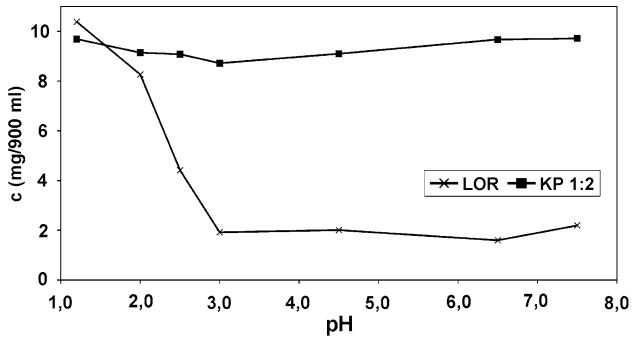

Fig. 6 pH-dependence of LOR and KP 1:2 product

Study of the effect of pH on the solubility

The defined daily dose of LOR is 10 mg. In view of the results of the preliminary studies, we chose DIMEB to examine how the solubility of LOR from the LOR:DIMEB 1:2 products depends on the pH. The PM products exhibited only 2–6% dissolution; both DSC and FT-IR proved that this preparation method did not result in inclusion complexes. The KP and SD methods gave products which displayed similar results in the dissolution tests. However, the KP method is simpler and easier to scale up than the SD method, so our choice was the KP 1:2 product. We then investigated the solubility of 10 mg of pure LOR and of the KP 1:2 product containing 10 mg of LOR in the different buffer solutions. The solubility of LOR has been reported to decrease with increasing pH [11]. As can be seen in Fig 6, the applied dose of pure LOR did not dissolve at the pH of intestines, from where it is absorbed. In contrast, virtually the whole quantity of LOR dissolved from the KP 1:2 product at each pH value. This clearly suggests an opportunity to ensure smooth dissolution for LOR, thereby achieving better and more uniform bioavailability.

Conclusions

CDs are capable of improving the solubility and dissolution of poorly water-soluble drugs such as LOR. Depending on the preparation method and molar ratio the solubility of LOR was enhanced ~200-fold. The DSC and FT-IR results demonstrated that DIMEB forms total inclusion complexes with LOR through hydrogen-bonding with the carboxyl group.

The solubility of LOR was made independent of the pH within the range of gastrointestinal pH through the application of DIMEB at a LOR:DIMEB composition of 1:2 with preparation by KP methods, so that the bioavailability will probably be better and smoother. For this purpose, we would like to carry out in vivo studies.

Acknowledgements This work was performed with the support of a Sanofi-Aventis Fellowship.

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Physico-chemical characterization and *in vitro*/*in vivo* evaluation of loratadine:dimethyl-β-cyclodextrin inclusion complexes

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ABSTRACT

A tricyclic, piperidine derivative of antihistamines, loratadine, which belongs in class II of the Biopharmaceutical Classification System, was investigated. It is an ionizable drug, whose solubility depends on the gastrointestinal pH, and the bioavailability is therefore very variable. Inclusion complexes were prepared by kneading method, containing loratadine (LOR) and dimethyl-β-cyclodextrin (DIMEB) in two different molar ratios in an attempt to achieve better dissolution and therefore the better bioavailability of loratadine. The formation and physicochemical properties of the inclusion complexes were investigated by means of dissolution tests, pH-dependent solubility studies, electrospray ionization mass spectrometry and diffusion-ordered ^{1}H NMR spectroscopy. The *in vivo* efficiency of the complexes was examined in rat animal experiments to confirm the better *in vitro* dissolution. The instrumental examinations proved the presence of total complexes in 1:1 ratio in both compositions. However, the *in vitro* pH-dependent solubility results, the *in vivo* blood levels and the greater pharmacological effect prove that excess DIMEB is needed to achieve the pH-independent and complete solubility of LOR, and therefore better and more consistent bioavailability.

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1. Introduction

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 [1]. These two factors form the basis of the Biopharmaceutical Classification System (BCS) [2]. If the drug is an ionizable compound, the intestinal absorption depends on its pKa, too [3]. Poorly water-soluble drugs (BCS classes II and IV) are associated with slow drug absorption, leading eventually to inadequate and variable bioavailability (BA) [2,4].

Loratadine (LOR), chosen for the current studies, is a BCS class II drug [5], with dissolution- or solubility-limited absorption. It is a second-generation antihistamine. LOR is a weak base; its pKa value at 25 °C has been reported to be 4.85–6.00 [5–8]. The solubility of bases increases with decreasing pH at pH values less than the pKa [9]. However, according to the modified Hendersson–Hasselbach equation [10], bases are totally ionized at lower pH values, and at ~pH 7 and higher they are totally non-ionized, which is the form able to be absorbed. Thus, LOR will probably be absorbed from the intestines, in which it exhibits poor solubility.

The main objective of the present study was to investigate the stoichiometry of two inclusion complexes (kneaded products (KPs) 1:1 and 1:2) and the anti-inflammatory effects of LOR and these complexes *in vitro* (Parallel Artificial Membrane Permeability Assay – PAMPA model) and *in vivo* (rat). Our earlier studies [11] revealed that complexation with dimethyl-β-cyclodextrin (DIMEB) (at some compositions) resulted in pH-independent solubility for LOR; at the pH, where LOR is totally non-ionized and hence able to be absorbed, the complexed LOR dissolves about 10 times better than in the absence of DIMEB. The aim of this study was to confirm this observation *in vivo*. Many studies have been published on the pharmaceutical applications of cyclodextrins (CDs) and primarily on their ability to improve the solubility and dissolution rate of poorly soluble drugs [12,13].

Electrospray ionization mass spectrometry (ESI-MS) and diffusion-ordered ^{1}H NMR spectroscopy (DOSY) provide powerful techniques to analyse the stoichiometry of supramolecular complexes like CD inclusion complexes [14]. By the PAMPA model the characteristics of passive diffusion can be investigated. Therewith

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performing an *in vivo* experiment *in vitro*/*in vivo* correlation can be predicted.

2. Materials and methods

2.1. Materials

LOR was kindly provided by TEVA Ltd., Hungary. Heptakis-2,6-di-O-methyl-β-CD (DIMEB-50, 50% isomeric purity, DS∼14) was purchased from Cyclolab Ltd., Hungary. Compound 48/80 (N-methyl-4-methoxy-phenethylamine) was supplied by Sigma–Aldrich Logistic GmbH, Germany. Other chemical reagents were of analytical grade purity.

2.2. Preparation of the products

The KPs were prepared in two molar ratios (LOR:DIMEB = 1:1 and 1:2). The physical mixtures were suspended in the same mass of 50% ethanol, and the solvent was evaporated off at room temperature. After drying, the products were ground. All of the samples were sieved (100 μm) and stored at room temperature under normal conditions.

2.3. Diffusion-ordered spectroscopy (DOSY)

Diffusion coefficients (D) were estimated via DOSY NMR experiments. In the DOSY spectra, chemical shifts were located along the F2 axis and D values along the F1 axis. From the D values, it is possible to infer the size of the species and therefore the absolute stoichiometry of the supramolecular complexes.

The NMR spectra were recorded at 25 °C on a Bruker Avance DRX 400 MHz spectrometer. For 2D DOSY ^{1}H NMR, pulsed field-gradient spin-echo NMR measurements were performed by using the stimulated echo and longitudinal eddy current delay (LED) sequence [15]. A time of 1.5 ms was used for the dephasing/refocusing gradient pulse length (δ), and 100 ms for the diffusion delay (Δ). The gradient strength was changed quadratically from 5% to 95% of the maximum value (B-AFPA 10 A gradient amplifier), and the number of steps was 16. Each measurement was run with 32 scans and 16 K time domain points. For the processing, an exponential window function and single zero filling were applied. During the diffusion measurements, the fluctuation of the temperature was <0.1 K. Prior to the NMR scans, all the samples were equilibrated for 30 min. The data were analysed by using XWINNMR 2.5 software.

2.4. Electrospray ionization mass spectrometry (ESI-MS) studies

The compounds were characterized by MS, using a Finnigan MAT 95S sector field mass spectrometer equipped with an electrospray ion source. Positive-ion ESI-MS spectra were obtained. The solutions were prepared in a 1:1 mixture of acetonitrile/water. The solutions were infused directly into the mass spectrometer at a rate of 200 μl min^{-1}. Data were collected for approximately 100 scans. The scan range was 100–3000 m/z. The spectrometer was used at a resolution of ∼1000–1500.

2.5. In vitro dissolution study

The modified paddle method with the USP dissolution apparatus (Erweka Type DT, Germany) was used to examine 200 mg samples of pure LOR or products containing 200 mg of LOR in 100 ml of simulated intestinal medium (SIM) (pH 7.0 ± 0.1; 14.4 g of $Na_2HPO_4{\cdot}2H_2O$ and 7.1 g of KH_2PO_4 made up to 1000 ml with distilled water). 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. At each sampling time, an equal volume of fresh medium was added, and the correction for the cumulative dilution was calculated. After filtration and dilution, the LOR contents of the samples were determined spectrophotometrically (λ_{SIM} = 248 nm).

2.6. Study of the effect of pH on the solubility

Seven buffer solutions were prepared with different pH values between 1.2 and 7.5 [16]. The defined daily dose of LOR is 10 mg, so 10 mg of LOR or a product containing 10 mg of LOR was examined in 900 ml of dissolution medium 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 ($\lambda_{pH\ 1.2,\ 2.0,\ 2.5,\ 3.0}$ = 276 nm; $\lambda_{pH\ 4.5}$ = 244 nm; $\lambda_{pH\ 6.5,\ 7.5}$ = 248 nm).

2.7. PAMPA

PAMPA "sandwiches" were formed from a donor 96-well microtitre plate (Millipore MATRNPS 50) and a matching filter plate (Millipore Multiscreen®-IP, MAIPNTR 10) with an apparent porosity of 0.45 μm, coated with 5 μl of a 1% (w/v) n-dodecane solution of lecithin. The initial donor sample concentrations were about 150 μM. The plate sandwich was allowed to incubate at 25 ± 1 °C for 16 h without stirring, in an atmosphere saturated in humidity. Afterwards, sample concentrations in both the acceptor and donor wells were determined by HPLC. Effective permeability coefficients (P_e) were determined by taking into account the apparent filter porosity and sample mass balance.

The donor (150 μl) and acceptor (300 μl) compartments both contained pH 7.4 buffer solutions. The permeability rates were calculated by using the following equation:

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

with

$$C = \frac{V_D \cdot V_A}{(V_D + V_A) \cdot A \cdot t}$$

where V_D, volume of the donor compartment (ml); V_A, volume of the acceptor compartment (ml); A, active surface area of the membrane (0.24 cm^2 in the case of the applied filter plate); t, incubation time for the assay (s); $[\text{drug}]_{\text{acceptor}}$, concentration of the compound in the acceptor compartment at the completion of the assay; $[\text{drug}]_{\text{donor}}$, concentration of the compound at theoretical equilibrium.

2.8. In vivo experiments

This study was approved by the Committee on Animal Research at the University of Szeged (IV./01758-6/2008). A colony of inbred 150 ± 5 g male Wistar rats (Charles River Laboratories, Germany) was used, fed commercial rodent pellets and tap water. The animals were housed in groups of 5 at a controlled room temperature (22 ± 1 °C) and maintained under an alternating 12 h light/12 h dark cycle (light on at 6:00 am).

The following materials were tested in this study: 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} (1.00 ml kg^{-1}).

The animals were divided into five groups, with 6 rats per group. Group I received the vehicle and served as a control. To study the influence of DIMEB on the oedema, the Group II animals received DIMEB. LOR, KP 1:1 and KP 1:2 were administered to the other three groups, respectively.

In these experiments, at first the animals were treated orally by the above mentioned suspensions. 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 [17,18]. In light isoflurane narcosis the intensity of the arising inflammatory reaction was measured after 30 min with the use of a plethysmometer (Ugo Basile, Harvard Apparatus, Germany) on the basis of the volume difference between the right hind leg treated with compound 48/80 and the left hind leg treated with vehicle (physiological saline). Immediately after measuring the extent of the oedema, blood samples were taken from rats by cardiac puncture and collected in tubes containing sodium ethylenediaminetetraacetate. Then these samples were centrifuged and the obtained plasmas were frozen until the measurement of LOR-content (see below).

A high-performance liquid chromatographic (HPLC) method has been developed for quantitative analyses of LOR in *in vitro* (PAMPA model) and *in vivo* samples.

HPLC measurements were performed with a JASCO PU-1580 binary pump (JASCO Inc., Japan) and a programmable variable UV–visible detector. LOR was chromatographed on a 100 mm × 4.6 mm i.d., 3 μm particle, Phenomenex Luna C8(2), 100 Å analytical column under reversed-phase conditions at 30 °C, protected with a SecurityGuard Cartridge (4.0 mm × 2.0 mm) precolumn. The degassed mobile phase was a 47:42:11 (v/v) mixture of acetonitrile, purified water and a phosphate buffer solution (0.5 M, pH 3.0 ± 0.1, adjusted by the addition of 85% orthophosphoric acid). The flow rate was 1.0 ml min^{-1} and the analyte was monitored at 250 nm.

Calibration plots were constructed by analysis of working solutions (concentrations of 5, 10, 25, 50 and 75 μg ml^{-1} (*in vitro*) and 5, 10, 50, 75 and 100 ng ml^{-1} (*in vivo*)) of LOR in the mobile phase and plotting concentration against peak-area response for each injection. The calibration curves were linear throughout the whole range tested and described by the equations $y = 20.318x - 23{,}775$ ($R^2 = 0.9912$) and $y = 1083.4x + 31.777$ ($R^2 = 0.9957$) for the *in vitro* and *in vivo* measurements, respectively. Unknown samples were quantified by reference to these calibration plots. Inter-day precision was calculated from results on the calibration sample of 5 μg ml^{-1} analysed on 20 consecutive days ($n = 5$). The mean amount found was 4.98 μg ml^{-1} and the RSD value was 2.47%. The limits of detection (LOD) and quantification (LOQ) were determined on the basis of the S.D. of the response (*y*-intercept) and the slope of the calibration plot. LOD and LOQ for LOR were 0.004 and 0.013 μg ml^{-1}, respectively.

500 μl of mobile phase was added to 500 μl of plasma, and this mixture was then centrifuged at 17,000 rpm for 15 min. The clear supernatant was next collected and filtered through a 0.22 μm membrane filter (Millipore). From this solution, a 100 μl aliquot was injected for HPLC analysis.

Statistical analyses were performed with Prism 4.0 software (GraphPad, San Diego, CA, USA). Differences in paw oedema between the treatment and control groups were determined by one-way analysis of variance (ANOVA) with the Newman–Keuls *post hoc* test. The criterion for statistical significance was taken as $p < 0.05$. An experimental group contained 6 rats. All values are expressed as mean ± S.E.

3. Results and discussion

3.1. ESI-MS results

ESI-MS is the most promising tool for the characterization of different kinds of host–guest complexes in the gas phase [19]. It can provide evidence of complexation and stoichiometry on the basis of the molecular weights of all vaporized species. The study of host–guest interactions in the gas phase allows the detection of specific interactions not necessarily present in solution, thereby giving a complementary picture of the intrinsic phenomena responsible for molecular recognition. Also, there are interpretation ambiguities as concerns the ESI-MS spectra of supramolecular assemblies, e.g. deciding whether the species present in the mass spectra correspond to those present in solution, or they rather result from processes occurring under high-vacuum conditions. Moreover, it is not clear whether the molecular ions observed are real inclusion complexes or only ion-dipole external adducts, i.e. "false positives" [20]. In our particular case, however, a comparison can easily be made with the aid of results obtained from independent solution-phase techniques.

The hydroxyl groups of CDs are not easily protonated or deprotonated; ESI-MS analyses are usually carried out in the presence of salts in order to enhance detection.

The positive ESI spectra of KP 1:1 and KP 1:2 are reported in Fig. 1a and b, respectively.

An interesting feature of the ESI-MS binding of the analytes with the CDs is the reproducible loss of water. This water loss is presumed to arise from displacement of water from the CD cavity.

Host–guest complexes formed in solution are also stable for characterization by ESI in the gas phase.

The ESI of DIMEB with positive ion detection leads to a series of protonated molecules, $[M+H]^+$, at *m*/*z* values depending on the number of methyl groups in each individual sugar unit of the CD derivative. The mass spectra reflect the average distribution of the methyl groups of DIMEB. It should be noted that the ion peak at *m*/*z* 1331 corresponds to 14 *O*-methyl groups (i.e. heptakis(2,6-di-*O*-methyl)cyclomaltoheptaose to a first approximation) [21].

As expected, the spectrum essentially involves 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.

When the mixtures with different molar ratios (1:1 or 1:2) of LOR and DIMEB were analysed by ESI-MS, only the 1:1 complex was found in all the mass spectra, which suggests that the DIMEB inclusion complex in the gas phase has a certain stoichiometry.

3.2. DOSY results

The *D* value observed in the NMR experiment (fast-exchange condition) is the weighted average of those of the bound and the free guest. The rationale behind the extraction of the bound fraction from diffusion NMR measurements is simple. The host and guest have their own *D* values in the free state, reflecting their molecular weight and shape. The guest molecules are significantly smaller than CD, and the *D* values of the bound guests were taken to be equal to that of CD; it was assumed that, for the binding of a small guest molecule to a large host molecule, the *D* value of the host is not greatly perturbed and that of the host–guest complex can be assumed to be the same as that of the non-complexed host molecule. In the case of a weak or negligible association, the *D* values of the host and the guest will remain unchanged. For any other case, assuming fast exchange on the NMR time scale, the observed (measured) *D* values are weighted averages of the free and bound *D* values [22].

In the 2D DOSY spectra (Fig. 2), the F2 dimension shows the chemical shift and F1 stands for log *D*. Groups belonging to the same molecule will therefore appear in almost the same F1 row.

In the molecule of DIMEB, there are $=CH_2$ groups and $-O-CH_3$ groups, which have chemical shifts (δ) in the range 1–5 ppm. In the molecule of LOR, there are aromatic protons which have higher δ values, at about 7–8 ppm. In the DOSY spectrum of KP 1:1, log *D*

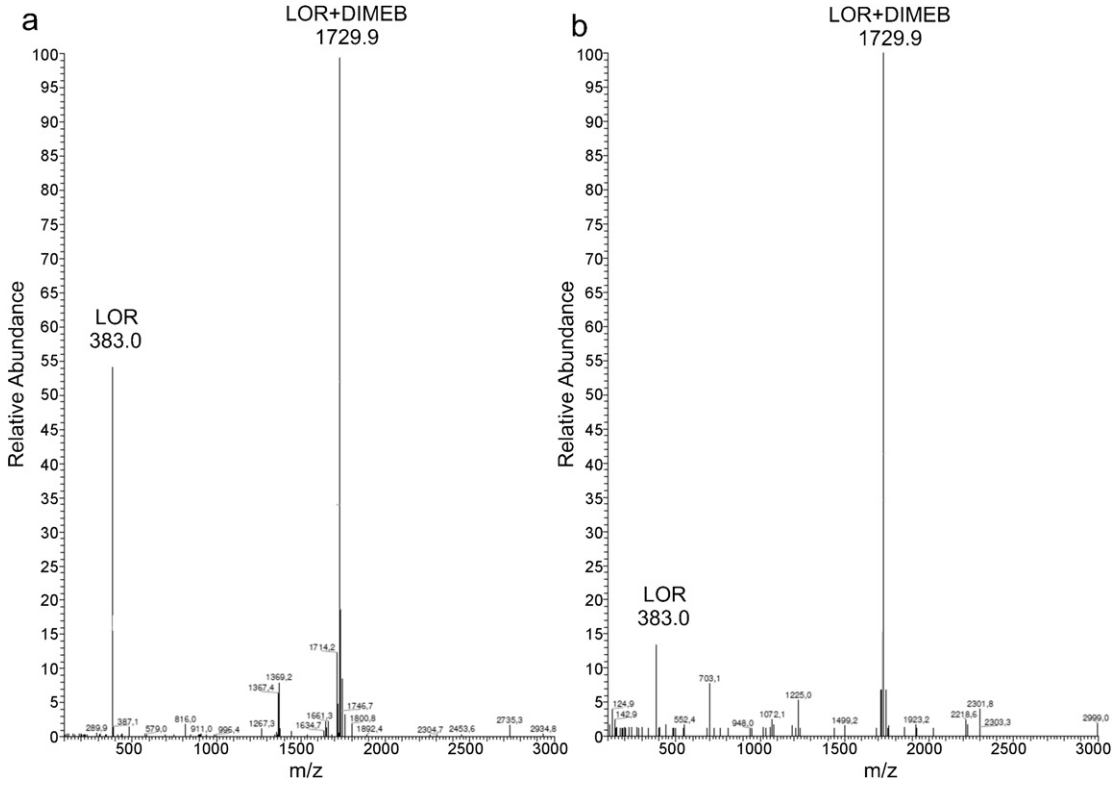

Fig. 1. ESI-MS results on KP 1:1 (a) and KP 1:2 (b).

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 complex indicates that it is best formulated as the 1:1 complex. For perfect spheres, theory predicts that increasing the molecular weight *n*-fold should lead to a *D* value decreased by a factor of $n_{-1/3}$. Unfortunately, in the absence of well-defined ^{1}H NMR spectra, X-ray crystallographic results or ESI-MS data, assignment of absolute stoichiometry to these aggregates is speculative [23].

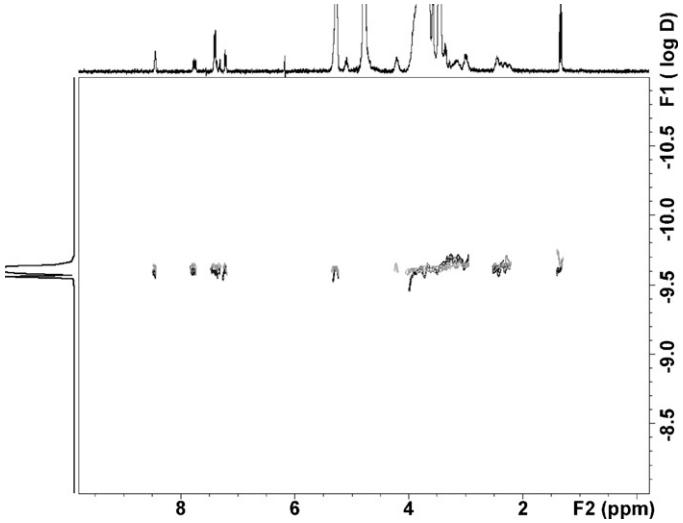

Fig. 2. Representative DOSY spectra of KP 1:1 (grey) and KP 1:2 (black).

3.3. In vitro dissolution study

According to the pKa value, the solubility of LOR depends on the pH:LOR can undergo protonation on the N of the pyridine ring in acidic media, forming salts with good solubility. It therefore exhibits good dissolution in acidic media, but dissolves poorly in alkaline media (e.g. SIM).

The rate of dissolution was improved for all of the products, but the extent of the increase depended on the molar ratio (see Fig. 3). For the KP 1:1 composition, the dissolution of LOR did not reach in 100%: only 70% of the LOR dissolved during 2 h. For KP 1:2, the whole of the investigated samples dissolved in the first 15 min, i.e. the dissolution in SIM was as good as in acidic media, which means that the same good dissolution would be attained at the extreme pH values of the gastrointestinal tract on the use of this DIMEB product. Accordingly, the rate-limiting step of absorption would not be the dissolution: the passage through the membrane would be regulated by the permeability. As LOR has good permeability, the application of LOR complexed with a CD such as DIMEB in 1:2 molar ratio would lead to a greater quantity of drug being absorbed, so that better BA would be obtained.

3.4. Effect of pH on solubility

The defined daily dose of LOR is 10 mg. The solubility of LOR has been reported to decrease with increasing pH [5]. As can be seen in Fig. 4, in the acidic range (to pH ~ 3) both the 1:1 and 1:2 compositions allow total dissolution of the applied dose. However, with increasing pH, DIMEB is not able to dissolve the whole quantity in the case of the 1:1 product, and the BA will therefore not be sufficient: only about 70% of the investigated amount of the 1:1 product dissolved at higher pH. In contrast, virtually the whole quantity of LOR dissolved from KP 1:2 at each pH value, proving the solubility of LOR is independent of the pH. This clearly suggests an oppor-

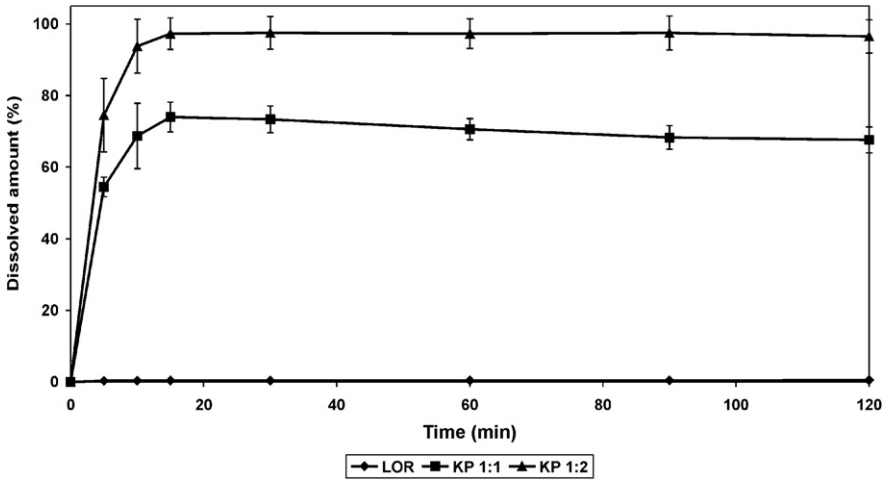

Fig. 3. Dissolution of LOR, KP 1:1 and KP 1:2 in SIM.

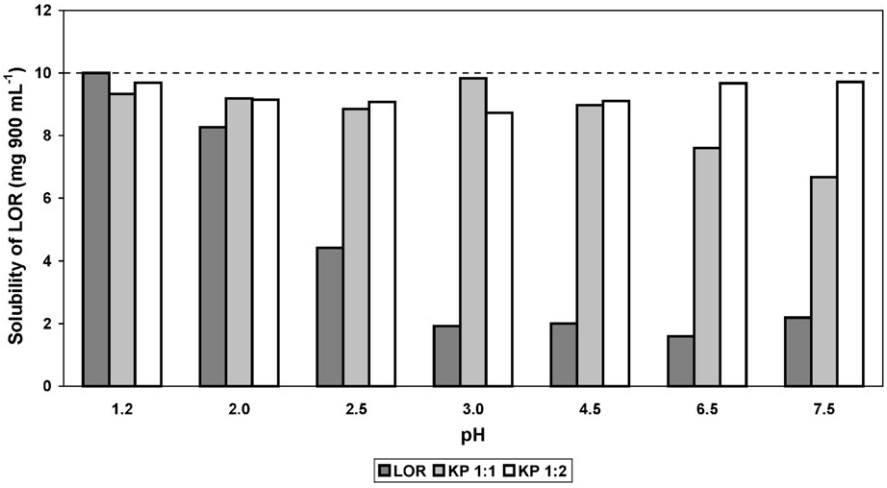

Fig. 4. Comparative solubility data of LOR, KP 1:1 and KP 1:2.

tunity to ensure consistent dissolution for LOR, thereby achieving better and more uniform BA.

3.5. PAMPA

LOR is a highly variable drug from the aspect of BA: its absorption depends greatly on whether the patient has eaten or not, and what was eaten. In the fasted state, different enzymes and natural solubilizers (e.g. bile acids) are released, which help to dissolve the drug. All forms of food have their own acidic (e.g. meats, cheese, egg, alcohol, mustard, sweeties, etc.), basic (e.g. potato, carrot, onion, mushroom or coconut) or natural characteristics, which influence the pH in the GI tract, and hence the solubility of this ionizable API. The pH of the stomach is known to differ significantly among individuals and is within the range of 1–3 or even higher (1–5) under fasting conditions. If undissolved LOR is emptied from the stomach, the absorption rate will be dramatically lower as compared with that in individuals where complete dissolution occurs in the stomach [5].

As LOR is a BCS class II drug, it readily passes through the intestinal wall, with a permeability value of 2.7×10^{-3} to 4.8×10^{-5} cm min^{-1} [5]. The PAMPA model represents only passive diffusion, and deceptive results can be observed if other mechanisms also play a role in the absorption of the API. LOR is a substrate of P-gp, which hinders its absorption, and thus the BA of this molecule. Fig. 5 indicates that LOR has higher permeability than the DIMEB-containing products.

The results demonstrate that, if passive diffusion were the main force of absorption, DIMEB would hinder this process. It is possible that the diffusion through this artificial membrane is bidirectional, and therefore the absorbed LOR can back-diffuse to the donor side and become recomplexed by DIMEB. Another explanation is that the stability of the complex is too high, and the association–dissociation balance is shifted to association. There is

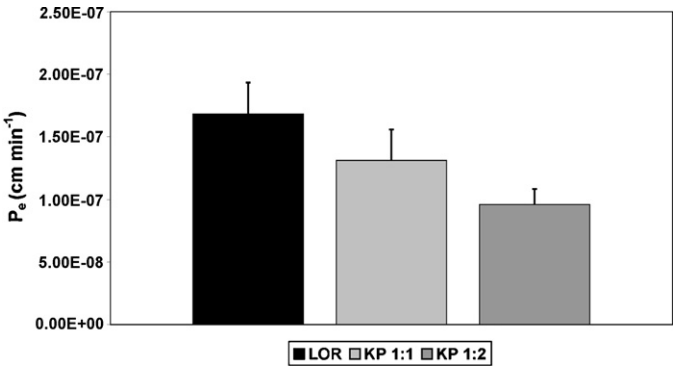

Fig. 5. Calculated effective permeability values of LOR, KP 1:1 and KP 1:2.

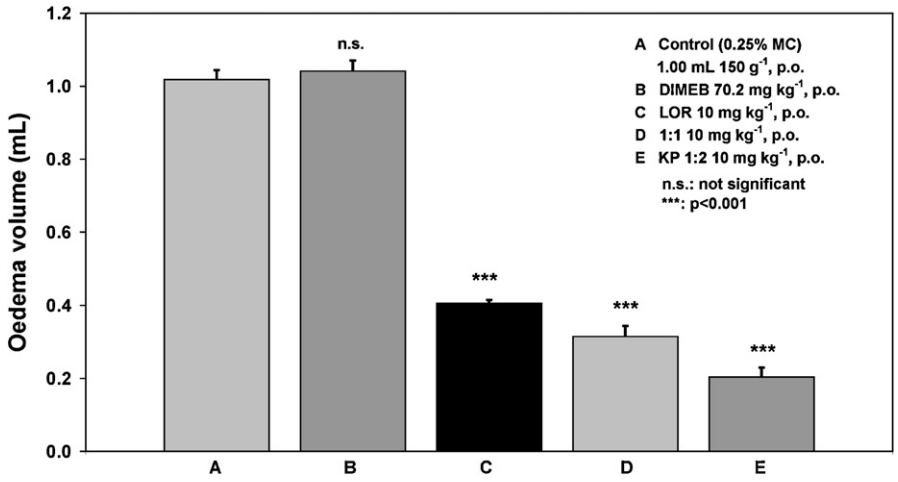

Fig. 6. Oedema volumes in the treated and the control groups (significance evaluations involve comparisons with LOR).

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. The statistical analysis reveals that the differences between the P_e values of LOR and KP 1:1, LOR and KP 1:2, and KP 1:1 and KP 1:2 are significant ($p < 0.05$).

The permeability of LOR increases with increasing pH: at acidic pH, LOR is in a protonated form, which cannot pass through the membrane, but at basic pH it is not protonated (non-ionized), and can be absorbed. This tendency also holds for KP 1:2, but the permeability is always lower at each pH value, which corresponds to the above discussion.

3.6. In vivo experiments

The main effect of the oedema inducer compound 48/80 is the release of histamine from the mastocytes, and hence the effect of the antihistamine LOR can be well examined with this model [17,18].

LOR, DIMEB and the inclusion complexes were investigated to establish to what extent they can reduce induced oedema. The volumes of the oedema induced in the right back paws are presented in Fig. 6. It was found that 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. The results of the Newman–Keuls multiple comparison test are presented in Table 1. 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.

We also measured the blood concentrations by HPLC. The data shown in Fig. 7 relate to the effects on oedema, except that the blood concentrations of LOR and KP 1:1 do not differ significantly, which demonstrates that KP 1:1 does not reach the desired BA. Excess DIMEB (as in KP 1:2) is needed to obtain pH-dependent solubility and more consistent and greater BA.

LOR was well separated from the biological background under the described chromatographic conditions, with mean RT = 7.50 min. No interference with plasma matrix constituents was observed. The mobile phase used guaranteed good repeatability of the retention times. The LOR concentration in samples from the animals is about a few ng ml^{-1}, and thus the analytical wavelength was selected according to the maximum LOR absorbance with respect to a stable baseline.

P-gp recognizes many compounds as substrates, and tends to have high affinity for hydrophobic and positively charged compounds at physiological pH. DIMEB appears not to be a substrate of P-gp because it is a hydrophilic and electrically neutral cyclic oligosaccharide with a relatively high molecular weight. Furthermore, DIMEB should not compete with P-gp substrates due to its lack of cell permeability. Thus, DIMEB must have an alternative inhibitory effect on P-gp activity, differing from the P-gp inhibitors that suppress the efflux by their surface activity. Some results suggested that DIMEB has a direct inhibitory effect on the P-gp level on the cell surface. DIMEB did not exhibit cytotoxicity on Caco-2 cells and had no effect on the paracellular and transcellular transport [24].

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

Table 1
Results of the Newman–Keuls multiple comparison test.

Comparisons	Mean difference	Q	p value
Control vs. DIMEB	−2.333	1.097	>0.05 ns.
Control vs. LOR	−60.83	30.08	<0.001***
Control vs. KP 1:1	−70.33	33.08	<0.001***
Control vs. KP 1:2	−81.43	35.98	<0.001***
LOR vs. KP 1:1	−9.50	4.015	<0.01**
LOR vs. KP 1:2	−20.60	8.273	<0.001***
KP 1:1 vs. KP 1:2	−11.10	4.311	<0.01**

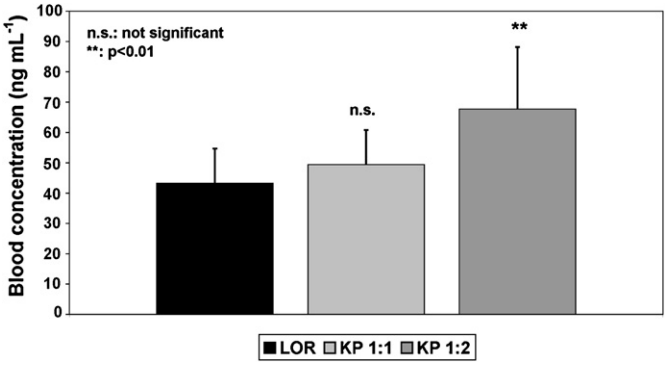

Fig. 7. Blood concentrations of LOR after oral administration of LOR and products (significance evaluations involve comparisons with LOR).

absorption, and therefore the greater BA. So these observations also suggested that the increase in the AUC value of LOR by DIMEB may be caused not only by the solubilizing activity of DIMEB, but also by inhibition of the efflux in the GI tract.

4. Conclusion

We have already demonstrated that DIMEB is able to make the solubility of LOR independent of the pH. It has now been established based on the ESI-MS and ^{1}H NMR DOSY results that the stoichiometry of the complex is 1:1. However, it is not enough to obtain pH-independence. For the 1:2 product, the solubility and the dissolution rate of LOR is higher and independent of pH against the KP 1:1 product. This is also indicated by the *in vivo* experiments, so better and more consistent BA can be achieved due to complexation in the appropriate molar ratio. The kneading method is one of the simplest complexation techniques; it can easily be scaled up and is often used in preclinical formulations.

Acknowledgements

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