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Host-guest inclusion complex of desloratadine with 2-(hydroxy)propyl-β-cyclodextrin (HP-β-CD): Preparation, binding behaviors and dissolution properties

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ABSTRACT: Desloratadine (DSL) is an anti-allergic agent that diminishes nasal and non-nasal allergic marks of seasonal allergic rhinitis. However, its efficiency of DSL is restricted with its low dissolution rate and aqueous solubility. The influence of 2-(hydroxy)propyl-β-cyclodextrin (HP-β-CD) on the DSL solubility was evaluated in respect of the phase solubility method. Following the formulation of inclusion complexes of DSL and HP-β-CD with various strategies (freeze-drying, spray-drying and kneading) (FD, SD and KN), compounds were investigated to evaluate the possibility of altering the physicochemical properties (solubility, release, stability, morphology) of DSL after complexation that are critical for subsequent formulation studies including the complexes prepared. Changes in DSC thermograms, FT-IR spectra and NMR spectra confirmed the constitution of a DSL-HP-β-CD complexes. *In vitro* release from inclusion complexes was compared to pure DSL by dialysis method. It was found that solubility enhancements of DSL-HP-β-CD complexes relies on the sort of the formulation technique. Higher release of DSL from complexes compared to pure DSL was ascribed to the interactive relations between HP-β-CD and DSL, high energetic amorphous status and inclusion complex constitution.

KEYWORDS: Desloratadine; 2-(hydroxy)propyl-β-cyclodextrin; inclusion complex; phase solubility diagram; *in vitro* dissolution; improved solubility.

1. INTRODUCTION

Desloratadine (DSL) is a non-sedating H_1 antihistamine, is strong and safe in the therapy of perennial allergic rhinitis and chronic idiopathic urticaria [1] (Figure 1). It suppresses significant cytokines and cellular activity, advising an anti-allergic and anti-inflammatory profile [2]. It is nearly 10-20 times more effective in H_1-receptor binding than loratadine and has 2.5-4 times stronger antihistaminic efficiency in animals. In addition, it has no significant cholinergic or H_2-receptor affinity [3]. DSL also inhibits only peripheral activity since it does not readily penetrate the blood-brain barrier; hence, it does not cause drowsiness because it does not penetrate the central nervous system [4].

From a chemical point of view, DSL is a base whose nonprotonated structures are inadequately soluble in water [5], eventuating in decreased drug dissolution rate in gastrointestinal system after oral administration, and attendantly, decreased bioavailability [2]. For the improvement of solubility and dissolution, pharmaceutical scientists have been used various approaches such as particle size reduction, salt formation, prodrug and drug derivatization, use of surfactants, cosolvency, change in crystal habit or cocrystals, self-emulsifying drug delivery systems, solid dispersions and inclusion in CDs [6]. CD complexation appear to be the most encouraging solubility increment, as it not only come through the limitations of the different methodologies but also improve the stability and bioavailability of such active agents.

CDs are the cyclic oligomers of glucose with a super molecular lattice structure with hydrophilic external surfaces and lipophilic inner cavity [7]. This structure allows the CD to reserve a guest molecule within the cavity so generating an inclusion complex [8]. Complexation with CDs defends drug from physical, chemical, and enzymatic disruption and also enhance the membrane permeability and bioavailability of drug [9]. Particularly, hydroxypropyl-β-cyclodextrin (HP-β-CD), which displays an acceptable inclusion ability, has high therapeutic values from the point of improving stability [10], enhancing aqueous solubility [11],

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maximizing bioavailability, modifying active agent release and decreasing drug toxicity [12], among others. It is remarkable to note that HP-β-CD presents relatively higher aqueous solubility and low toxicity than other derived forms of CD such as methyl-β-CD and β-CD [13]. HP-β-CD has been used to enhance dissolution rate and the solubility of many active agents with lower solubility and different pharmacological activity, such as posaconazole [12], sulconazole [14], apiprazole [15] , trimethoprim [16], daidzein [17], fluconazole [18], glabridin [19], telmisartan [20], miconazole [21] and curcumin [22]. The studies in which inclusion complexes were prepared with different methods, an increase in efficacy and bioavailability have been shown in addition to increasing water solubility and dissolution rate [12,19,22].

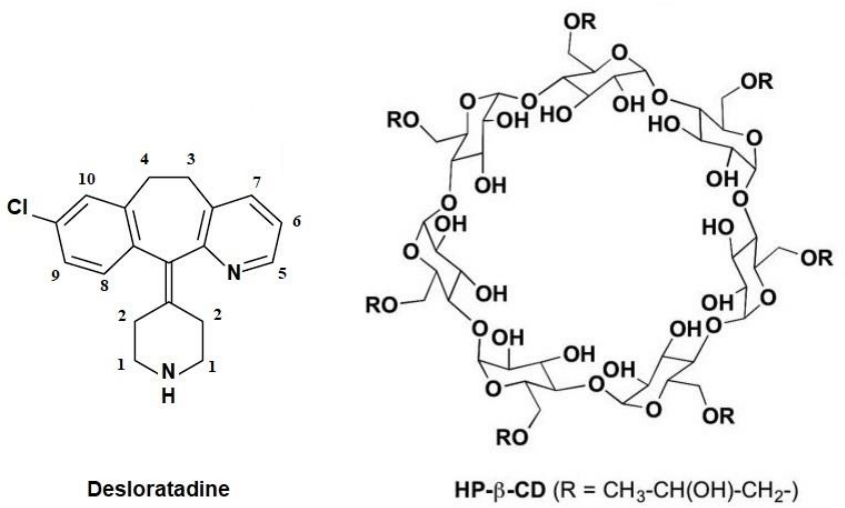

Figure 1. Chemical structure of desloratadine and 2-(hydroxy)propyl-β-cyclodextrin.

The aim of the study was to design inclusion complexes of DSL and HP-β-CD to enhance the biopharmaceutical and physicochemical characteristics of DSL. Recently, solid dispersions [2] and complexes of DSL [1] with biocompatible carbohydrate units were reported to enhance the physicochemical characteristics of DSL. However, HP-β-CD has not been used expect the established β-CD that is usually hampered by its relatively lower aqueous solubility and hemolytic effects than derived forms such as methyl-β-cyclodextrin, HP-β-CD and sulfobutyl ether-β-cyclodextrin were produced to come through such disadvantages of β-CD. The DSL-HP-β-CD complexes were formulated by kneading (KN), freeze-drying (FD) and spray-drying (SD) methods and complexation efficiency in comparison to different methods were analyzed. The apparent stability constant and inclusion stoichiometry of complexes were ascertained according to phase solubility diagrams. The binding behaviors, thermal stability, aqueous solubility and dissolution properties of the inclusion complexes was analysed by thermal analysis (DSC), fourier-transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), nuclear magnetic resonance (NMR) and high-performance liquid chromatography (HPLC). This could supply a beneficial advance to novel DSL-based formulations with higher dissolution rate and aqueous solubility.

2. RESULTS and DISCUSSION

2.1. Determination of DSL by HPLC

The calibration curve was developed by plotting the area/retention time ratio of the DSL. The linearity of the method was investigated within the range of 1-100 µg.mL^{-1} and the calibration curve was selected in this interval. DSL linearity formula was determined as y = 14356.9887x + 1022.013 (r^2= 0.9999) as a result of HPLC process validation studies. HPLC method used in this study resulted in high levels of recovery (101-102%). Measurements fulfilled on three different concentrations (low, medium and high) assessing the repeatability and reproducibility of the analytical process tended to confirm precision of the method as the percentage of relative standard deviation (RSD) was found to be below 2% [23] within the targeted range in this study (1.97- 0.30%). It was determined that the limit of detection (LOD) and limit of quantification (LOQ) values were 0.4115 µg.mL^{-1} and 1.2469 µg.mL^{-1}. Consequently, the HPLC quantification method for DSL was ascertained to be validated for data accuracy [24].

2.2. Phase solubility studies

Phase solubility analysis supplies information about not only the solubilizing potential but also the apparent stability constant of the complex by examining the diagrams of phase solubility [25]. DSL's phase solubility curve was shown in Figure 2 in the presence of HP-β-CD at 25°C. The curves revealed a linear relationship between the solubilized amount of DSL and the concentration of HP-β-CD (r^2= 0.9958). The linear relation between DLS solubility and HP-β-CD concentration indicates a Higuchi and Connors A_L type phase diagram [25]. This diagram is typical of 1:1 complexation indicating the development of water-soluble complex between the substrate (DLS) and the ligand (HP-β-CD). Furthermore, the slope was lower than 1 (0.3873) suggesting that the molar ratio of 1:1 between the guest and host molecule was obtained an inclusion complex formation [26].

DSL solubility was increased 30-fold when HP-β-CD was used in the concentration of 20 mM. The apparent stability constant was calculated as 1859.34 M^{-1}, suggesting relatively strong interaction between molecules [7]. The value of K 1:1 is most commonly between 50 and 2000 M^{-1} with an average value of 129, 490 and 355 M^{-1} for α-, β- and γ-CD, respectively [27]. In the literature, the constant of the stability of the HP-β-CD complexes were significantly higher than those of the parent β-CD [20]. The improved performance is attributed to the increment of inclusion capacity and enhancement of physicochemical properties of native CDs [21].

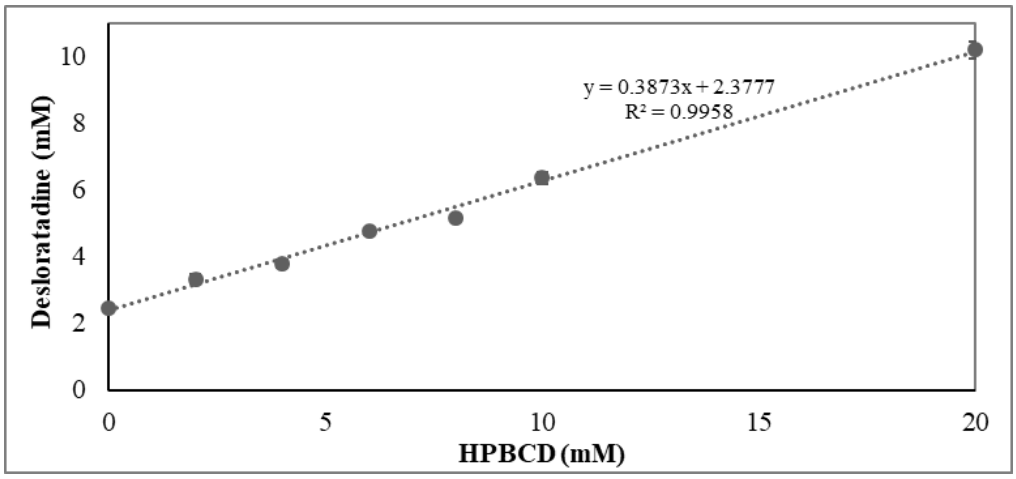

Figure 2. Phase solubility diagram of DSL in the presence of HP-β-CD.

2.3. Characterization of inclusion complexes

2.3.1. DLS content, yield and aqueous solubility

The practical yield (complexation performance) was determined as the ratio of the dried complex and the sum of the DSL and HP-β-CD masses. The findings were presented in Table 1. The lowest practical yield was observed with spray drying method. The loading contents were found to be 17.96, 17.47 and 17.54% for FD, SD and KN complexes, respectively. The embedding efficiency varied from 95.91% to 98.59% for the complexes prepared.

It has been stated that CD molecules are capable to enhance solubility of the guest molecule. Table 1 demonstrates the aqueous solubility of DSL in this work as a result of various formulation techniques of DSL-HP-β-CD complexes. Regarding solubility experiments at 25°C, an enhancement of DSL solubility was occurred in the presence of HP-β-CD. When the aqueous solubility of pure DSL is 0.11 ± 0.01 $mg.mL^{-1}$, the solubility of inclusion complexes 132.46 ± 1.92 $mg.mL^{-1}$, 115.35 ± 3.37 $mg.mL^{-1}$ and 11.03 ± 0.01 $mg.mL^{-1}$ for FD, SD and KN complexes, respectively. The increase of DSL solubility was absolutely associated with the extent of the interactive relation of the active agent with HP-β-CD according to preparation method. The remarkable increment of the aqueous solubility that occurred with FD and SD complexes has been ascertained to the inclusion complexation with high energetic amorphous status/decrease of post-complexation crystallinity [7,28].

Table 1. Properties of pure DSL and DSL/HP-β-CD complexes.

Code	Practical yield (%)	EE% ± SE	Loading content % ± SE	Aqueous solubility ± SE (mg.mL^{-1})
DSL	-	-	-	0.11 ± 0.01
FD	78.97 ± 2.52	98.59 ± 0.82	17.96 ± 0.09	132.46 ± 1.92
SD	29.58 ± 3.15	95.91 ± 2.34	17.47 ± 0.25	115.35 ± 3.37
KN	95. 63 ± 1.65	96.29 ± 0.96	17.54 ± 0.10	11.03 ± 0.01

DSL: Desloratadine; FD: Freeze-drying; SD: Spray-drying; KN: Kneading; SE: Standard error; EE: Embedding efficiency

2.3.2. Morphology

SEM is a principle technique for assessing quantitatively alterations in particle size and the surface morphology of materials, such as inclusion complexes [12]. The SEM photographs of the samples were illustrated in Figure 3.

Pure DSL showed crystalline structure with a flaky morphology (Figure 3a) and HP-β-CD (Figure 3b) appeared as a spherical shape with inside cavities of various sizes [29]. In the physical mixture (Figure 3c) of both components, the crystalline DSL was combined with the HP-β-CD microspheres through adherence to the CD layer. On the other hand, inclusion complexes prepared with different methods (Figure 3d-f) took a typical shape, which was not only structurally distinct from the free components but also from the physical mixture. The freeze-dried material (Figure 3d) appeared in a typical morphology with a soft and fluffy structure [30]. The spray-dried complexes demonstrated spherical shape with smooth surfaces that are the common morphology of spray-dried amorphous materials [31]. The kneaded complexes presented irregular morphology with plate-like structure [14]. These results are consistent with the formation of a solid phase with different morphologies hold a candle to the starting materials suggesting that DSL was deposited into the HP-β-CD non-polar cavity, revealing an obvious solid-state interaction [14,29].

2.3.3. Differential scanning calorimetry (DSC)

DSC is an important analytical technique for the analysis of solid materials as it can supply exhaustive information on their physical and energy characteristics [32]. DSC is therefore commonly utilized to investigate the interactions between drug substances and CDs in solid form [11]. We can elicit about solid-state modifications and interactions between the components and verify complexation mechanism by comparison with the thermograms of individual constituents, their physical blend and the prepared inclusion complexes.

The DSC thermograms of pure components and inclusion complexes were presented in Figure 4. DSL exhibited a sharp endotherm at 165.18°C corresponding to the melting endotherm and verifying its crystalline nature (Figure 4a). The thermogram of HP-β-CD demonstrated two broad endotherms; one, appeared around 80°C, which can be related to the release from the CD cavity of crystallized water molecules [12], and the other wide endothermic peak at 280°C owing to the thermal degradation [21] (Figure 4b). The physical mixture revealed a simple superposition of pure DSL and HP-β-CD endothermic peaks, thereby indicating that the host and guest molecules do not interact in physical mixture [11] (Figure 4c). The absorption peak of DSL at 165.18°C disappeared in the inclusion complex thermograms (Figure 4c-e). Complete absence of the melting endotherm of the active agent implies amorphization of the crystalline substance in the presence of an amorphous structure and verifies constitution of inclusion complex [7,9,11].

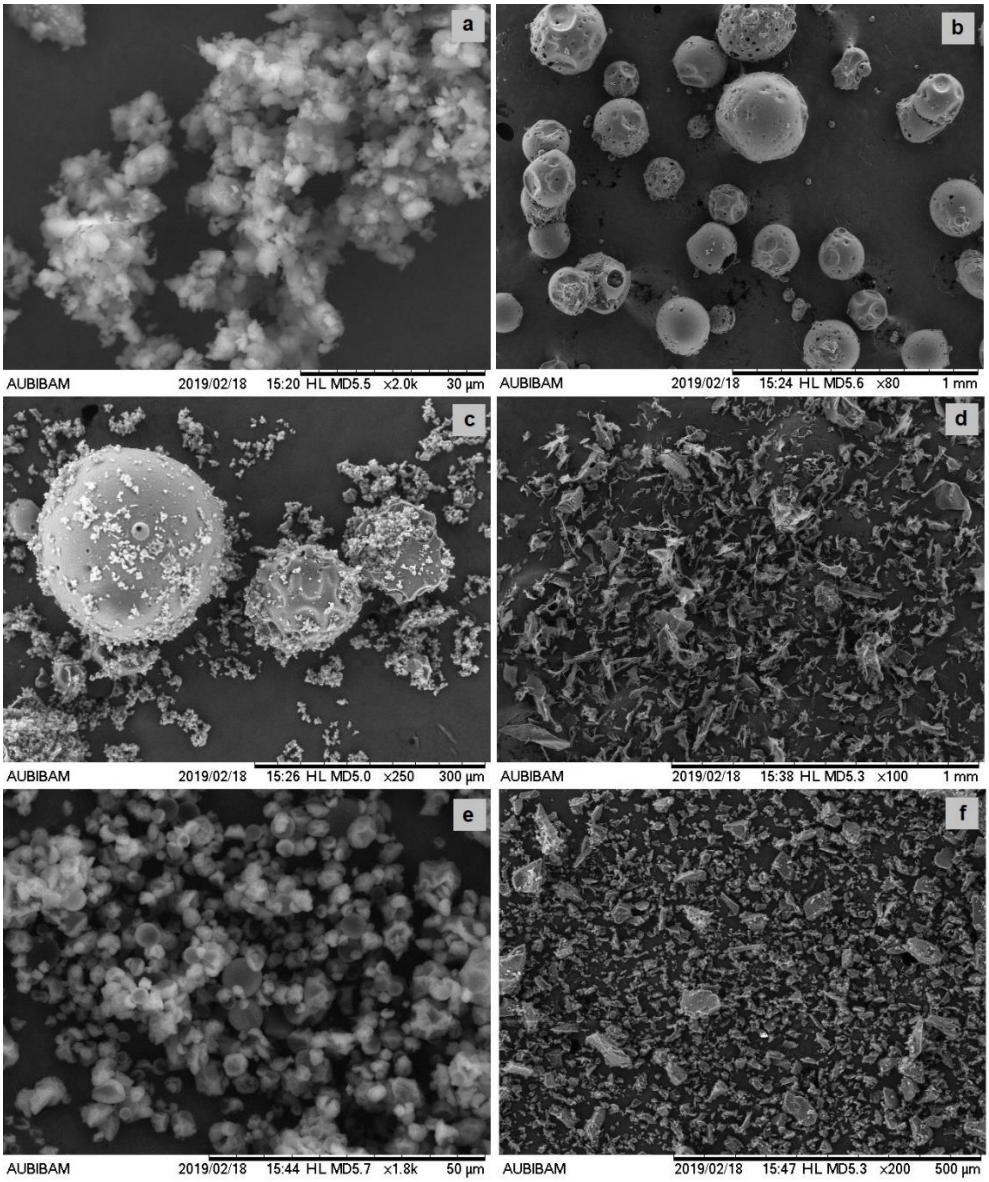

Figure 3. SEM images of pure DSL, pure HP-β-CD, physical mixture and DSL/HP-β-CD complexes. (a: DSL; b: HP-β-CD, c: PM, d: FD, e: SD, f: KN).

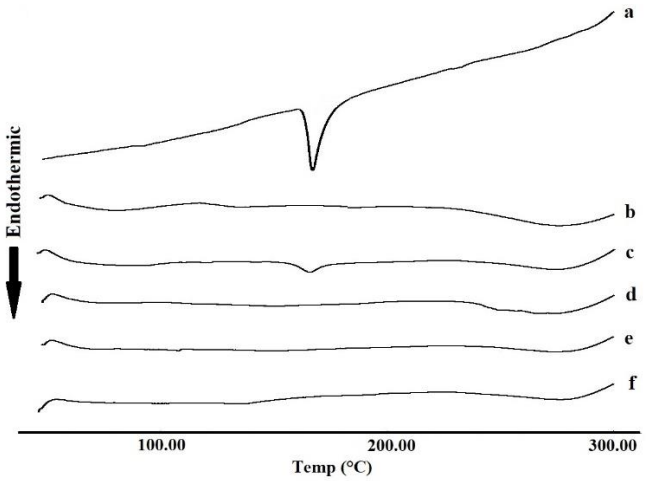

Figure 4. DSC thermograms of pure DSL, pure HP-β-CD, physical mixture and DSL/HP-β-CD complexes. (a: DSL; b: HP-β-CD, c: PM, d: FD, e: SD, f: KN).

2.3.4. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectroscopy is commonly utilized in order to investigate inclusion complexes of drugs with CDs. This approach makes it possible to decide what bands of stretching vibrations of the pure compounds and CD changes in the complexation mechanism. Change, widening, disappearance in intensity of the peaks and their shifts declare the inclusion complex constitution. This might arise from a reduction in the stretching vibrations of the guest molecule by the reason of well accommodation into the CD cavity or by weak interatomic bonds [33].

The pure DSL spectrum demonstrated peaks at 3302 cm^{-1} and 1585 cm^{-1}, belongs to the N-H stretch and N–H bend vibrations of secondary amine group [2]. The spectra also showed alkene [C=C stretching (conjugated)] at 1610-1640 cm^{-1}, imines (R_2C=N-R stretching) at 1640-1690 cm^{-1}, aromatic C-H (stretching) at 3000-3020 cm^{-1} and alkanes (C-H stretching) at 2980-2850 cm^{-1} [34]. Other peaks observed at 1176 cm^{-1} and 779 cm^{-1} corresponded to C-N and C-Cl stretching, respectively and given in Figure 5 [35].

The FT-IR of HP-β-CD includes wide band at 3343 cm^{-1} that ascribed to H-bond stretching vibrations of hydroxyl groups. The other bands are organized as follows: 2910 cm^{-1} (C-H) stretching vibrations of O-H bonds in C-O-H groups and water molecules; 1362 and 1149 cm^{-1} (C-H, deformation vibrations in groups of CH_2OH and CHOH); and adsorption peak at 1080 cm^{-1} (C-O-C) [11,19]. FT-IR spectrum of the physical mixture (Figure 5c) is a combination of HP-β-CD and DSL's spectra. This observation showed that there was no intermolecular interaction in the physical mixture [7].

The unique pattern in the FT-IR spectra of the all complexes indicated the constitution of inclusion complexes. In particular, unlike the physical mixture, there were no DSL absorption peaks in the spectra of the SD and FD inclusion complexes suggested that DSL was embedded inner cavity of the HP-β-CD molecule upon inclusion complex constitution (Figure 5d-e). When examining the FT-IR spectra of the KN complex, one of the DSL main peaks at 1585.49 cm^{-1} were sighted to be appear with lower intensity (Figure 5f). However, disappearance of other distinctive DSL peaks as a result of partial interaction drug and HP-β-CD could be confirmation of the alteration [9].

3.3.5. Nuclear magnetic resonance (NMR)

Additional evidence of inclusion complex generation can be provided by NMR spectroscopy, which is the most efficient technique for evaluating constitution of inclusion complexes and to prove the potential mode of inclusion [12,36]. Analysis of chemical shift changes in NMR spectra (^{1}H NMR and ^{13}C NMR) of both host and guest molecules can not only demonstrate the complex constitution but also provide beneficial knowledge on the complex's stability, stoichiometry, mechanism and geometry. Calculations of chemical shift alterations of the guest molecule as a function of increasing CD concentration enable the investigation of the complex association constant while supplying insight into the stoichiometry and conformation of the generated system [37].

Figure 6 demonstrated the ^{1}H-NMR spectroscopy DSL before and after generation of the inclusion complex (as ppm). The formation of inclusion complex resulted in a significant downfield or upfield shifts for DSL protons as described in Table 2. A remarkable shifts of DSL protons in complexes suggested that the aromatic core of guest compound had penetrated to the HP-β-CD cavity [29]. Since the lowest chemical shifts values were obtained with the KN complex (H-5, H-7, H-10, H-9), indicating that the part of the DSL carrying these protons may not be included the HP-β-CD central cavity [7]. This suggested that there is a partial interaction DSL and HP-β-CD in the KN complex.

Herein, the ^{1}H-NMR spectra of HP-β-CD, before and after generation of the inclusion complex (as ppm) is illustrated in Figure 6. As far as we know, H-3 and H-5 protons of CDs are situated in the internal cavity. Also, H-3 and H-5 protons are close to the secondary and primary face, respectively. Remarkable alterations were observed in the H-3 and H-5 protons' chemical shifts when host molecules occurred inclusion complexes with DSL by different preparation methods. Upfield shifts H-3 of complexes FD (0.0010 ppm) and SD (0.0071) was emerged whereas H-3 of KN complex did not show any changes. While the resonance of H-5 protons demonstrated a relative greater upfield shift upon complex formation for all complexes (0.0021, 0.0018 and 0.0038 for FD, SD and KN complexes, respectively). (Table 3). This information suggested constitution of stable inclusion complexes between DSL and HP-β-CD [8,12,17].

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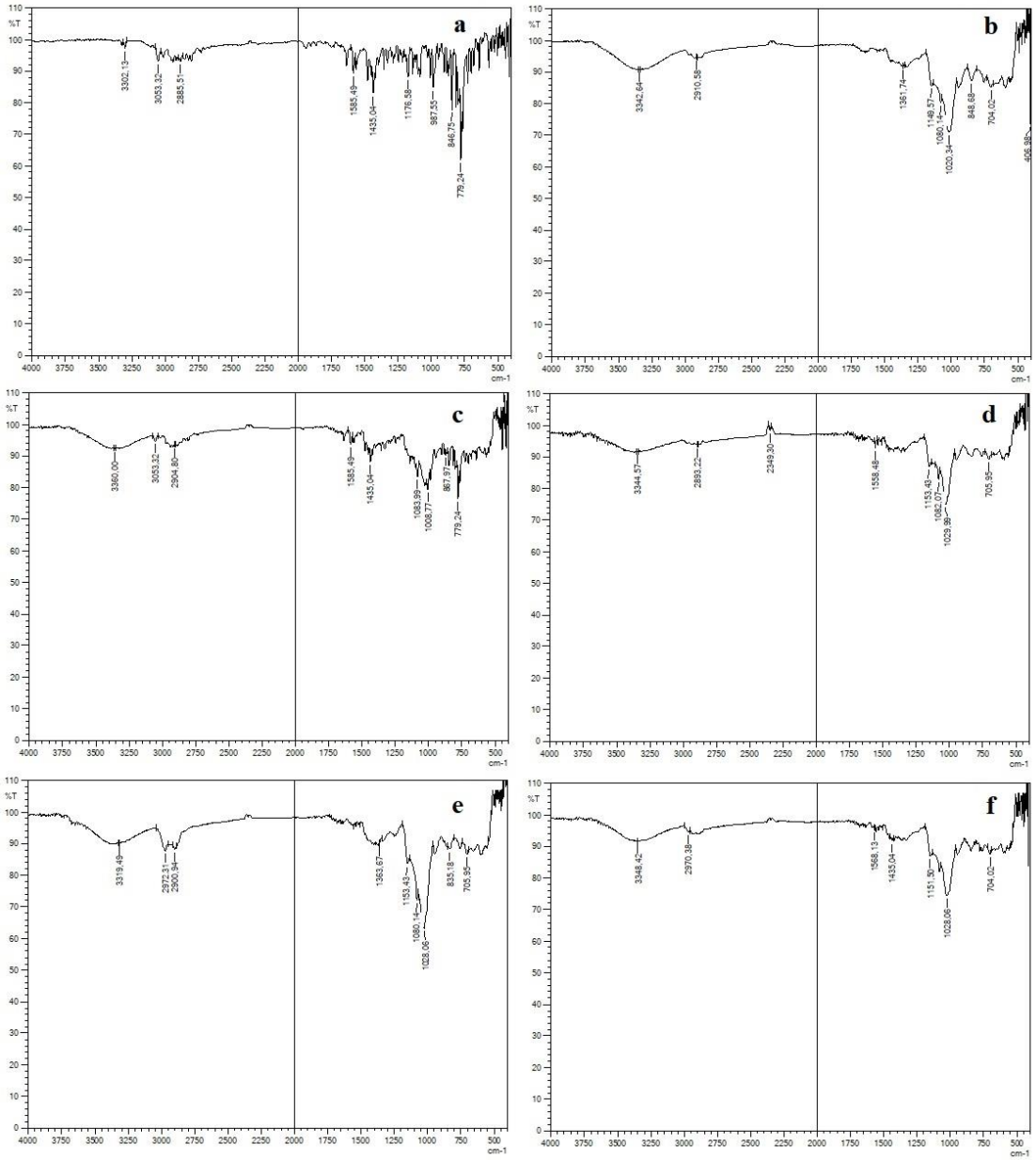

Figure 5. FT-IR spectra of pure DSL, pure HP-β-CD, physical mixture and DSL/HP-β-CD complexes. (a: DSL; b: HP-β-CD, c: PM, d: FD, e: SD, f: KN).

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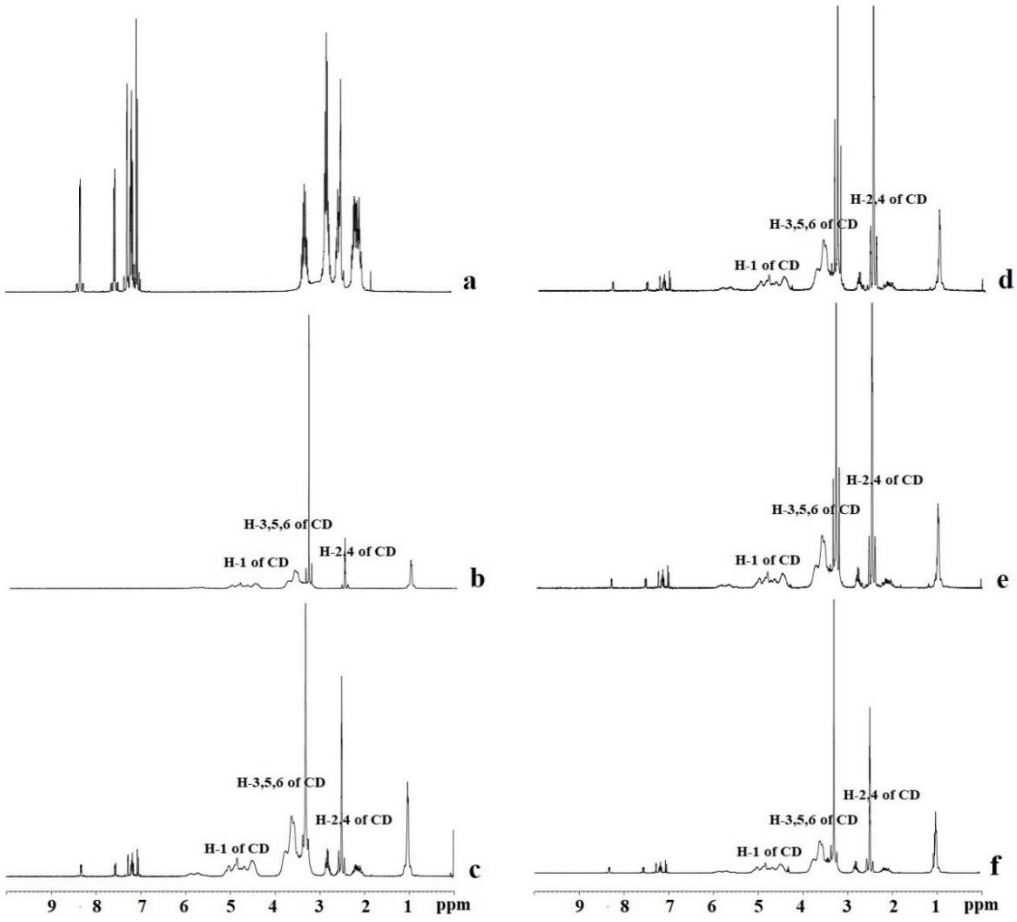

Figure 6. [1]H NMR spectra of pure DSL, pure HP-β-CD, physical mixture and DSL/HP-β-CD complexes. (a: DSL; b: HP-β-CD, c: PM, d: FD, e: SD, f: KN).

Table 2. Variation of the [1]H-NMR chemical shifts of DSL in free and complex status.

Protons	DSL (free) (δ)	FD (δ)	FD (Δδ)	SD (δ)	SD (Δδ)	KN (δ)	KN (Δδ)
H-5	8.3179	8.3139	**-0.0040**	8.3196	**-0.0017**	8.3194	**-0.0015**
H-7	7.5437	7.5507	**+0.0070**	7.5509	**-0.0072**	7.5451	**-0.0014**
H-10	7.2768	7.2736	**-0.0032**	7.2745	**+0.0023**	7.2749	**+0.0019**
H-8	7.2002	7.2044	**+0.0042**	7.2121	**-0.0119**	7.2044	**-0.0042**
H-6	7.1709	7.1758	**+0.0049**	7.1763	**-0.0054**	7.1760	**-0.0041**
H-9	7.0668	7.0685	**+0.0017**	7.0689	**-0.0021**	7.0680	**-0.0012**
H-4	3.3102	3.3011	**-0.0091**	3.3029	**+0.0073**	3.3025	**+0.0057**
H-1	2.8393, 2.5338	2.8221, 2.4994	**-0.0172, -0.0344**	2.8220, 2.4999	**-0.0173 -0.0339**	2.8126, 2.4999	**-0.0267 -0.0339**
H-3	2.8170	2.7989	**-0.0181**	2.7996	**-0.0174**	2.7990	**-0.0180**
H-2	2.1923, 2.0706	2.1947, 2.0876	**+0.0024, +0.0170**	2.1946, 2.0838	**+0.0023 +0.0182**	2.1945, 2.0881	**+0.0022 +0.0175**

DSL: Desloratadine, FD: Freeze-drying, SD: Spray-drying, KN: Kneading

Table 3. Variation of the chemical shifts of H-3 and H-5 protons of HP-β-CD in free and complex status.

Protons	HP-β-CD (free) (δ)	FD (δ)	SD (δ)	KN (δ)
H-3	3.7396	3.7406	3.7457	3.7396
H-3 (Δ δ)	-	+ 0.0010	+ 0.0071	-
H-5	3.6145	3.6166	3.6163	3.6183
H-5 (Δ δ)	-	+ 0.0021	+ 0.0018	+ 0.0038

FD: Freeze-drying, SD: Spray-drying, KN: Kneading

2.4. *In vitro* dissolution study

CDs have a critical function in delivery of poorly water-soluble drugs by enhancing dissolution and the apparent solubility of drug through generation an inclusion complex [38]. The enhanced aqueous solubility and dissolution rates might be detected from solubility phase diagram and kinetics of drug dissolution, respectively. As a principal rule, it can be assumed that the enhanced dissolution rate of CD-embedded active agents ensures different factors such as advanced wettability, enhanced solubility and molecular dispersion [9]. Figure 7 demonstrates the dissolution profiles of DSL and DSL: HP-β-CD complexes. The dissolution rate of HP-β-CD complexes is obviously faster than pure material. This might be on the occasion of the high energetic amorphous state of complexes caused by a faster dissolution rate. As the decrease of drug crystallinity with complexation with CDs supplies to the improved apparent drug solubility and the rate of dissolution [38]. Moreover, CDs have a surfactant-like characteristics that might decrease the interfacial tension between the dissolution medium and water insoluble active agent and, resulting in a higher dissolution rate [8].

It was observed that dissolution increment of DSL: HP-β-CD complexes relies on the preparation method. The higher rate of dissolution of DSL from complexes compared to pure form was attributed to the interactions between active agent and HP-β-CD, the entrapment of the drug in the CD cavity and CD's capability to enhance its wettability. Furthermore, as shown by DSC and FT-IR analyses generation of the amorphous state of drug also promoted to the improved dissolution of complexes [8,39].

The resulting dissolution profiles of the products under different test conditions can be hold a candle using model-independent or model dependent statistical procedures. The model-independent similarity factor (f_2) method is a relatively simple and commonly confirmed approach for comparing profiles of dissolution [40]. Most international regulatory authorities advocate the methodology of the f_2 similarity factor as a way of demonstrating similarity in dissolution. The guidelines state that, f_1 values up to 15 (0–15) and f_2 values over 50 (50-100) ensure that the two profiles are "same" or "equivalent." [41].

f_1 and f_2 values for comparison of dissolution profiles were shown at Table 4. According to f_1 and f_2 factor, FD and SD complexes, which showed complete inclusion complex formation confirmed by characterization methods, were found to be different according to the reference values. However, the KN complex, which has been proved to be partially complexation by FT-IR and NMR studies, was found to be similar to the reference according to the f_2 factor.

Thus, the enhanced dissolution rate and suitable statistical comparison values were obtained from the inclusion complexes prepared by FD and SD techniques. This may be attributed to the reduction in crystallinity and complete constitution of inclusion complex.

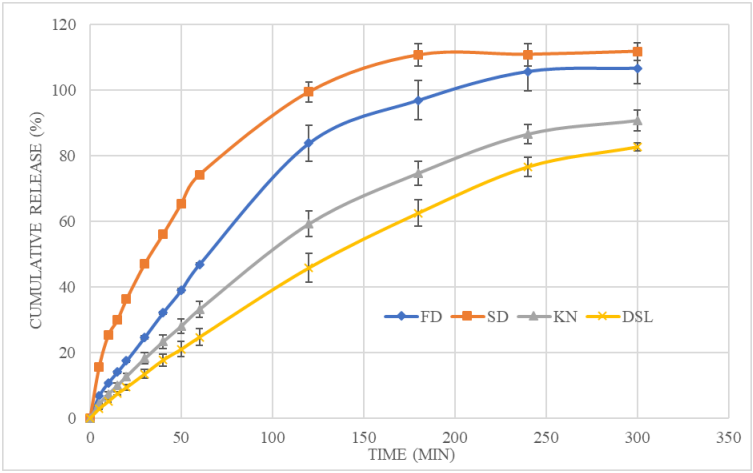

Figure 7. *In vitro* dissolution profiles of DSL and HP-β-CD complexes (mean ± SE) (n=3).

Table 4. Difference and similarity factors for comparison of dissolution profiles of DSL and DSL-HP-β-CD complexes.

	FD	SD	KN
f_1 (difference factor)	58.25	111.83	21.38
f_2 (similarity factor)	34.61	22.71	56.63

FD: Freeze-drying, SD: Spray drying, KN: Kneading

3. CONCLUSION

The current study shows that DSL can form inclusion complexes with HP-β-CD in the 1:1 stoichiometric ratio, leading to an A_L type phase-diagram. The apparent stability constant was calculated as 1859.34 M^{-1}, suggesting relatively strong interaction between DSL and HP-β-CD. Based on the experimental data, inclusion process was occurred all the complexation methods with different levels of interaction. Aqueous solubility analyses indicate that HP-β-CD is the most appropriate tool for the DSL, as the aqueous solubility of DSL, which is 0.11 $mg.mL^{-1}$ for the pure DSL increases to 132.46 $mg.mL^{-1}$, 115.35 $mg.mL^{-1}$ and 11.03 $mg.mL^{-1}$ for the inclusion complexes generated by FD, SD and KN methods, respectively. The structure of complexes was verified by SEM, DSC, FT-IR and NMR analyses, which all confirmed formation of the inclusion complexes with high energic amorphous state. Also, the present results indicated that KN complex showed lowest interaction of DSL and HP-β-CD. As a consequence, evaluation of DSL-HP-β-CD complexes prepared demonstrated that bioavailability drawbacks might be come through by improving the dissolution rate and aqueous solubility.

4. MATERIALS AND METHODS

4.1. Materials

DSL was Neutec's kind gift (İstanbul, Turkey). HP-β-CD was obtained from Sigma-Aldrich (Germany). Potassium dihydrogen phosphate, sodium hydroxide, ethanol, methanol and acetonitrile were the product of Merck (Germany). O-phosphoric acid was purchased from Sigma-Aldrich (Germany).

4.2. Determination of DSL by HPLC

A modified HPLC method (Shimadzu 20-A, Japan) was utilized for the quantification of DSL using reversed-phase Nucleosil 120-5 column (4.0 mm x 150 mm, 5 µm) with a photodiode array detector at 242 nm [42]. The mobile phase with a flow rate of 0.75 $mL.dk^{-1}$ included 40:60 (v/v) acetonitrile: bidistilled water (1% o-phosphoric acid) was utilized following tests for the detection of DSL. 20 µL fixed volume of samples was injected by automatic injector (Shimadzu, Japan). The temperature of the column was arranged to 30°C. Validation tests of the HPLC process have been carried out for data accuracy.

4.3. Phase solubility studies

The phase solubility diagram is a crucial and extensively acclaimed technique for assessing the influence of CD on the active agent solubility and the apparent stability of complexation [18]. The phase solubility studies were conducted using a horizontal shaker according to Higuchi-Connors method [25]. The equilibrium establishment was confirmed after 24, 48 and 72h by comparing the phase solubility of HP-β-CD. The findings did not show any major differences. The equilibrium time was therefore set at 24 hours. Excess amount of DSL (30 mg) were put into 5 mL of 1:4 ethanol: distilled water (v/v) with increased HP-β-CD (0-20 mM) concentration. The obtained suspensions were shaken at room temperature in a 24h at horizontal shaker that is considered adequate to achieve the equilibrium that confirmed by a preliminary study. Following equilibration, the suspensions were filtered through a 0.45 µm polyamide filter to remove undissolved active agents. The concentration of DSL in the filtrates were analysed by HPLC (n=3). Plotting the quantity of dissolved DSL (mM) against the quantity of added HP-β-CD (mM) was used to construct the phase solubility diagram. The K_s value of complexation can be estimated according to the following equation (Eq. 1) from the phase solubility diagrams:

$$K_s = \text{Slope}/S_o \times (1-\text{Slope}) \qquad \text{[Eq. 1]}$$

Where slope is the value calculated from linear adjustment of the data where S_o is the solubility of DSL without HP-β-CD [16].

4.4. Preparation of physical mixtures (PM)

DSL and HP-β-CD physical mixtures in 1:1 molar ratio was prepared thoroughly in a mortar by gentle dry mixing of precisely weighted quantities of HP-β-CD (0.279 g) with DSL (0.062 g) until a homogeneous mixture was obtained.

4.5. Preparation of DSL-HP-β-CD complexes

The inclusion complexes of DSL-HP-β-CD was prepared by various methods which are explained in detail below. The molar ratio of 1:1 was predicated on the previous studies of phase solubility.

4.5.1. Spray drying method (SD)

DSL (0.311g) was dissolved in ethanol and HP-β-CD (1.396g) was dissolved in ultra-pure water, separately. The mixture was shaken at 25°C in a 24h horizontal shaker after mixing the solutions. The resultant solution was spray-dried using a Buchi B-190 mini spray dryer (BUCHI, Switzerland) with an inlet and outlet temperatures of 120 ± 1°C and 55 ± 1°C, respectively. Carbon dioxide gas was used with a flow rate of 120 L/min. The system's level of residual oxygen was regulated below 4% [8,43].

4.5.2. Freeze drying method (FD)

DSL (0.311g) and HP-β-CD (1.396g) were dissolved in ethanol and water, respectively. The mixture was shaken at room temperature in a 24-hour horizontal shaker after adding the aqueous solution of HP-β-CD to the ethanol-based solution of DSL. The resultant solution was frozen at -86°C for 24h, and then lyophilized (Labconco Frezone 6 Plus, USA) to collect solid inclusion complexes [7,8,26].

4.5.3. Kneading method (KN)

Complexes were produced by adding 3 mL of a water-ethanol (50:50) (v/v) solution to the mixture of DSL and HP-β-CD (1:1 DSL- HP-β-CD molar ratio) and kneading vigorously with a pestle until get a homogeneous paste. The sample was kept in a drying incubator at 50°C for 24h to remove the solvent traces [8,44].

4.6. Characterization of inclusion complexes

4.6.1. Embedding efficiency and loading content (%)

Accurately amount of complex (1.5 mg) was dissolved in mobile phase (1 mL) to determine the amount of DSL in solid complexes. The solutions were analysed and the DSL quantity was determined by HPLC (n=3). The loading content and the embedding efficiency were determined using Eq. (2) and Eq. (3), respectively [30]:

$$EE\ \% = \frac{amount\ of\ DSL\ entrapped}{initial\ DSL\ amount}\ x\ 100 \qquad \text{[Eq. 2]}$$

$$Loading\ content\ \% = \frac{amount\ of\ DSL\ content\ entrapped}{amount\ of\ inclusion\ complex}\ x\ 100 \qquad \text{[Eq. 3]}$$

4.6.2. Determination of aqueous solubility

Aqueous solubility of complexes was determined thorough the preparation of saturated solutions. Excess quantities of pure DSL (~5mg) and complexes (~800mg) were added to 1 mL of water, and the dispersions were stirred at 25°C for 24h at room temperature. To extract the insoluble compounds, the collected suspensions are processed through a polyamide filter of 0.45 µm. The DSL concentration was determined on the basis of the validated HPLC method previously mentioned (n=3).

4.6.3. Morphology

The surface properties and particle shapes of the freshly prepared complexes, physical mixture, HP-β-CD and DSL were investigated by SEM (HITACHI TM3030 Plus Tabletop Microscope, Japan) after spreading the formulation on a carbon coated copper grid.

4.6.4. Differential scanning calorimetry (DSC)

Differential calorimetry (DSC; DSC-60, Shimadzu Scientific Instruments, USA) was used to analyse crystallinity and any structural changes in DSL and HP-β-CD. Analyses were performed against the aluminum reference under nitrogen at a flow rate 50 mL/min at 50-300°C with an increment rate of 10°C/min. Pure DSL and pure HP-β-CD were also analysed to reveal the reference thermograms of materials.

4.6.5. Fourier transform infrared spectroscopy (FT-IR)

Fourier transform infrared spectrophotometric (FT-IR) analysis spectra were collected using Shimadzu IR Prestige-21 (Japan) and the complexes were analysed to study the generation and structure of inclusion complexes within the 4000-500 cm^{-1} wavelength range. Also obtained and used as references were FT-IR spectra of pure DSL and pure HP-β-CD.

4.6.6. Nuclear magnetic resonance (NMR)

^{1}H-NMR analyses were carried out on Ultrashield CPMAS NMR (Bruker, Germany). Dissolving formulations of deuterated dimethyl sulfoxide were used to prepare samples. Pure DSL and pure HP-β-CD were also analysed and used as references.

4.7. *In vitro* dissolution study

Two compartment systems were used for the *in vitro* dissolution test, separated by dialysis (Sigma) membranes with exclusion diameter of 12-14 kDa [44]. Samples of the complex or an equivalent amount of DSL were placed in a dialysis bag along with 1 mL of the dissolution medium. The dialysis bag was immersed into 70 mL of the dissolution medium (PBS, pH 7.4) held at 37°C ± 0.5 and stirred at 80 rpm continuously. Samples were collected at fixed time intervals and replaced with the fresh dissolution medium. Samples were analysed by the validated HPLC technique and dissolution profile of the pure DSL was used as reference for better evaluation of the profiles. Each experiment was carried out in triplicate.

In order to examine the difference of the DSL and inclusion complexes prepared by different methods, difference and similarity factors were estimated by DD solver software.

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