# Cyclodextrin-Covered Organic Microrods and Microsheets Derived from Supramolecular Self Assembly of 2,4-Dihydroxyazobenzene and 4-Hydroxyazobenzene Inclusion Complexes

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The self assembly behavior of 2,4-dihydroxyazobenzene (DHAB) and 4-hydroxyazobenzene (HAB) inclusion complexes were studied by SEM and TEM. Formation of inclusion complexes were characterized by FT-IR, DSC, PXRD, <sup>1</sup>HNMR, UV–visible, fluorescence, time-resolved fluorescence, and molecular modeling techniques. Micrometer-sized rods were observed in DHAB: $\alpha$ -cyclodextrin ( $\alpha$ -CD) and DHAB: $\beta$ -cyclodextrin ( $\beta$ -CD) inclusion complexes while microsheets were obtained in HAB/ $\alpha$ -CD and HAB/ $\beta$ -CD complexes. In DHAB/CD and HAB/CD individual "barrel" type (head to head arrangement) of 2:2 inclusion complexes form microrods and microsheets through intermolecular hydrogen bonding between the neighboring complexes. Molecular modeling calculations suggest that (i) DHAB and HAB form stable complexes with  $\alpha$ -CD and  $\beta$ -CD, (ii) intermolecular hydrogen bonds form between the guests and CDs. Investigations of energetic, thermodynamic, and electronic properties by PM3 method confirmed the stability of the inclusion complex.

Developments in nano- and microscale supramolecular self assembly materials have become great initiation for new technology with potential applications to photoelectric conversion devices,<sup>1</sup> separation and filtration media,<sup>2</sup> nonlinear optics,<sup>3</sup> single molecular devices,<sup>4</sup> and organic memory devices.<sup>5</sup> Different morphologies of supramolecular materials with nano- and microstructure have been reported from a wide range of guest and different cyclodextrins (CDs).<sup>6-8</sup> The homologous series of cyclic oligomers (CDs) consists of 6 to 8  $\alpha$ -1,4-linked D-(+)-glucopyranose residues. In particular 6, 7, and 8 member homologous molecules named  $\alpha$ -,  $\beta$ -, and  $\gamma$ -CD have been a focus for important supramolecular host materials. Among them  $\beta$ -CD is a familiar interesting host material for numerous applications in the area of catalysis, molecular recognition, increasing solubility, bioavailability, drug delivery, and environmental protection.9 Due to their wide applications in a vast area, a number of studies have been undertaken to understand the nature of CD inclusion complexes.<sup>10</sup>

It is well known that CDs self aggregate through intermolecular hydrogen bonding to form nano- or microstructures. Absorption, fluorescence, and infrared spectra (FT-IR), differential scanning colorimetriy (DSC), powder X-ray diffraction (PXRD), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) are general tools exploited to characterize these nano- or microstructures. Many reports have demonstrated these supramolecular architectures through involvement of appropriate guest molecules.<sup>11–13</sup> Chen et al. reported carbonaceous nanofiber membrane functionalized by  $\beta$ -CD for molecular filtration.<sup>11</sup> Mandal et al. reported that  $\gamma$ -CD host–guest complex nanotubes aggregate by fluorescence correlation spectroscopy.<sup>12</sup> Sun et al. reported controlled transformation from nanorods to vesicles induce by cyclomaltoheptose.<sup>13</sup> St. Dennis et al. reported their results in the simple, one-step synthesis of carbon nanorod-like structure from direct pyrolysis of  $\alpha$ -CD.<sup>14</sup> From the above reports it has been shown that the construction of supramolecular architecture can be achieved by interfacial supramolecular interactions. All these recent studies establish that CDs formed supramolecular architecture through host-guest aggregates.<sup>11-14</sup> With these reports on hand, the intension of our present work is to characterize the formation of nanorod aggregate and to find the role of the dimension of CDs in the self assembly. We have already reported formation of inclusion complexes of 2,4-dihydroxyazobenzene (DHAB) and 4-hydroxyazobenzene (HAB) with  $\beta$ -CD in aqueous solution,<sup>15</sup> but in this paper we analyze the micro self assembly of  $\alpha$ -CD and  $\beta$ -CD induced by DHAB and HAB using SEM, TEM, FT-IR, DSC, PXRD, <sup>1</sup>HNMR, absorption, fluorescence, fluorescence decay spectroscopy, and molecular modeling.

#### **Results and Discussion**

Nanomaterial Observations. SEM images clearly show the different morphologies of the inclusion complex nanomaterials: (i)  $\alpha$ -CD has prismatic, (ii)  $\beta$ -CD has sheeted and platted form, and (iii) DHAB and HAB dyes have uniform cubic crystal shape and agglomerated irregular shape, respectively. As seen from the SEM images, DHAB/ $\alpha$ -CD (Figure 1) inclusion complex present in agglomerated uniform crystal shape, whereas DHAB/ $\beta$ -CD (Figure 1) presents in plate form and this morphology differs from that of the pure DHAB compound. Similarly, HAB/ $\alpha$ -CD and HAB/ $\beta$ -CD inclusion complexes were also present in different shapes than that of pure HAB. Modification of these morphologies can be taken as a proof for the formation of inclusion complex nanomaterials.

TEM images of HAB/ $\alpha$ -CD, HAB/ $\beta$ -CD, DHAB/ $\alpha$ -CD, and DHAB/ $\beta$ -CD are shown in Figure 2. The thickness for



Figure 1. SEM photographs of (a)  $\alpha$ -CD, (b)  $\beta$ -CD, (c) DHAB, (d) DHAB/ $\alpha$ -CD, (e) DHAB/ $\beta$ -CD, (f) DHAB, (g) HAB/ $\alpha$ -CD, and (h) HAB/ $\beta$ -CD inclusion complex.

DHAB/β-CD (Figures 2c and 2d) microrod was approximately in the range of ca.  $1.46 \,\mu\text{m}$  and height is about ca.  $9.8 \,\mu\text{m}$ , we observed that the microrod has uniform surface and the corner was smoothly ended, whereas, DHAB/a-CD (Figures 2a and 2b) microstructure has different morphology i.e., many microrod-agglomerated structures. The typical average dimension of DHAB/a-CD microrods is ca. 360 nm width and ca. 3 µm length. We already reported that both DHAB and HAB bound with the CD cavity to form a 1:1 (host:guest) inclusion complexes then these 1:1 inclusion complexes are further developed into a higher order 2:2 (host:guest) "barrel" type of inclusion complexes.<sup>15</sup> Both DHAB/α-CD and DHAB/ β-CD microstructures suggest that the individual "barrel" type (head-to-head arrangement) of 2:2 inclusion complexes form various dimensions of microrods based on supramolecular pseudorotaxane mechanism. The considerable torsion angle flexibility of the azo functional group (-N=N-) lead to both azo dye molecules (DHAB and HAB) being present in the inside of the CD cavity forming 2:2 inclusion complexes. Liu et al. reported that a linear structure was attained from 4aminoazobenzene with β-CD complex by intra- and interdimer hydrogen bonding. Intradimer  $\pi$ - $\pi$  interactions and wave-type structure were attained respectively.<sup>16</sup>

A proposed pseudorotaxane mechanism shown in Figure 3. There are no massive end groups in either complex (2:2 barrel type) of barrel arrangements are self assembled into a (DHAB/ α-CD and DHAB/β-CD) different template of microrod making possible formation of a single line head to head arrangement 2:2 inclusion complex (Figure 3d). The small individual nanorods are secondary self-assembled through intermolecular hydrogen bonds by CD hydroxy groups, these types of higher stoichiometry (2:2) inclusion complexes have the tendency to form linear CD pseduopolyrotaxanes.<sup>17-19</sup> Further, the key to the exploitation of the microscale rod structure is believed to be related to the various forces and hydrogen bonding. According to previous reports<sup>8,10</sup> the self aggregation proceeds mainly from the contribution of hydrogen bonding or electrostatic interactions, however these reports indicates that the aggregation between CD inclusion complexes is not only hydrogen bonding and nonbonding van der Waals forces are also responsible for the formation of microrod structures. Therefore the above two forces beyond doubt exist between the DHAB/CD inclusion complexes and the resultant microrods are formed.

HAB/ $\alpha$ -CD (Figures 2e and 2f) complexes form a selfassembled monolayer whereas HAB/ $\beta$ -CD (Figures 2g and 2h) forms a thick microsheet. HAB/ $\beta$ -CD complexes show the strong agreement between the center and margin, which is a distinctive typical microsheet structure (Figures 2g and 2h). Since CDs having two types of hydroxy groups on the end of the cavities, one is a primary hydroxy group (tail) and another one is a secondary hydroxy group (head). Hence, there is a possibility of three types of arrangement modes which CDs



Figure 2. TEM images of (a and b) DHAB/ $\alpha$ -CD, (c and d) DHAB/ $\beta$ -CD, (e and f) HAB/ $\alpha$ -CD, and (g and h) HAB/ $\beta$ -CD inclusion complex.

can have interacted with each others, namely head-to-head, tailto-tail, and head-to-tail.<sup>18,19</sup> From the above argument monolayer architecture formed by HAB/ $\alpha$ -CD, which related to the weaker supramolecular interaction, whereas microsheets were formed by HAB/ $\beta$ -CD due to the stronger van der Waals force and hydrogen bond between neighboring complex.

**Infrared Spectral Studies.** FT-IR is useful to identify host–guest interactions, Figures S1 and S2 shows the FT-IR spectra of the inclusion complex nanomaterials, physical mixtures, and pure azo dyes (DHAB and HAB). The above figures indicate that the physical mixture of the guests and hosts are different from the inclusion complex nanomaterials. For pure DHAB, the absorption peak at 3380 cm<sup>-1</sup> can be attributed to the –OH stretching vibration and 628 cm<sup>-1</sup> is reflected –OH out plane deformation. A substantial increase in intensity as well as broadening of the –OH band was noted in the DHAB/α-CD (Figure S1b) and DHAB/β-CD (Figure S1c) complexes suggest that the –OH group of the DHAB molecule interacts with



Figure 3. Proposed mechanism for the formation of microrod from inclusion complex. (a) DHAB, (b) CD, (c) 2:2 barrel type inclusion complexes through head-to-head arrangement, (d) 2:2 inclusion complexes secondary self assembled to form smaller nanorod, (e) individual nanorods are again self assembled with another nanorods through hydrogen bonding to form cluster of microrod.

CDs. The aromatic C-H stretching of DHAB appeared at 2920 cm<sup>-1</sup> moved to a higher frequency in the DHAB/ $\alpha$ -CD (2926 cm<sup>-1</sup>) and DHAB/ $\beta$ -CD (2925 cm<sup>-1</sup>) complexes. Azo group (-N=N-) stretching appeared at 1455 cm<sup>-1</sup> moved to lower frequency at 1420 cm<sup>-1</sup> in the inclusion complex nanomaterials. The FT-IR spectrum of HAB shows the absorption frequency at 3397 cm<sup>-1</sup> for the -OH stretching and 678 cm<sup>-1</sup> frequency due to -OH deformation vibration (Figure S1d). The frequency at 1452 cm<sup>-1</sup> can be attributed to N=N stretching vibration. The -OH stretching appeared as a single broad band at  $3377\,cm^{-1}$  in HAB/\alpha-CD (Figure S1e) and  $3382\,cm^{-1}$  in HAB/ $\beta$ -CD (Figure S1f) complex nanomaterials. This result suggests that the formation of a monomeric dispersion of the HAB molecule is a consequence of the interaction with CDs, which can result from inclusion of HAB in the hydrophobic cavities. Further, DHAB and HAB hydroxy group stretching band shapes changed in the corresponding inclusion complex nanomaterials spectra indicating that -OH of these molecules interacts strongly with CDs.

**DSC Thermograms.** DSC profiles of  $\alpha$ -CD,  $\beta$ -CD, DHAB, HAB, and the corresponding inclusion complexes are presented in Figure 4. The thermal curve of DHAB shows a sharp endothermic peak at 170.6 °C, which corresponds to the melting point of the DHAB. DSC curves of  $\beta$ -CD show a broad endothermic peak at 128.6 °C and  $\alpha$ -CD shows three endothermic peaks at 79.2, 109.1, and 137.5 °C, these endothermic peaks



Figure 4. DSC thermograms of (a) α-CD, (b) β-CD,
(c) DHAB, (d) DHAB/α-CD, (e) DHAB/β-CD, (f) HAB,
(g) HAB/α-CD, and (h) HAB/β-CD inclusion complexes.

are attributed to crystal water loss from CDs. A broader endothermic effect was recorded for  $\alpha$ -CD,  $\beta$ -CD, and respective inclusion complex nanomaterials as a consequence of water loss from the CDs. When compared with isolated DHAB and CDs, DHAB/CD (Figures 4d and 4e) inclusion complexes did not show the entire distinguishable peak which indicates the formation of inclusion complex nanomaterials. The DSC thermogram of DHAB/CD complexes did not show peaks corresponding to the isolated DHAB and CD, instead new peaks appeared at 98.6 and 118.5 °C for DHAB/α-CD and DHAB/β-CD complex nanometers respectively. In the case of HAB/ $\alpha$ -CD (Figure 4g) and HAB/ $\beta$ -CD (Figure 4h) the absence of the characteristic HAB endothermic peak at 152.7 °C, in the thermograms, indicates trapping of HAB in the CDs cavities. The disappearances of endothermic peaks of DHAB and HAB in the DSC curves of all the respective inclusion complexes give strong evidence for the formation of inclusion complex nanomaterials.<sup>20</sup>

**Powder X-ray Diffractograms.** Powder X-ray diffractometry (PXRD) is a useful method for detection of CD complexation in powder or microcrystalline states. The diffraction patterns of the complex nanomaterials are distinct from that of the pure components (free guest and CD) is taken as evidence for the inclusion complex formation.<sup>21</sup> As shown in Figure 5, PXRD patterns of DHAB show intense, sharp peaks that prove the crystalline nature of the compounds (Figure 5a). DHAB had strong crystallinity peaks at  $2\theta$  of 13.8, 17.1, 24.8, and



**Figure 5.** PXRD patterns of (a) DHAB, (b) DHAB/ $\alpha$ -CD, (c) DHAB/ $\beta$ -CD, (d) HAB, (e) HAB/ $\alpha$ -CD, and (f) HAB/ $\beta$ -CD inclusion complex.

 $26.1^{\circ}$  and several minor peaks at  $2\theta$  of 14.1, 19.8, 20.9, 28.6, and 32.1°. On the other hand, DHAB/ $\alpha$ -CD (Figure 5b) and DHAB/β-CD (Figure 5c) inclusion complex patterns are clearly distinct diffraction patterns when compared to free DHAB (Figure 5a) suggesting the formation of inclusion complex nanomaterials. Moreover many sharp peaks appeared in the range of 10 to 25°, confirming the formation of DHAB/CD inclusion complex nanomaterials. In the case of HAB, major intense diffraction peaks appear at  $2\theta$  of 11.8, 23.6, 25.2, and 27.8° is shown in Figure 5d. Many of these peak intensities are dramatically increased and new peaks appeared in the HAB/a-CD (Figure 5e) and HAB/ $\beta$ -CD (Figure 5f) inclusion complex diffraction patterns. The above results are taken as evidence for the formation of HAB/CD inclusion complex nanomaterials. Overall, the variation in the peak intensities of the DHAB/CD and HAB/CD complex diffraction patterns from the corresponding free molecule diffraction patterns suggest that new complex nanomaterials were formed.

**Proton Magnetic Resonance Spectral Studies.** Generally, the chemical shift values of the guest protons tend to show appreciable changes if the guest molecules are included in the CDs cavities.<sup>22</sup> Hence, <sup>1</sup>H NMR spectra of DHAB and HAB complexes are acquired at 25 °C in DMSO- $d_6$  and compared with those of pure compounds, which are shown in Figures S3 to S5. As can be seen from Table S1, the  $\delta$  value of H<sub>a</sub> and H<sub>b</sub> are the hydroxy protons of DHAB, shifts up field in  $\alpha$ -CD



Figure 6. Absorbance spectra of DHAB and HAB in different α-CD concentrations (M): (1) 0, (2) 0.001, (3) 0.002, (4) 0.004, (5) 0.006, (6) 0.008, and (7) 0.01. Insert figure absorbance vs. α-CD concentrations.

complex and shifts down field in  $\beta$ -CD complex. The aromatic protons of the DHAB molecules (H<sub>c</sub>, H<sub>d</sub>, H<sub>e</sub>, and H<sub>f</sub>) shift up field as compared with the corresponding free DHAB. These results indicate that these above aromatic protons interact with CD cavity protons. Further, considering the higher chemical shift of the H<sub>f</sub> protons (DHAB/ $\alpha$ -CD = -0.024 ppm and  $DHAB/\beta\text{-}CD = -0.026\,\text{ppm})$  suggests that the  $H_{\rm f}$  protons of the DHAB molecule have more interaction with the CDs. As can be seen from the Table S1 the chemical shift data for the inclusion complex nanomaterials was different from the free compound. In particular, the resonance of the H<sub>a</sub> (-OH) protons of HAB molecule showed remarkable up field shift in the HAB/ $\alpha$ -CD = -0.078 ppm and HAB/ $\beta$ -CD = -0.089 ppm inclusion complex nanomaterials. This higher chemical shift value of the H<sub>a</sub> proton of the HAB indicates that this proton may be hydrogen bonded with the CD hydroxy protons. In HAB molecule, the aromatic ring protons H<sub>b</sub> and H<sub>d</sub> are up field shifted in the complex, which suggests that the aromatic ring is shielded largely in the complex and it must penetrate deeply into the CDs cavities.

Absorption and Fluorescence Spectral Studies. Figures 6 and S6 depicts the absorption spectral data of DHAB, HAB, and AB in aqueous solution containing different concentrations of  $\alpha$ -CD and  $\beta$ -CD respectively. The absorption spectrum of DHAB ( $\lambda_{abs}$  ca. 382, 249, 220 nm to 379, 256, 226 nm) and HAB molecules ( $\lambda_{abs}$  ca. 342, 228 nm to 340, 240 nm) shows a slight blue shift with increasing concentration of both CDs. The absorbance of CD solutions recorded after 12 h remains the



**Figure 7.** Fluorescence spectra of DHAB and HAB in different  $\alpha$ -CD concentrations (M): (1) 0, (2) 0.001, (3) 0.002, (4) 0.004, (5) 0.006, (6) 0.008, and (7) 0.01. Insert figure fluorescence intensity vs.  $\alpha$ -CD concentrations.

same indicating that both azo molecules were present in the CD cavities in solution without decomposition due to the formation of inclusion complex. The absorbance of DHAB and HAB increases in both CDs solutions indicating that the encapsulation of these molecules into the CD cavities. Figures 7 and S7 represent the fluorescence spectra of DHAB and HAB in different concentrations of  $\alpha$ -CD and  $\beta$ -CD respectively. In both CD solutions, the fluorescence spectra of the above guest molecules are more sensitive than the absorption spectra. As the CD concentrations increased, the emission maxima of both hydroxy azo compounds were largely red shifted from 430 to 460 nm for DHAB and 390 to 450 nm for HAB. Such a change in the emission maxima of both molecules caused by the introduction of both CD indicates the formation of an inclusion complex nanomaterials. The above unusual red shift emission maxima due to the formation of 2:2 inclusion complexes were already explained in our earlier reports.<sup>15,23-26</sup>

To determine the stoichiometry of the inclusion complexes, a Job's method<sup>27</sup> for the absorption/fluorescence was applied keeping the sum of initial concentration of the guests and CDs constant and the molar ratio of CD changing from 0 to 1. The absorption/fluorescence intensity of the guest in the absence of ( $I_0$ ) and presence of CD (I) were determined respectively. A plot of  $(A - A_0)/A_0$  and  $(I - I_0)/I_0$  versus the molar fraction of CDs were provided in Figure 8. It shows that,  $(A - A_0)/A_0$ and  $(I - I_0)/I_0$  values versus molar fraction gives the straight line, indicating a 1:1 stoichiometry of the azodyes with CDs in the inclusion complex.

In order to further characterize Fluorescence Lifetime. the properties of inclusion complex nanomaterials of DHAB and HAB, we measured fluorescence lifetimes of the above fluorophores in water as well as in 0.01 M  $\alpha\text{-CD}$  and  $\beta\text{-CD}.$  The fluorescence decay profile for the DHAB and HAB molecules were measured in the excitation wavelength of 360 and 340 nm respectively. Multiexponential decays were significant for organic compounds in solution, and it is often difficult to mechanically assign the various components of decay. In the aqueous medium, DHAB and HAB have multiple hydrogen bonding sites in the ground and excited states. The above behavior gives rise to different charge distribution structures with varying fluorescence decay lifetimes. As an alternative of giving importance to individual decay components, we define the secondorder average lifetime  $\langle \tau \rangle$  for these azo molecules in solution as described by Guchhait and his co-workers.<sup>28</sup> The typical decay





profiles for DHAB and HAB molecules are shown in Figure S8 and the fitted lifetime values are displayed in Table 1. The decay profile for the DHAB and HAB molecules in water shows a two exponential decay, but in the CD solutions it fits with three exponentials. From Table 1 it can be observed that in CD solution DHAB and HAB molecules show higher lifetime than compared with water medium. This suggests that fluorescence emission of these azo molecules was stabilized by both CDs cavities. For example, in water, DHAB is found to be 1.22 ns is less than that of DHAB/ $\alpha$ -CD (1.85 ns) and DHAB/ $\beta$ -CD (3.01 ns) inclusion complexes. The fluorescence decay profile of DHAB and HAB are shown triexponential fit, indicates that three different species were present in solutions.

**Molecular Modeling.** In addition to providing experimental results we also applied the molecular modeling studies, which offer a molecular level explanation with ability to estimate the participation of specific functional groups and their interaction in the complex nanomaterials stabilization. As stated in the earlier studies<sup>15,23</sup> azo form only exists in the aqueous solution, in order to confirm this we also performed molecular optimization of both azo and hydrazo tautomers of the DHAB and HAB molecules with CDs. The minimum energies of the optimized DHAB/CD and HAB/CD complexes given in Table 2 and Table 3, and the minimized energy structure of these guests and the complex nanomaterials are shown in Figures 9 to 11.

Two different structures could be written corresponding to the molecular composition of DHAB and HAB molecules namely, azo and hydrazo (Figure 9). This was due to the migration of the hydrogen atom of a hydroxy group (which is attached to C4 carbon) to the azo nitrogen atom. A further tautomeric structure was also possible in the case of DHAB as a result of the transfer of the -OH proton to the nitrogen atom of azo group. Azo as well as hydrazo forms have been geometrically optimized using PM3 level and the results are compiled in the Table 2 and Table 3 respectively. Analysis of the optimized molecular geometries reveals that the azo and hydrazo tautomers were essentially nonplanar in the ground states since significant torsion angles were detected between the plane of the azo linkage and the  $C_1$  and  $C_7$  carbons (Figure 9). We optimized both azo and hydrazo inclusion complex nanomaterials and the energy change accompanying the formation of DHAB/CD and HAB/CD complex nanomaterials calculated using the following equation

$$\Delta E = E_{\text{complex}} - (E_{\text{CD}} + E_{\text{dye}}) \tag{1}$$

where  $E_{\text{complex}}$ ,  $E_{\text{dye}}$ , and  $E_{\text{CD}}$  are the energies of the complex nanomaterials, the free guest,  $\alpha$ -CD, and  $\beta$ -CD (host) respec-

Table 1. Fluorescence Decay Parameters of DHAB and HAB in Water and 0.01 M CD Solution

Dyes	Medium	Lifetime/ns			Pre exponential factor			(7)
		$ au_1$	$ au_2$	$ au_3$	$a_1$	$a_2$	<i>a</i> <sub>3</sub>	$\langle l \rangle$
DHAB	Water	0.96	3.35		0.56	0.02		1.22
	α-CD	0.31	1.09	4.65	0.86	0.51	0.04	1.85
	β-CD	0.11	1.79	4.65	0.55	0.30	0.11	3.01
HAB	Water	0.47	4.97		0.26	0.02		2.44
	α-CD	0.36	1.10	4.69	0.16	0.14	0.02	2.04
	β-CD	0.31	2.11	5.08	0.88	0.39	0.13	2.94

Properties	DHAB	HAB	α-CD	β-CD	DHAB/α-CD	DHAB/β-CD	HAB/a-CD	HAB/β-CD
$E_{\rm HOMO}/{\rm eV}$	-8.79	-8.97	-10.37	-10.35	-8.90	-8.80	-8.96	-8.90
$E_{\rm LUMO}/{\rm eV}$	-0.86	-0.79	1.26	1.23	-0.98	-0.65	-0.83	-0.67
$E_{\rm HOMO} - E_{\rm LUMO}/{\rm eV}$	-7.93	-8.18	-11.63	-11.58	-7.93	-8.15	-8.13	-8.23
Dipole moment/D	1.13	1.17	11.34	12.29	11.61	11.87	10.69	10.86
$E^{\mathrm{a})}$	9.68	45.02	-1247.62	-1457.63	-1253.99	-1467.84	-1210.47	-1421.62
$\Delta E^{a)}$					-16.05	-19.89	-7.87	-9.01
$G^{\mathrm{a})}$	114.06	150.39	-676.37	-789.52	-568.13	-686.02	-527.51	-643.75
$\Delta G^{ m a)}$					-5.83	-10.56	-1.54	-4.62
$H^{\mathrm{a})}$	147.07	175.52	-570.84	-667.55	-440.97	-541.97	-401.10	-500.23
$\Delta H^{ m a)}$					-17.20	-20.76	-5.78	-8.20
$S^{\mathrm{b})}$	0.110	0.117	0.353	0.409	0.426	0.483	0.417	0.481
$\Delta S^{\mathrm{b})}$					-0.037	-0.036	-0.053	-0.045
ZPE <sup>a),c)</sup>	129.08	121.97	635.09	740.56	761.73	867.14	759.29	863.33
Mulliken charge	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

**Table 2.** Energetic Features, Thermodynamic Parameters, and HOMO–LUMO Energy Calculations for Azo Form of DHAB, HAB, AB,and Its Inclusion Complexes by PM3 Method

a) kcal mol<sup>-1</sup>. b) kcal mol<sup>-1</sup> K<sup>-1</sup>. c) ZPE: zero point vibration energy.

**Table 3.** Energetic Features, Thermodynamic Parameters, and HOMO–LUMO Energy Calculations for Hydrazo Form of DHAB, HAB,and Its Inclusion Complexes by PM3 Method

Properties	DHAB	HAB	DHAB/a-CD	DHAB/β-CD	HAB/α-CD	HAB/β-CD
$E_{\rm HOMO}/{\rm eV}$	-8.46	-8.53	-8.63	-8.56	-9.28	-8.68
$E_{\rm LUMO}/{\rm eV}$	-1.29	-1.44	-1.45	-1.27	-0.77	-1.36
$E_{\rm HOMO} - E_{\rm LUMO}/{\rm eV}$	-7.16	-7.30	-7.18	-7.29	-8.51	-7.32
Dipole moment/D	1.12	1.17	9.82	10.64	11.12	11.07
$E^{\mathrm{a})}$	12.25	45.02	-1241.03	-1453.86	-1164.06	-1404.01
$\Delta E^{a)}$			-5.66	-8.48	-6.93	-9.49
$G^{\mathrm{a})}$	111.45	150.39	-552.10	-627.37	-494.43	-633.19
$\Delta G^{ m a)}$			12.81	50.70	-1.10	-2.4
$H^{\mathrm{a})}$	146.34	175.52	-428.38	-528.49	-359.16	-483.28
$\Delta H^{ m a)}$			-3.88	-7.28	-5.12	36.35
$S^{\mathrm{b})}$	0.117	0.117	0.414	0.482	0.410	0.469
$\Delta S^{\mathrm{b})}$			-0.056	-0.044	-0.056	-0.054
ZPE <sup>a),c)</sup>	125.19	121.97	762.16	867.01	755.20	863.19
Mulliken charge	0.00	0.00	0.00	0.00	0.00	0.00

a) kcal mol<sup>-1</sup>. b) kcal mol<sup>-1</sup> K<sup>-1</sup>. c) ZPE: zero point vibration energy.



Figure 9. PM3 optimized structures of (a) DHAB-azo, (b) HAB-azo, (c) DHAB-hydrazo, and and (d) HAB-hydrazo tautomers.



**Figure 10.** PM3 optimized azo tautomers structures of (a) DHAB/α-CD, (b) DHAB/β-CD, (c) HAB/α-CD, and (d) HAB/β-CD inclusion complex.

tively. This provides quantitative measures of the interaction forces driving the complexation process. In overall azo tautomer complex nanomaterials were most stable because it has higher negative  $\Delta E$  value. For example in HAB azo/ $\alpha$ -CD complex nanomaterials has an energy of  $-7.87 \text{ kcal mol}^{-1}$  which was more negative than that of corresponding hydrazo tautomer  $(-6.93 \text{ kcal mol}^{-1})$ . Similar results were observed in other DHAB/CD and HAB/CD inclusion complex nanomaterials. Azo tautomer inclusion complex nanomaterials were energetically favorable, otherwise in the process of energy minimization these HAB and DHAB molecules pass the apolar cavity of CD, neither formatting hydrogen bonds between the CD and guest molecules (Figures 10 and 11). This suggested that the complexes were stabilized predominantly by dispersion of van der Waals interactions but as one would expect additional hydrogen bond lowers the energies of DHAB/CD and HAB/CD inclusion complex nanomaterials and thus increased the stability.

Changes in the dipole moment as a result of complexation for each tautomeric form are quite clear. The dipole moment changes accompanying complexation in the case of azohydrazo forms are not very high compared to corresponding isolated host molecules, but all the values are positive in both tautomers. This implies that after inclusion complexation, the polarity of the host (CD) molecule decreased. These substantial changes in the dipole moment were due to the specific interactions of the guest molecules with the CD.<sup>29</sup> In view of these facts, the formed inclusion complex nanomaterials are more stable than the corresponding isolated host and guest molecules. The HOMO/LUMO orbital picture of both azo and hydrazo tautomers of the DHAB, HAB, and AB molecules was shown in Figure S9. Generally the difference between this HOMO and LUMO is responsible for the stability of the complexes.<sup>30</sup> Hence HAB/ $\beta$ -CD complex was more stable because it has higher energy difference between the HOMO and LUMO (-8.23 eV) than the other complexes. The net Mulliken charge distribution analysis reveals that all studied complexes were not having charge-transfer character.

Thermodynamics of Inclusion Complexes. To investigate the thermodynamics of the most stable inclusion complex nanomaterials, we applied statistical thermodynamic calculations at 1 atm and 298.15 K by PM3 method and the results are listed in Tables 1 and 2. In the light of our data obtained from PM3, the azo forms were found to be more thermodynamically stable than the hydrazo tautomers. The negative enthalpy changes suggest that these inclusion processes are enthalpy driven in nature. As compared to hydrazo tautomer, the enthalpy change ( $\Delta H$ ) of azo tautomer of HAB/ $\alpha$ -CD is more negative  $(-5.78 \text{ kcal mol}^{-1})$ , suggesting that the azo inclusion complex nanomaterials are more stable and that these inclusion process were enthalpy driven. In the case of DHAB/ $\beta$ -CD complexes, the high negative  $\Delta H$  values (-20.76  $kcal mol^{-1}$ ) indicated that this complex nanomaterial is more stable and occurred spontaneously at room temperature.<sup>31</sup>

Gibbs free energy changes are negative for complexation azo tautomer in both HAB and DHAB molecules, which imply that these occur spontaneously at room temperature. But in the case



**Figure 11.** PM3 optimized hydrazo tautomers structures of (a) DHAB/α-CD, (b) DHAB/β-CD, (c) HAB/α-CD, and (d) HAB/ β-CD inclusion complex.

of DHAB the hyrazo tautomer has positive  $\Delta G$  of value 12.81 and 50.70 kcal mol<sup>-1</sup> for  $\alpha$ -CD and  $\beta$ -CD respectively, this suggests that they hardly occur spontaneously at room temperature. DHAB/ $\beta$ -CD inclusion complexes show high negative  $\Delta G$  value -10.56 kcal mol<sup>-1</sup> which implies that the DHAB/ $\beta$ -CD complex nanomaterials formed more spontaneously than other complex nanomaterials.

The geometric parameters like bond length, bond angles, and dihedral angles of the guest molecule changed drastically before and after complexation (Tables S2 and S3). The dihedral angle between the azo groups was more affected by the complexation, while only small changes were observed in the bond length and bond angle, which indicated that the DHAB and HAB adopted a specific conformation to form a stable complex nanomaterials. The internal diameter of  $\beta$ -CD is approximately 6.5 Å and  $\alpha$ -CD is approximately 5.0 Å and the height of the both CD is 7.8 Å. Considering the shape and dimensions of CD, both molecules cannot be completely embedded into the CD nanocavity. The vertical distance and length of the DHAB, HAB, and AB were greater than the upper/lower rim of the CD. Hence, the entire molecule cannot be fully encapsulated in the CD cavity.

#### Conclusion

The self assembly behavior of 2,4-dihydroxyazobenzene (DHAB) and 4-hydroxyazobenzene (HAB) inclusion complex

nanomaterials were studied by SEM and TEM. The inclusion complex nanomaterials of  $\alpha$ -CD and  $\beta$ -CD with DHAB and HAB were investigated by UV-vis, steady-state and timeresolved fluorescence, FT-IR, DSC, PXRD, and <sup>1</sup>H NMR molecular modeling techniques. DHAB/CD and HAB/CD microstructure formed by individual "barrel" type (head-to-head arrangement) of 2:2 inclusion complexes through intermolecular hydrogen bonding between the neighboring complexes. The thickness for DHAB/β-CD microrod was approximately in the range of ca. 1.46 µm and height is about ca. 9.8 µm, whereas, typical average dimension of DHAB/α-CD micro rod is ca. 360 nm width and ca. 3  $\mu$ m length. HAB/ $\alpha$ -CD complexes form a self-assembled monolayer and HAB/β-CD forms a thick microsheet. From the computational study, we find that negative Gibbs energy and enthalpy changes for the inclusion complexes indicate that the formation of these complexes is spontaneous and exothermic and hydrogen-bonding interactions play a major role in the inclusion process.

### Experimental

**Materials.** DHAB, HAB,  $\alpha$ -CD, and  $\beta$ -CD were purchased from Sigma-Aldrich chemical company and used without further purification. The purity of the compound was checked for similar fluorescence spectra when excited with different wavelengths.

**Preparation of Nanomaterials.** CD (1 mmol) was dissolved in 40 mL of distilled water and DHAB or HAB (1 mmol) in 10 mL of methanol and was slowly added to the CD solution. This mixture was stirred at 50 °C overnight. Then the final solution was refrigerated overnight at 5 °C. The precipitated DHAB/CD and HAB/CD complexes were recovered by filtration and washed with a small amount of ethanol and water to remove uncomplexed DHAB, HAB, and CD, respectively. This precipitate was dried in vacuum at room temperature for two days and stored in an airtight bottle. This powder sample was used for further analysis.

**Preparation of CD Solution.** The concentration of stock solution of DHAB and HAB was  $2 \times 10^{-3}$  M. The stock solution (0.2 mL) was transferred into 10 mL volumetric flasks. To this, varying concentration of CD solution  $(1.0 \times 10^{-3}$  to  $1.0 \times 10^{-2}$  M) was added. The mixed solution was diluted to 10 mL with triply distilled water and shaken thoroughly. The final concentration of DHAB and HAB in all the flasks was  $4 \times 10^{-5}$  M. The experiments were carried out at room temperature.

Instruments. Scanning electron microscopy (SEM) photographs were collected on a JEOL JSM 5610LV instrument. The morphology of DHAB and HAB encapsulated CDs inclusion complexes was investigated by transmission electron microscopy (TEM) using a TECNAI G2 microscope with accelerating voltage 200 kV, for the TEM analysis using carbon-coated copper TEM grid (200 mesh). FT-IR spectra of DHAB, HAB, both CDs and the inclusion complexes were measured between 4000 and 400 cm<sup>-1</sup> on a Nicolet Avatar 360 FT-IR spectrometer by using KBr to make pellets. One-dimensional <sup>1</sup>HNMR spectra for DHAB, HAB, and CDs were recorded on a Bruker AVANCE 400 MHz spectrometer (Germany) using DMSO- $d_6$ (99.98%) as a solvent. The differential scanning calorimetry (DSC) was recorded using a Mettler Toledo DSC1 fitted with STR<sup>e</sup> software (Mettler Toledo, Switzerland), temperature scanning range was from 25 to 220 °C with a heating rate of 10 °C min<sup>-1</sup>. Powder X-ray diffraction (PXRD) spectra were recorded with a BRUKER D8 advance diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) and the patterns were measured in the  $2\theta$  angle range between 5 and  $80^{\circ}$  with a scan rate 5° min<sup>-1</sup>. Absorption spectral measurements were carried out with a Shimadzu (model UV 1650 PC) UV-visible spectrophotometer and steady-state fluorescence measurements were made by using a Shimadzu spectrofluorimeter (model RF-5301). pH of the solution was measured on a Elico pH meter (model LI-120). The fluorescence lifetime measurements were performed using a picosecond laser and single photon counting setup from Jobin-Vyon IBH (Madras University, Chennai).

**Molecular Modeling Studies.** The theoretical calculations were performed with the Gaussian 03W package. The initial geometry of the DHAB, HAB,  $\alpha$ -CD, and  $\beta$ -CD were constructed with the aid of Spartan 08 and then optimized by the semiempirical PM3 method. The CD was fully optimized by PM3 without any symmetry constraint.<sup>32</sup> The glycosidic oxygen atoms of CD were placed onto the *XY* plane and their center was defined as the center of the coordinate system. The primary hydroxy groups were placed pointing toward the positive *Z* axis. The inclusion complex was constructed from the PM3 optimized CD and guest molecules. The longer dimension of the guest molecule was initially placed onto the Z axis. The position of the guest was determined by the Z coordinate of one selected atom of the guest. The inclusion process was simulated by putting the guest on one end of the CD and then letting it pass through the CD cavities. Since the semiempirical PM3 method has been proven to be a powerful tool in the conformational study of CD inclusion complexes and has high computational efficiency,<sup>33</sup> we selected semiempirical PM3 method to study the inclusion process of CDs with the DHAB and HAB.

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## **Supporting Information**

In the Supporting file we are provide three tables and nine figures. Table contains <sup>1</sup>H NMR chemical shift values and geometrical parameters of azo and hydrazo forms for the DHAB and HAB molecules with  $\alpha$ -CD and  $\beta$ -CD. Figures contain FT-IR spectra, <sup>1</sup>H NMR spectra, absorption and fluorescence spectra, and fluorescence decay curves for DHAB and HAB with  $\alpha$ -CD and  $\beta$ -CD inclusion complexes. Further, HOMO and LUMO pictures of DHAB and HAB are also provided in the figures. This material is available free of charge on the web at http://www.csj.jp/journals/bcsj/.

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