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Correlation of structure and properties of loratadine complexes with β-cyclodextrin and its derivatives in solution. Integrated spectroscopic, thermodynamic and computational study

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Corresponding Author:	Nives Galić University of Zagreb Faculty of Science Zagreb, Croatia CROATIA
First Author:	Marijana Pocrnić
Order of Authors:	Marijana Pocrnić
	Marta Hoelm
	Anna Ignaczak
	Ana Čikoš
	Ana Budimir
	Vladislav Tomišić
	Nives Galić
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Suggested Reviewers:	Janez Plavec janez.plavec@ki.si expert in NMR spectroscopy
	Luis Fernando Cabeça luiscabeca@utfpr.edu.br preparation and characterization of inclusion complexes with beta-cyclodextrins and its derivatives
	Christian Schonbeck jechsc@ruc.dk solubility studies and stability constants determination of inclusion complexes with beta-cyclodextrins and its derivatives
	Dariusz Maciej Pisklak dariusz.pisklak@wum.edu.pl DFT calculations

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Dear Editor,

We are submitting the manuscript entitled ″Correlation of structure and properties of loratadine complexes with β-cyclodextrin and its derivatives in solution. Integrated spectroscopic, thermodynamic and computational studies″ for publication in *Carbohydrate Polymers*.

Loratadine (LOR) is a second-generation antihistaminic administered mostly orally for the treatment of allergies. The main drawback of LOR is related to its poor water solubility, which can be efficiently addressed by inclusion complexation with cyclodextrins (CD). Both natural and chemical modified CDs are known to act as multifunctional excipients which enhance *in vitro* dissolution of drugs by changing their chemical and physical properties and thereby provide alternative ways of drug delivery.

To the best of our knowledge, there are several papers describing the phase-solubility study of LOR in aqueous solution upon the addition of β-CD, and hydroxypropyl-β-CD, but the results are not conclusive. There are also significant discrepancies in the reported values of stoichiometry of the complexes, as well as their stability constants. Molecular modeling was performed only for LOR:β-CD complex *in vacuo*. In addition, techniques like mass spectrometry (MS) and isothermal titration calorimetry (ITC) were not used so far for the investigation of such systems.

In this work we carried out measurements involving β-cyclodextrin (β-CD), 2-hydroxypropyl-β-cyclodextrin (HPβCD), randomly methylated β-cyclodextrin (RMβCD) and sulfobutylether sodium salt β-cyclodextrin (SBEβCD) in unbuffered aqueous solution, as well as in simulated biorelevant media at pH = 4.5 and 6.8, to investigate the influence of β-CD and its derivatives on LOR solubility. In addition to phase solubility measurements, we have employed various experimental techniques such as ITC, HRMS, and NMR, followed by quantum chemical computations. Microcalorimetric titrations were used to determine thermodynamic parameters (K, $\Delta_r G$, $\Delta_r H$, $\Delta_r S$,) for drug-CDs systems at pH = 2.8. Comprehensive analysis of NMR spectra suggested the mode of LOR inclusion in the β-CD cavity, which was corroborated by the results of DFT study.

The results obtained by the applied integrated approach comprising different methods enabled the verification of all hypotheses posed in the Introduction section. In our opinion the manuscript fits well in the scope of *Carbohydrate Polymers*. We believe that the results presented

in the submitted paper will be of interest to a wide audience of chemists and pharmacists, particularly those dealing with different applications of polysaccharides in diverse fields. Therefore, we would very much appreciate it if you would consider the manuscript for publication in *Carbohydrate Polymers*.

Thank you in advance.

Yours sincerely,

Nives Galić

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Correlation of structure and properties of loratadine complexes with β-cyclodextrin and its derivatives in solution. Integrated spectroscopic, thermodynamic and computational study

Marijana Pocrnić[a], Marta Hoelm[a,b], Anna Ignaczak[b], Ana Čikoš[c], Ana Budimir[d], Vladislav Tomišić[a], Nives Galić[a,*]

[a]*Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia*
[b]*Theoretical and Structural Group, Department of Physical Chemistry, Faculty of Chemistry, University of Lodz, Pomorska 163/165, Lodz, 90-236, Poland*
[c]*Ruđer Bošković Institute, NMR Centre, Bijenička cesta 54, Zagreb, Croatia*
[d]*Department of General and Inorganic Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, Zagreb, Croatia*

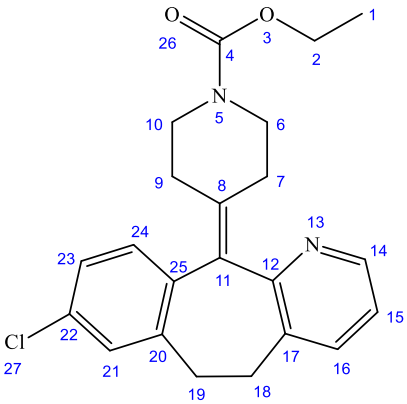
Abstract

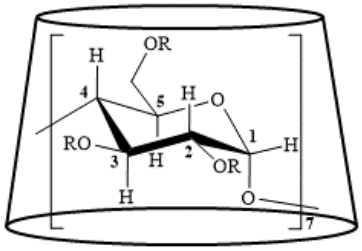
The complexation of loratadine (LOR) by β-cyclodextrin (β-CD) and its derivatives (hydroxypropyl β-CD (HP-β-CD), randomly methylated β-CD (RM-β-CD) and sulfobutylether β-CD sodium salt (SBE-β-CD)) in water and simulated biorelevant media was thoroughly studied by means of an integrated approach comprising phase solubility experiments, ITC, MS, NMR spectroscopy, and computational methods. The addition of all investigated cyclodextrins considerably increased the LOR solubility, the most effective being RM-β-CD. The formation of 1:1 and 1:2 (LOR:CD) inclusion complexes was observed for all derivatives, and the corresponding reactions were thermodynamically characterized by determining and discussing the complex stability constants (and derived standard reaction Gibbs energies), standard reaction enthalpies and entropies. The structures of β-CD complexes with non-protonated and protonated ($LORH^+$) loratadine forms were elucidated by comprehensive 1D and 2D NMR spectroscopy. Additional information regarding the structures as well as the energetic and thermodynamic stabilities of LOR and $LORH^+$ complexes was obtained by carrying out the DFT calculations, which corroborated the experimental results. It was found that protonation had minor effect on the complex stability.

Keywords: loratadine, β-cyclodextrin derivatives, inclusion complexes, solubility, mode of binding

1. Introduction

The solubility and permeability of drugs are determining factors for their bioavailability. In recent years a significant rise of low solubility drugs is noticed on the market, with 70 % of novel drugs classified as class II according to the Biopharmaceutics Classification System (BCS). This class of drugs is characterized by low solubility and high permeability (Benet, 2023; Bhalani, Nutan, Kumar, & Chandel, 2022). Loratadine (LOR) is a second-generation antihistaminic administered mostly orally for the treatment of allergies (El-Gawad, Soliman, Shams, & Maria, 2013). It is a weak base (Fig. 1) with pK_a value in the range 4.85–6.00, classified as a BCS type II drug (Szabados-Nacsa et al, 2011). The low LOR solubility in water can be increased by lowering the pH, increasing the temperature or by addition of organic solvent (Yang, Wang, & Wang, 2017; Wang et al., 2017), but none of these solutions is applicable for human use. Another approach is to use biocompatible and biodegradable materials such as cyclodextrins (CDs) and their derivatives (Kali, Haddadzadegan, & Bernkop-Scnürch, 2024). However, before implementing the new formulations, an extensive and detailed study of the system is needed. That is why in this work the comprehensive investigation of LOR interaction with β-CD and its derivatives (Fig. 1) which have already been registered as pharmaceutical excipients (hydroxypropyl β-CD (HP-β-CD), randomly methylated β-CD (RM-β-CD) and sulfobutylether β-CD sodium salt (SBE-β-CD)), was performed.





β-CD: R = H_{21}

HP-β-CD: R = H_{21-n} or $(–CH_2CH(OH)CH_3)_n$, n = 2–8; DS = 4.5

RM-β-CD: R = H_{21-n} or $(–CH_3)_n$, n = 9–15; DS = 12

SBE-β-CD: R = H_{21-n} or $(–(CH_2)_4SO_3Na)_n$, n = 3–7; DS = 6.5

Figure 1. Structures and numbering of loratadine and β-cyclodextrins

Natural cyclodextrins (CDs) are cyclic oligosaccharides with six (α-), seven (β-) or eight (γ-) glucopyranose units. They are known for their ability to form inclusion complexes with different molecules, which can affect physico-chemical properties of host molecules, such as solubility and stability (Kali et al, 2024; Matencio et al., 2021).

Improvement of LOR solubility in the presence of CDs has already been studied to some extent. Natural cyclodextrins, as well as derivatives of β-CD, such as HP-β-CD, have proved to increase the solubility of LOR in water and different buffered media. However, the results are not conclusive, especially for β-CD, and there are significant discrepancies in the reported values of stability constants. Both 1:1 and the mixture of 1:1 and 1:2 LOR-CD complexes were suggested (El—Gawad et al., 2013; Lin, Hsu, & Sheu, 2010; Lin et al, 2012; Nacsa, Berkesi, Szabó-Révész, & Aigner, 2009; Omar et al, 2007; Szabados-Nacsa et al, 2011).

Mass spectrometry and NMR spectroscopy, as well as molecular modeling, are important tools in analysis of stoichiometry of inclusion complexes with drugs, as well as elucidating the mode of inclusion (Klarić, Kelrajter, Čikoš, Budimir, & Galić, 2024; Mazurek & Szeleszczuk, 2022). However, to the best of our knowledge, inclusion complexation of LOR with CDs has not been extensively studied by these methods. Previously, formation of inclusion complex of LOR and dimethyl β-CD in 1:1 ratio was confirmed by mass spectrometry and diffusion-ordered ^{1}H NMR spectroscopy (Szabados-Nacsa et al, 2011). Molecular modeling was only performed on LOR:β-CD complex *in vacuo*, which confirmed complex formation in 1:1 ratio by inclusion of chlorophenyl moiety, as well as 1:2 complex ratio (Omar et al, 2007). Similar results were obtained for structurally alike desloratadine, active LOR metabolite. Results of ^{1}H NMR studies in D_2O showed that desloratadine forms complexes with β-CD in 1:1 stoichiometric ratio by inclusion of chlorophenyl moiety (Mashhood Ali, Upadhyay & Maheshwari, 2007).

Besides aforementioned methods, isothermal titration calorimetry (ITC) can provide new perspective in understanding the interaction of drugs with CDs. It can be used to simultaneously determine the stoichiometry of the inclusion complexes and thermodynamic parameters such as complex stability constant (K) and derived standard reaction Gibbs energy ($\Delta_r G°$), standard reaction enthalpy ($\Delta_r H°$) and entropy($\Delta_r S°$) (Bouchemal & Mazzaferro, 2012; Grimm et al., 2023). However, to the best of our knowledge, ITC has not been used to study interactions between LOR and CDs.

As a part of our research on drug supramolecular complexes (Kezele Špehar et al., 2021; Klarić et al., 2024), in this work we present the results on LOR-CDs systems in solution

obtained by phase solubility study, high resolution mass spectrometry (HR MS), NMR spectroscopy, ITC and computational methods.

The working hypotheses are (*i*) complexation with β-CD and its derivatives increase LOR solubility in water and biorelevant media, (ii) the results obtained from different techniques provide multi-perspective view on structure-property relationships, (iii) this research is a good foundation for subsequent process of developing new drug formulations.

2. Material and methods

2.1. Chemicals and materials

LOR was purchased from Biosynth (United Kingdom). All cyclodextrins (β-CD, HP-β-CD, with an average degree of substitution, DS = 4.5, RM-β-CD, DS = 12 and SBE-β-CD, DS = 6.5) were obtained from CycloLab (Hungary). Ammonium acetate and acetic acid were purchased from Sigma Aldrich (USA), HPLC grade acetonitrile was purchased from J. T. Baker (Netherlands). MS grade formic acid and acetonitrile were purchased from Carlo Erba (Germany). Ultrapure water was obtained by Mili-Q Advantage A10 purification system (Merck, USA). Deuterated solvents (DMSO-d_6 and D_2O) were purchased from EurIsotop (Cambridge Isotope Laboratories, Inc). Phosphate buffers for simulated biorelevant media and ITC titrations were prepared according to European Pharmacopeia.

2.2. Instrumentation

UV-Vis spectra were recorded on a Specord 200 plus spectrophotometer (Analytik Jena AG, Germany) over the range 190–500 nm using standard quartz cell (l = 1 cm). Chromatographic measurements were carried out on Agilent 1220 Infinity LC using Zorbax Eclipse XDB-C18 (5 µm, 150×4.6 mm) column. NMR and ESI HRMS spectra were recorded on Bruker Avance AV600 spectrometer and Agilent 6550 Q-TOF in positive ion mode, respectively. For microcalorimetric titrations, PEAQ-ITC MicroCal (Malvern Panalytical Ltd, UK) was used.

2.3. Methods

2.3.1. Phase solubility studies

Solubility measurements were carried out in water, simulated duodenal (SDM, pH 4.5) and intestinal media (SIM, pH 6.8), which were prepared according to the European Pharmacopeia. Experiments were performed by weighing excess amounts of LOR and adding

5 mL of appropriate solvent. Cyclodextrins were added into each sample so that their final concentration was 0 – 12.5 mM (for β-CD) or 0 – 40 mM (for other CDs). Samples were shaken for 72 hours on vortex shaker at ambient temperature, filtered using 0.45 μm Chromafil Xtra H-PTFE filters and diluted to achieve finals solvent composition of methanol:aqueous solvent 40:60 (*v*/*v*). The LOR concentration was determined by previously developed UV-Vis or HPLC methods (Supporting Information, SI, Table S1.1). After solubility diagrams were constructed (Brewster & Loftsson, 2007), the apparent stability constant ($K_{1:1}$) and complexation efficacy (CE) were calculated using formulas: $K_{1:1}$ = slope / S_0(1-slope), and CE = slope / (1-slope). All experiments were performed in triplicate.

2.3.2. High resolution mass spectrometry

Samples for HR MS analysis were prepared by mixing stock solutions of LOR and CDs (γ = 1 mg mL^{-1}) in molar ratios 1:1 (β-CD), 1:2 (RM-β-CD) or 1:5 (HP-β-CD and SBE-β-CD) to give final concentrations of LOR 4.4×10^{-5} M or 2.2×10^{-4} M in MeOH:water 1:1. The ESI-MS spectra were obtained in positive mode in the range from *m/z* 390–3200. All details can be found in SI.

2.3.3. Isothermal microtitration calorimetry (ITC)

Microcalorimetric measurements were conducted using a PEAQ-ITC MicroCal isothermal titration calorimeter (Malvern Panalytical Ltd, UK) at 25.0 °C. All solutions were prepared in phosphate buffer ($c(NaH_2PO_4)$ = 0.065 M, pH = 2.8) and briefly degassed before use. Enthalpy changes obtained upon stepwise, automatic addition of a solution of β-CDs to LOR (c = 5.00 × 10^{-4} M or 6.80 × 10^{-4} M) were recorded. Control experiments were performed to make corrections for the enthalpy changes corresponding to titrant dilution. The dependence of successive enthalpy changes on the reactant concentrations was processed using a sequential binding site model available in the MicroCal PEAQ-ITC Analysis software (version 1.30). Titrations for each studied system were repeated at least three times. All details can be found in SI.

2.3.4. NMR

The final concentration of LOR, β-CD, and LOR:β-CD 1:1 mixture in 600 μL of DMSO-d_6 was 22 mM, while 12 mM in D_2O at pH 1.9. The complete NMR analysis was based

on one- and two-dimensional NMR spectra (^{1}H, ^{13}C, COSY, NOESY, 1D and 2D ROESY, DOSY, HSQCe and HMBC. All details can be found in SI.

2.3.5. Computational details

The initial models of the β-CD:LOR complex (Fig. S5.1) were built using HyperChem program (HyperChem, 2011), creating the two sets: β-CD_EXP:LOR and β-CD_CCCW:LOR. The geometry of β-CD in β-CD_EXP:LOR was based on the crystallographic structure 3CGT (Schmidt, Cottaz, Driguez & Schulz, 1998), while that in β-CD_CCCW:LOR on its ground state reported in the paper by Snor et al, 2007. Both structures were reoptimized in water described by the polarizable continuum model (PCM) (Tomasi, Mennucci, & Cammi, 2005) using the density functional theory (DFT) method M06-2X-GD3/6-31G(d,p) (Grimme, Antony, Ehrlich & Krieg, 2010; Rassolov, Ratner, People, Redfern, & Curtiss, 2001; Zhao & Truhlar, 2008). The calculations were done in the Gaussian16 program (Frisch et al., 2016). In both sets the same conformer of LOR was used (denoted as LOR-1; Fig. S5.3) found from the final step of the conformational analysis described in Procedure S1 and Chart S1. Next, for each configuration shown in Fig. S5.1, the three-step configurational analysis was performed, the description of which is provided in Procedure S2 and Chart S2 (SI). The protonated complexes (β-CD_EXP:LORH^{+} and β-CD_CCCW:LORH^{+}) were constructed using the most stable structures obtained from the final step of the configurational search conducted at the M06-2X-GD3/6-31G(d,p) theory level in water (PCM; see Procedure S2). In these molecules, the proton was added to the pyridine nitrogen of LOR, and then they were optimized using the same DFT theory level. Similarly, LORH^{+} was constructed, for which a full three-step conformational analysis, similar to that performed for the neutral LOR, was conducted (see Procedure S1).

The energetically favored complexes were characterized by several energetic factors, such as the complexation energy (E_{compl}), interaction energy (E_{int}), enthalpy (H_{compl}), and corrected Gibbs energy ($G_{\mathrm{corr\_compl}}$). The influence of the basis set superposition error (BSSE) on the energetic parameters was assessed using the counterpoise correction (Boys & Bernardi, 1970). Additionally, the ^{1}H and ^{13}C NMR chemical shifts were calculated from the isotropic values obtained at the M06-2X/6-31++G(d,p)//M06-2X-GD3/6-31G(d,p) theory level in water (PCM) by applying the procedure proposed by Tantillo in 2019. The appropriate equations and a detailed description of the method used to obtain these results are given in Procedure S3 in the SI.

3. Results and discussion

3.1. Phase solubility studies

Solubility studies of LOR and CDs were performed in water, simulated duodenal (SDM, pH 4.5), and simulated intestinal media (SIM, pH 6.8). Concentration of LOR in the samples was determined by previously validated UV-Vis and HPLC methods (Table S1). Observed phase solubility diagrams of LOR in the presence of β-CD (except at pH 6.8) and SBE-β-CD are identified as A_L (Figs. 2 and S1–S2 in SI), indicating formation of 1:1 complex, which is in accordance with previously published work (Lin et al., 2012; Ramesh, Yadav, Sarheed, Elmarsafawy, & Islam, 2020).

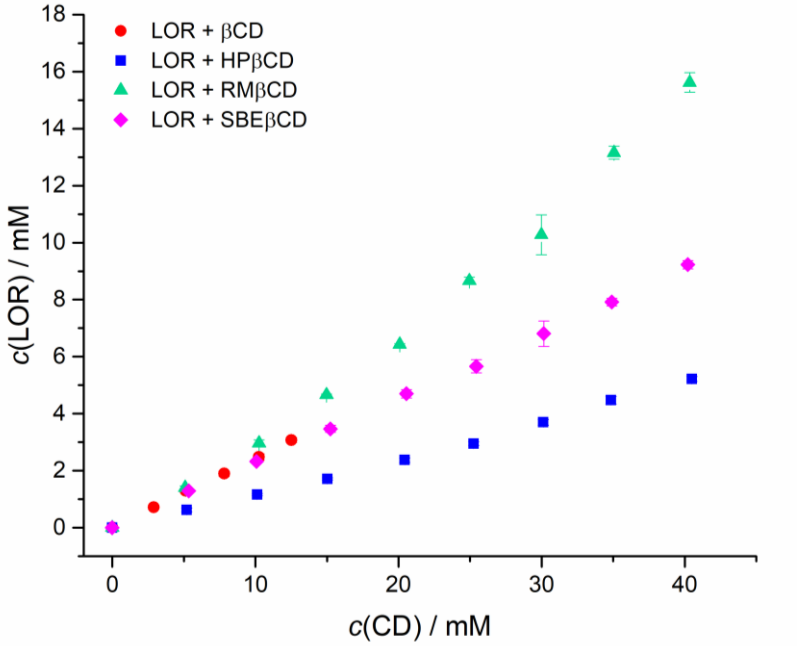


Figure 2. Phase solubility diagram of LOR with β-CD (●), HP-β-CD (■), RM-β-CD (▲) and SBE-β-CD (♦) in water at ambient temperature.

However, phase solubility diagram of LOR and β-CD at pH 6.8 was identified as A_P, indicating the additional formation of complex with 1:2 stoichiometry. Type A_P phase solubility diagrams were also observed with RM-β-CD in all media, and with HP-β-CD in water and in simulated duodenal medium. That differs from previously reported results, where HP-β-CD formed 1:1 complex in water (Lin et al., 2010; Lin et al., 2012). The disparity of the results could be due to the higher concentration range of HP-β-CD (0–40 mM) used in our work, since the relation of concentration of dissolved LOR and added CDs is linear up to 20 mM CDs, which was maximal concentration used in the reported studies. However, our results agree with those reported by Omar et al. (2007) who observed both 1:1 and 1:2 LOR:β-CD and LOR:HP-β-CD

complexes in phosphate buffer at pH = 7. Regardless of A_L or A_P profile, only the stability constants for 1:1 LOR:CDs complexes were determined, based on the linear part of the curves in the phase solubility diagrams, and using experimentally obtained S_0 values. Since the value of K is strongly dependent on S_0, which also depends on the method used for its determination, a better way to compare the solubilizing effects of CDs is to compare their complexation efficiency (CE) (Brewster et al., 2007). Results of phase solubility measurements, including $K_{1:1}$, CE and S_{max}/S_0 values, are summarized in Table 1. As can be seen, all four cyclodextrins greatly improve solubility of LOR in all three media, particularly RM-β-CD.

Table 1. Stability constants, complexation efficacies and solubility enhancement of LOR in the presence of CDs in water and simulated biorelevant media.

log $K_{1:1}$ / M			
CD	**Water**	**SDM (pH 4.5)**	**SIM (pH 6.8)**
β-CD	4.82 ± 0.01	4.19 ± 0.01	5.23 ± 0.02 ***
HP-β-CD	4.42 ± 0.02 **	3.92 ± 0.02 **	4.84 ± 0.02
RM-β-CD	5.14 ± 0.01 *	4.40 ± 0.02 **	5.20 ± 0.01 **
SBE-β-CD	4.95 ± 0.01	4.20 ± 0.04	5.03 ± 0.03
CE			
β-CD	0.323 ± 0.015	0.381 ± 0.011	0.526 ± 0.021
HP-β-CD	0.131 ± 0.004	0.207 ± 0.002	0.222 ± 0.006
RM-β-CD	0.462 ± 0.013	0.499 ± 0.012	0.485 ± 0.024
SBE-β-CD	0.293 ± 0.009	0.331 ± 0.025	0.294 ± 0.009
S_{max}/S_0			
β-CD	619 ± 27	141 ± 4	1431 ± 43
HP-β-CD	1048 ± 22	298 ± 18	2288 ± 49
RM-β-CD	4690 ± 237	619 ± 21	5886 ± 446
SBE-β-CD	2770 ± 58	480 ± 39	3382 ± 192

* linear range 0–20 mM CDs; ** linear range 0–25 mM CD; *** linear range 0–7,5 mM CD
All measurements were performed in triplicate.

Since both A_L and A_P type phase-solubility profiles were noticed, the high resolution mass spectrometry was used to shed more light on the investigated systems, and to determine the stoichiometries of the complexes.

3.2. High resolution mass spectrometry

High resolution mass spectrometry can be used to confirm the formation of inclusion complexes and to determine their stoichiometry in a gas phase, which can be correlated with the behavior in the solution (Gabelica, Galić, & De Pauw, 2002; Klarić et al., 2024; Silion et al., 2019; Špehar et al., 2021). The results of HR MS analysis of LOR and CDs solutions are presented in Table 2 and SI (Figs. S2.1–S2.6 and Tables S2.1–S2.5). As can be seen, the 1:1 complexes were observed for all CDs, except for SBE-β-CD, with excellent agreement between theoretical and observed *m/z* values. Substituted cyclodextrins are a mixture of cyclodextrins with different degrees of substitution. Therefore, their MS spectra are much more complex, with ions that correspond to protonated forms of each degree of substitution, as well as to sodium adducts. The MS spectra of SBE-β-CD is even more complex due to the ions corresponding to differently charged species (Grard, Elfakir & Dreux, 2001; Ma, Zhang & Xu, 2016). This could be the reason that inclusion complexes with SBE-β-CD were not observed, both in positive and negative mode (Figs. S2.5; S2.6 and Tables S2.4; S2.5). The 1:2 complexes, which were supposed to exist in solution based on phase solubility study, were not observed in MS spectra since they were probably too labile to survive ionization process.

Table 2. Calculated and observed *m/z* values of most abundant $[M+H]^+$ ions of inclusion complexes of LOR and CDs.

	Calculated *m/z*	Observed *m/z*	Error/ppm
LOR+β-CD	1517.5219	1517.5237	1.19
LOR+HP-β-CD (DS = 5)*	1807.7312	1807.7272	–2.21
LOR+RM-β-CD (DS = 11)	1671.6940	1671,6960	1.20
LOR+SBE-β-CD (DS = 4)	2127.5453	–	–

*DS – degree of substitution

3.3. Isothermal titration calorimetry

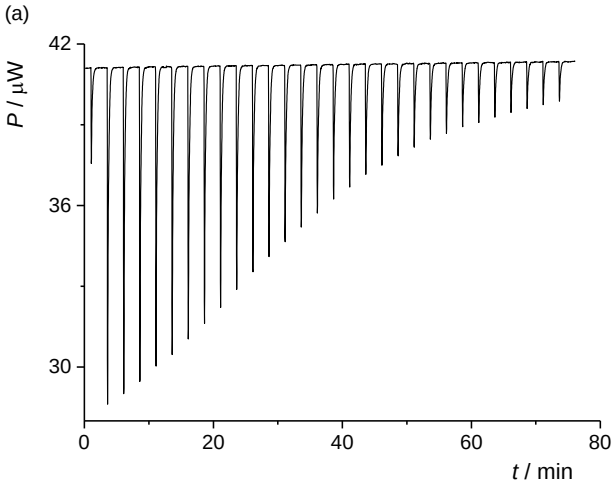
The results of ITC studies of the LOR inclusion into the cyclodextrin derivatives are summarized in Table 3. It should be noted that calorimetric experiments were carried out at pH

= 2.8 because of the limited LOR solubility at higher pH values. The obtained thermograms and titration curves are shown in Figs. 3 and S3.1-S3.3. In all cases, the formation of complexes with 1:1 and 1:2 (LOR:CD) stoichiometries was observed. Having in mind that the experimental conditions were different with respect to pH, the values of equilibrium constants for the reactions of formation of 1:1 complexes (Table 3) are in satisfactory agreement with those determined by phase solubility experiments (Table 1).

Table 3. Thermodynamic parameters of LOR stepwise complexation reactions with β-cyclodextrins in phosphate buffer (c = 0.065 M, pH = 2.8) at 25 °C. Complex stoichiometries (LOR:CD) are given in parentheses.

Cyclodextrin	$\log\left(\frac{K}{\mathrm{M}^{-1}}\right) \pm \mathrm{SE}$	$\frac{(\Delta_r G^\circ \pm \mathrm{SE})}{\mathrm{kJ\ mol^{-1}}}$	$\frac{(\Delta_r H^\circ \pm \mathrm{SE})}{\mathrm{kJ\ mol^{-1}}}$	$\frac{(T\Delta_r S^\circ \pm \mathrm{SE})}{\mathrm{kJ\ mol^{-1}}}$
β-CD	4.47 ± 0.04 (1:1)	-25.5 ± 0.2	-15.3 ± 0.2	10.2 ± 0.4
	3.49 ± 0.02 (1:2)	-19.9 ± 0.1	-10.1 ± 0.3	9.8 ± 0.5
HP-β-CD	3.61 ± 0.02 (1:1)	-20.6 ± 0.1	-8.8 ± 0.3	11.8 ± 0.3
	2.99 ± 0.02 (1:2)	-17.0 ± 0.1	-7.5 ± 0.3	9.5 ± 0.4
RM-β-CD	4.12 ± 0.05 (1:1)	-23.5 ± 0.2	-4.0 ± 0.2	19.5 ± 0.3
	3.05 ± 0.05 (1:2)	-17.4 ± 0.3	-7.3 ± 0.4	10.1 ± 0.7
SB-β-CD	4.55 ± 0.06 (1:1)	-26.1 ± 0.4	-9.2 ± 0.3	16.9 ± 0.6
	3.48 ± 0.06 (1:2)	-19.9 ± 0.3	-7.6 ± 0.6	12.3 ± 0.9

SE – standard error of the mean (N = 3).



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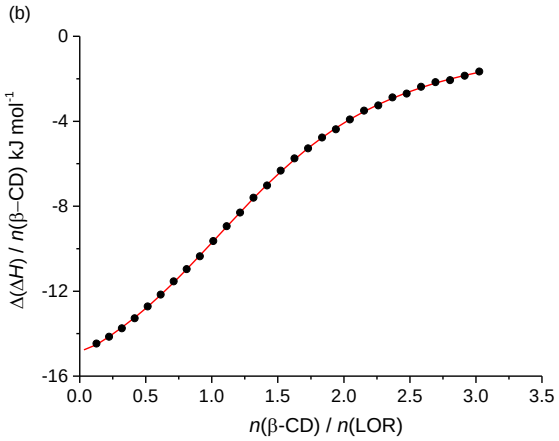

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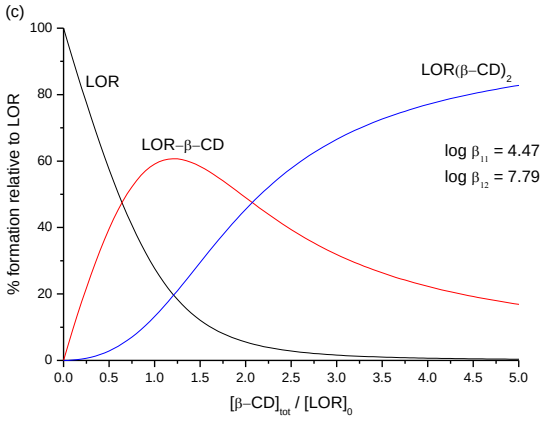

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271 **Figure 3**. (a) Microcalorimetric titration of LOR (c_0 = 5.00 × 10^{-4} M, V_0 = 200 μL) with β-CD
272 (c_0 = 8.11 × 10^{-3} M) in phosphate buffer (c = 0.065 M, pH = 2.8) at 25 °C. (b) Dependence of
273 normalized successive enthalpy changes on β-CD/LOR molar ratio. ● experimental; —
274 calculated. (c) Distribution diagram for LOR: β-CD system, c(LOR) = 5.00 × 10^{-4} M.

All studied reactions are both enthalpically and entropically favorable (Table 3). In the case of β-CD reaction with LOR, the enthalpic contribution to the standard reaction Gibbs energy is slightly dominant whereas for the other β-CD derivatives the entropic contribution prevails. The binding of the first cyclodextrin molecule is almost in all cases more exothermic (the only, but not remarkable, exception is the reaction with RM-β-CD) and more entropically favorable than that of the subsequent one. The exothermicity of the reactions is expected and common for cyclodextrins (Biedermann, Uzunova, Scherman, Nau, & Simone, 2012; Biedermann, Nau, & Schneider, 2014; Connors, 1997; Rekharsky & Inoue, 1998). Namely, it is known that the water molecules included in the smaller cyclodextrin cavity are less hydrogen bonded (on average 2.96 H-bonds per molecule) compared to those in the bulk solvent (3.62 H-bonds per molecule) (Biedermann et al., 2014). As in the course of the guest-binding process these molecules are released from the cavity, the binding is enthalpically beneficial, and that should be more pronounced for the formation of 1:1 complex compared to 1:2, as observed in this work. On the other hand, the release of solvent molecules from the cavity is predicted to be disadvantageous for the reaction from the entropic point of view (Usenik et al., 2023a). However, the guest (LOR) desolvation, taking place upon its inclusion in cyclodextrin, should also be taken into account (Usenik et al., 2023a,b). This process is envisaged to be entropically favorable, particularly having in mind that at working pH = 2.8 LOR is protonated and positively charged (pK = 5.25 according to Popović, Čakar, & Agbaba, 2009), again probably more so for the first β-CD molecule binding. If the entropic effect of drug desolvation overcomes that of water-molecules release from the cyclodextrin cavity, the reaction entropy will be positive, as is the case for the herein studied inclusion reactions. Although the enthalpic and entropic contributions to the reaction Gibbs energies are somewhat different for the reactions of the investigated cyclodextrin derivatives, there is overall no significant variation in their affinities towards LOR, as a consequence of a partial enthalpy-entropy compensation (Table 3).

To get an insight into the composition of complexes responsible for the increase of loratadine solubility in presence of β-cyclodextrins, by using stability constants listed in Table 3 we have prepared the corresponding distribution diagrams shown in Figs. 3c and S3.4-S3.6. It can be seen that in all cases at equimolar amounts of the drug and receptor the most abundant complex is that of 1:1 stoichiometry, whereas at higher molar ratios the 1:2 complex is the dominant species in solution. This finding is in accordance with the results of phase solubility studies which indicated the presence of higher-stoichiometry complex at higher cyclodextrin concentrations (Fig. 2).

To get an insight into the structure of LOR/β-CD complexes, a comprehensive NMR study was performed.

3.4. NMR spectroscopy

For NMR measurements the solutions obtained by dissolving solid LOR, β-CD, and LOR: β-CD mixture in 1:1 molar ratio were used.

3.4.1. Experiments in DMSO-d_6

The results of NMR analysis, including chemical shift perturbations (^{1}H and ^{13}C), intermolecular interactions through space (NOESY), and diffusion coefficients calculations (DOSY), showed that under given conditions (DMSO-d_6, 25 °C) the only evidence of complex formation was found in DOSY spectrum. The details of NMR analysis in DMSO-d_6 are presented in SI (Tables S4.1-S4.3, Figs. S4.1-S4.16).

3.4.2. Experiments in D_2O

3.4.2.1. Structure elucidation

Full NMR analysis of LOR (Figure 1) in D_2O at 25 °C could not be performed due to the low solubility of the compound. The ^{1}H NMR spectrum of LOR (Figure S4.17) was only tentatively assigned. The solubility of LOR was significantly increased by dissolving the solid mixture comprising equimolar amounts of LOR and β-CD. Therefore, full proton and carbon signal assignment was performed only on LOR:β-CD complex and free β-CD in D_2O (Table S4.4, Figs S4.18-S4.26).

To increase the free LOR solubility in water, solution of DCl in D_2O was added to all analyzed samples resulting in the final pH of 1.9. Under this condition, all samples dissolved completely, which enabled full proton and carbon signal assignment, as well as further NMR analyses (Figs. S4.27-S4.38).

3.4.2.2. Proton and carbon chemical shift perturbations

The comparison of ^{1}H and ^{13}C chemical shifts for free *vs.* complexed β-CD is shown in Table 4 for D_2O and Table 5 for acidic D_2O, revealing the largest perturbation for atoms B3 and B5 (B stands for β-CD atoms). This is a strong indication that the complex between LOR and β-CD has formed in both neutral and acidic D_2O. In fact, the perturbation is much larger in acidic D_2O suggesting that complexation is stronger when LOR is protonated. Moreover, the fact that the most pronounced difference was observed for atoms B3 and B5 (positioned on the

inner side of the cyclodextrin cavity), suggested that LOR entered the cavity thus forming an inclusion complex.

The large perturbation between proton chemical shifts for atoms L15 and L16 (L stands for LOR atoms) in D_2O *vs*. acidic D_2O (Fig. 4) was observed as expected, confirming that the protonation is at pyridine nitrogen (position L13).

The comparison of ^{1}H and ^{13}C chemical shifts for free and complexed LOR is given in Tables S4.4 and S4.5. It reveals the formation of the complex through appearance of the second set of LOR signals, the most noticeable in the aromatic region (Fig. 4). The existence of two sets of signals suggest that LOR occupies the β-CD cavity adopting two different conformations which are stable enough in D_2O at 25 °C to be visible on the NMR timescale. A similar effect was noticed earlier for desloratadine (Mashhood Ali et al., 2007). The variable temperature experiments performed on the complex in acidic D_2O revealed signal coalescence at 80 °C showing these two conformations to be in chemical exchange (Figure S4.38).

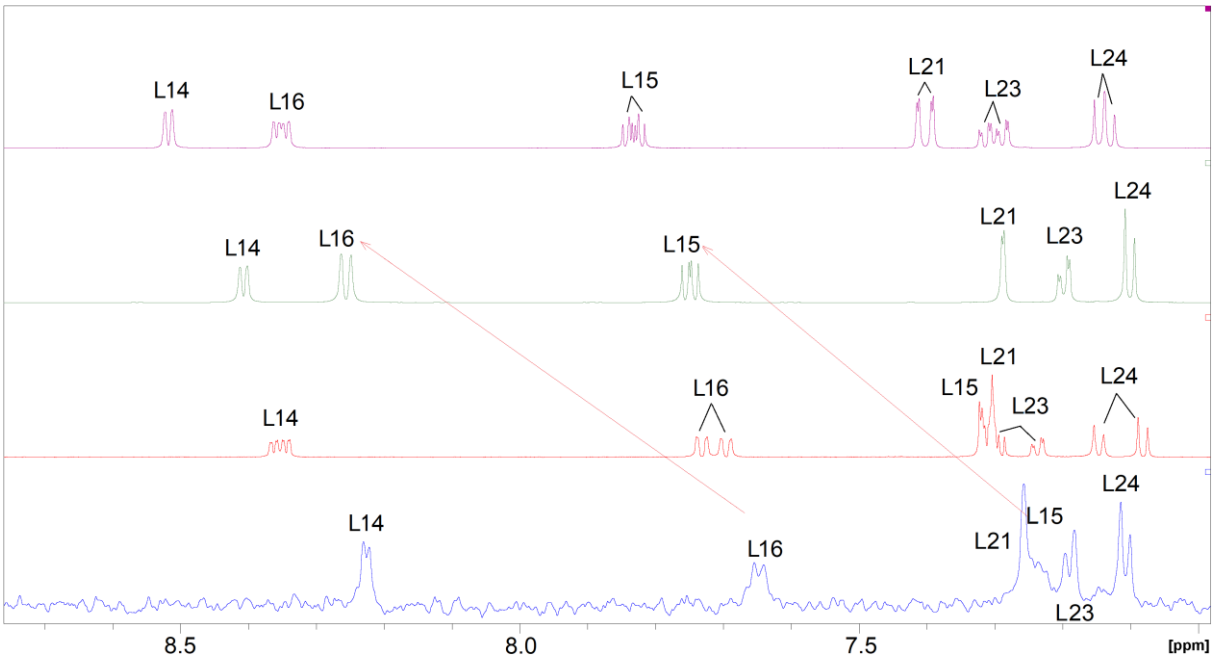

Figure 4. Comparison of the ^{1}H NMR aromatic region for LOR: free in D_2O (blue), complexed with β-CD in D_2O (red), free in acidic D_2O (green) and complexed with β-CD in acidic D_2O (magenta)

Table 4. Comparison of proton and carbon chemical shifts for free β-CD and in complex with LOR in D_2O at 25 °C; atoms with largest detected difference are marked with red.

Proton	Δδ (free and complex)	Free βCD			βCD in complex with LOR			Carbon	Δδ (free and complex)	Free βCD	βCD in complex with LOR
		δ / ppm	multiplet	*J* / Hz	δ / ppm	multiplet	*J* / Hz			δ / ppm	δ / ppm
1	-0.02	5.00	d	3.7	4.98	d	3.8	1	0.17	101.8	102.0
2	0.00	3.58	dd	9.9; 3.7	3.58	dd	10.0; 3.5	2	-0.09	72.0	71.9
3	-0.14	3.89	t	9.5	3.75	m	-	3	0.09	73.0	73.1
4	0.00	3.51	t	9.2	3.51	t	9.7	4	0.08	81.1	81.2
5	-0.11	3.79	m	-	3.68	m	-	5	0.03	71.7	71.8
6	-0.02	3.81	m	-	3.79	m	-	6	-0.16	60.2	60.0

Table 5. Comparison of proton and carbon chemical shifts for free β-CD and in complex with LOR in acidic D_2O at 25 °C; atoms with largest detected difference are marked with red.

Proton	Δδ (free and complex)	Free βCD			βCD in complex with LOR			Carbon	Δδ (free and complex)	Free βCD	βCD in complex with LOR
		δ / ppm	multiplet	*J* / Hz	δ / ppm	multiplet	*J* / Hz			δ / ppm	δ / ppm
1	-0.04	4.99	d	3.8	4.95	d	3.8	1	0.32	101.8	102.1
2	0.02	3.57	dd	9.7; 4.1	3.59	dd	9.7; 3.5	2	0.18	71.7	71.9
3	-0.42	3.88	t	9.7	3.46	m	-	3	1.41	72.0	73.4
4	0.00	3.50	t	10	3.50	s	-	4	0.25	81.1	81.3
5	-0.28	3.78	m	-	3.50	s	-	5	-1.25	73.0	71.8
6	-0.04	3.80	m	-	3.76	m	-	6	-0.38	60.2	59.8

3.4.2.3. Analysis of the NOESY and DOSY spectra

Analysis of LOR:β-CD NOESY spectrum in D_2O (Figs. S4.22 and S4.23) showed the formation of the complex, since all observed LOR and β-CD inter- and intra-molecular interactions were of the same negative sign as the spectrum diagonal. This occurred because LOR through complexation adopted the properties of much larger and slower moving β-CD. The same behavior was observed for the acidic conditions as well, where free $LORH^+$ showed positive NOEs (Fig. S4.31, blue interactions) characteristic for smaller and faster moving molecules, while $LORH^+$ in the complex showed negative NOEs (Fig. S4.36, red interactions) characteristic for larger and slower moving molecules.

A set of inter-molecular interactions was observed in NOESY spectrum of LOR:β-CD complex in D_2O (Fig. S4.23), most importantly L9, L21 and L24 with both B3 and B-5, while L23 only interacted with B6. These were observed for both conformations. Also, there were some other interactions present, but they could not be unambiguously assigned due to the signal overlap (L21 signals overlapped with two L15 signals and one of the L23, while B6 was overlapped with L10a, 6a).

Fortunately, the LOR:β-CD complex NOESY spectrum in acidic D_2O showed much better signal separation. The key NOE intermolecular interactions are shown in Fig. 5. The proton closest to the wide rim of β-CD (B3) exhibits interactions with L9, L21 and L24. B5 buried in the β-CD cavity also shows interactions with these three, but also with L23. Finally, the group which is lining the narrow rim of the β-CD (B6) interacts with L23 (medium) and L24 (weak). From this we can conclude that the LOR enters the β-CD cavity from the wider rim side and ends up with the chlorine atom near the narrow rim. These findings are in agreement with those obtained for desloratadine-β-CD complex by Mashhood Ali et al. in 2007.

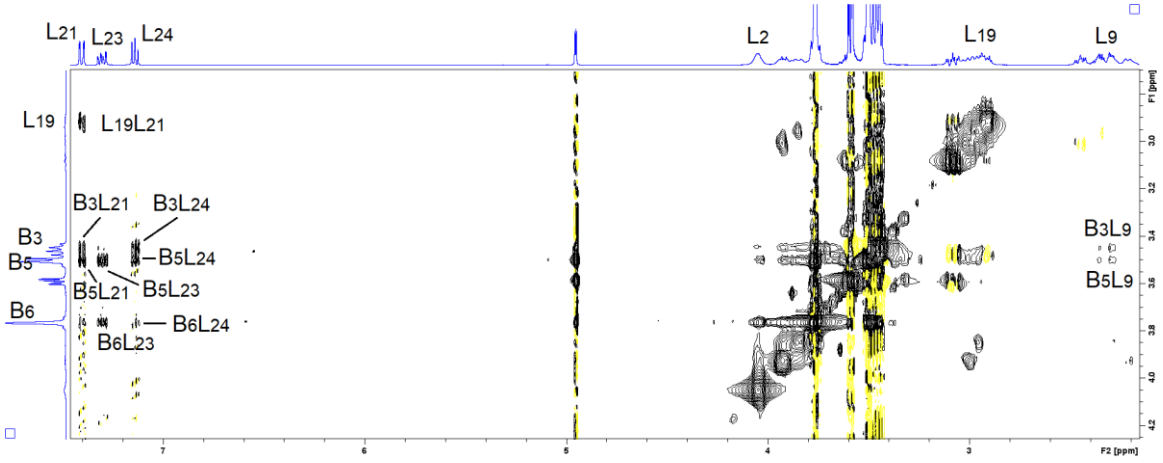

Figure 5. Region of NOESY spectrum showing interactions between LOR and β-CD in acidic D_2O at 25 °C.

The diffusion coefficients in acidic D_2O, measured by diffusion spectroscopy (Fig. S4.39), of free LOR, free β-CD, and the species in their mixture amounted to: 10^{10} D/m^2 s^{-1}: LOR, 3.51; β-CD, 2.44; complex, 2.31, additionally confirming the complex formation.

3.5. Configurational analysis, structural and energetical parameters of the complexation process

In addition to comprehensive experimental work, theoretical studies were performed to find out the most important structural and energetic parameters of the molecules. The smallest, β-CD was used as a model system for all CDs. Since experiments in solution were performed at different pH values, both neutral and protonated forms of LOR were considered.

The analysis below is related to the computational results obtained only for the complexes. The results of the conformational analysis performed for the free molecules (β-CD, LOR and $LORH^+$), such as the most stable conformers, as well as a discussion about the LOR spatial geometry and the energetic parameters, are presented in Figs. S5.2, S5.3 and Comment S1 in the SI.

The stability of different complex configurations is reflected by the BSSE corrected complexation energies ($E_{BSSEcompl}$) presented in Fig. 6. As can be seen, the formation of both β-CD:LOR and β-CD:$LORH^+$ complexes is energetically very profitable. The overall effect of protonation on the complex stability is rather minor, as the differences in the complexation energies between the neutral and protonated forms are relatively small. Nevertheless, it has some influence on which configuration is energetically more favorable: while for both β-CD_EXP:LOR and β-CD_EXP:$LORH^+$, the same configuration K01a is favored, for β-CD_CCCW:LOR and β-CD_CCCW:$LORH^+$, they are different (K03a and K05a, respectively). It should be mentioned that, for all investigated complexes both enthalpy and Gibbs energy (Table S5.3) have negative values, indicating that the formation of both β-CD:LOR and β-CD:$LORH^+$ is an exothermic and spontaneous process, in accordance with the ITC results (Table 3).

The $E_{BSSEcompl}$ values suggest that the β-CD_EXP:LOR complexes are more stabilized than β-CD_CCCW:LOR. This can be reasoned by the fact that β-CD_CCCW has a highly symmetrical geometry which is undoubtedly deformed upon complexation, and that may lead to an increase in energy. Therefore, in the following discussion we focus only on the complexes formed with β-CD_EXP.

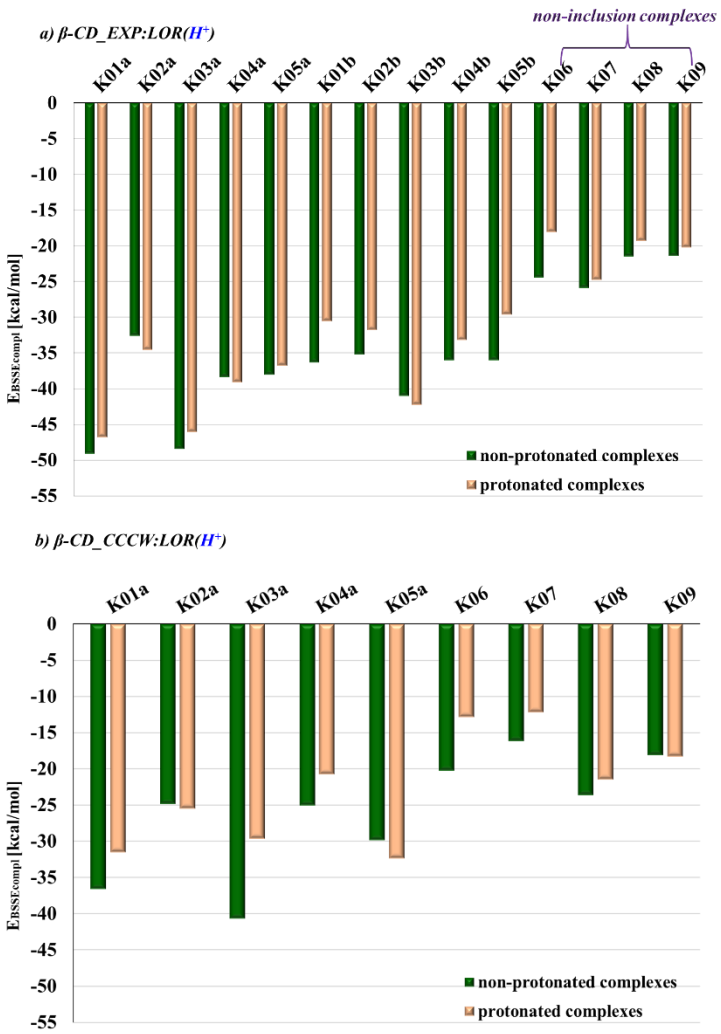

Figure 6. Comparison of the BSSE corrected complexation energies obtained from the M06-2X-GD3/6-31G(d,p) calculations in water (PCM) for: a) the fourteen configurations of β-CD_EXP:LOR and β-CD_EXP:LORH$^+$, b) the nine configurations of β-CD_CCCW:LOR and d β-CD_CCCW:LORH$^+$.

As mentioned above, among the complexes with β-CD_EXP, for both LOR and LORH$^+$, the most stable configuration is K01a, having $E_{BSSEcompl}$ equal to -49.1 (β-CD_EXP:LOR) and -46.8 kcal/mol (β-CD_EXP:LORH$^+$). The corresponding structures are shown in Fig. 7. The structures of complexes are not significantly disturbed by LOR protonation. This is not surprising since the geometries of protonated complexes were formed by adding a proton to the pyridine nitrogen. In the K01a configuration, both aromatic rings are close to the wider rim of CD, while the remaining part of LOR is placed inside the β-CD cavity. However, the K01a structure differs from that indicated by the NOESY spectrum analysis described above, in which the chlorine atom is near the narrower rim. Such an arrangement appears in the structure K04a,

which is included into Fig. 7 for comparison. Although the complexation energies for β-CD_EXP:LOR and β-CD_EXP:LORH$^+$ in the K04a configuration are significantly higher (~11 and ~8 kcal/mol, respectively) than for K01a, the $E_{BSSEcompl}$ value for K04a is still quite large (-38.4 kcal/mol), indicating high stability of this form. Note also that, the strength of interaction between LOR(H$^+$) and β-CD_EXP in both K01a and K04a is very similar (Table S5.3). The preference of the K01a structure over K04a indicated by the DFT results may result from describing the solvent with the PCM model, which does not take into account the hydrogen bonds that the complex can form with water molecules. This effect may enhance the stability of K04a, in which, unlike in K01a, the carbonyl oxygen is exposed to the solvent and can participate in interactions with its molecules.

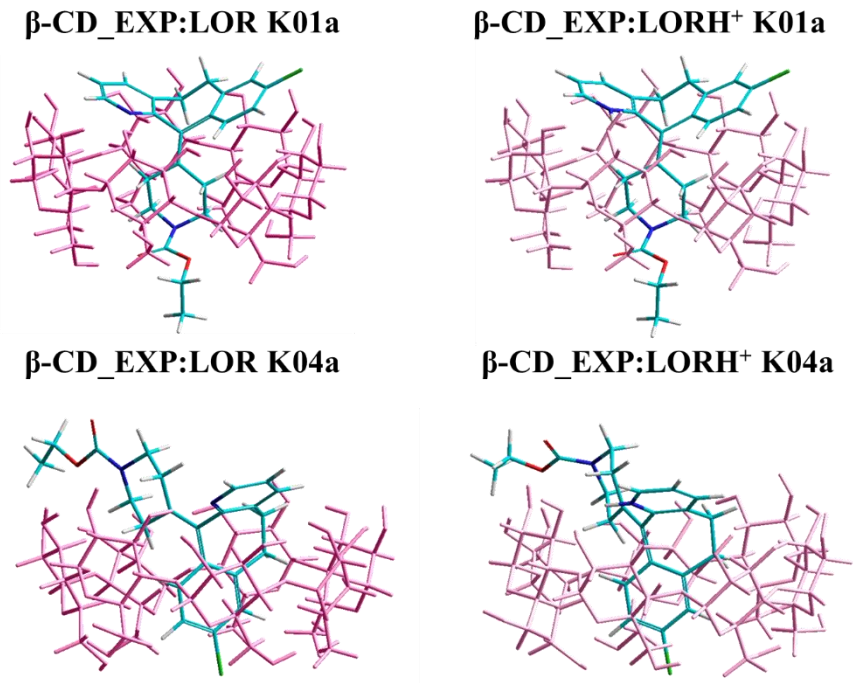

Figure 7. Structures of selected configurations of the complexes β-CD_EXP:LOR and β-CD_EXP:LORH$^+$ obtained by the M06-2X-GD3/6-31G(d,p) calculations in water (PCM): the most stable K01a (a,b) and the configuration K04a indicated by the experimental results (c,d). Atom colors: carbon – cyan, oxygen – red, nitrogen – dark blue, hydrogen – grey, chlorine-green, β-CD_EXP – pink. The coordinates of the neutral complexes are listed in Table S4.

3.5.1. Calculated NMR spectra for selected structures of LOR complexes

The NMR chemical shifts obtained from the M06-2X/6-31++G(d,p)//M06-2X-GD3/6-31G(d,p) calculations in water for the free substrates and the complexes K01a and K04a

corroborate the experimental conclusion that, in the aqueous solution, the complex has rather the K04a-type structure. The computed ^{1}H NMR chemical shifts in the aromatic region for free LOR and its complexes K01a and K04a are shown in Fig. 8 and they can be directly compared to the experimental spectra displayed in Fig. 4. The relative positions of experimentally and computationally obtained chemical shifts are similar for all LOR protons. The exact match of δ values is not to be expected, since the DFT calculations were performed for single structures, while in the real solution various configurations co-exist.

The DFT results obtained for the K01a and K04a complexes clearly show that the positions of chemical shifts for protons in K01a are very different than in K04a, and the pattern found for K04a fits much better to the experimental data. The discrepancy is especially striking for LOR-H24 chemical shift, which in K01a has a very large value.

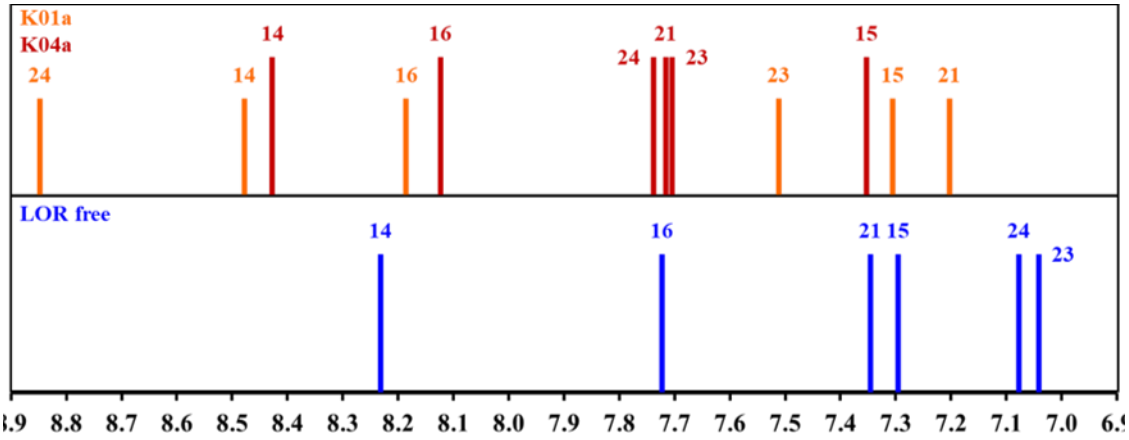

Figure 8. The computed proton chemical shifts obtained from the M06-2X/6-31++G(d,p)//M06-2X-GD3/6-31G(d,p) calculations performed in water (PCM) for H in aromatic rings in: a) free LOR and b) K01a and K04a configurations of β-CD_EXP:LOR. For the complexes, the shorter bars correspond to K01a, while longer to K04a. The numerical data is provided in Tables S5.6 and S5.7 in the SI, while the atom numbering is given in Fig. 1.

The proton chemical shifts obtained from the DFT calculations for β-CD_EXP in the complex K04a (Table S5.5) also show better agreement with the measured values (Tables 4 and 5) than those for K01a. Similarly to the experimental results, the large perturbation of the averaged chemical shifts was found for protons BDC-H3 and BCD-H5. More detailed data for ^{1}H and ^{13}C NMR chemical shifts obtained from the DFT calculations for free molecules and two complex configurations, K01a and K04a, is given in Tables S5.6 and S5.7.

4. Conclusion

By carrying out the comprehensive study of loratadine complexation with different β-cyclodextrin derivatives, the working hypotheses posed in the Introduction section were verified:

(*i*) all cyclodextrins studied substantially increase LOR solubility in the investigated media

(*ii*) by means of an integrated approach which included the use of several experimental and computational methods, the binding reactions were thermodynamically characterized and the mode of LOR-CD interactions as well as complex structures suggested

(*iii*) the obtained results can serve as a basis for further investigations of the LOR-CD systems aiming to develop new drug formulations with improved properties.

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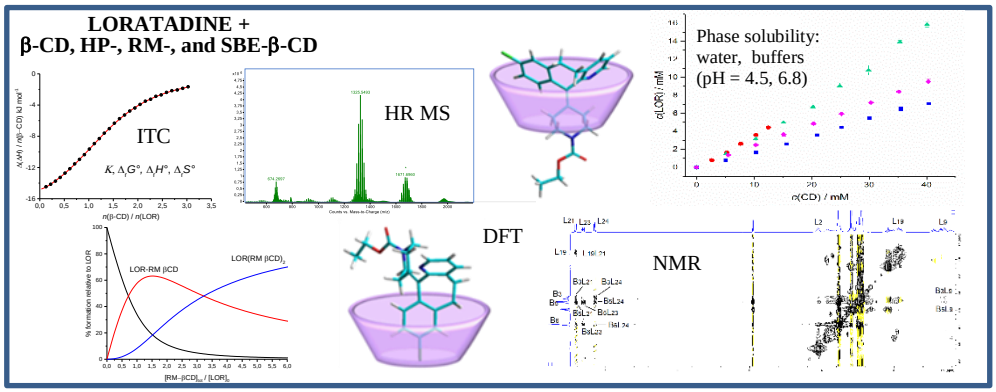

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