Carbohydrate Polymers 114 (2014) 558–566

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Spectral investigation and characterization of host–guest inclusion complex of 4,4′-methylene-bis(2-chloroaniline) with beta-cyclodextrin

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ARTICLE INFO

Article history:
Received 7 July 2014
Received in revised form 5 August 2014
Accepted 6 August 2014
Available online 14 August 2014

Keyword:
4,4′-Methylene-bis(2-chloroaniline)
β-Cyclodextrin
Inclusion complex
Atomic force microscope
UV–fluorescence spectrum
Molecular docking

ABSTRACT

The host–guest inclusion complex of 4,4′-methylene-bis(2-chloroaniline) (MBCA) with beta-cyclodextrin (β-CD) was prepared by co-precipitation method and characterized using absorption, fluorescence, fourier transform infrared, differential scanning calorimetry, scanning electron microscopy, atomic force microscopy and X-ray diffraction. The spectral shifts revealed that the aniline ring of 4,4′-methylene-bis(2-chloroaniline) was entrapped in the beta-cyclodextrin cavity. Nano second time resolved fluorescence studies revealed that 4,4′-methylene-bis(2-chloroaniline) exhibits single exponential decay in aqueous medium and bi-exponential in beta-cyclodextrin medium confirmed the formation of 1:1 inclusion complex. The Gibbs free energy change of the complexation process was determined and the complexation process was spontaneous. The differential scanning calorimetry analysis showed that the thermal stability of 4,4′-methylene-bis(2-chloroaniline) was altered in the presence of beta-cyclodextrin. The implementation of molecular docking test confirmed that the complexation could reduce the energy of the system. A mechanism was proposed to explain the mode of inclusion in the inclusion process.

1. Introduction

Cyclodextrins (CDs) are macro cyclic oligosaccharides consisting of six to twelve α,1,4-linked D-glucopyranose units joined in a truncated cone shaped structure (Tiwari, Tiwari, & Rai, 2010). Among these CDs, α, β and γ-cyclodextrins (α-CD, β-CD and γ-CD) consisting of six, seven and eight glucopyranose units are most frequently used and studied (Inoue et al., 1993). They are produced as a result of intermolecular trans glycosylation reaction from degradation of starch by cyclodextrin glycanotransferase enzyme (Szetjli, 1998). From the X-ray structures, it appears that in cyclodextrins the secondary hydroxyl groups are located on the wider edge of the ring and the primary hydroxyl groups on the other edge, and that the apolar hydrogens and ether-like oxygens are at the inside of the torus-like molecules. This result in a molecule with a hydrophilic outside, which can dissolve in water and an apolar cavity which provides a hydrophobic matrix, described as a micro heterogeneous environment (Szetjli, 1989).

The most notable features of cyclodextrins are their ability to form solid inclusion complexes (host–guest complexes) with a wide range of solid, liquid and gaseous compounds by a molecular complexation. Complex formation is a dimensional fit between host cavity and guest molecule (Muñoz-Botella, Del Castillo, & Martyn, 1995). The lipophilic cavity of cyclodextrin molecules provide a microenvironment into which appropriately sized non-polar moieties can enter to form inclusion complexes (Loftsson & Brewster, 1996). No covalent bonds are broken or formed during formation of the inclusion complex (Schneiderman & Stalcup, 2000). The main driving force of complex formation is the release of enthalpy-rich water molecules from the cavity. Water molecules are displaced by more hydrophobic guest molecules present in the solution to attain an apolar–apolar association and decrease of cyclodextrin ring strain resulting in a more stable lower energy state (Villiers, 1891).

Inclusion in cyclodextrins exert a profound effect on the physicochemical properties (solubility enhancement, degradative effects

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http://dx.doi.org/10.1016/j.carbpol.2014.08.006
0144-8617/

of oxidation, visible or UV light and heat and chromatographic separations etc.) of guest molecules as they are temporarily locked or caged within the host cavity giving rise to beneficial modifications of guest molecules. Thus CDs have been widely used in various fields like food (Fujishima, Kusaka, Umino, Urushinata, & Terumi, 2001) and pharmaceuticals (Bhardwaj, Dorr, & Blanchard, 2000).

Earlier we have reported the effect of inclusion complexation on spectral and photo-prototropic behaviors of some amino compounds with β-CD (Rajamohan, Kothainayaki, & Swaminatan, 2008; Rajamohan, Kothainayaki, & Swaminathan, 2011). 4,4′-methylene-bis(2-chloroaniline) (MBCA) is widely used as a curative extender in the production of isocyanate based polymers and epoxy resins (International Agency for research on Cancer, 1975) It is an aromatic amine with structural similarity to known human bladder carcinogen (International Agency for research on Cancer, 1972). There are so many evidences reported for its mutagenic and carcinogenic activities (Mc Cann, Choi, Yamasaki, & Ames, 1975; U.S. Dept. of Health and Human Services, 1983). MBCA is used to make polyurethane products that are a significant component of many common appliances. These are widely used in such items as gear systems in modern office and home appliances, sporting goods, mouldings for motor vehicle body parts and military equipment.

Previously DNA binding study of MBCA in explant cultures of Human and dog bladder (Narayan Shivapurkar et al., 1987) and the additive effect of MBCA on Flexible molded Foam properties (Zhang, Bertsch, & Macosko, 1998) were studied. In our present work, we report the preparation and characterization of inclusion complex formed by MBCA with β-CD. We utilized absorption and fluorescence spectral titration techniques to determine the inclusion stoichiometry and binding constant of MBCA:β-CD complexes. The solid inclusion complex was characterized by FT-IR, DSC, SEM, powder XRD and AFM. Further the most probable structure of 1:1 inclusion complex was proposed by molecular docking studies.

2. Experimental

2.1. Reagents

4,4′-Methylene-bis(2-chloroaniline) (MBCA) purchased from Sigma Aldrich, was purified by repeated recrystallization from methanol. A stock standard solution of MBCA was prepared by using methanol and it is in the order of 3×10^{-4} mol dm^{-3}. β-Cyclodextrin (β-CD) obtained from SD fine chemicals was used as received and the stock solutions were prepared in water for spectral measurements. Sodium hydroxide and sulphuric acid were dissolved in distilled and deionized water to make a solution of pH 6.8. Triply distilled water was used for the preparation of experimental solutions. The solutions were prepared just before taking each measurement.

2.2. Instruments

The absorption spectral (UV–visible spectrum) measurements were carried out with Hitachi model U-2001 spectrophotometer. Fluorescence measurements were made using a JASCO FP-750 spectrofluorimeter. pH values were measured on ELICO pH meter (model L1-10T). Fluorescence lifetimes were measured by using a time-resolved single photon counting picoseconds spectrofluorimeter (Tsunami, Spectra physics). FT-IR spectra of powder sample MBCA, β-CD and the inclusion complexes were measured between 4000 cm^{-1} and 400 cm^{-1} on Avtar-330 FTIR spectroscopy using KBr pellet. Powder X-ray diffraction patterns were obtained by Xpert PRO analytical diffractometer. Microscopic morphological structure measurements were performed with JEOL-JSM 5610LV scanning electron microscope. Differential scanning calorimetry (DSC) analysis was carried out in the temp range of 20–200 °C in steam of nitrogen atmosphere on DSC-50 thermal analyzer (shimadzu.Japan). Surface morphology was also recorded using AGILENT-N 9410 A SERIES 5500 AFM.

2.3. Job's continuous variation method

Stoichiometry of the inclusion complex was determined by using continuous variation technique (Job's plot) based on the differences in absorbance of MBCA. Equimolar solutions of MBCA and β-CD were prepared and mixed to standard volumes such that the total concentration remained constant ([MBCA] + [β-CD] = M). Absorbance differences were calculated by measuring the absorbance of MBCA in the absence (A_0) and presence (A) of the corresponding concentration of β-CD. An equimolar solution of β-CD was used as a blank to take into account its refractive index.

2.4. Preparation of inclusion complex in solutions

A 3×10^{-4} mol dm^{-3} concentration of stock solution of MBCA was prepared using methanol, and 0.6 ml of this solution was added into 10 ml volumetric flasks. It was made up to the mark using the following concentration of β-CD solutions 0, 0.002, 0.004, 0.006, 0.008, 0.010 and 0.012 mol dm^{-3}, respectively. It was shaken thoroughly so that the final concentration of MBCA in each flask is 1.8×10^{-5} mol dm^{-3}.

2.5. Preparation of inclusion complex in solid state

The solid complex of β-CD and MBCA was prepared by co-precipitation method (Rajamohan, Kothainayaki, & Swaminathan, 2011). β-CD was dissolved in triply distilled water under warm condition till a clear solution was obtained. MBCA was dissolved in methanol. This solution was added to β-CD solution in drops with constant stirring at room temperature and allowed to stir for 24 h and then freezed for 24 h. The white precipitate obtained was filtered and washed with triply distilled water. Then it was allowed to dry for 24 h at room temperature.

2.6. Preparation of physical mixture

The physical mixture of MBCA and β-CD with 1:1 molar ratio was prepared by grinding in a ceramic mortar.

2.7. Molecular docking study

The most probable structure of the MBCA:β-CD inclusion complex was also determined by molecular docking studies using the Patch Dock server. The 3D structural data of β-CD and MBCA was obtained from crystallographic databases. The guest molecule (MBCA) was docked in to the host molecule (β-CD) cavity using Patch Dock server by submitting the 3D co-ordinate data of MBCA and β-CD molecules. Docking was performed with complex type configuration settings. Patch Dock server follows a geometry-based molecular docking algorithm to find the docking transformations with good molecular shape complementarity. Patch Dock algorithm separated the Connolly dot surface representation (Connolly, 1983) of the molecules into concave, convex and flat patches. These divided complementary patches were matched in order to generate candidate transformations and evaluated by geometric fit and atomic desolvation energy scoring (Zhang, Vasmatzis, Cornette, & DeLisi, 1997) function. RMSD (root mean square deviation) clustering was applied to the docked solutions to select the non-redundant results and to discard redundant docking structures.

2.8. Semi empirical quantum mechanical calculations

The ground state of MBCA molecule was optimized using Argus Lab program by AM1 method. MolSoft MolBrowser tool was used to visualize the 3D structural data.

3. Results and discussion:

3.1. Interaction of 4,4′-methylene-bis(2-chloroaniline) with β-cyclodextrin in solutions

3.1.1. Absorption spectral studies

Spectrophotometric analysis was carried out to determine the molecular encapsulation behavior of β-CD with MBCA in aqueous solution. It has been observed that the shape and position of peak maximum and molar absorption coefficient of the compound strongly depends on the CD cavity environments. The absorption spectral data of MBCA with different concentration of β-CD at pH 6.8 is given in Table 1. Two spectral bands are observed for MBCA at 243.5 nm and 291 nm. On increasing the concentration of β-CD it is observed that there is a slight red shift in the absorption maximum (Fig. 1a) with a gradual increase in the absorbance up to the concentration of β-CD (1.2×10^{-2} mol dm^{-3}) The β-CD concentration dependent, the hyper chromic shift in spectral bands of MBCA to higher intensity resulted due to host guest interaction between β-CD and MBCA (Gibuad, Ben Zirar, mutzenhardt, Fries, & Astier, 2005). This behavior suggests the enhanced dissolution of guest molecule through detergent action of β-CD during inclusion complexation reaction (Rajamohan, Kothainayaki, & Swaminatan, 2008; Muthu Vijayan Enoch & Swaminathan, 2006).

The binding constant K and stoichiometric ratio of the inclusion complex of MBCA with β-CD can be determined from the changes in the absorbance of MBCA molecule with the increasing concentration of β-CD using Benesi–Hildebrand equation (Benesi & Hildebrand, 1949). Benesi–Hildebrand equations for 1:1 and 1:2 complexes are shown by Eqs. (1) and (2), respectively.

$$\frac{1}{(A-A_o)} = \frac{1}{\Delta\varepsilon}1 + \frac{1}{K}[\text{MBCA}_o]\,\Delta\varepsilon\left[\beta\text{-CD}\right]_o \quad (1)$$

$$\frac{1}{(A-A_o)} = \frac{1}{\Delta\varepsilon} + \frac{1}{K}[\text{MBCA}]_o\,\Delta\varepsilon\left[\beta\text{-CD}\right]_o^2 \quad (2)$$

where $(A-A_o)$ is the difference between the absorbance of MBCA in the presence and absence of β-CD. $\Delta\varepsilon$ is the difference between the molar absorption co-efficient of MBCA in the presence and absence of β-CD. $[\text{MBCA}]_o$ and $[\beta\text{-CD}]_o$ are the initial concentrations of MBCA and β-CD, respectively. K is the Binding constant. A good linear correlation ($R^2 = 0.9954$) is obtained when $1/(A-A_o)$ is plotted against $1/[\beta\text{-CD}]$ (Fig. 1a inset) indicating that the stoichiometry of the complexe is 1:1 (Negi & Sing, 2013). Moreover, the plot of $1/(A-A_o)$ against $1/[\beta\text{-CD}]^2$ (Fig. 1b) gives down ward curve. The non linearity of the plots ruled out the possibility of 1:2 stoichiometry between MBCA and β-CD (Rajendiran & Siva, 2014). Thus we have concluded that the stoichiometry of the inclusion complex was 1:1 as evidenced by fitting a double-reciprocal plot of $1/(A-A_o)$ vs $1/[\beta\text{-CD}]$. The binding constant K for the inclusion complex formation obtained from the slope of the Benesi–Hildebrand plot according to Eq. (3) is found to be 63 ±3 M^{-1} at 303 K.

$$K = \frac{1}{\text{Slope}\,[A' - A_o]} \quad (3)$$

3.1.2. Fluorescence spectral studies

The fluorescence emission spectral data of MBCA with different concentration of β-CD at pH 6.8 is given in Table 1. Fig. 1c shows Fluorescence spectra of MBCA at pH 6.8 with different concentration of β-CD in aqueous solution. The effects of β-CD on the emission spectra of MBCA are more pronounced than the corresponding effect on the absorption spectra with respect to the concentration of β-CD. From this figure it is observed that fluorescence signal increases with increasing concentration of β-CD and fluorescence maximum was slightly red shifted. The results suggest that a stable inclusion complex is formed between MBCA and β-CD. The increase in fluorescence intensity while increasing β-CD concentration during the formation of inclusion complex may be due to lowering of solvent polarity provided by β-CD cavity and has been reported earlier (Al.Hassan, Klein, & Sawaiyan, 1993). The β-CD dependence of MBCA fluorescence can be analyzed by the Benesi–Hildebrand plots (Benesi & Hildebrand, 1949). Benesi–Hildebrand equations for 1:1 and 1:2 complexes are given as Eqs. (4) and (5), respectively.

Benesi–Hildebrand equation for 1:1 complex

$$1/I - I_o = 1/I' - I_o + 1/K(I' - I_o)[\beta\text{-CD}] \quad (4)$$

Benesi–Hildebrand equation for 1:2 complex

$$\frac{1}{I - I_o} = \frac{1}{I' - I_o} + \frac{1}{K}\left(I' - I_o\right)\left[\beta\text{-CD}\right]^2 \quad (5)$$

where K is binding constant, I_o is the fluorescence intensity of MBCA without β-CD. I is the fluorescence intensity of certain concentration of β-CD, and I' is the fluorescence intensity of MBCA with the highest concentration of β-CD. A good linear regression ($R^2 = 0.9918$) is obtained when $1/I - I_o$ is plotted against $1/[\beta\text{-CD}]$ (Fig. 1c inset). The linearity of the plot reveals that the stoichiometry of the complex between MBCA and β-CD is 1:1. Whereas a non linear relationship is obtained when $1/I - I_o$ is plotted against $1/[\beta\text{-CD}]^2$ (Fig. 1d). The non-linearity of the plots ruled out the possibility of 1:2 stoichiometry between MBCA and β-CD. The binding constant 'K' calculated from the slope of Benesi–Hildebrand plot using the Eq. (6) is found to be 83 ± 3 at 303 K.

$$K = \frac{1}{\text{Slpoe}\,[I' - I_o]} \quad (6)$$

3.1.3. Jobs plot

The 1:1 stoichiometry of the host–guest inclusion complex between MBCA and β-CD is supported by the plot of Job's continuous variation method using the absorption data. Fig. 1e shows the plot of absorbance change against mole fraction of MBCA. In this plot absorbance difference is maximum at 0.5 which indicates that the stoichiometry of the complex is found to be 1:1 (Negi & Sing, 2013).

3.1.4. Analysis of fluorescence decay curve

The fluorescence decay of MBCA at different concentrations of β-CD was recorded. The excitation wavelength was fixed at 300 nm and the emission wavelength was fixed at 330 nm. The bi-exponential function is given by the following equation,

$$I(t) = B_1 \exp\left(\frac{-t}{\tau_1}\right) + B_2 \exp\left(\frac{-t}{\tau_2}\right) \quad (7)$$

where τ_1 (free guest) and τ_2 (complex) are life times of the two components, B_1 and B_2 are the pre-exponential factors of the same, and $I(t)$ is the intensity of fluorescence at time t.

The life-times of MBCA along with their amplitude and χ^2 values without and with different concentration of β-CD are given in Table 2. The χ^2 values for the single and double exponential fittings are less than 1.2. In water, the fluorescence decay of MBCA obtained from monitoring the emission at 330 nm is a single exponential with life time value 2.2 ns (τ_1). By the addition of β-CD the single exponential becomes bi-exponential. Two life times were obtained in the presence of β-CD [τ_1 in the range of 7.6×10^{-10} to 2.1×10^{-10} s and τ_2 in the range of 2.7–9.6 ns]. The above data reflect that the observed lifetime values of the complexed form

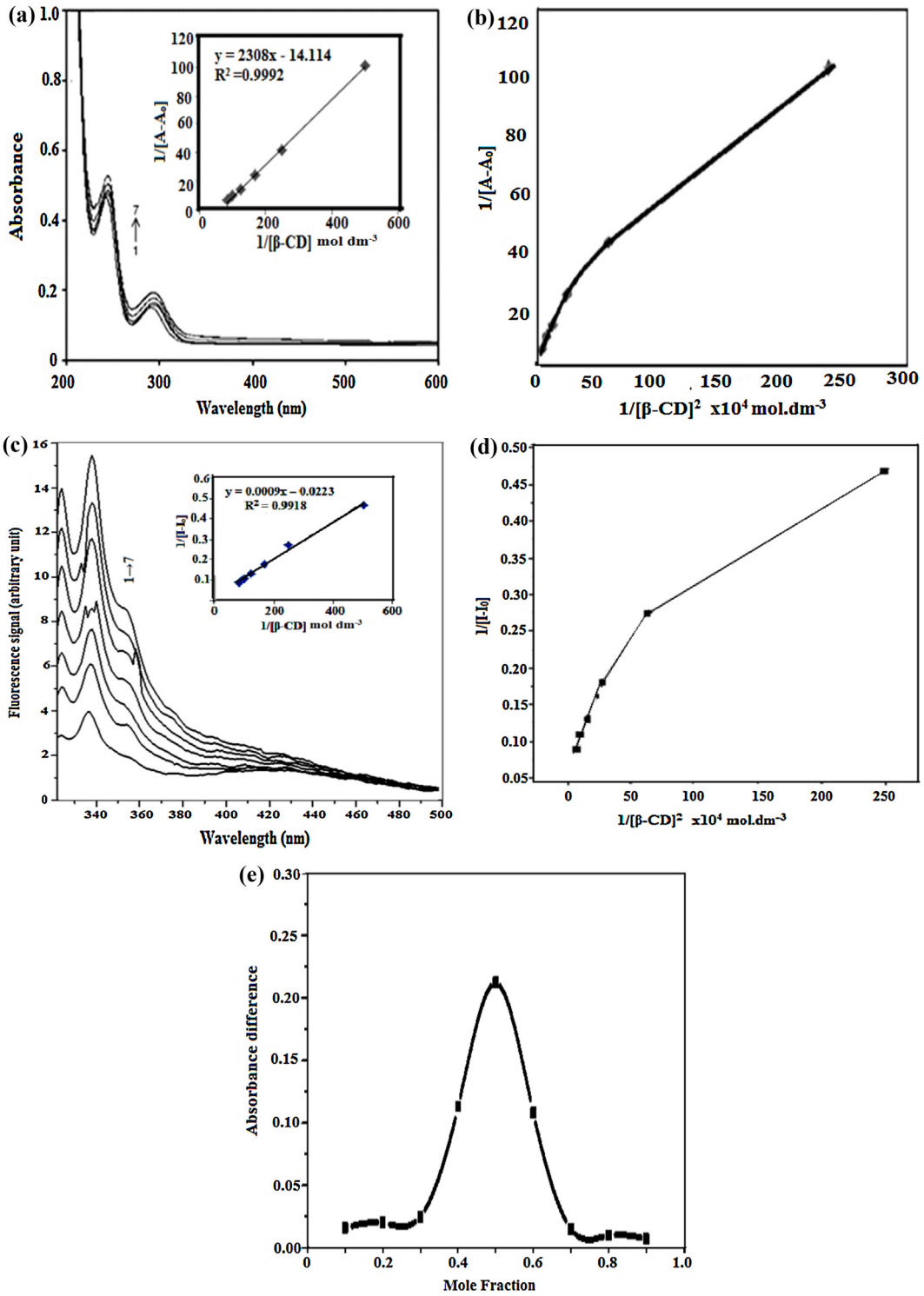

Fig. 1. (a) Absorption spectra of MBCA with increasing concentration of β-CD [(1) without β-CD, (2) 0.002, (3) 0.004, (4) 0.006, (5) 0.008, (6) 0.010 and (7) 0.012 mol dm^{-3} β-CD]. [Inset: Benesi–Hildebrand absorption plot of MBCA with β-CD (λ_{abs} = 245 nm]. (b) Benesi–Hildebrand absorption plot of $1/(A - A_o)$ against $1/[\beta\text{-CD}]^2$ for MBCA with β-CD (λ_{abs} = 245 nm). (c) Fluorescence spectra of MBCA with increasing concentration of β-CD [(1) Without β-CD, (2) 0.002, (3) 0.004, (4) 0.006, (5) 0.008, (6) 0.010 and (7) 0.012 mol dm^{-3} β-CD]. [Inset: Benesi–Hildebrand fluorescence plot of MBCA with β-CD (λ_{exi} = 338 nm).] (d) Benesi–Hildebrand fluorescence plot of I/I_o against $1/[\beta\text{-CD}]^2$ (λ_{emi} = 338 nm), (e) Job's plot ΔOD vs Mole fraction of MBCA with β-CD (λ_{abs} = 245 nm).

increases with the addition of β-CD. The increase in the life time of complexed component with the increase in β-CD concentration confirms the encapsulation of the MBCA in β-CD cavity. The longer life time is ascribed to a deep encapsulation of MBCA into the β-CD cavity. (El-Kemary, El-Gezawy, El- Baradie, & Issa, 2002; El-Kemary & El-Gezawy, 2003). The amplitude of the complexed component also increases with the addition of β-CD, while that of the free species decrease (Table 2). This shows that MBCA is included in the β-CD cavity. (Rajamohan et al., 2008). Triple exponential analysis was also attempted, but χ^2 was not improved.

Table 1
The absorption and fluorescence spectral maxima of MBCA in different concentration of β-CD at pH 6.8.

Concentrations of β-CD (mol dm^{-3})	pH 6.8					
	λ_{max} (nm)	abs	λ_{max} (nm)	abs	λ_{flu} (nm)	λ_{flu} (nm)
0	243.5	0.471	291.0	0.152	336	–
2×10^{-3}	244.0	0.478	292.0	0.160	337	354(s)
4×10^{-3}	244.5	0.489	292.5	0.166	338	352(s)
6×10^{-3}	245.0	0.507	293.0	0.180	338	352(s)
8×10^{-3}	245.0	0.530	293.5	0.195	338	352(s)
10×10^{-3}	245.0	0.538	294.0	0.196	338	353(s)
12×10^{-3}	245.0	0.542	294.0	0.205	338	353(s)

Table 2
Fluorescence lifetime and amplitude of MBCA with increasing concentrations of β-CD (excitation wavelength 300 nm, detection wavelength 320 nm).

Concentration of β-CD (mol dm^{-3})	Lifetime (s)	Relative amplitude (%)	$\chi 2$	Standard deviation
0	2.2×10^{-9}	100	1.018	7.4×10^{-11}
0.002	$7.6 \times 10^{-10} 2.7 \times 10^{-9}$	69.5530.45	1.020	$5.4 \times 10^{-11} 2.02 \times 10^{-10}$
0.004	$7.0 \times 10^{-10} 3.5 \times 10^{-9}$	65.7234.28	0.966	$5.84 \times 10^{-11} 2.94 \times 10^{-10}$
0.006	$4.7 \times 10^{-10} 8.1 \times 10^{-9}$	50.9149.09	1.030	$5.85 \times 10^{-11} 2.59 \times 10^{-10}$
0.008	$4.1 \times 10^{-10} 8.3 \times 10^{-9}$	49.5350.47	1.080	$4.71 \times 10^{-11} 1.6 \times 10^{-10}$
0.010	$3.7 \times 10^{-10} 9.2 \times 10^{-9}$	32.1567.85	1.050	$4.5 \times 10^{-11} 1.2 \times 10^{-10}$
0.012	$2.1 \times 10^{-10} 9.6 \times 10^{-9}$	24.00v76.00	1.066	$5.94 \times 10^{-11} 1.72 \times 10^{-10}$

3.1.5. Spontaneity of inclusion complexation

The determination of thermodynamic parameter, free energy change for the host–guest inclusion process can be calculated from the binding constant values, '*K*' by the following equation

$$\Delta G = -RT \ln K \quad (8)$$

ΔG values for the binding of MBCA with β-CD using absorption and fluorescence data are −10.4 and −11.1 kJ mol^{-1}, respectively.

Negative value of ΔG suggests the spontaneity of host–guest complexation reaction at 303 K.

3.2. Characterization of inclusion complex in solid state

3.2.1. Fourier transform infra red spectral analysis

The interaction between the guest and host in the inclusion complexation process has been determined by FT-IR spectral study (Farcas, Karrpixb, Farcas, Harabagiu, &Guegan, 2006). β-CD and MBCA form a solid inclusion complex by the non-covalent interactions between β-CD and MBCA such as hydrophobic interactions, van der Waals interactions and hydrogen bonding. When the guest molecule is encapsulated by means of inclusion complexation reaction, the absorption bands resulted from the included part of guest molecule are generally shifted in their position. The FT-IR spectrum of pure MBCA, inclusion complex and physical mixture is shown in Fig. 2. The changes in the shape and position of absorption bands of guest (MBCA), inclusion complex and the physical mixture are observed. The O—H stretching frequency appears 3361 cm^{-1} in β-CD (Fig. 2a), it appears at 3361 cm^{-1} in physical mixture. It is shifted to 3408 cm^{-1} in the case of solid inclusion complex. Similarly ring C—H bending vibration of MBCA appeared at 771.55 cm^{-1} and 589.59 cm^{-1} are shifted to 764. 40cm^{-1} and 587. 85cm^{-1} in the case of solid inclusion complex. The N—H stretching frequency appeared at 3440.9 cm^{-1} in pure MBCA (Fig. 2b) and in physical mixture at 3441.10 cm^{-1} (Fig. 2d) is shifted to 3411.15 cm^{-1} in the solid complex (Fig. 2c). The C—Cl stretching frequency is appeared at 722.65 in pure MBCA and physical mixture is significantly shifted to 714.40 in the solid complex. The C—H aromatic stretching frequency is appeared at 3039.8 cm^{-1} in pure MBCA and at 3041 cm^{-1} in the physical mixture. Due to complexation it is significantly shifted to 3035.6 cm^{-1} Further the broader peak at 3356.5 cm^{-1} is due to the presence of larger number of hydrogen bonded —OH groups introduced by β-CD during complex formation. The observed changes in the FT-IR spectra of MBCA/β-CD inclusion complex are due to the restriction of the vibration of free MBCA molecule as the benzene ring of MBCA with Cl and NH_2 groups is encapsulated into the β-CD cavity. The FT-IR experimental result is also confirmed by carrying out Molecular Docking studies (Fig. 7). FT-IR and Molecular Docking studies results are in good correlation. Since no new bonds are formed which reveals the non-covalent interaction between host and guest (Schneiderman & Stalcup, 2000). That is due to van der Waals interaction between MBCA and β-CD.

3.2.2. Scanning electron microscopy image analysis

The SEM images of β-CD, pure MBCA, physical mixture of MBCA with β-CD and solid complex are shown in Fig. 3. These images were

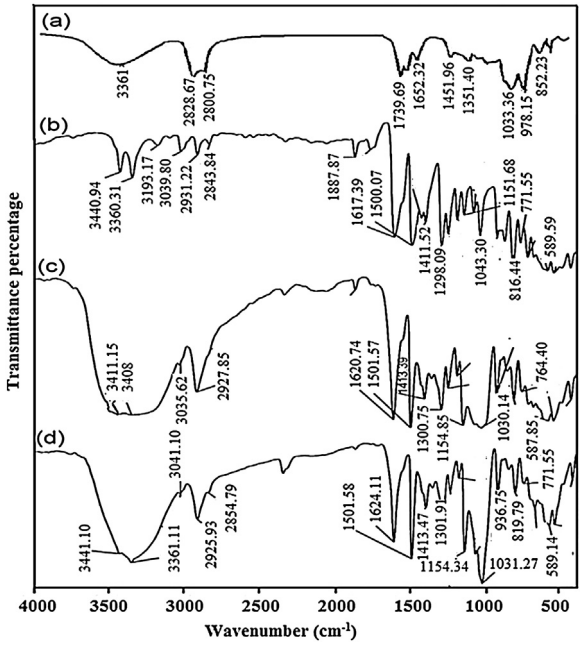

Fig. 2. Fourier transform infra red spectra of (a) β-CD, (b) MBCA, (c) solid complex of MBCA with β-CD and (d) physical mixture of MBCA and β-CD.

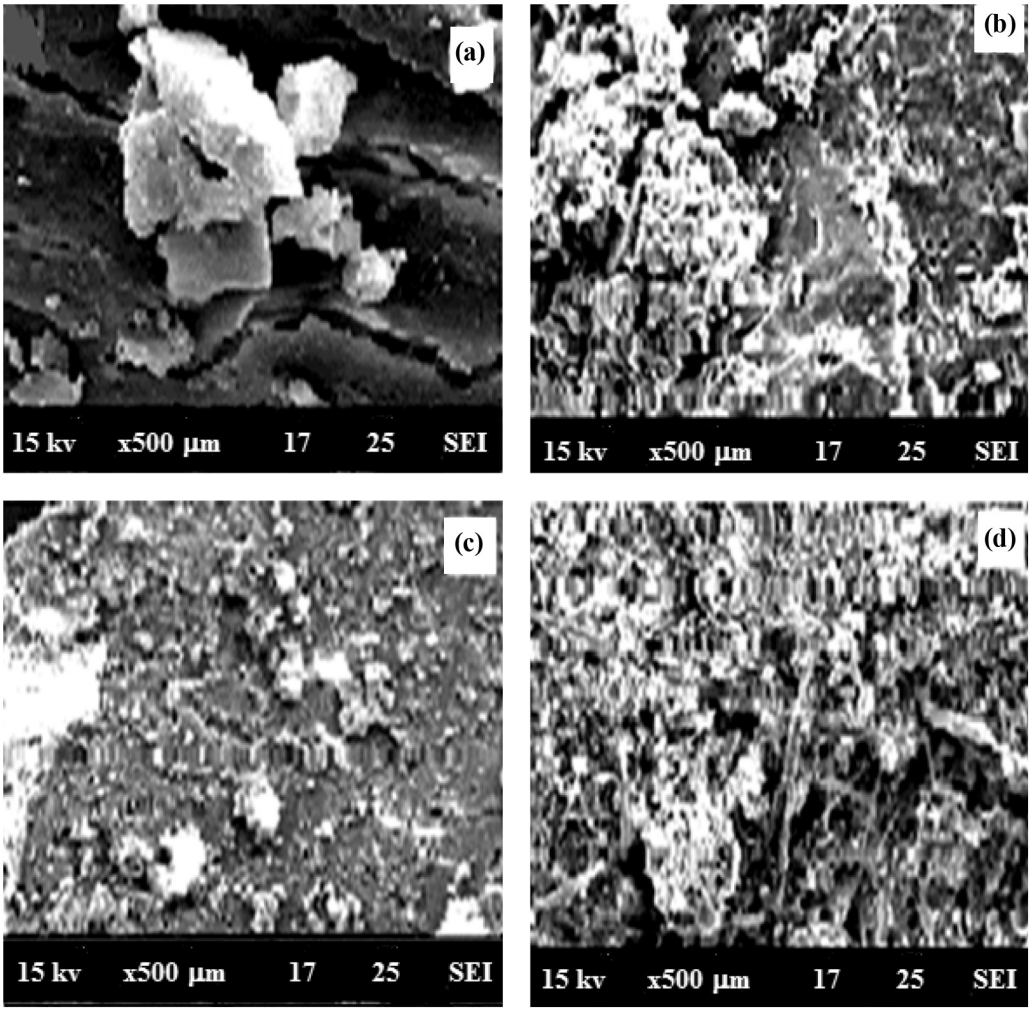

Fig. 3. Scanning electron microscopy images of (a) β-CD, (b) MBCA (×500), (c) physical mixture of MBCA and β-CD (× 500) and (d) solid complex of MBCA with β-CD (×500).

utilized to evaluate the effect of co-precipitation process on the morphology of the solids used for the formation of solid systems. The SEM photographs clearly elucidate the difference between the pure components and their inclusion complexes. β-CD (Fig. 3a) is observed as plate shaped crystals. Pure MBCA (Fig. 3b) appeared as irregular shaped crystals. The SEM image of the physical mixture (Fig. 3c) clearly shows the characteristic MBCA crystals, mixed with β-CD particles are adhered to their surface, thus confirming the presence of crystalline MBCA. In contrast the morphology and shape of particles of 1:1 solid inclusion complex (Fig. 3d) are changed drastically. That is, there is aggregation of needle like structures in amorphous state which demonstrates an apparent interaction between MBCA and β-CD. Actually the original morphology of both pure components disappeared, and therefore it is not possible to differentiate individual components. These modifications of crystal structure can be taken as a proof for the formation of solid inclusion complex (Rajamohan et al., 2008).

3.2.3. Powder X-ray diffraction analysis

XRD is a useful method for the detection of β-CD encapsulation and it has been used to assess the degree of crystallinity of the given sample. Generally the crystalline nature of the guest molecule is reduced and more number of amorphous structures are increased in the solid inclusion complex (Eid et al., 2011.). Hence the solid complexes usually exhibit less numbered as well as less intense peaks.

Fig. 5d clearly shows the solid inclusion complex has more amorphous character than crystalline one as in MBCA. MBCA exhibits characteristic peaks at 2θ values of 12.33°, 14.49°, 23.31°, 25.72°, 28.78°, 31.75°, and 33.88° (Fig. 4b). The diffraction pattern of β-CD exhibited important peaks at 2θ values of 8.95°, 10.56°, 12.45°, 18.60°, 22.4°, 31.8°, and 35.01° (Fig. 4a). Most of the characteristic peaks of MBCA and β-CD are present in the diffraction pattern of physical mixture (Fig. 4c). The X-ray diffraction pattern of the solid inclusion complex (Fig. 4d) is evidently different from that of pure MBCA, β-CD, and physical mixture. XRD pattern of MBCA and physical mixture displayed crystalline pattern whereas in the complex the diffraction peaks are larger in number but it is not possible to distinguish the characteristic peaks. An amorphous pattern lacking crystalline peaks is observed for the complex and peak intensity is reduced significantly in the diffraction pattern of the complex also suggested the reduction in crystallinity. The differences observed among these patterns indicate that the solid inclusion complex is formed due to the nano hydrophobic interaction of β-CD with MBCA (Srinivasan, Stalin, & Sivakumar, 2012).

3.2.4. Differential scanning calorimetry study of complex

DSC is a powerful qualitative analytical technique for physico chemical characterization of encapsulation systems in solid. This technique is widely used for a rapid preliminary qualitative investigation of the thermal behavior of the isolated components and the inclusion complexes prepared according to a standard procedure

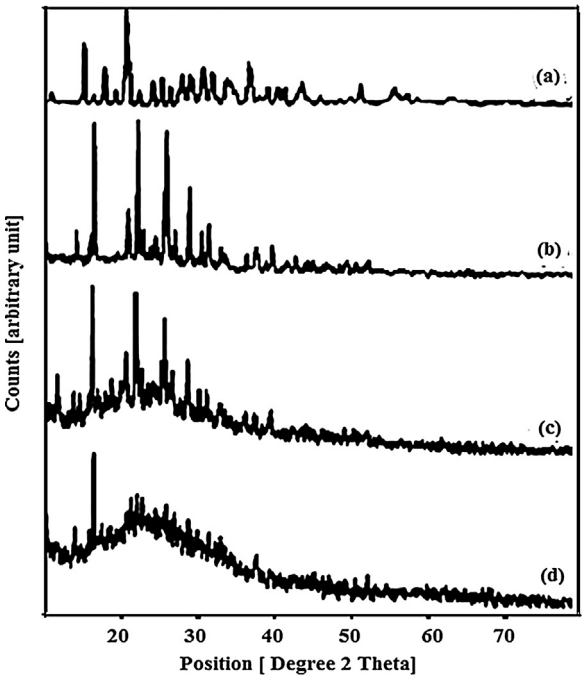

Fig. 4. X-ray diffraction pattern of (a) β-CD, (b) MBCA, (c) physical mixture of MBCA and β-CD and (d) solid complex of MBCA with β-CD.

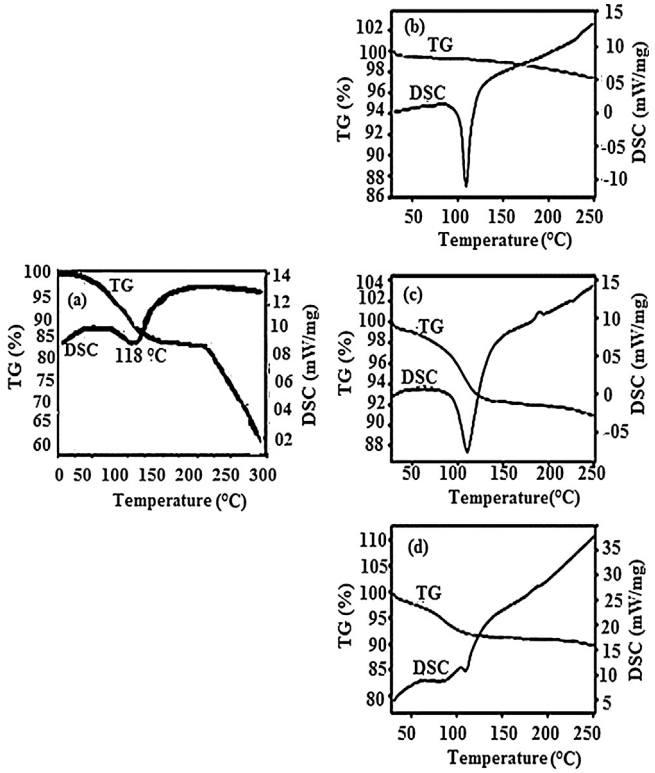

Fig. 5. Differential scanning calorimetry (DSC) and Thermo gravimetric (TG) graphs of (a) β-CD, (b) MBCA, (c) physical mixture of MBCA and β-CD and (d) solid complex of MBCA with β-CD.

such as co-precipitation. When guest molecule is included in CD cavity, their melting point, boiling point and sublimation points may shift to different temperature or disappear.

The DSC thermo gram for β-CD, MBCA, physical mixture and MBCA:β-CD solid inclusion complex are represented in Fig. 5. The DSC profile of β-CD (Fig. 5a) shows a broad endothermic peak at 118 °C. This endothermic peak is mostly associated to crystal water losses from β-CD cavities. The thermo gram of MBCA (Fig. 5b) showed a sharp endothermic peak at 110 °C corresponding to its melting point. In the physical mixture (Fig. 5c), the peak is found at 110 °C without significant reduction in intensity, whereas in MBCA:β-CD solid complex (Fig. 5d) the peak is observed at 108 °C with strong reduction in its intensity. The lower melting points of inclusion complex is due to melting point depression by the β-CD (Nerome et al., 2013). Similar observation was found when felodipine and carbamazepine were complexed with β-CD, the endothermic temperature of inclusion complex was lowered by 3 and 5 °C, respectively (Mielcarek, 1998). This confirms the interaction between MBCA and β-CD molecules in the host–guest inclusion complex.

3.2.5. Atomic force microscopy analysis

AFM analysis is well suited for visualizing the surface topography of deposited films when the surface texture sizes are far below three microns. Using adequate software, it is possible to evaluate the characteristics such as roughness, porosity, average size, and particle size distribution, which influence directly the optical, mechanical, surface, magnetic and electrical properties of thin films. The surface topography of MBCA and the inclusion complex is shown in Fig. 6.

Visualization of surface topography of MBCA and the complex revealed a significant difference in the surface morphology of MBCA when encapsulated within the β-CD. Modification of crystal structure can be taken as a proof for the formation of solid inclusion complex .The peak to valley distance in these images is used as an indicator of the surface roughness. When the distance between the peak and the valley is more which means that it has rougher surface and if the distance is less it indicates the smoothness of surface (Karim and Adnan, 2012). Based on this peak and valley distance, the AFM image of pure MBCA (Fig. 6a) shows a rough surface and uneven distribution. But in the case of solid complex (Fig. 6b) the rough surface of MBCA is changed into a smooth surface. The surface texture

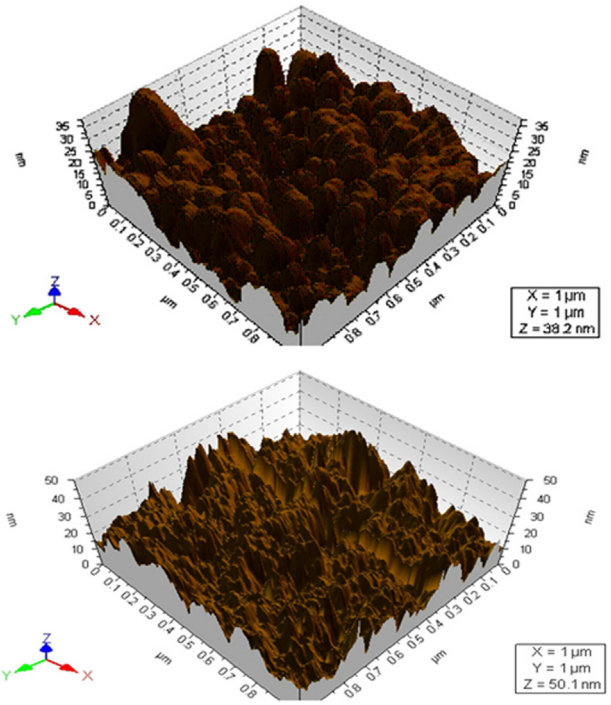

Fig. 6. Atomic force microscope photographs of (a) MBCA and (b) solid complex of MBCA with β-CD.

Table 3
Scores of the top 10 docked models of MBCA and β-CD inclusion complex computed using patch dock server.

Model	Geometric shape complementarity score	Approximate interface area size of the complex (Å^2)	Atomic contact energy (kcal/mol)
1	3472	418.4	−263.1
2	3440	427.9	−254.9
3	3402	399.0	−246.7
4	3380	356.9	−229.0
5	3378	394.9	−293.3
6	3298	394.8	−239.3
7	3288	407.7	−255.8
8	3274	377.3	−189.1
9	3148	396.2	−249.7
10	3040	397.1	−253.1

of MBCA and the complex was measured with horizontal length scale of less than 3 μm and a vertical length scale of 38.2 nm and 50.1 nm, respectively. Hence, host–guest complexation using β-CD finds important application in the preparation of nano materials.

3.3. Molecular docking study of inclusion process

The 3D structure of β-CD and MBCA obtained from crystallographic databases are shown in Fig. 7a and b. The guest molecule, MBCA was docked into the cavity of β-CD using Patch Dock server. The Patch Dock server program gave several possible docked structures for the most probable structure based on the energetic parameters; geometric shape complementarity score (Duhovny, Nussinov, & Wolfson, 2002) approximate interface area size and atomic contact energy (Zhang et al., 1997) of the MBCA:β-CD inclusion complex (Table 3). The docked MBCA:β-CD 1:1 structure (Fig. 7c) with the highest geometric shape complementarity score 3472, approximate interface area size of the complex 418.4 Å^2 and atomic contact energy −263.1 kcal/mol is the highly probable and energetically favorable model and it is in good correlation with results obtained through experimental methods.

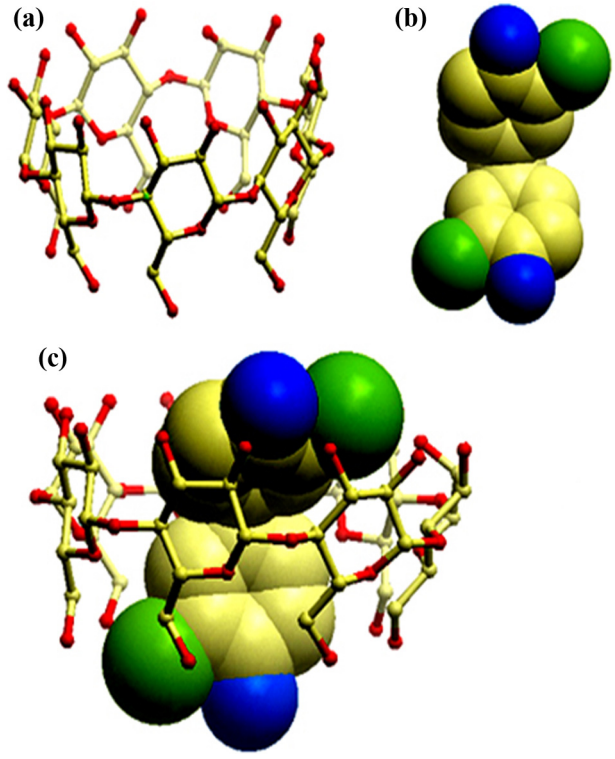

Fig. 7. Ball and stick representation of (a) β-CD, (b) MBCA, (c) 1:1 inclusion complex; the oxygen atoms are shown as red, nitrogen as blue, chlorine as green balls, carbon atoms are shown as golden balls and sticks, and hydrogen atoms are not shown.

3.4. Semi-empirical quantum mechanical calculations

The ground state of MBCA molecule was optimized using Argus Lab program by AM1 method. MolSoft MolBrowser tool was used to visualize the 3D structural data. The internal diameter of the β-CD cavity is approximately 6.5 Å and its height is 7.8 Å (Fig. 8a). Considering the shape and dimensions of β-CD, it is clear that the MBCA molecule cannot be completely included in the β-CD cavity. Because, the overall height of MBCA is 10.2 Å (Fig. 8b) (i.e., the vertical distance between H_{27}–H_{28} and H_{26}–H_{29}), but an overall height of β-CD cavity is only 7.8 Å. The horizontal length of MBCA molecule is 4.9 Å (i.e., the horizontal distance between Cl_{15}–H_{24} and Cl_{16}–H_{19}) (Table 4). Hence, there is possibility for partial accommodation of MBCA molecule i.e. along axial orientation inside the β-CD cavity. It is also confirmed by low binding constant value '*K*'. Based on the above findings, the structure of host–guest inclusion complex of MBCA:β-CD is proposed and it is shown in Fig. 8.

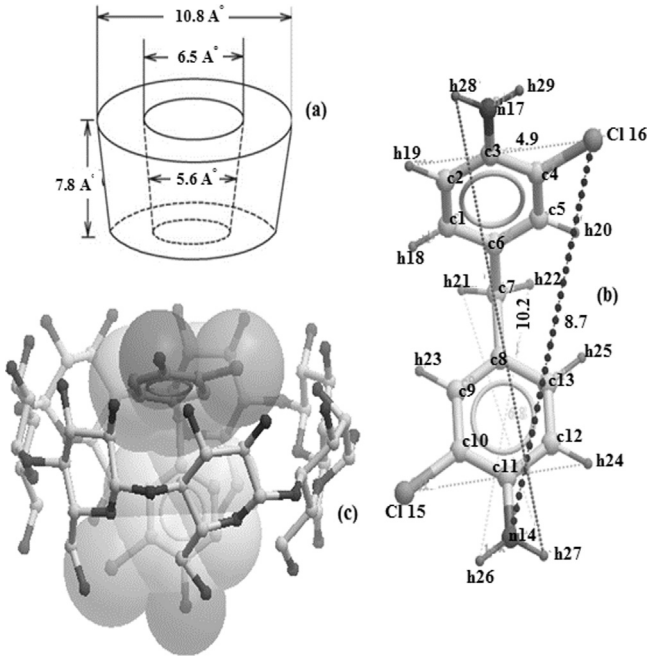

Fig. 8. (a) Structure of β-CD, (b) structure of MBCA, (c) structure of 1:1 host–guest inclusion complex of MBCA:β-CD.

Table 4
Bond distances and orientations of MBCA.

MBCA bond distance in Å	Orientation	
H_{27}–H_{28}	10.2	Vertical
H_{26}–H_{29}	10.2	Vertical
N_{14}–Cl_{16}	8.7	Vertical
H_{21}–H_{27}	6.8	Vertical
H_{22}–H_{26}	6.8	Vertical
Cl_{15}–H_{24}	4.9	Horizontal
Cl_{16}–H_{19}	4.9	Horizontal

4. Conclusions

The following conclusion can be arrived from the above studies.

1. The linearity of the Benesi–Hildebrand plot $1/(A - A_o)$ against $1/[\beta\text{-CD}]$ and $1/I - I_o$ against $1/[\beta\text{-CD}]$ indicates that the stoichiometry of the MBCA:β-CD inclusion complex is 1:1.
2. From the time resolved fluorescence decay curve, bi-exponential decay shows the presence of two species of MBCA namely free (τ_1) and complexed form $(\tau_{2)}$ with β-CD.
3. FT-IR, DSC, XRD, SEM and AFM analysis of solid complex supports the formation of host–guest inclusion complex between MBCA and β-CD. It is also inferred that there is no complex formation in the physical mixture.
4. The energetically favorable complex obtained by molecular docking study agrees with the mode of inclusion process predicted through FT-IR observations.

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