Study of the Inclusion Complexes Formed Between Cetirizine and α-, β-, and γ-Cyclodextrin and Evaluation on Their Taste-Masking Properties

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ABSTRACT: Complexation properties of cetirizine dihydrochloride (cetirizine) with α-, β-, and γ-cyclodextrin (CD) were investigated by ultra violet (UV) and nuclear magnetic resonance (NMR) spectroscopies and isothermal titration calorimetry (ITC). The use of the continuous variation method, applied on UV and NMR data, demonstrated 1:1 complex stoichiometry for cetirizine–α-CD, cetirizine–β-CD, and cetirizine–γ-CD, respectively. NMR two-dimensional Rotational nuclear Overhauser Effect SpectroscopY experiments revealed that for α- and β-CD, the complexation takes place by including either the phenyl or chlorophenyl ring of the cetirizine into the CD cavity, whereas in the case of γ-CD, both rings can be included simultaneously. Association constants (K_a) determined by UV spectroscopy demonstrated that cetirizine forms more stable complex with β-CD ($K_a = 5641 \pm 358\,M^{-1}$) than α-CD ($K_a = 1434 \pm 60\,M^{-1}$). No information could be extracted from the UV spectroscopic analysis of cetirizine–γ-CD solutions. ITC results for association constant determination were in compliance with UV results and confirmed that the highest association constant was found for the cetirizine–β-CD complex ($2540 \pm 122\ M^{-1}$). The association constants from ITC measurements for cetirizine–γ-CD and cetirizine–α-CD complexes were found to be 1200 ± 50 and $800 \pm 22\,M^{-1}$, respectively. Taste-masking studies revealed that β-CD is the only native CD recommendable for oral pharmaceutical formulations. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:3177–3185, 2011

Keywords: cetirizine; cyclodextrins; complexation; structure; taste masking; NMR spectroscopy; UV/Vis spectroscopy; Calorimetry (ITC).

INTRODUCTION

Cetirizine is a second-generation antihistaminic drug with selective affinity to H_1 receptors. Its selectivity in the inhibition of the peripheral histamine H_1 receptors provides minimal antihistaminic adverse effects such as dry mouth and sedation.[1] Cetirizine dihydrochloride (cetirizine) is a relatively water-soluble drug with a very bitter taste, and its use in some oral pharmaceutical dosage forms such as syrups, chewing tablets, or gums may be limited by this property. In these cases, an appropriate taste-masking agent is needed in order to reduce or eliminate the unpleasant bitter taste.[2,3]

One approach to reduce the unpleasant bitter properties of the active pharmaceutical ingredients (APIs) is by inclusion complex formation with cyclodextrins (CDs).[4] First-generation or parent CDs such as α-, β-, and γ-CD are composed of six, seven, and eight α-(1,4)-linked glucosyl residues, respectively. The glucose units of the CDs form a cyclic structure with a hydrophilic outer surface and a less polar inner cavity. As a result of this, CDs are capable of accommodating various molecules or hydrophobic parts of the molecules inside their cavity, whereas more polar groups remain exposed to the bulk solution (water).[5] The ability of CDs for the formation of inclusion complexes with other molecules makes them potential solutions to several problems encountered in drug formulation. The complexes most often display altered physical, chemical, and biological properties compared with the uncomplexed active compound itself. This includes improved stability,

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increased aqueous solubility and bioavailability, decreased side effects, and masking of unpleasant tastes.[6–14] The use of CDs for taste masking of bitter APIs has been a subject of many studies,[4] and CDs have been shown to be able to mask the taste of propantheline bromide, oxyphenonium bromide,[15,16] primaquine phosphate,[17] famotidine,[18] doxylamine,[19] thiamine,[20] nicotine.[21] The reduction in unwanted taste is a direct consequence of the CDs inclusion complex formation with the unpleasant tasting component. In addition, it has been speculated that interaction and blockage of the gatekeeper proteins of the taste buds by the CDs also adds to the taste-masking effect.[4] All these features of the CDs make them multifunctional excipients for drug formulation. Very little has been reported in literature on the complex formation between cetirizine and CDs. So far, only nuclear magnetic resonance (NMR) studies on cetirizine–β-CD complex[22] have been performed, and as we will substantiate in the discussion section, these results are in part inconclusive. A couple of patents describe pharmaceutical formulations of cetirizine containing CDs as agents for the reduction of the unpleasant bitter taste.[23,24] Szejtli and Szente[4] also discuss the taste-masking effect of β-CD in cetirizine–β-CD complexes. No detailed study of the inclusion complexes formed between CDs and cetirizine has been published. Here, we present a thorough characterization of the complexes formed between cetirizine and α-, β-, and γ-CDs in aqueous solution, providing detailed information on the thermodynamics, stoichiometry, and structure of the formed complexes. The taste-masking properties of α-, β-, and γ-CDs on cetirizine were examined. This study gives useful information for further development of different cetirizine pharmaceutical dosage forms.

In the present paper, an ultra violet (UV) spectroscopic study was applied for observing the changes in the spectral properties, determination of the stoichiometry, and the association constants of the complexes. NMR spectroscopy was used to examine the stoichiometry and structure of the complexes. The thermodynamics of the complex formation was described by enthalpy and entropy changes obtained from isothermal titration calorimetry (ITC) measurements. The gustatory sensory study was performed by healthy volunteers.

MATERIALS AND METHODS

Materials

Two batches of cetirizine were used. One batch of cetirizine dihydrochloride (certified content 100%) was obtained from Sigma–Aldrich (Steinheim, North Rhine-Westphalia, Germany) and another batch was obtained from Dr. Reddy's Laboratories Ltd. (Hyderabad, Andhra Pradesh India) with a certified content of 99.77%. α-, β-, and γ-CDs were purchased from Wacker Chemie (Burghausen, Bavaria, Germany). Deuterium oxide (D_2O, 99.9%), used for the NMR experiments, was purchased from Larodan Fine Chemicals, Malmö, Sweden.

UV Spectroscopic Studies

For all UV spectroscopy studies, a Shimadzu UV–visible spectrophotometer, model UV-1601, was used with 1 cm matched quartz cells and wavelength scanning speed of 370 nm/min. Cetirizine from Sigma–Aldrich was used for all UV spectroscopic studies. Stock solution of cetirizine was prepared by dissolving 100 mg of cetirizine in 100 mL demineralized water. From the stock solution, by further dilution with demineralized water, standards were prepared in the concentration range of 1–30 $\mu g\ mL^{-1}$. Absorption spectra were measured in the wavelength range between 200 and 250 nm. In the concentration range of 1–30 $\mu g\ mL^{-1}$, cetirizine obeyed Beer's law and the standard curve demonstrated very good linearity. Room temperature (21°C) was used for all UV studies.

Continuous Variation Method (Job's Plot) for UV Spectroscopic Determination of The Complex Stoichiometry

Continuous variation method[25] was used for complex stoichiometry determination. Stock solutions of 10 mM cetirizine, α-, β-, and γ-CDs, in demineralized water were prepared. From the stock solutions, a set of solutions of cetirizine with α-, β-, and γ-CDs were prepared to a constant volume. This was performed by adding various amounts of the respective CD solution to the cetirizine solution. Thus, molar fractions of cetirizine and CDs were varied continuously from zero to one with a rate of 0.1, maintaining a constant accumulative concentration (10 mM) of cetirizine and CDs.

The absorbance maximum of the samples was measured at 230 nm. By measuring the absorbance of samples containing cetirizine with (denoted A) and without CD (denoted A_0), the absorbance difference $\Delta A = A_0 - A$ could be determined. Complex stoichiometry was determined from the plot of the product ΔA [cetirizine] as function of ratio R = [cetirizine]/[cetirizine + CD].

UV Spectroscopic Determination of the Association Constant

Determination of the association constant (K_a) was carried out according to Connor's[26] mole ratio method. This was performed by the addition of various amounts of α-, β-, and γ-CDs, respectively, to a cetirizine solution resulting in cetirizine–CD solutions with constant concentration of cetirizine

(3.25×10^{-5} M) and a molar ratio of cetirizine–CD from 1:1 to 1:100.

Equation 1 is the binding isotherm for 1:1 complex stoichiometry and describes the hyperbolic dependence of K_a in excess CD.

$$\Delta A = \frac{[\text{cetirizine}]_t \cdot K_a \cdot \Delta\varepsilon_{1:1} \cdot [\text{CD}]}{1 + K_a \cdot [\text{CD}]} \quad (1)$$

It was used for association constant determination with fitting procedure by nonlinear regression from the plot of $\Delta A = f([\text{CD}]_t)$ (Origin® SR2 v 7.0383 software (OriginLab Corporation, Northampton, MA, USA)).

In the isotherm 1, [CD] is approximated to the known total CD concentration $[\text{CD}]_t$, as [CD] >>> [cetirizine − CD].[26]

NMR Spectroscopic Studies

All NMR experiments were carried out at 20°C on a BRUKER DRX 600 NMR spectrometer (Bruker Corporation, Billerica, MA, USA), equipped with a TXI (H/C/N) probe and operating at field strength of 14.1 T. ^{1}H NMR, correlation spectroscopy (COSY), and Rotational nuclear Overhauser Effect SpectroscopY (ROESY) experiments were performed in D_2O. For the ROESY experiment of γ-CD, cetirizine from Sigma–Aldrich was used, and for the rest of NMR experiments, cetirizine from Dr. Reddy's Laboratories Ltd. was used.

Continuous Variation (Job's Plot) Method for NMR Spectroscopic Determination of the Complex Stoichiometry

The continuous variation method was also applied on NMR data to determine the complex stoichiometry. ^{1}H NMR spectra were recorded and changes in the observed chemical shift ($\Delta\delta_{obs}$) of cetirizine protons were used. Complex stoichiometry was found from the plot of the product $\Delta\delta_{obs}$ [cetirizine] as function of the ratio R = [cetirizine]/([cetirizine] + [CD]).[25]

NMR Spectroscopic Investigation of the Complex Structure

For assignment of the ^{1}H NMR signals of cetirizine–α-, cetirizine–β-, and cetirizine–γ-CDs, double-quantum filtered COSY experiments were recorded in D_2O. ROESY experiments with a 250 ms spin-lock of 2.1 kHz strength were used for observing through-space intermolecular interactions between the protons of cetirizine and CD, allowing the subsequent determination of the complex structure. Prior to the COSY and ROESY experiments, several ^{1}H NMR experiments were performed in order to determine the ratio of both components that would give well resolved signals and optimal separation of peaks. This is performed to simplify the interpretation of the COSY and ROESY spectra. Optimum conditions were found to be: 30 mM cetirizine and 70 mM α-CD, 4 mM cetirizine and 6 mM β-CD, and 40 mM cetirizine and 20 mM γ-CD.

Isothermal Titration Calorimetry

Calorimetric measurements were performed using a VP-ITC microcalorimeter (MicroCal Inc., Northampton, Massachusetts) controlled by MicroCal's VP viewer software (version 5.0, MicroCal Software Inc., Northampton, Massachusetts). All experiments were conducted at 30°C. The reaction cell (1.4095 mL) and the injection syringe were filled with an aqueous solution of 1 mM cetirizine and 10 mM CD solution, respectively. Thirty portions, 10 μL each, of the CD solution were injected into the cetirizine solution with intervals of 250 s and the heat change in the sample cell was recorded. First injection (1 μL) was discarded to eliminate material diffusion from the syringe into the calorimetric cell. For data analysis, Origin®SR2 v 7.0383 software (OriginLab Corporation, Northampton, Massachusetts) with the MicroCal LLC ITC add-on was used. The data were processed by fitting with nonlinear regression analysis. Using "one set of sites" model in the Origin® software, based on the Wiseman isotherm,[27] K_a and ΔH were kept as varying parameters. Heat of dilution for both cetirizine and CDs was determined by the titration of cetirizine with water and the addition of CD to pure water. In both cases, it was found to be insignificant and did not affect the values obtained.

Cetirizine–CD Sensory Study

Thirteen healthy volunteers (10 men and three women) rinsed their mouth with 5 mL (1 mg/mL cetirizine) cetirizine–CD solutions, kept it for 10 s, and provided information about the bitterness. Thereafter, the solutions were thrown out (spit out). The time between the tasting of each sample was 3 min, when the volunteers rinsed their mouth thoroughly with water. The scale used for grading of the bitterness had following levels: 1 = no bitterness, 2 = slightly bitter, 3 = moderate bitter, 4 = bitter, and 5 = strongly bitter. Because of different personal perception of taste, all panelists were calibrated by giving them a pure solution of cetirizine and designating it as strongly bitter. In order to avoid suggestion, the study was performed in blind manner where only the examiner knew the composition of the solutions. The examined solutions differed in the CD used (α-, β-, and γ-CD) and the molar ratio of cetirizine–CD (1:2 and 1:5). In total, following six solutions were tested: cetirizine–α-CD 1:2 and 1:5, cetirizine–β-CD 1:2 and 1:5, and cetirizine–γ-CD 1:2 and 1:5 molar ratio of cetirizine–CD.

RESULTS AND DISCUSSION

UV Spectroscopic Determination of the Association Constant and the Complex Stoichiometry

UV spectroscopy was used to observe the behavior of the cetirizine absorption spectra with addition of excess CD, determination of the stoichiometry, and the association constant of the complex formation. Changes, such as increase in UV absorption with a bathochromic shift at wavelengths below the isosbestic point and a decrease in UV absorption with a hypsochromic shift at wavelengths above the isosbestic point with the addition of excess CD, provide information that maybe used in the calculation of the association constant K_a or the determination of the complex stoichiometry. These changes occur as a result of partial shielding of the excitable electrons in the CD cavity.[3] UV absorption of cetirizine standards demonstrated very good correlation of 0.9995 in a range between 1 and 30 μg mL^{-1}. Gradual addition of CD to cetirizine solutions resulted in both concentration-dependent hypsochromic shift and decrease in the UV absorption maximum, which are in good accordance with the expected complex formation. Isosbestic points appeared at 221.8 and 220 nm for the solutions of cetirizine with α- and β-CD, respectively, suggesting complex formation. Figures 1a and 1b show the effect of excess α- and β-CD on the UV absorption of cetirizine.

The stoichiometry of the complexes was determined by the continuous variation method. According to the method, the relationship between some physical properties (in this case, UV absorption) and the concentration enables the determination of the sample with the maximum concentration of the complex, when a set of samples with continuous variation of its fractions is used. The sample with the maximum concentration of the complex is the one wherein the molar ratio R corresponds to the complexation stoichiometry.[25]

Figure 2 presents Job's plot for the complex formed between cetirizine and α- and β-CD. In both curves, the position of the maximum is at R = [cetirizine]/([cetirizine] + [CD]) = 0.5, corresponding to 1:1 complex stoichiometry.

For both α- and β-CD, complex stoichiometries were found to be 1:1, and for association constant determination, a binding isotherm 1 for 1:1 complex was applied. Association constants for both α- and β-CD were calculated by the mole ratio method as described in "*UV Spectroscopic Determination of the Association Constant*" by nonlinear regression analysis from the plot $\Delta A = f([CD]_t)$ (Figs. 3a and 3b). The association constant was found to be $1434 \pm 60\ M^{-1}$ for α-CD and $5641 \pm 358\ M^{-1}$for β-CD.

Because the complex formed between γ-CD and cetirizine did not display any significant difference in

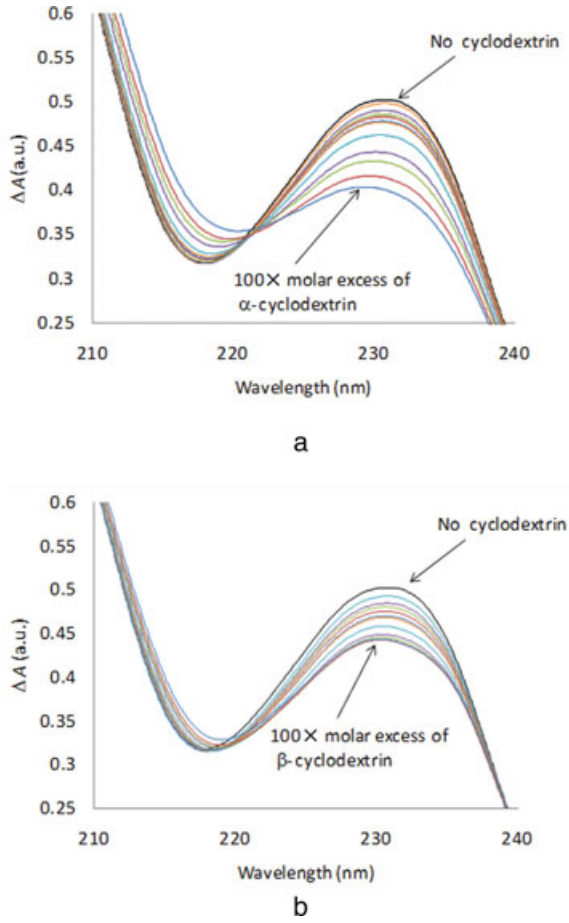

Figure 1. UV absorption spectra of cetirizine (3.25×10^{-5} M) in addition of excess α-cyclodextrin (CD) (a) and β-CD (b) with molar ratio of cetirizine–α-CD and cetirizine–β-CD from 1:1 to 1:100 (1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:10, 1:20, 1:30, 1:50, and 1:100). Both spectra for cetirizine–α-CD and cetirizine–β-CD demonstrate presence of an isosbestic point, concentration dependent hypsochromic shift and decrease in the UV absorption maximum.

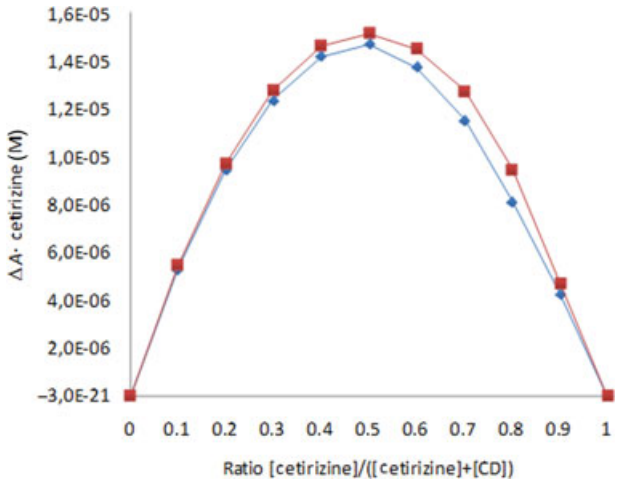

Figure 2. Continuous variation plot (Job's plot) from UV absorbance measurements for the complexation of cetirizine by α-cyclodextrin (CD) (◆) and β-CD (□).

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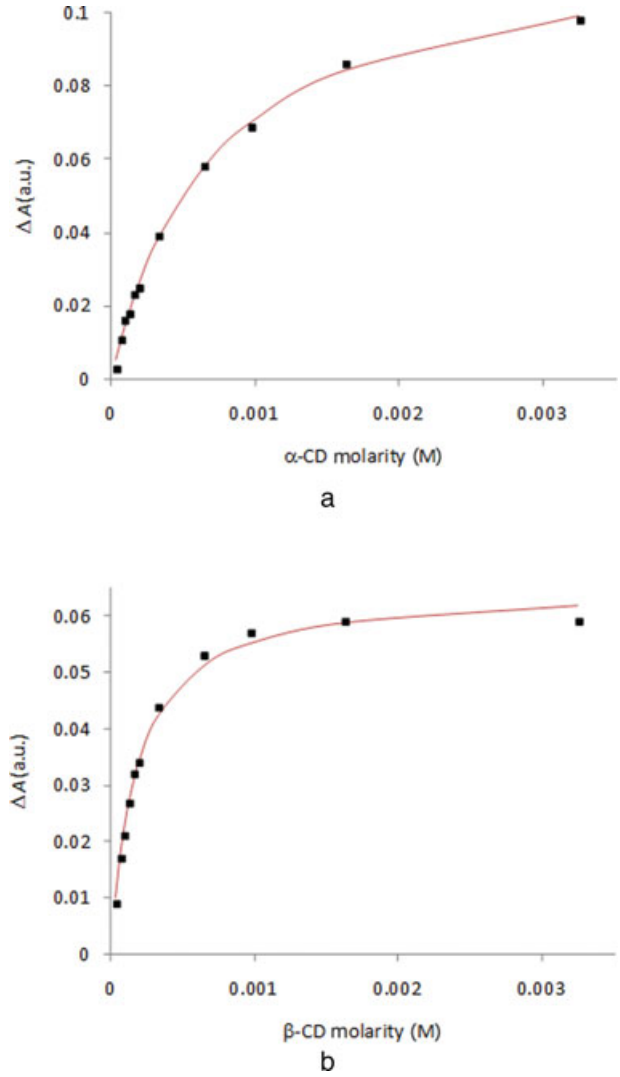

Figure 3. Mole ratio titration plot for cetirizine-α-CD (a) and cetirizine-β-CD (b) complex (cetirizine 3.25×10^{-5} M). Black dots represent the experimental data and the line describes the theoretical function with the fitted data.

the UV-absorption spectrum compared with that of free cetirizine, no information on the strength and stoichiometry of the complex could be extracted using this method.

The association constant determination by UV spectroscopy was performed in diluted solutions of cetirizine–CD that assumable behaved as ideal solutions in which the interaction between formed complexes, free cetirizine, and free CD molecules is neglected. It is already known that the formation of aggregates in particularly concentrated CD-based drug formulations is possible,[28,29] and that they might influence the precision of the method used for the association constant determination.[30] Therefore, the CD concentration should be taken into consideration when various methods are used for association constant determination. It has been reported that α- and β-CD form self-assembled structures, and at concentrations of 10 mM, the aggregates are with radiuses around 194 and 200 nm.[31] Another study reports that for β-CD at a concentration of 3 mM, the aggregate radius is about 90 nm.[32] In our UV spectroscopic determinations of the association constants of cetirizine–α-CD and cetirizine–β-CD, respectively, the CD concentrations used were between 3.25×10^{-5} and 3.25×10^{-3}M, and thus the influence of aggregate formation can be neglected.

Isothermal Titration Calorimetry

Isothermal titration calorimetry was used for association constant determination and to reveal the thermodynamics of the complex formation (Table 1).

Standard formation enthalpies (ΔH°) for all three complexes demonstrated large negative values, indicating an exothermic process and strong energy release during complexes formation. The exothermic process is due to release of the cavity-bound water molecules to the bulk water, a solvophobic effect due to the aromatic rings of the cetirizine and van der Waals interactions between cetirizine and the CD cavity.[33,34] The entropy effect ($T\Delta S^\circ$) for the three complexes is significantly different. Negative values of the entropy effect for cetirizine–α-CD and cetirizine–β-CD are unfavorable for the complex formation, but at typical consequence of the strong enthalpic association (enthalpy–entropy compensation). On the contrary, a positive entropy effect of cetirizine–γ-CD (3546.85 kJ mol^{-1}) is highly favorable for the complex formation and makes a larger contribution to the negative change of standard Gibbs energy (ΔG°) than the negative enthalpy effect. This indicates a predominantly entropy-driven complexation process in the case of γ-CD. The high negative value for the entropy effect of cetirizine–γ-CD complex can be explained partly by the large size of the γ-CD ring, giving a looser fit between cetirizine and γ-CD and a greater freedom of rotation of the cetirizine molecule inside the γ-CD cavity. In addition, the larger cavity of γ-CD results in a much lower enthalpic contribution from cavity-"bound" water, and thus the thermodynamics of classical hydrophobic interaction is prevalent. The highest association constant was determined for β-CD, followed by α- and γ-CDs.

Table 1. Thermodynamic Parameters for the Inclusion Complex Formation Between Cetirizine and α-, β-, and γ-CD Determined by ITC

Complex	ΔH°	$T\Delta S^\circ$	ΔG°	K_a
Cetirizine	($kJ \cdot mol^{-1}$)	($kJ \cdot mol^{-1}$)	($kJ \cdot mol^{-1}$)	(M^{-1})
α-CD	-5514 ± 72	-1485.43	-4029	800 ± 22
β-CD	-4733 ± 65	-9.79	-4724	2540 ± 122
γ-CD	-723 ± 12	3546.85	-4269	1200 ± 50

The values of the association constants determined by UV spectroscopy were a bit higher. The differences in the association constants determined by both methods are due to using solutions with different cetirizine concentrations. This resulted in cetirizine solutions with different pH values. In both cases, the pH of the cetirizine solutions was not controlled. Anyhow, both methods demonstrated the highest value for cetirizine–β-CD.

NMR Spectroscopic Determination of Structure and Stoichiometry of the Complex

The change in the observed ($\Delta\delta_{obs}$) chemical shift of CD and the guest molecule protons assures the existence of the complex and subsequent determination of structure and stoichiometry of the complex. In the case of cetirizine–CD solutions, chemical shift changes for both CD (Table 2) and cetirizine (Fig. 4) protons are observed, which is evidence of host–guest interaction. The largest chemical shift changes at CD were observed for the H-3 and H-5 cavity protons, resulting from intermolecular interactions with guest protons residing in the CD cavity. This is based on the expectation that inclusion complex formation of the guest with CD will alter the chemical shift of CDs H-3 and H-5 protons because they are oriented toward the CD cavity.[35,36]

Chemical shift changes were observed to some extent for all cetirizine protons, and the highest change was observed at the chlorophenyl and phenyl ring protons (Fig. 4).

The large changes in chemical shifts of both host and guest proton signals indicate that the CDH-3 and H-5 protons and aromatic phenyl and chlorophenyl ring protons from the guest molecule are located in close proximity in the complex. This displacement of the chemical shift of CD atoms is due to the magnetic anisotropy of the guest aromatic group, suggesting inclusion complex formation with the integration of the aromatic rings into the CD cavity. The chemical formula of cetirizine molecule is given in Figure 5.

The changes in the observed chemical shift ($\Delta\delta_{obs}$) of cetirizine protons were used for determination of the complex stoichiometry. Figure 6 presents the Job's plot obtained for the complexes formed between cetirizine and α-, β-, and γ-CD. In all cases, Job's plots show a maximum value at R = [cetirizine]/[cetirizine + CD] = 0.5, corresponding to 1:1 complex stoichiometry.

In order to access the structure of the complex, two-dimensional (2D) ROESY experiments were performed (Fig. 7). The presence of cross peaks between H-3 and H-5 protons from the CDs and H-7,7′, H-8,8′, and H-9 phenyl and H-10,10′ and H-11,11′chlorophenyl protons from the cetirizine molecule indicates the penetration of the aromatic rings into the CD cavity.

Figure 7a shows a 2D ROESY spectrum of α-CD with cetirizine. Cross peaks from CD signals to the chlorophenyl ring protons had a higher intensity compared with the cross peaks to the phenyl ring protons. This observation shows that the binding of chlorophenyl ring is stronger and that the inclusion of chlorophenyl ring into the CD cavity is preferable over the phenyl ring. The relative cross peak intensities suggest preferred inclusion geometry with the

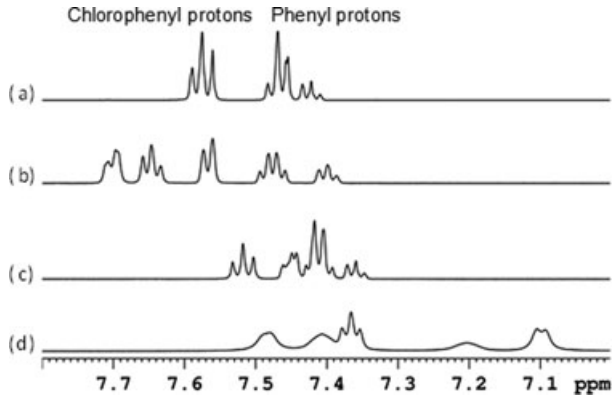

Figure 4. Partial ^{1}H NMR spectra demonstrating chemical shift change of cetirizine phenyl and chlorophenyl protons as a result of the presence of cyclodextrins. (a) 10 mM cetirizine, no CD present (b) 10 mM cetirizine and 4 mM α-CD, (c) 10 mM cetirizine and 4 mM β-CD, and (d) 10 mM cetirizine and 4 mM γ-CD.

Table 2. Chemical Shifts Changes ($\Delta\delta_{obs}$ ppm) of α-, β-, and γ-CD Protons (10 mM CD) in the Presence of 4 Mm Cetirizine

	$\Delta\delta_{obs}$α-CD (ppm)	$\Delta\delta_{obs}$β-CD (ppm)	$\Delta\delta_{obs}$γ-CD (ppm)
H-1	0.033	0.028	−0.038
H-2	0.023	−0.003	−0.005
H-3	0.288	0.161	0.061
H-4	0.024	0.024	0.017
H-5	−0.102	0.156	0.102
H-6	−0.031	0.015	0.044

The change in observed chemical shift is expressed as: $\Delta\delta_{obs} = \delta_{free} - \delta_{complex}$, where δ_{free} and $\delta_{complex}$ are the chemical shifts from observed molecule protons in native and complex form, respectively.

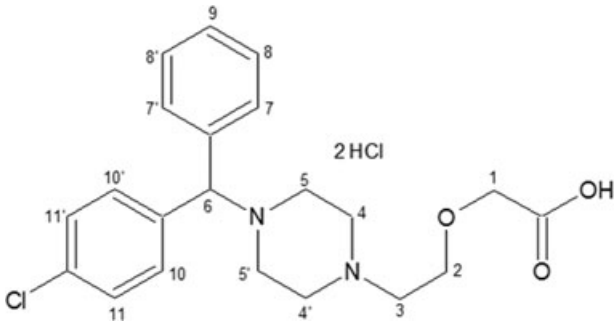

Figure 5. Structure of cetirizine with arbitrary numbering used in the text.

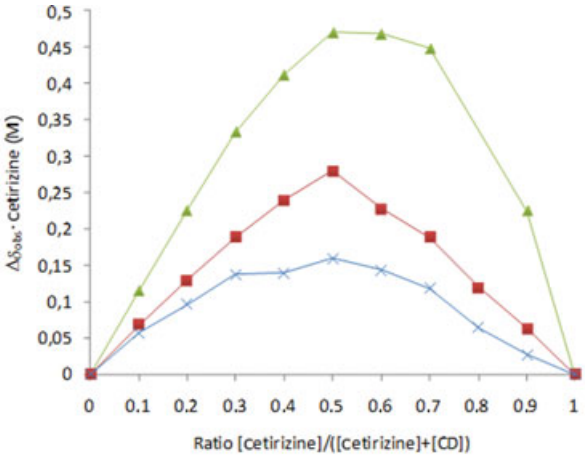

Figure 6. Continuous variation plot (Job's plot) from ^{1}H NMR observed chemical shift ($\Delta\delta_{obs}$) of cetirizine protons for the complexation of cetirizine by α-cyclodextrin (CD) (×), β-CD (□), and γ-CD (△).

phenyl ring entering the CD cavity from the wide rim. However, the NMR line shape of the phenyl ring signals is more complicated in the bound form than in the free form (Fig. 4), suggesting the simultaneous existence of more than one distinct complex geometry with a conformational exchange rate that is lower than the difference in resonance frequencies. Also for the chlorophenyl ring, the extremely weak interaction with H-6 would still point at an interaction, wherein the chlorophenyl ring enters the CD cavity from the wide rim, while we would expect much stronger cross peaks to H-6, if cetirizine was entering the cavity from the narrow rim. However, the relative intensities of H-10,10′ and H-11,11′ cross peaks to CD H-3 and H-5 are not indicative of a single preferred inclusion complex structure, and thus rather point at a multitude of different structures. This is also supported by the observed line shape. The NMR signals of the chlorophenyl ring do not consist of two doublets, but form a more complicated pattern looking like two sets of two doublets each, suggesting two distinctly different complex structures exhibiting slow (on the NMR timescale) conformational interchange.

At β-CD, signal overlap between H-8,8′ and H-11,11′ is somewhat complicating the interpretation of the data; however, it still seems that both the chlorophenyl ring and the phenyl ring enter the CD cavity from the wide rim. A clear conclusion on preferred complex geometry is not possible. We also observed weak interactions with CD H-2 and H-4 on the outside of the CD molecule. The distance of an aromatic ring inside the CD cavity to H-2 and H-4 of CD is on the order of 5 Å, where weak cross peaks can be observed. Again, changes in the line shape suggest the existence of several conformations exchanging slowly on the NMR timescale.

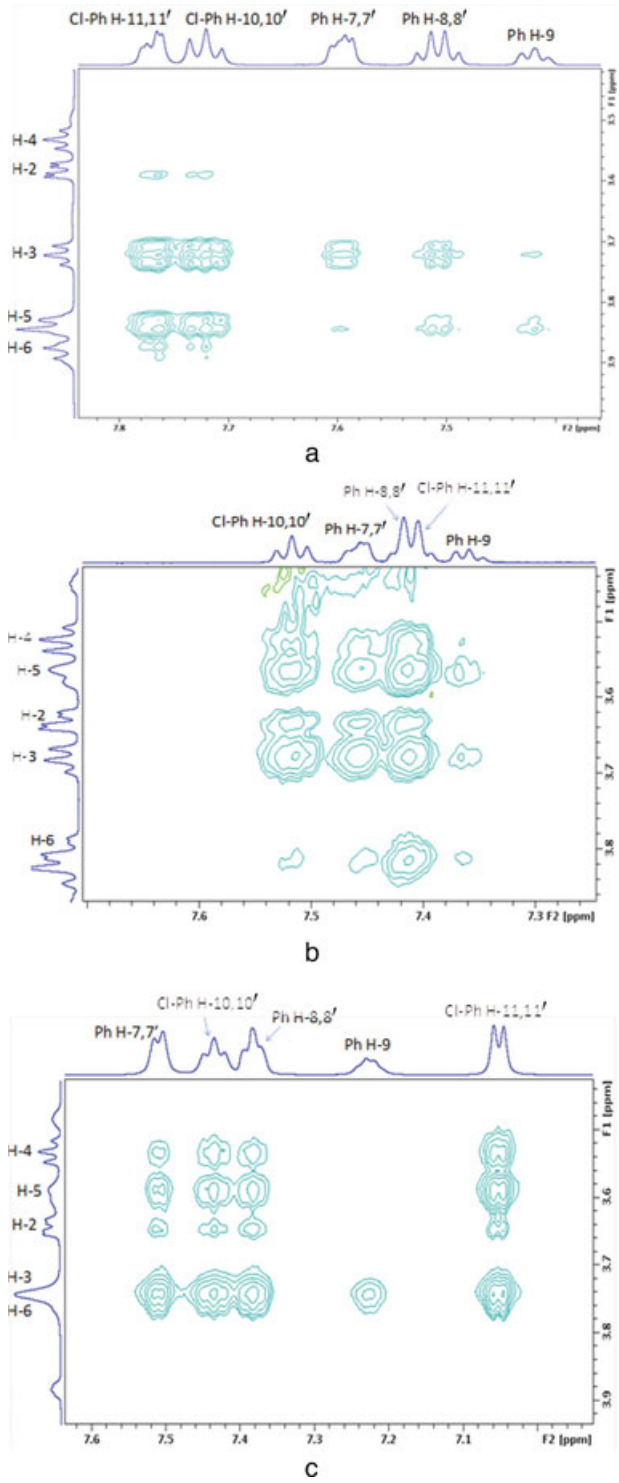

Figure 7. Partial contour plot of two-dimensional ROESY spectrum of a solution containing: (a) 30 mM cetirizine and 70 mM α-cyclodextrin (CD), (b) 4 mM cetirizine and 6 mM β-CD, and (c) 40 mM cetirizine and 20 mM γ-CD.

Very similar conclusions can be drawn for γ-CD; also the biggest of the CDs investigated here accommodates both phenyl and chlorophenyl moieties, although with a slight preference for the chlorophenyl

Table 3. Evaluation of the Bitterness of Cetirizine–CD Solutions by the Healthy Volunteers

	Number of Volunteers Designating the Bitterness of the Solutions As Level:				
	No Bitterness (1)	Slightly Bitter (2)	Moderate Bitter (3)	Bitter (4)	Strongly Bitter (5)
CTZ–α-CD 1:2		3	1	8	1
CTZ–β-CD 1:2	1	6	5	1	
CTZ–γ-CD 1:2			1	1	11
CTZ–α-CD 1:5	1	4	5	3	
CTZ–β-CD 1:5	5	7	1		
CTZ–γ-CD 1:5			3	2	8

CTZ: cetirizine.

ring. Also here, no single complex geometry can explain the observed ROE (Rotational nuclear Overhauser Effect) patterns. As for β-CD, significant cross peaks to H-2 and H-4 on the outside of the CD molecule occur. Poor separation of CDs H-3 and H-6 peaks for cetirizine–γ-CD prevents conclusions as to whether the aromatic rings enter from the wide or the narrow rim. The larger cavity of γ-CD also allows the incorporation of both rings simultaneously.

Earlier results on the inclusion complex of β-CD and cetirizine reported by Syed et al.[22] cannot be compared with the results presented here because the ROESY spectrum presented in Figure 4 of Syed et al.[22] suffers from an insufficient digital resolution prohibitive of extracting any details about the structure of the inclusion complex.

Cetirizine–CD Sensory Study

Pure cetirizine itself is very bitter and very sour due to the dihydrochloride salt of the cetirizine. With the addition of CD, depending on the ratio, the bitter taste decreases but the sourness is still present. The results from the bitterness evaluation using healthy volunteers are presented in Table 3. The perception for taste is very subjective; therefore, some outliers were expected.

Generally, all examiners demonstrated overall same pattern of bitterness perception for the cetirizine–CD solutions. The highest taste-masking effect was observed for the cetirizine–β-CD solutions, followed by cetirizine–α-CD. Cetirizine–γ-CD formulations demonstrated the poorest taste-masking effect compared with the one of pure cetirizine. The reason for this is probably due to the relatively low association constant. The ratio of the CD used as well had an influence on the taste masking, where solutions with ratio 1:5 cetirizine–CD demonstrated better taste compared with the one with 1:2 ratio. This is probably due to the excess of CD, which ensures that cetirizine is predominantly complexed. The best taste-masking properties were achieved with cetirizine–β-CD solutions in a molar ratio of 1:5 of cetirizine–CD, where five and seven of 13 persons designated it as there is no bitterness or as slightly bitter, respectively. The exceptional taste-masking property of the β-CD could be prescribed to its sweet taste and good association with the cetirizine molecule, which is described by the relatively high association constant when compared with the other two native CDs (α- and γ-CD). The worst taste-masking properties exhibited cetirizine–γ-CD solution, 1:5 molar ratio of cetirizine–CD, in which 11 of 13 panelists described it as strongly bitter. Even though, all three native CDs demonstrate good complexation with cetirizine, when it comes to the taste masking, only β-CD is suitable for formulation of oral pharmaceutical dosage forms.

CONCLUSION

Considering that the complexation of APIs with CDs might mask their bitter taste, a study was performed to investigate the complexation properties of cetirizine. The data from all three used methods (UV, NMR, and ITC) confirm the formation of cetirizine–α-, cetirizine–β-, and cetirizine–γ-CD inclusion complexes. 2D NMR spectroscopic studies revealed two possible CD binding sites in the cetirizine structure, the phenyl and chlorophenyl rings. NMR and UV spectroscopic studies for the determination of the complex stoichiometry demonstrated a 1:1 complex ratio between cetirizine and CD molecules. This indicates that both aromatic rings cannot be occupied simultaneously by one CD molecule each. For α- and β-CD, there can only occur complexation of either the phenyl or chlorophenyl ring at any given time, whereas in the case of γ-CD, which is big enough, both aromatic rings can be included simultaneously, but also this leads only to a 1:1 complex. ITC demonstrated that for cetirizine–α- and–β-CD, the complexation process is completely enthalpy driven, whereas for cetirizine–γ-CD, the major contributor to the binding affinity is the entropy change. Both UV and ITC results indicated that cetirizine forms more stable complexes with β-CD than α- and γ-CD. Association constants for cetirizine–β-CD complex are 5641 ± 358 and $2540 \pm 122\,M^{-1}$ obtained by UV and ITC, respectively, and are very close to reported values in the literature (3292 and 3587 M^{-1} for competition experiment with crystal violet and methyl orange, respectively[23]). Even though β-CD was found to form

most stable complex with cetirizine, this study reveals that α- and γ-CD may also be possible candidates for pharmaceutical dosage forms of cetirizine. Concerning the taste-masking properties, β-CD with a 1:5 molar ratio of cetirizine–CD is the preferred candidate for the formulation of oral pharmaceutical dosage forms.

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