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ORIGINAL ARTICLE

Detailed investigation of a γ-cyclodextrin inclusion complex with L-thyroxine for improved pharmaceutical formulations

Jaya Lakkakula · Rui Werner Maçedo Krause · Derek Tantoh Ndinteh · S. P. Vijaylakshmi · Ashok M. Raichur

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Abstract Thyroxine is a naturally occurring human hormone produced by the thyroid gland. Clinical applications of thyroxine to treat several chronic disorders are limited by poor water solubility and instability under physiological conditions. An inclusion complex of levo-thyroxine (L-thyroxine), the active form of the hormone with *gamma* cyclodextrin (γ-CD) has been obtained and studied with the aim of improving oral delivery rather than the injection formulation of the sodium salt. In addition to greater patient acceptability, inclusion complexes often improve aqueous solubility and bioavailability, stability, and reduce toxicity of drugs, thus providing enhanced pharmaceutical formulations. Physicochemical characterization of the inclusion complex was carried out using Fourier transform infrared spectroscopy, X-ray diffractometry, differential scanning calorimetry, scanning electron microscopy and proton nuclear magnetic resonance spectroscopy. Intermolecular dipolar interactions for the inclusion complex were also studied using 2 dimensional ROESY experiments. Formation of the inclusion complex between the protons H3 and H5 of cyclodextrin with aromatic protons of thyroxine was confirmed by their dipolar interaction. Molecular modelling was used to understand the basis for the complex formation and predict the formation of other complexes. Interestingly, we found that L-thyroxine forms an inclusion complex only with the larger γ-CD and not with other available *alpha* and *beta* forms.

Keywords γ-Cyclodextrin · Levo-thyroxine · Hypothyroidism · Inclusion complex

J. Lakkakula · R. W. M. Krause (✉) · D. T. Ndinteh
Department of Applied Chemistry, Center for Nanomaterials Science, University of Johannesburg, Doornfontein, Johannesburg 2028, South Africa
e-mail: rkrause@uj.ac.za

S. P. Vijaylakshmi · A. M. Raichur
Department of Materials Engineering, Indian Institute of Science, Bangalore 560 012, India

Introduction

Levo-thyroxine (L-thyroxine) is a synthetic and natural hormone (Fig. 1) used for treating hypothyroidism and other thyroid conditions, and is commonly administered as a daily oral tablet or in the ionized form as an injection formulation. In hypothyroidism, the thyroid gland does not produce enough thyroid hormone or the body metabolises the hormone too rapidly. This hormone is essential for many vital metabolic functions in the body, especially for energy. Some of the symptoms of hypothyroidism therefore include mood swings, lethargy, increased weight and intolerance to cold [1]. L-Thyroxine referred to as T4 (tetraiodothyronine), is secreted by the thyroid gland and transported around the body. The action of the deiodinase enzymes on T4 in peripheral tissues leads to the loss of an iodine atom, forming T3 (triiodothyronine), which acts on the thyroid receptor in cell nuclei [2]. Patients suffering from hypothyroidism are treated with both T3 and T4. Absorption of the drugs occurs mostly in the intestine, with some 62–82 % of L-thyroxine being absorbed within the first three hours of passing into the lower intestine (i.e. jejunum and ileum) [3]. Patients are under daily medication as the drug is not stored in the body, and is often rapidly degraded in the stomach. To overcome these problems and for better patient compliance efforts have been put to design drug delivery systems for controlled and sustained release of these and other hormones.

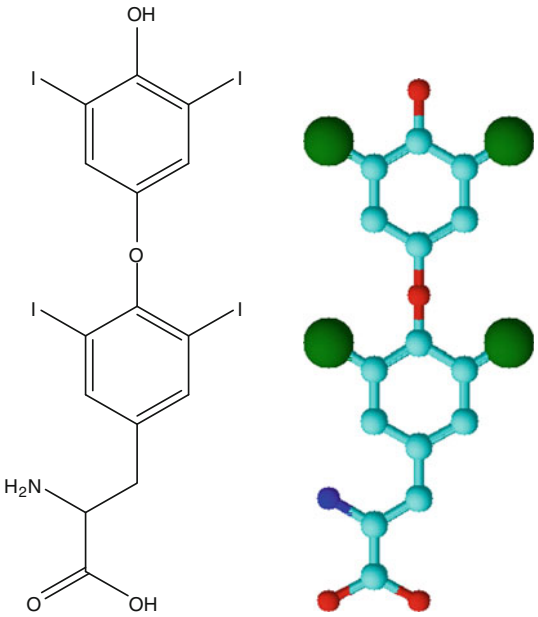

Fig. 1 Plane and spatial representations of L-thyroxine molecular structure

There is a need for pharmaceutical formulations that will increase the duration of the availability of the hormones in the body. In this way there would be a reduction in the frequency of administration of the drugs and hence an increased patient compliance, but also this process avoids the potentially dangerous effects of too much hormone in the body.

A possible way to approach the above stated goal may be oral drug formulation that will enable delivery of the drug in a controlled manner. Carriers have always played an important role in designing proper drug formulation for prolonged release and action of drug. For controlled release of dosage forms for various drugs, cyclodextrin has played a pivotal role as a carrier material [4]. Cyclodextrins can also be used as complexing molecules as they have a unique property of forming inclusion complexes in solid and liquid states [5].

Cyclodextrins (CDs) are cyclic, torus-shaped non-toxic oligomers of amylose with external hydrophilic surface and internal hydrophobic interiors. The most commonly available CDs are α-, β-, and γ-cyclodextrins that have 6, 7 and 8 glucose units, respectively (Fig. 2). The characteristics of cyclodextrin allow the formation of inclusion complexes with many organic molecules where polarity of guest molecule plays a key role in the complex formation [6, 7]. The driving forces are both the stabilisation of a hydrophobic guest, and the entropically favourable expulsion of water molecules from the lipophilic CD cavity. However, steric interactions start to operate as the size of side included guest increases, thereby also reducing the number of molecules that can be complexed [6]. Entry of lipophilic guest molecule into the hydrophobic cavity of cyclodextrin leads to formation of inclusion complex with the displacement of water molecules seated inside the torus [8]. Improved aqueous solubility and membrane permeation have been studied by formulating thyroxine with cyclodextrin [9]. Yet no complete characterisation of the inclusion complex between thyroxine and gamma cyclodextrin has been reported.

Generally, guest/host systems of various compounds with cyclodextrins have been prepared and characterised. The formation of inclusion complexes of phenols with β-CD have been used for waste water treatment and studied using mass spectrometry, FTIR, surface tension and ultraviolet visible spectroscopy [10], infra-red spectroscopy, XRD and DSC was used to characterize the inclusion of aromatic benzene moieties such as those of miconazole nitrate into the cavity of β-CD [11].

The aqueous inclusion complex between substituted β-CDs and ebastine was studied using ^{13}CNMR, 2D NMR, and ^{1}H NMR [12]. Emission spectra, stoichiometry, change in entropy and enthalpy of inclusion complex was studied using fluorescence and molecular mechanics [13], while

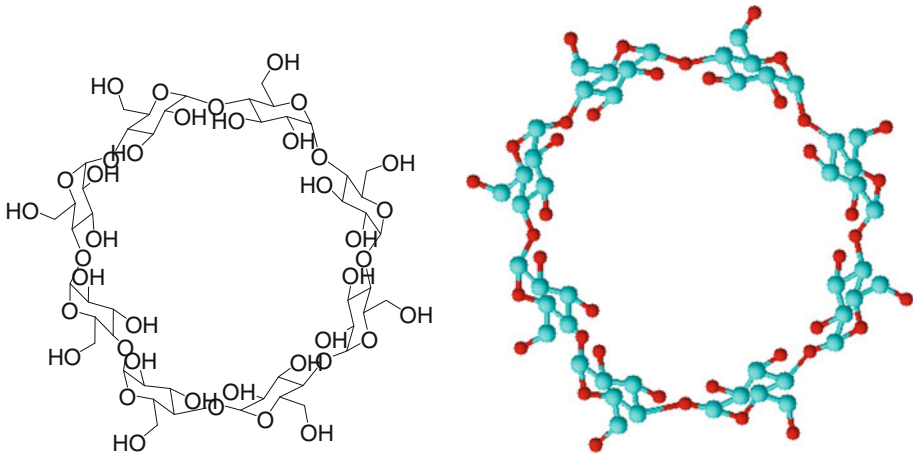

Fig. 2 Plane and spatial representations of molecular structure γ-CD

crystal structure, packing and intermolecular hydrogen bonding of inclusion complex was calculated using X-ray crystallography [14]. Spectroscopy remains amongst the most important tools for investigating various cyclodextrin phenomena [15] and in this work we investigate the complexation of L-thyroxine and γ-CD and characterise the complex using NMR, 2D NMR, XRD, FTIR, DSC, SEM and molecular modelling.

Materials and methods

Materials

Cyclodextrin was obtained from Wacker Chemie and L-thyroxine purchased from Sigma-Aldrich (Germany) and both were used as received. All reagents were of analytical grade. Deionized water was used throughout the experiment.

Methods

Preparation of inclusion complexes

The inclusion complex of γ-cyclodextrin with L-thyroxine was affected using a method similar to that adopted for making pharmaceutical compositions containing the same, with minor changes [9]. The inclusion complexes were formed by dissolving 20 ml of a 10 % γ-CD in water and adding 500 mg of L-thyroxine under intensive stirring (600 rpm) at room temperature for 12 h. The resulting opalescent solution was then filtered using a 0.22 μm cellulose acetate filter and clear filtrate was freeze-dried yielding a white solid.

Solid state characterization of inclusion complex

PXRD

The powder X-ray diffraction patterns were recorded using X-Pert PRO, PANalytical diffractometer system, operated at a voltage of 40 kV and a current of 30 mA. The pure γ-CD, L-thyroxine and inclusion complex were analyzed in the 2θ angle range of $3–60^0$.

^{1}HNMR

^{1}HNMR was recorded with BRUKER Avance 400 MHz or 500 MHz spectrometer in deuterated DMSO and referenced to residual solvent.

2D-ROESY experiments were carried out in phase sensitive mode, set up applying a continuous wave (CW) with spin lock for mixing. Spectra were obtained on the inclusion complex using spin lock of $180x\ 180{-}x$ pulses using purge pulses before d1 with a 90° high power pulse.

DSC

DSC was carried out in a temperature range of 25–350 °C under an argon flow maintained at 80 ml/min and the scanning rate was 5 °C/min. The sample was weighed and placed in an aluminium pan whereas empty aluminium pan was used as a reference.

FTIR

Infrared spectra of the inclusion complex, host (γ-CD) and the guest molecule (L-thyroxine) was studied using a BRUKER ALPHA-P spectrometer. The spectra were recorded from 3,600 to 600 cm^{-1} in attenuated total reflectance (ATR) mode.

SEM

Vacuum dried formulations were prepared on silicon wafers and coated with gold. The samples were examined using SIRION High resolution FEI scanning electron microscope (SEM) at an accelerating voltage of 20 kV.

Molecular modelling

Molecular mechanics and dynamics were performed using Allinger's MM2 force field [16, 17] on Cambridge soft Chem3D software™ on a Pentium CPU.

Gamma cyclodextrin was geometry-optimised using a combination of a Truncated Newton and Polak Ribiere optimiser, to a Root-Mean-Square (RMS) gradient of 0.01. Optimisation of the geometry of the L-thyroxine and the complex was performed similarly. All parameters in the forcefield are from the "MM2 (1991) Parameter Set", as provided by N. L. Allinger, University of Georgia, apart from the $C(sp_2)–O–C(sp_2)$ bond angle in thyroxine, which was modelled on the $C(sp_2)–O–(Csp_3)$ bond angle.

Modelling of the γ-CD/L-thyroxine complexes were completed by manually docking the optimised L-thyroxine structure inside each of the optimised γ-CD structures as a starting geometry and then performing a full-geometry optimization inside a static solvent (water) cage. Manual docking was performed for both possible arrangements of the drug (i.e. amino-acid towards the CD primary or secondary face). The dielectric constant for electrostatic interactions was set at 1.

The optimised structures were subjected to a series of simulated annealing heating and cooling cycles between 46 and 370 K, in steps of 2 ps and a heating rate of 1.00 kcal/atom/ps and allowing for energy minimization after each 10 steps.

Results and discussions

Powder X-ray diffraction (XRD) study

The inclusion complex of γ-CD and L-thyroxine was studied using powder XRD. If a molecule forms an inclusion complex with γ-cyclodextrin the diffraction pattern of the inclusion complex formed will be different from that of the pure host and guest molecules. The powder XRD patterns of γ-CD and L-thyroxine showed strong sharp peaks, affirming their crystalline properties. The most intense peak of γ-CD (Fig. 3a) is shown at $2\theta = 12.28^{\circ}$, 16.34°, 18.79° and 21.79°. These peaks disappear in the diffractogram of the inclusion complex. Similarly the most intense peaks for the L-thyroxine (Fig. 3b) appear at $2\theta = 14.53^{\circ}$, 17.56° and 23.21° and these peaks also are not found in the inclusion complex. The changes in diffractogram confirm the formation of inclusion complex [18] (Fig. 3c) and has been observed in other complexes [19]. Further diffraction studies on the conformational changes induced by the inclusion complex formation are ongoing with the assistance of collaborators.

Differential scanning calorimetry (DSC)

DSC analysis is an important tool that gives an idea about the interactions that can occur in a host–guest complex. The appearance, disappearance or shift of peaks is an indication of the kind of interactions that exist between the guest and the host.

The DSC curves of the pure drug, γ-CD and inclusion complex are shown in (Fig. 4). Pure γ-CD exhibits a broad endothermic peak from 50 to 102 °C, corresponding to dehydration process, while degradation occurred at 270° in accordance with the expected decomposition temperature [20].

The hydrophobic interior of the cyclodextrin is the main driving force for attracting the guest molecule to form inclusion complex, but additional factors such as hydrogen bonding between the guest and the host CD are also important [21]. In the inclusion complex (Fig. 4c), the endothermic peak for L-thyroxine at 223 °C disappears due to the L-thyroxine being sheltered in the cavity of γ-CD.

Fourier transmission infrared spectroscopy

FTIR spectroscopy is another important tool to recognize the formation of inclusion complexes. Analysis of changes in the signal intensity or frequency of the inclusion complex gives information about complex formation.

The broad band around 3,300 cm^{-1} is assigned to symmetric and asymmetric –OH stretching modes for both cyclodextrin and thyroxine. The FTIR spectrum of γ-CD (Fig. 5a) is characterized by an intense band at 3,284 cm^{-1} for the primary and secondary OH groups. The position of this band is shifted to higher frequency and is broadened after the formation of inclusion complex due to interaction of γ-CD with L-thyroxine. This is later confirmed by the molecular modelling, indicating the formation of a close-contact hydrogen bond. Vibrations of –CH range in between 2,800 and 2,950 cm^{-1}, and band at 2,924 cm^{-1} confirm the presence of L-thyroxine in the complex, as well as a peak for the aromatic C–H at 3,057 cm^{-1} (Fig. 5b). Aromatic C–C bands appear as sharp bands around 1,620–1,575 and 1,525–1,475 cm^{-1} in L-thyroxine and are also present in the γ-CD/L-thyroxine inclusion complex, although somewhat depressed (Fig. 5c). The IR spectrum of the inclusion complex showed variations compared to

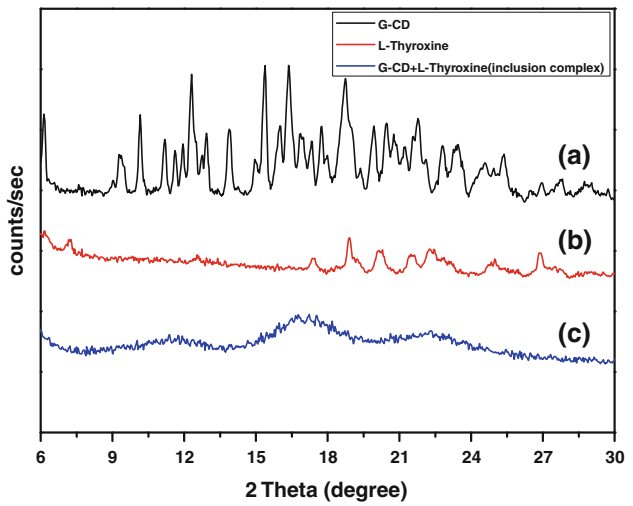

Fig. 3 X-ray powder diffractogram of **a** γ-CD, **b** L-thyroxine and **c** γ-CD/L-thyroxine (inclusion complex)

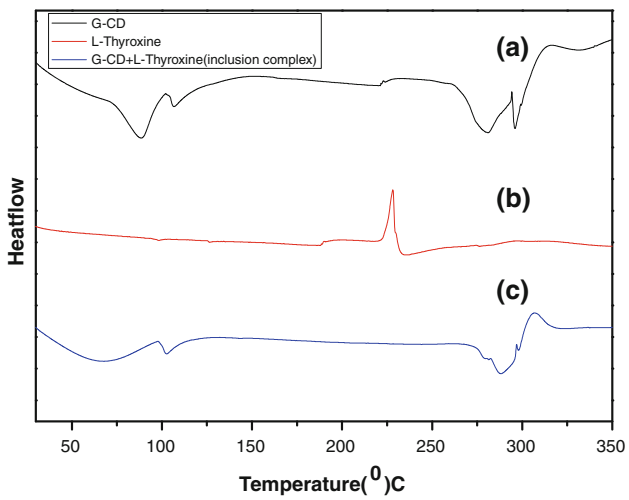

Fig. 4 Differential scanning calorimetry thermograms of **a** γ-CD, **b** L-thyroxine and **c** γ-CD/L-thyroxine (inclusion complex)

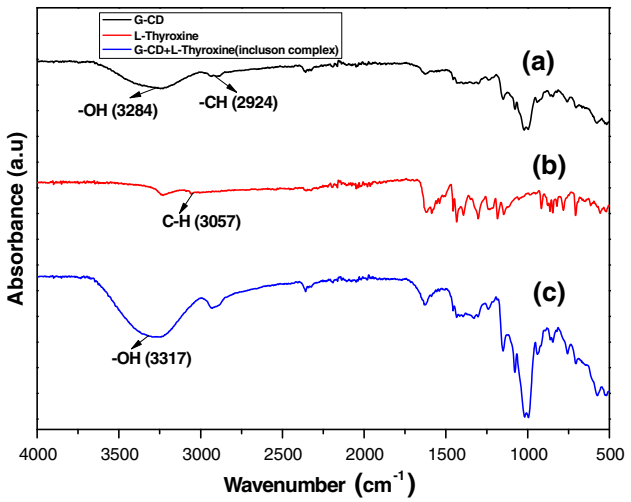

Fig. 5 FT-IR spectra of **a** γ-CD, **b** L-thyroxine and **c** γ-CD/L-thyroxine (inclusion complex)

γ-CD especially in hydrogen bonded-OH region. The noticed changes in the spectrum can be attributed to interstitial and intracavity interactions between the drug and γ-CD. As observed the spectra of γ-CD/L-thyroxine inclusion complex resembles that of γ-CD, another indication of successful complex formation [22, 23].

Scanning electron microscopy

The morphological changes that occur are indicated by the SEM study. The SEM micrographs of γ-CD and γ-CD/L-thyroxine inclusion complex prepared using the freeze drying method, are shown in Fig. 6a and b, respectively. The morphology of γ-CD/L-thyroxine inclusion complex was found to be totally different from that of γ-CD and L-thyroxine. The γ-CD showed presence of small "florets" of 10 to 20 nm in diameter, while the inclusion complex shows an amorphous structure. This morphology change is currently being probed in further experiments, but the presence of amorphous phase allows for more dissolution kinetics.

Nuclear magnetic resonance spectroscopy

^{1}H NMR is considered a most important tool for studying the behaviour and microscopic structure of inclusion complexes. ^{1}H NMR spectra of γ-CD (Fig. 7a), L-thyroxine (Fig. 7b), γ-CD/L-thyroxine complex (Fig. 7c) were studied. Comparing the spectra of the inclusion complexes with those of host and guest tells a lot about the inclusion complex formed. The chemical shift change ($\Delta\delta$) is defined as the difference in chemical shift in the presence and absence of the other molecules. In cases of chemical shift

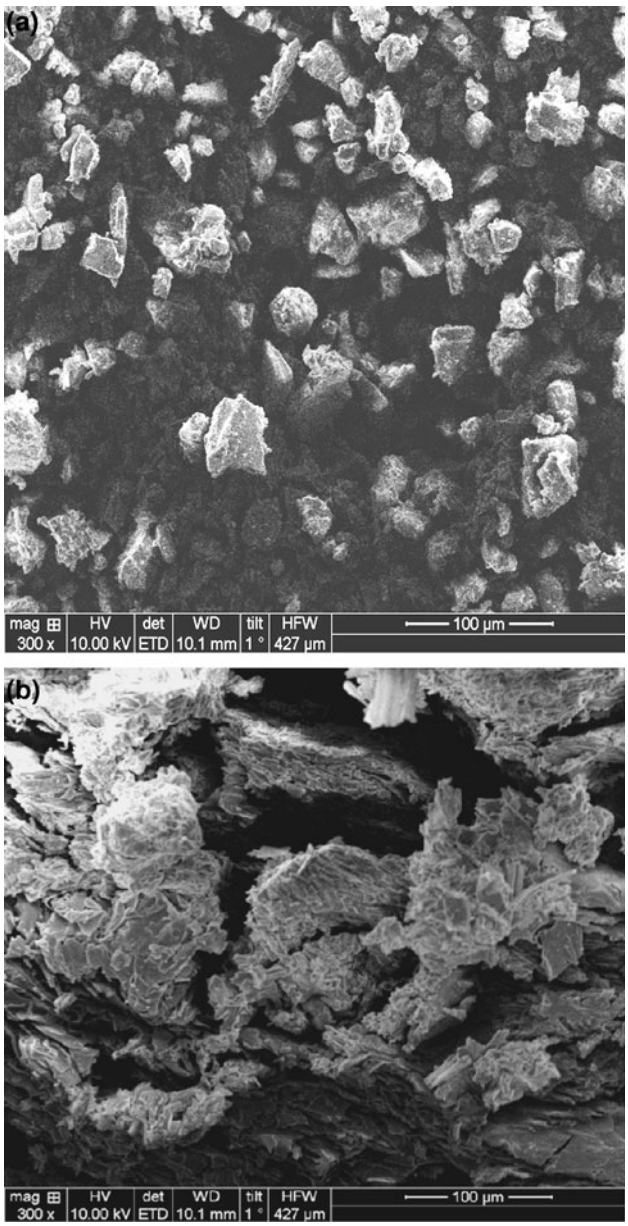

Fig. 6 SEM micrograph of **a** γ-CD and **b** γ-CD/L-thyroxine (inclusion complex)

changes, a positive sign means a downfield shift and a negative sign means an upfield shift. The values of chemical shift for different protons can be seen in the Table listed below for $\gamma\text{-CD}_{free}$ and γ-CD/L-thyroxine complex (Table 1) as well as for L-thyroxine_{free} and γ-CD/L-thyroxine complex (Table 2).

In the NMR spectra of inclusion complex (Fig. 7c) we can see the peaks appearing at position δ (ppm) = 7.1 and 7.8, which are attributed to the aromatic protons of the L-thyroxine moiety at positions 2′6′ and 2″6″. Comparing the spectrum of L-thyroxine with that of the inclusion complex we noticed that the signals for protons 2″6″ undergo a slight shift, suggesting, as expected that the

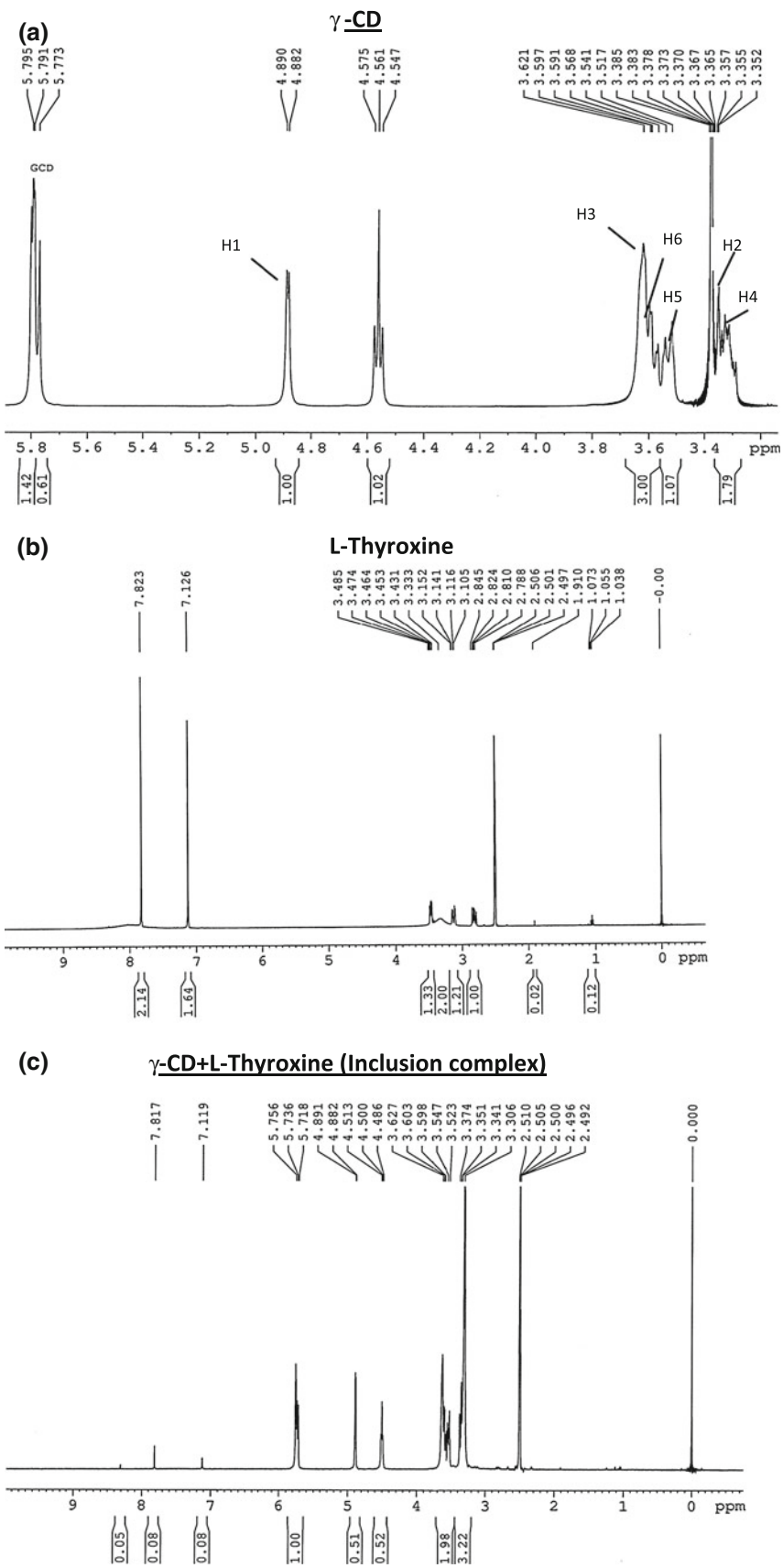

Fig. 7 **a** ^{1}H NMR spectra of γ-CD. **b** ^{1}H NMR spectrum of L-thyroxine. **c** ^{1}H NMR spectrum of γ-CD/L-thyroxine (inclusion complex)

Table 1 ^{1}H NMR chemical shift of protons of γ-CD free or complexed with L-thyroxine in DMSO

No.	^{1}H assignment	δ γ-CD$_{free}$ (/ppm)	δ γ-CD$_{complexed}$ (/ppm)	Δ (γ-CD$_{complexed}$−γ-CD$_{free}$) (/ppm)
1	H1	4.890	4.891	0.001
2	H3	3.621	3.603	−0.018
3	H6	3.597	3.598	0.001
4	H5	3.541	3.521	−0.020
5	H2	3.373	3.374	0.001
6	H4	3.352	3.351	−0.001

Table 2 ^{1}H NMR chemical shift of protons of L-thyroxine free or complexed with γ-CD in DMSO

No.	^{1}H assignment	δ L-thyroxine$_{free}$ (ppm)	δ L-thyroxine$_{complexed}$ (ppm)	Δ (L-thyroxine$_{complexed}$−L-thyroxine$_{free}$) (ppm)
1	2′6′	7.126	7.119	−0.007
2	2″6″	7.823	7.817	−0.006
3	Ar–OH	7.997	8.322	0.325

aromatic portion part of L-thyroxine is inside the cyclodextrin cavity. Similarly, the broad phenolic proton located at $\delta = 8.0$ in the free thyroxine is shifted to 8.3. The H3 and H5 protons on the inside edge the CD cavity also undergo a shift due to the formation of the complex [19]. They experience a deshielding effect that shows increase in the chemical shift possible because they are sitting in the deshielding zone of anisotropic ring(s) of the benzene ring in the L-thyroxine.

With this knowledge at hand, it could be suggested that quite a deep inclusion occurs between the hydrophobic part of L-thyroxine and lipophilic truncated cavity of cyclodextrin, which is held in place via the hydrogen bonding. Evidence for the hydrogen bonding comes also from the ROESY data (see below) and the molecular modelling.

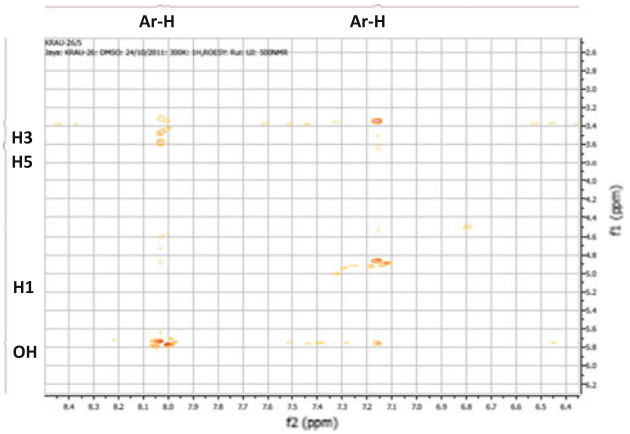

Fig. 8 ROESY spectrum of γ-CD/L-thyroxine inclusion complex, showing cross peaks between aromatic protons (Ar–H) and various protons on the γ-CD

Two dimensional ^{1}H-NMR studies (ROESY)

Detailed intermolecular dipolar correlations between the γ-CD and L-thyroxine were studied using 2D ROESY experiments to understand the structural analysis of the intermolecular interactions between the host and guest.

The ROESY data demonstrate through-space interactions of the aromatic protons with the cyclodextrin, indicating a successful inclusion complex. These data give clear evidence between the spatial proximities of the protons from the host, γ-CD (H3 and H5 at ca. $\delta = 3.5$ ppm) placed inside the toroidal cavity and the guest aromatic protons.

Protons H3 and H5 are located inside the primary and secondary rim of cavity, so dipolar interactions with these protons gives a clear picture about the interaction of the guest due to the change in the microenvironment. The cross-peaks (Fig. 8) between the aromatic protons (7.2 and 8 ppm) and the H3 and H5 protons (3.5 ppm) are the only visible through-space interactions apart from the interactions with the rim hydroxyl protons (5.8 ppm). This again was expected since the bulky thyroxine is predicted to protrude from the cyclodextrin. These interactions match well with the predicted geometries obtained by molecular modelling (see below), and suggest hydrogen bonding may be holding the complex together.

Molecular modelling

The molecular modelling studies were carried out in order to visualise the likely three-dimensional interactions taking place in the complexes, and in order to confirm the analytical data obtained for the complex.

The full geometry optimisation of the complex showed the aromatic portion within the CD cavity (Fig. 9) and the

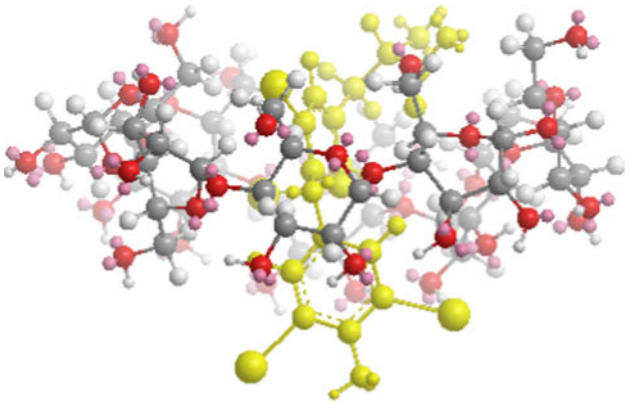

Fig. 9 γ-CD + L-thyroxine inclusion complex (*side on view*) showing the drug molecule (*in yellow*) deep inside the cavity, but protruding

amino-acid moiety in close proximity with at least one of the hydroxyl groups of the CD rim.

The inclusion complex formed with the larger γ-CD was much more stable throughout the molecular dynamics simulations, reaching a geometry minimum with the aromatic portions deep within the CD cavity. This was confirmed using NMR and XRD data obtained on this complex. The structure of the γ-CD complex before simulated annealing shows a very closest contact between the iodine atoms of the thyroxine and the CD wall (closest contact distances of between 3.1 and 3.2 Å).

In addition, the structure of γ-CD-thyroxine complex after simulated annealing remained almost the same, indicating that the complex formation was stable. By comparison, the beta-CD complex (not shown) was not able to reach a minimum energy conformation after simulated annealing.

The structure suggests that the drug is well inside the cavity but protrudes sufficiently to allow an interaction between the drug (OH) and the rim hydroxyl groups. The γ-CD/L-thyroxine complex also appears to be stabilised by an intermolecular H-bond between the COOH and lone pairs at one of the acetal positions of the CD. The measured intermolecular distances of 2.12 and 2.14 Å in the geometry optimised structure reveals that this may be a symmetrical H-bond, (3-atom–4-electron interaction) and further crystallographic and spectroscopic investigations are underway to confirm this.

Conclusion

The growing importance of drug delivery systems has led to the need for novel pharmaceutical compositions as a preparation strategy. In this study we demonstrated and characterised the formation of γ-CD/L-thyroxine inclusion complex that could potentially be used instead of injectable formulations for the treatment of thyroid conditions. NMR clearly demonstrates that the hydrophobic L-thyroxine is embedded deeply inside the truncated cone of γ-CD, with significant changes in the microenvironment occurring between free and bound states. The up-field shift for protons H3 and H5 of the cyclodextrin confirms the formation of the complex and molecular modelling indicates that the size of the cavity of γ-CD is large enough to accommodate L-thyroxine with four iodine atoms, inducing a strong steric effect [24]. The modelling also confirms that L-thyroxine interacts with secondary alcohol functions of γ-CD via hydrogen bonding [25]. DSC; XRD; and FTIR give supportive data for the formation of inclusion complex. Further studies on the solubility, phase kinetics and slow release of thyroxine under physiological conditions are now underway to determine the suitability of this complex for pharmaceutical application as a sustained release formulation.

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References

1. Chemburkar, S.R., Deming, K.C., Reddy, R.E.: Chemistry of thyroxine: an historical perspective and recent progress on its synthesis. Tetrahedron **66**, 1955–1962 (2010)
2. Duggan, B.M., Craik, D.J.: Conformational dynamics of thyroid hormones by variable temperature nuclear magnetic resonance: the role of side chain rotations and cisoid/transoid interconversions. J. Med. Chem. **40**, 2259–2265 (1997)
3. Liwanpo, L., Hershman, J.M.: Conditions and drugs interfering with thyroxine absorption. Best. Pract. Res. Clin. Endocrinol. Metab. **23**, 781–792 (2009)
4. Bruschi, M.L., De Freitas, O.: Oral bioadhesive drug delivery systems. Drug Dev. Ind. Pharm. **31**, 293–310 (2005)
5. Numata, M.: Creation of unique supramolecular nanoarchitectures utilizing natural polysaccharide as a one-dimensional host. J. Incl. Phenom. Macrocycl. Chem. **68**(1–2), 25–47 (2010)
6. Malpezzi, L., Fronza, G., Fuganti, C., Melea, A., Bruckner, S.: Crystal architecture and conformational properties of the inclusion complex, neohesperidin dihydrochalcone-cyclomaltoheptaose (β-cyclodextrin), by X-ray diffraction. Carbohydr. Res. **339**, 2117–2125 (2004)
7. Nu′ñez-Agüero, C.-J., Escobar-Llanos, C.-M., Diaz, D., Jaime, C., Garduno-Juarez, R.: Chiral discrimination of ibuprofen isomers in β-cyclodextrin inclusion complexes: experimental (NMR) and theoretical (MD, MM/GBSA) studies. Tetrahedron **62**, 4162–4172 (2006)
8. Loftsson, T., Masson, M.: Cyclodextrins in topical drug formulations: theory and practice. Int. J. Pharm. **225**, 15–30 (2001)

9. Fischer, W., Bracher, D.: Thyroxine/cyclodextrin complexes and pharmaceutical compositions containing the same. United States Patent (1999)
10. Bonenfant, D., Niquette, P., Mimeault, M., Furtos-Matei, A., Hausler, R.: UV–Vis and FTIR spectroscopic analyses of inclusion complexes of nonylphenol and nonylphenol ethoxylate with β-cyclodextrin. Water Res. **43**, 3575–3581 (2009)
11. Wang, J.-H., Cai, Z.: Investigation of inclusion complex of miconazole nitrate with β-cyclodextrin. Carbohydr. Polym. **72**, 255–260 (2008)
12. Maddens, T., Vélaz, I., Machín, R., Isasi, J.R., Martín, C.: Complexation of ebastine with β-cyclodextrin derivatives. J. Incl. Phenom. Macrocycl. Chem. **70**(3–4), 415–419 (2011)
13. Serna, L., Marino, A.D., Mendicuti, F.: Inclusion complexes of a bichromophoric diester containing anthracene and naphthalene groups with α- and β-cyclodextrins: thermodynamics and molecular mechanics. Spectrochim. Acta Part A **61**, 945–1954 (2005)
14. Wang, E.-J., Liana, Z.-X., Cai, J.: The crystal structure of the 1:1 inclusion complex of β-cyclodextrin with benzamide. Carbohydr. Res. **342**, 767–771 (2007)
15. Zhao, W., Chao, J., Du, R., Huang, S.: Spectroscopic studies on the inclusion behavior between caffenic acid and γ-cyclodextrin, Online First™ (2011). doi:10.1007/s10847-011-9930-9
16. Allinger, N.L.: Conformational analysis. 130. MM2. A hydrocarbon force field utilizing V1 and V2 torsional terms. J. Am. Chem. Soc. **99**, 8127–8134 (1977)
17. Sprague, J.T., Tai, J.C., Yuh, Y., Allinger, N.L.: The MMP2 calculational method. J. Comput. Chem. **8**, 581–603 (1987)
18. Xua, D., Wanga, X., Ding, L.: Spectroscopic studies on the interaction of γ-cyclodextrin–daunorubicin inclusion complex with herring sperm DNA. Carbohydr. Polym. **83**(3), 1257–1262 (2010)
19. Anselmi, C., Centini, M., Ricci, M., Buonocore, A., Granata, P., Tsuno, T., Facino, R.M.: Analytical characterization of a ferulic acid/γ-cyclodextrin inclusion complex. J. Pharm. Biomed. Anal. **40**, 875–881 (2006)
20. Kawasaki, J., Satou, D., Takagaki, T., Nemoto, T., Kawaguchi, A.: Structural features of inclusion complexes of γ-cyclodextrin with various polymers. Polymer **48**, 1127–1138 (2007)
21. Wulff, M., Alden, M.: Solid state studies of drug–cyclodextrin inclusion complexes in PEG 6000 prepared by a new method. Eur. J. Pharm. Sci. **8**, 269–281 (1999)
22. Stancanelli, R., Crupi, V., Luca, L.D., Ficarra, P., Ficarra, R., Gitto, R., Guardo, M., Iraci, N., Majolino, D., Tommasini, S., Venuti, V.: Improvement of water solubility of non-competitive AMPA receptor antagonists by complexation with β-cyclodextrin. Bioorg. Med. Chem. **16**, 8706–8712 (2008)
23. Lu, J., Shin, I.D., Nojima, S., Tonelli, A.E.: Formation and characterization of the inclusion compounds between poly(e-caprolactone)-poly(ethylene oxide)-poly(e-caprolactone) triblock copolymer and α- and γ-cyclodextrin. Polymer **41**, 5871–5883 (2000)
24. Peani, C., Creminon, C., Grassi, J., Pradelles, P., Perly, B., Djedaini-Pilard, F.: NMR investigations of the inclusion of thyroxine and derivatives in natural cyclodextrins. J. Incl. Phenom. Macrocycl. Chem. **33**, 307–319 (1999)
25. Shahgaldian, P., Hegner, M., Pieles, U.: A cyclodextrin self-assembled monolayer (SAM) based surface plasmon resonance (SPR) sensor for enantioselective analysis of thyroxine. J. Incl. Phenom. Macrocycl. Chem. **53**, 35–39 (2005)