J Incl Phenom Macrocycl Chem (2009) 64:249–254
DOI 10.1007/s10847-009-9558-1

ORIGINAL ARTICLE

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

Á. Nacsa · O. Berkesi · P. Szabó-Révész · Z. Aigner

Received: 21 January 2009 / Accepted: 27 February 2009 / Published online: 13 March 2009

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

Á. Nacsa · P. Szabó-Révész · Z. Aigner (✉)
Department of Pharmaceutical Technology, University of Szeged, Eötvös u. 6, 6720 Szeged, Hungary
e-mail: aigner@pharm.u-szeged.hu

O. Berkesi
Department of Physical Chemistry, University of Szeged, Rerrich B. tér 1, 6720 Szeged, Hungary

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

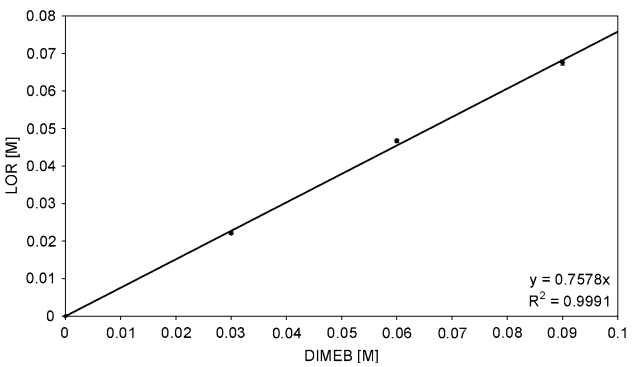

Fig. 1 Phase-solubility diagram of LOR at pH 7.5

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.

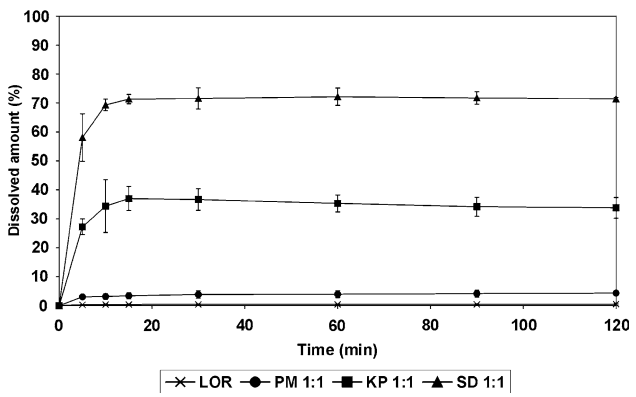

Fig. 2 Dissolution of 1:1 products in SIM

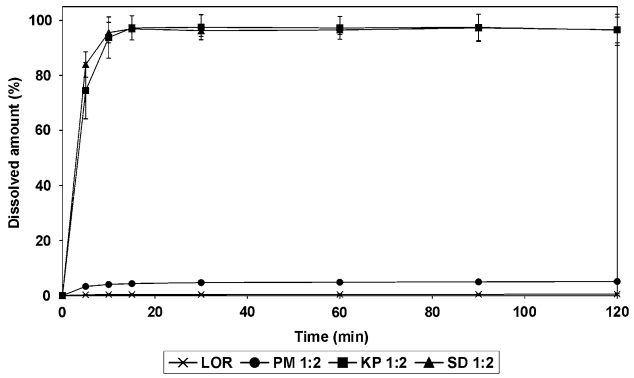

Fig. 3 Dissolution of 1:2 products in SIM

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

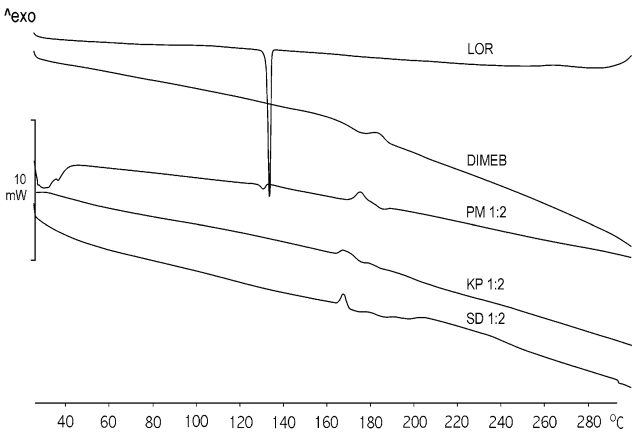

Fig. 4 DSC curves of LOR and 1:2 products

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.

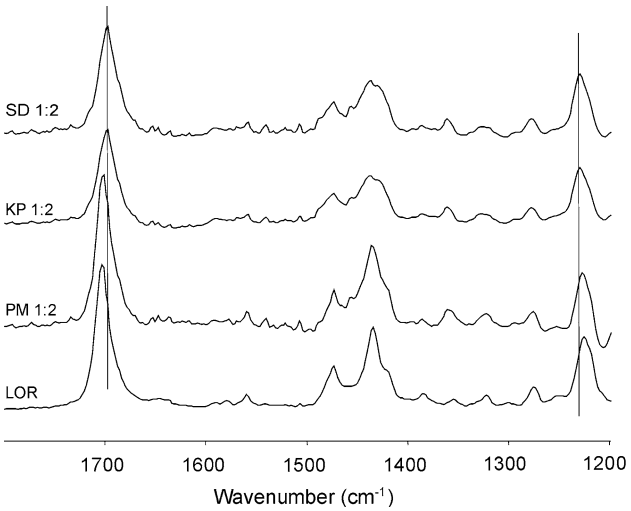

Fig. 5 FT-IR spectras of LOR and 1:2 products

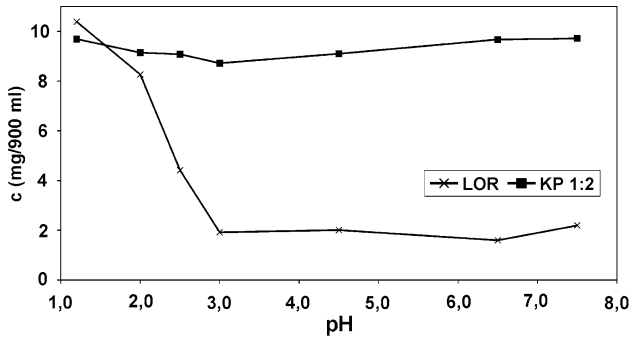

Fig. 6 pH-dependence of LOR and KP 1:2 product

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.

References

1. Merisko-Liversidge, E., Liversidge, G.G., Cooper, E.R.: Nanosizing: a formulation approach for poorly-water-soluble compounds. Eur. J. Pharm. Sci. **18**, 113–120 (2003). doi:10.1016/S0928-0987(02)00251-8
2. Loftsson, T., Jarho, P., Másson, M., Järvinen, T.: Cyclodextrins in drug delivery. Expert Opin. Drug Deliv. **2**, 335–351 (2005). doi:10.1517/17425247.2.1.335
3. Higuchi, T., Connors, K.A.: Phase-solubility techniques. Adv. Anal. Chem. Instrum. **4**, 117–212 (1965)
4. Pouton, C.W.: Formulation of poorly water-soluble drugs for oral administration: physicochemical and physiological issues and the lipid formulation classification system. Eur. J. Pharm. Sci. **29**, 278–287 (2006). doi:10.1016/j.ejps.2006.04.016
5. Bhattachar, S.N., Deschenes, L.A., Wesley, J.A.: Solubility: it's not just for physical chemists. Drug Discov. Today **11**, 1012–1018 (2006). doi:10.1016/j.drudis.2006.09.002
6. Hörter, D., Dressman, J.B.: Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. Adv. Drug Deliv. Rev. **46**, 75–87 (2001). doi:10.1016/S0169-409X(00)00130-7
7. Dressman, J.B., Amidon, G.L., Reppas, C., Shah, V.P.: Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. Pharm. Res. **15**, 11–22 (1998). doi:10.1023/A:1011984216775
8. Zimmermann, T., Yeates, R.A., Laufen, H., Pfaff, G., Wildfeuer, A.: Influence of concomitant food intake on the oral absorption of two triazole antifungal agents, itraconazole and fluconazole. Eur. J. Clin. Pharmacol. **46**, 147–150 (1994). doi:10.1007/BF00199879
9. Dashevsky, A., Kolter, K., Bodmeier, R.: pH-independent release of a basic drug from pellets coated with the extended release polymer dispersion Kollicoatw SR 30 D and the enteric polymer dispersion Kollicoatw MAE 30 DP. Eur. J. Pharm. Biopharm. **58**, 45–49 (2004). doi:10.1016/j.ejpb.2004.03.013
10. Estelle, F., Simons, R.: Comparative pharmacology of H1 antihistamines: clinical relevance. Am. J. Med. **113**(9), 38–46 (2002)
11. Khan, M.Z., Raušl, D., Zanoški, R., Zidar, S., Mikulčić, J.H., Krizmanić, L., Eškinja, M., Mildner, B., Knežević, Z.: Classification of loratadine based on the biopharmaceutics drug classification concept and possible in vitro–in vivo correlation. Biol. Pharm. Bull. **27**(10), 1630–1635 (2004). doi:10.1248/bpb.27.1630
12. Omar, L., El-Barghouthi, M.I., Masoud, N.A., Abdoh, A.A., Al Omari, M.M., Zughul, M.B., Badwan, A.A.: Inclusion complexation of loratadine with natural and modified cyclodextrins: phase solubility and thermodynamic studies. J. Solution Chem. **36**, 605–616 (2007). doi:10.1007/s10953-007-9136-3
13. ter Laak, A.M., Tsai, R.S., Donné-Op den Kelder, G.M., Carrupt, P.-A., Testa, B., Timmermann, H.: Lipophilicity and hydrogen-bonding capacity of H_1-antihistaminic agents in relation to their central sedative side-effects. Eur. J. Pharm. Sci. **2**, 373–384 (1994). doi:10.1016/0928-0987(94)00065-4
14. Moffat, A.C., Osselton, M.D., Widdop, B.: Clarke's Analysis of Drugs and Poisons, 3rd ed. edn. Pharmaceutical Press, London (2004)
15. Caliaro, G.A., Herbots, C.A.: Determination of pK_a values of basic new drug substances by CE. J. Pharm. Biomed. Anal. **26**, 427–434 (2001). doi:10.1016/S0731-7085(01)00423-X
16. Peterson, M.L., Hickey, M.B., Zaworotko, M.J., Almarsson, Ö.: Expanding the scope of crystal form evaluation in pharmaceutical science. J. Pharm. Pharm. Sci. **9**, 317–326 (2006)
17. Carrier, R.L., Miller, L.A., Ahmed, I.: The utility of cyclodextrins for enhancing oral bioavailability. J. Control. Release **123**, 78–99 (2007). doi:10.1016/j.jconrel.2007.07.018
18. Badr-Eldin, S.M., Elkheshen, S.A., Ghorab, M.M.: Inclusion complexes of tadalafil with natural and chemically modified β-cyclodextrins. I: preparation and in vitro evaluation. Eur. J. Pharm. Biopharm. **70**, 819–827 (2008). doi:10.1016/j.ejpb.2008.06.024
19. Kata, M., Ambrus, R., Aigner, Z.: Preparation and investigation of inclusion complexes containing nifluminic acid and cyclodextrins. J. Incl. Phenom. **44**, 123–126 (2002). doi:10.1023/A:1023074025175
20. Hassan, H.B., Kata, M., Erős, I., Aigner, Z.: Preparation and investigation of inclusion complexes containing gemfibrozil and DIMEB. J. Incl. Phenom. **50**, 219–225 (2004). doi:10.1007/s10847-004-3124-7
21. Amidon, G.L., Lennernas, H., Shah, V.P., Crison, J.R.: A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. **12**, 413–420 (1995). doi:10.1023/A:1016212804288
22. Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J.: Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. **46**, 3–26 (2001). doi:10.1016/S0169-409X(00)00129-0
23. Marttin, E., Verhoef, J.C., Merkus, F.W.: Efficacy, safety and mechanism of cyclodextrins as absorption enhancers in nasal delivery of peptide and protein drugs. J. Drug Target. **6**, 17–36 (1998)
24. Loftsson, T., Brewster, M.E., Másson, M.: Role of cyclodextrins in improving oral drug delivery. Am. J. Drug Deliv. **2**, 261–275 (2004). doi:10.2165/00137696-200402040-00006
25. Desiraju, G.R.: Crystal and co-crystal. Cryst. Eng. Commun. **5**, 466–467 (2003). doi:10.1039/b313552g