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Synthesis of 2,4 Ditertiary Butylphenol: Hydroxypropyl-α-Cyclodextrin inclusion complex and its characterization

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Abstract
2,4-Ditertiary Butylphenol, also known as 2,4-bis(1,1-dimethylethyl)-phenol (2,4-DTBP), is a natural compound that is very poisonous to almost all test species. 2,4-DTBP is a lipophilic phenol that has a low water solubility. This compound is present in the leaves of Ficus auriculata Lour. (F.auriculata), which is isolated by TLC & Column chromatography. The water solubility is improved by complexation with Hydroxypropyl α-Cyclodextrin (HP-α-CD). HP-α-CD was used to create inclusion complexes in both the liquid and solid states, and they were then examined using FT-IR, ^{1}H NMR, UV-Vis, Fluorescence spectroscopy and SEM studies. Phase solubility investigations showed that when 2,4 DTBP is complexed with HP-α-CD, the complex shows 1:1 stoichiometry and their solubility was increased. Further molecular docking investigations support the most popular and advantageous model of the complex.

Keywords: 2,4-DTBP, HP-α-CD, inclusion complex, Molecular docking, stability.

Introduction

2,4 DTBP is known to occur in 169 species of organisms [1]. It exhibits cytotoxicity in human cells [2] and animals, insecticidal [3] and nematicidal activities [4], antimicrobial activities [5,6] and phytotoxicities [7,8]. It is insoluble in water but soluble in acetone, methyl ethyl ketone, methanol, isopropanol, benzene, petroleum ether, toluene and ethanol. Cyclodextrins (CDs) are cyclic oligosaccharides which contains hydrophobic cavity. With a variety of guest molecules, the hydrophobic cavity forms inclusion compounds. Inclusion complex formation enhances the physicochemical properties such as solubility, stability, and bioavailability of poorly water-soluble drug [9]. The non-covalent bonding of a macromolecule and small molecule is studied using molecular docking [10]. Auto Dock Tools (ADT) 1.5.6 is used to prepare the host as well as the guest [11]. PyMOL is used as a molecular viewer due to its vast visualization properties [12]. The present study aims to investigate the possibility of improving the solubility of the drug via complexation with Hydroxypropyl α-Cyclodextrin.

Materials and Methods

Prepaation of Extract

The collected leaf is washed, dried and powdered. This is extracted with Ethanol solvent using Soxhlet apparatus.

Reagents

2,4 DTBP isolated and purified from Ficus auriculata *Lour.* and Analytical grade of HP-α-CD from Sigma-Aldrich were used for the studies. The solvents used were of analytical grade. The experiment is done using double distilled water.

Instruments

i. Systronics Smart Double beam Spectrophotometer-2203
ii. JASCO Spectrofluorometer FP-8200

Column Chromatography

Column is packed using Silica gel (mesh size 230-400). Ethyl Acetate: Hexane (30:70) is used as the mobile phase, and the eluted fractions were collected and subjected to TLC.

Thin Layer Chromatography

The readymade TLC sheet (Silica gel 60F25420 cmx20 cm) is placed in the beaker containing Ethyl acetate: Hexane (6:14). The sample spot is elevated and then removed. From this, R_f value can be determined by:

R_f = Distance travelled by the solute / Distance travelled by the solvent

Preparation of solid inclusion complex

By dissolving 0.062 g of 2,4 DTBP in 30 ml Ethanol with 0.354 g of HP-α-CD in 30 ml water in a beaker and stirring constantly for 48 hours, solid inclusion complexes of 2,4 DTBP with HP-α-CD is prepared. After evaporation, the precipitate is collected, dried, and used for characterisation.

Preparation of liquid inclusion complex

In a 100 ml beaker, 0.004 g of 2,4 DTBP is dissolved in 10 ml of ethanol, and 0.07 g of HP-α-CD is dissolved in 30 ml of double-distilled water. It is possible to prepare liquid inclusion complex by adjusting the concentrations of HP-α-CD.

The UV-Visible & Fluorescence Spectroscopy were recorded usingSystronics Double Beam Spectrophotometer-2203 and JASCO Spectrofluorometer FP-8200 respectively, in Scott Christian College, Nagercoil. The FT-IR for solid inclusion complex is recorded using Shimadzu FT-IR Spectrometer in ANJAC, Sivakasi. ^{1}H-NMR spectroscopy studies were carried out using Bruker 300MHz FT NMR Spectrometer, in Gandhigram Rural University, Dindugal. Samples were prepared by dissolving 2,4 DTBP in dimethyl sulphoxide and the solid inclusion complexes in D2O.

Phase Solubility Studies

HP-α-CD in aqueous solution, at various concentrations are agitated with excess amounts of guest until equilibrium. A plot of solubility of the guest as a function of HP-α-CD concentration, gives the Phase solubility diagrams. This study was conducted in accordance with Higuchi and Corners.

Molecular Docking Study

Molecular docking study is carried out using AutoDock tools 1.5.6. The hosts as well as the ligand was prepared in PDBQT format and the output is viewed using PyMol software.

Results and Discussion

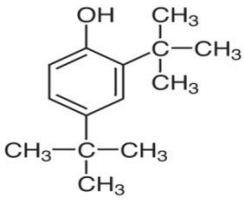
Column Chromatography

Solvent	Ratio	Fractions
Ethyl Acetate: Hexane	6:14	7

On subjecting the Ethanolic extract to column chromatography, 7 fractions were eluted, which is subjected to TLC.

Thin Layer Chromatography

From the R_f value, the IV fraction is subjected to GC-MS and specral analysis. The results obtained, confirms the structure of 2,4 DTBP to be:



Absorption Study

The absorption spectra of the drug 2,4 ditertiary butylphenol (2,4 DTBP) with various concentrations of HP-α-CD is shown in Table.1 and Figure.1. Drug's interaction with HP-α-CD is thought to be demonstrated by their change in wavelength and absorption intensities. In this instance, a shift in peak towards higher wavelength and increase in absorbance is observed. This findings demonstrate that the drug 2,4-ditertiary butylphenol forms an inclusion complex with HP-α-CD.

Table.1 Absorption Maxima of drug 2,4 ditertiary butylphenol with HP-α-CD at different concentrations.

[HP-α-CD]	λmax	Absorbance
0	214.4	1.255
0.002	219.2	1.338
0.004	221.6	1.408
0.006	224	1.587
0.008	226.4	1.471
0.01	228.8	1.624

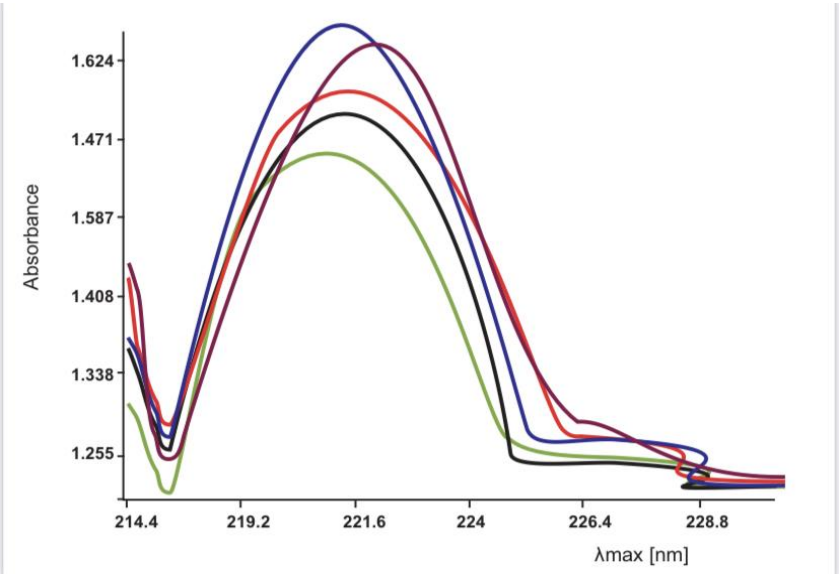


Fig.1 Absorption Spectrum of 2,4 DTBP with HP-α-CD

Fluorescence Study

The drug 2,4 ditertiary butylphenol's emission spectra with various concentration of hydroxy propyl α-CD is shown in Table.2 and Figure.2. In this case, an intensified bathochromic (Red) shift is seen.

Table.2 Fluorescence Maxima of drug 2,4 ditertiary butylphenol withHP-α-CD at different concentrations.

[HP-α-CD]	λmax nm	Intensity
0	398	293.61
0.002	404	304.53
0.004	409	323.86
0.006	410	348.61
0.008	413	350.23
0.01	415	354.32

The emission maxima for 2,4 DTBP increases from 398 nm to 415 nm with the addition of HP-α-CD. With HP-α-CD, the emission wavelength and intensity rise as the concentration rises. This demonstrates that the drug is present in the HP-α-CD cavity.

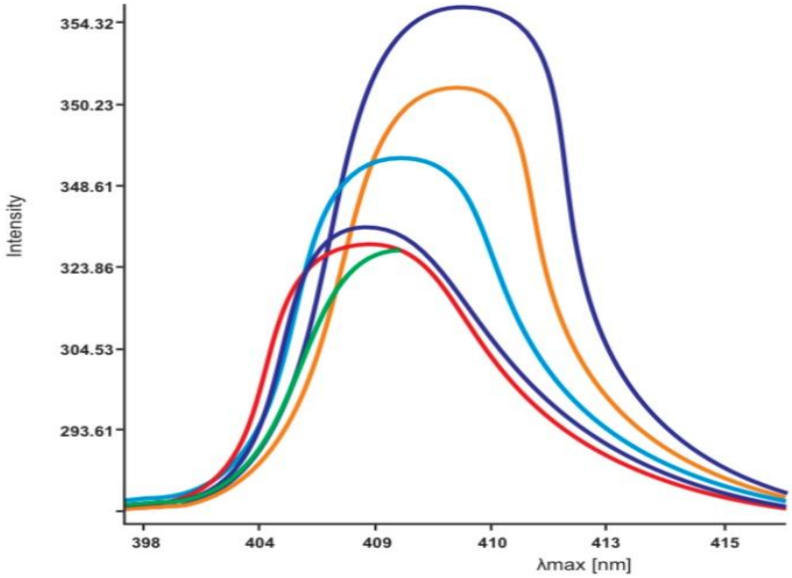


Fig.2 Emission Spectra of 2,4 DTBP: HP-α-CD complex

The Association constant (K) for the formation of inclusion complexes is determined using **Benesi-Hildebrand equation.** Fig.3 depicts the Benesi-Hildebrand plot for absorption and emission spectra.The equation for 1:1 complexes are :

Absorption

$$\frac{1}{A - A_0} = \frac{1}{A - A_0} + \frac{1}{(A - A_0)[\text{HP-}\alpha - CD]}$$

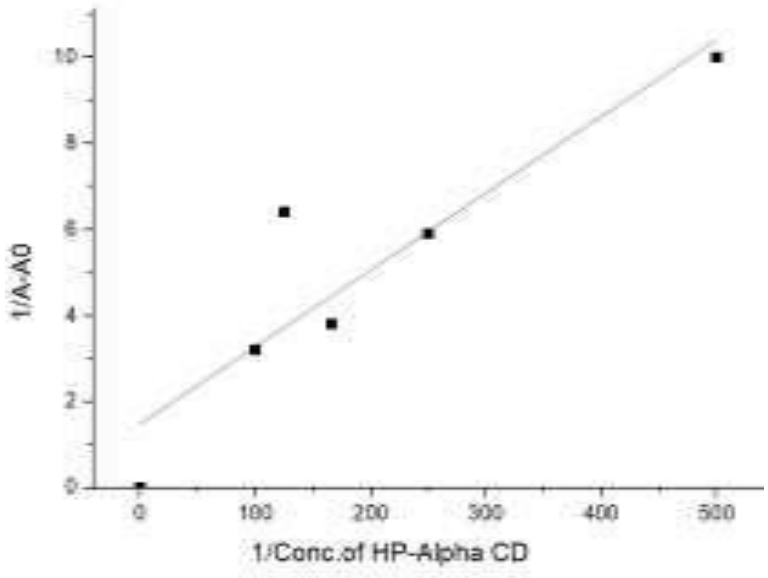
Fluorescence

$$\frac{1}{I - I_0} = \frac{1}{I - I_0} + \frac{1}{(I - I_0)[\text{HP-}\alpha - CD]}$$

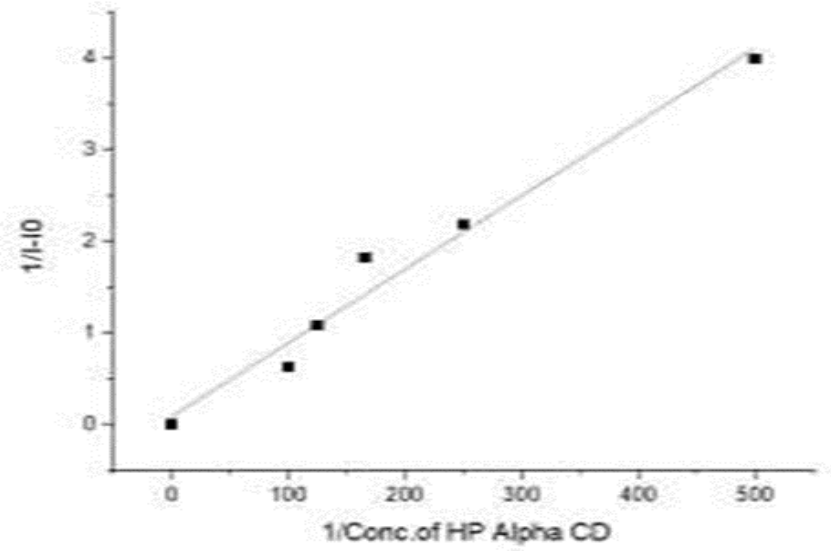
Where,

A_0/I_0 is the absorbance/intensity of the drug 2,4 ditertiary butylphenol without HP-α-CD,
A/I is the absorbance/intensity of the drug at a particular concentration of HP- α-CD,
A good linear correlation is obtained on plotting the concentration of HP-α-CD with absorbance/emission intensity. The Association constant (K) for absorption and emission is determined from the slope of the graph.

For Absorption
K = 182.1 for 2,4 ditertiary butylphenol: HP-α-CD inclusion complex.
For Emission
K = 156.25 for 2,4 ditertiary butylphenol: HP-α-CD inclusion complex.



(a)



(b)

Fig.3 Bensi-Hildebrand plot of (a) Absorption spectra (b) Emission spectra

The association constant reveals the stability of the complex. The association constant of absorption is higher than emission. This shows that the complex 2,4 DTBP : HP-α-CD is highly stable. The higher values of association constants, confirms the formation of inclusion complex.

Phase Solubility Study

The phase solubility diagram of 2,4-ditertiary butylphenol: HP-α-CD inclusion complex is shown in Figure 4. It is obvious from the diagram that when the concentration of HP-α-CD rises, solubility also rises linearly. The stability constant for the Higuchi and Cornors model, which categorises the diagrams as A_L type, is found to be 200 M^{-1}.

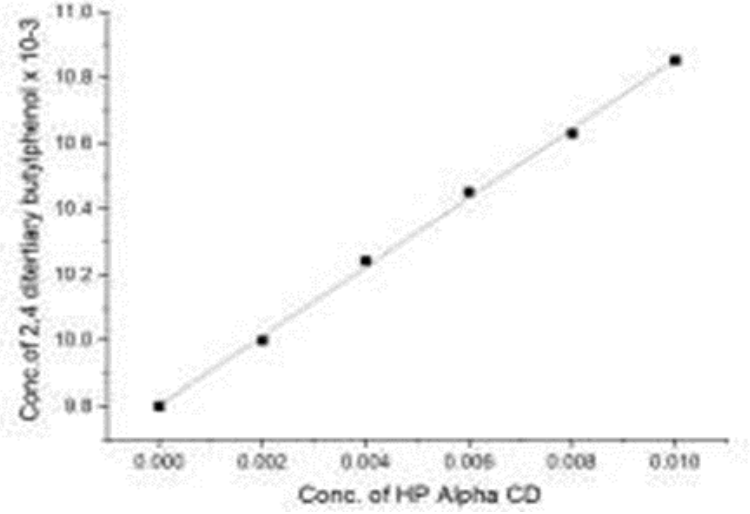
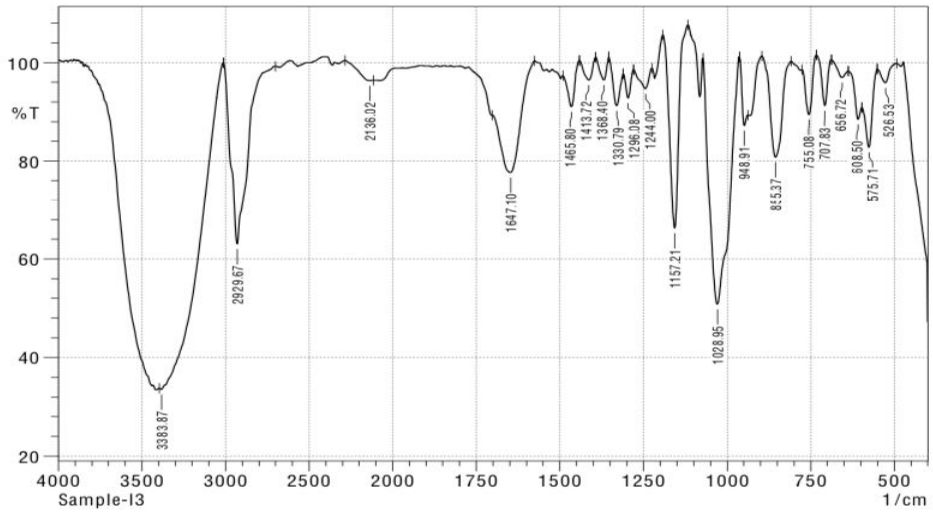


Fig.4 Phase Solubility diagram of 2,4 DTBP : HP-α-CD inclusion complex

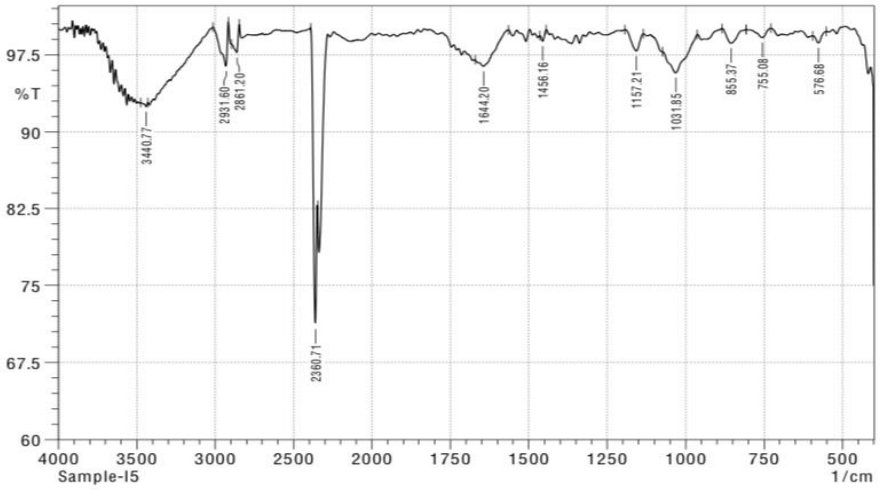
Fourier Transform Infrared (FTIR) Spectroscopic Study

The FT-IR Spectra of HP-α-CD and its solid inclusion complex with 2,4 DTBP is shown in the following fig.5 (a) & (b) respectively. The original -OH Stretching frequency of the host HP-α-CD at 3383.87 cm^{-1} is shifted to 3440 cm^{-1}. C-H Stretching frequency at 2929.67 cm^{-1} is shifted to 2931.60 cm^{-1}. The H-O-H bending frequency at 1647.10 cm^{-1} is shifted to 1644.20 cm^{-1}. C-O-C stretching frequency at 1028.95 cm^{-1} is shifted to 1031.85 cm^{-1}. The C-O stretching frequency at 1157.21 cm^{-1}

remains unaltered. The above changes in the FTIR Spectra of HP-α-CD, and its inclusion complex indicates that the guest molecule 2,4 ditertiary butylphenol is included in the cavity of the host HP-α-CD.



(a)



(b)

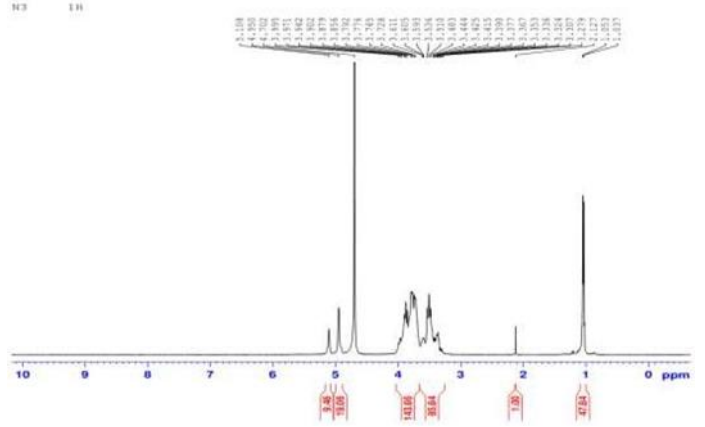
Fig.5 FT-IR Spectroscopy of (a) HP-α-CD (b) 2,4 DTBP: HP-α-CD

^{1}H NMR Spectroscopy

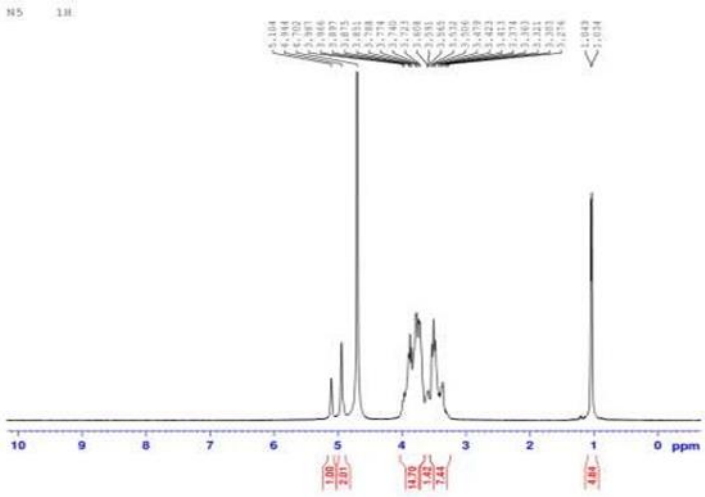
Inclusion complex formation may potentially alter the spectrum of the guest. Fig.6 shows the chemical shifts of various protons. From Table.3, it can be concluded that H3 & H5 protons are located at the interior of the cavity. In the complex 2,4 DTBP with HP-α-CD, H3 & H5 shows upfield shift, and other protons shows minimal downfield shift. This confirms that 2,4 DTBP forms an inclusion complex with HP- α-CD.

Table.3 Chemical Shifts (ppm) for the protons of HP-α-CD and Inclusion complex of 2,4DTBP with HP-α-CD

	H1	H2	H3	H4	H5	H6
HP-α-CD	5.108	3.483	3.942	3.367	3.856	3.510
2,4 DTBP : HP-α-CD	5.104	3.479	3.966	3.363	3.875	3.506
δ	0.004	0.004	0.024	0.004	0.019	0.004



(a)

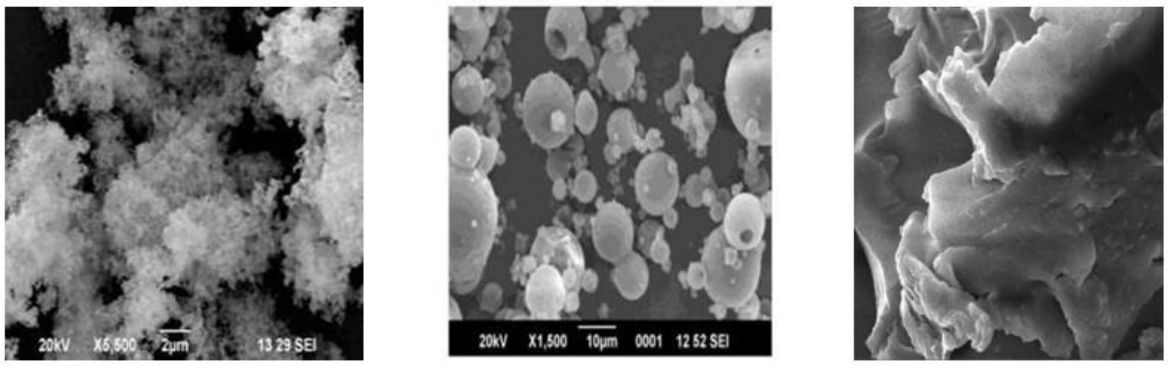


(b)

Fig.6 ^{1}H NMR of (a) HP-α-CD (b) 2,4 DTBP : HP-α-CD complex

SEM Image

Fig.7 depicts the morphology of the guest, hosts and the inclusion complexes. From the SEM images, it can be concluded that, HP- α-CD is present in Spherical shape and pure drug is present in irregular shaped crystal. The SEM images concludes the morphology of the inclusion complex to be completely different from the pure drug as well as the host, which can be taken as a proof for the formation of inclusion complex.



(a) (b) (c)

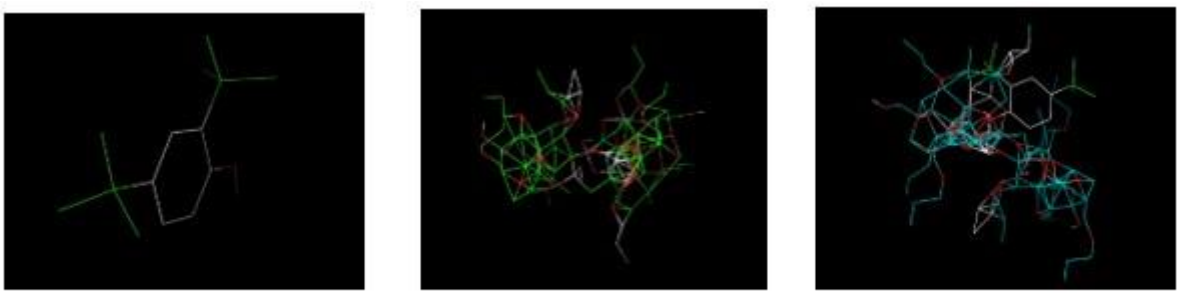
Fig.7 SEM Image of (a) 2,4 DTBP (b) HP-α-CD (c) 2,4 DTBP: HP-α-CD complex

Molecular Docking Study

Table.4 shows the affinity and RMSD mode of 2,4 DTBP : HP-α-CD.Greater negative binding energy corresponds to improved complex stability. If the complex is more stable, the energy is less. Lesser the binding energy, better is the binding of the ligand and protein. From the above data, it is concluded that -5.5 kcal/mol is the best binding mode for 2,4 DTBP with HP-α-CD complex. The 3-D Structures of the host, ligand and the stable complex are shown in fig.8.

Table.4 Set of results showing binding mode of 2,4 DTBP with HP-α-CD.

S.No	Affinity (Kcal/mol)	Dist RMSD l.b.	Best mode RMSD u.b.
1	-5.5	0.000	0.000
2	-5.4	12.425	13.924
3	-5.3	1.281	4.749
4	-5.0	2.587	4.747
5	-5.0	1.970	5.229
6	-5.0	1.812	3.497
7	-4.5	3.159	5.379
8	-4.4	11.660	12.833
9	-4.3	1.822	3.549



(a) (b) (c)

Fig.8 3-D structure of (a) 2,4 DTBP (b) HP-α-CD (c) inclusion complex of 2,4 DTBP: HP-α-CD

Conclusion

2,4 DTBP possess various pharmacological activities, but its application is limited because of its poor water solubility and low bioavailability. Inclusion with HP-α-CD improves the solubility as well as the bioavailability of the compound. The absorption, fluorescent spectral studies, FT-IR, ^{1}H-NMR and SEM images confirms the formation of 2,4 DTBP: HP- α-CD inclusion complex. The In-Silico studies also confirms that the complex 2,4 DTBP: HP- α-CD is highly stable.

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