Supramolecular Complexes of Azocellulose and α-Cyclodextrin: Isothermal Titration Calorimetric and Spectroscopic Studies

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ABSTRACT: Isothermal titration calorimetry (ITC) and UV−visible spectroscopy were used to study the supramolecular complexes of α-cyclodextrin (α-CD) and azocellulose (azobenzene functionalized hydroxypropyl methylcellulose, AZO−HPMC) in aqueous solutions. The equilibrium constant K, enthalpy change ΔH, Gibbs free energy ΔG, entropy change ΔS, and the stoichiometric number n for the inclusion complexation were determined. α-CD forms stable inclusion complexes with AZO−HPMC with the stoichiometry of 1:1. The inclusion complexation is exothermic. Both negative enthalpy and entropy changes are observed, suggesting that the inclusion complexation is enthalpy driven and entropy opposed. AZO−HPMC with higher azobenzene content (DS_{azo}) is less favored to form inclusion complexes with α-CD due to strong H-aggregation of azobenzene groups. ITC studies confirmed that the cis azobenzene groups is unable to form an inclusion complex with α-CD. The red-shift in the absorption spectra of AZO−HPMC aqueous solution upon addition of α-CD also confirmed the formation of 1:1 complex.

Introduction

Cyclodextrins (CDs) are well-known in supermolecular chemistry as molecular hosts.[1,2] They are cyclic oligosaccharides composed of six, seven, and eight glucose units, which are called α-, β-, and γ-cyclodextrin, respectively. The CD molecule is often described as a shallow truncated cone with a hydrophilic exterior and a hydrophobic interior cavity. Because of this structure and its constrained size, CDs can selectively form inclusion complexes with a variety of guest molecules in aqueous solution.[3] In the early 1990s, Harada et al. first reported that CDs could interact with various polymers of high specificity to give crystalline inclusion complexes in which CD molecules are threaded by polymer chains.[4] However, all these complexes are crystalline precipitates which are water-insoluble. CDs have also been used together with hydrophobically modified (HM) polymers with the purpose of reducing the viscosity of polymer aqueous solutions.[5−7] In these systems, CD molecules form complexes with the hydrophobic groups in HM-polymers, which reduce the possibility of hydrophobic association.

Low molecular weight azobenzene compounds can also form inclusion complexes with CDs.[8−15] It has been proven that *trans*-azobenzene forms stable inclusion complexes with α-CD, while *cis*-azobenzene does not form any inclusion complex with α-CD due to strong steric hindrance.[13,14] Although the inclusion complexation between azobenzene compounds and CDs have been extensively investigated, very few studies on the inclusion complexation of azo polymers with CDs have been reported in the literature.[16,17] We have recently prepared a series of novel photoresponsive cellulose derivatives, azobenzene functionalized hydroxypropyl methylcellulose (AZO−HPMC) and constructed supramolecular complexes with α-CD.[18,19] The azobenzene moieties in AZO−HPMC not only act as photoresponsive triggers but also as guests which can form inclusion complexes with α-CD.

Isothermal titration calorimetry (ITC) is a thermodynamic technique suitable for monitoring any reaction initiated by the addition of a binding component. When substances bind, heat is either generated or absorbed. Measurement of this heat allows the very accurate determination of binding constant (K), reaction stiochiometry (n), enthalpy (ΔH) and entropy (ΔS) change. Thus, ITC provides a complete thermodynamic profile of the molecular interaction in a single experiment.[20] ITC has been developed primarily for the study of biomolecular interactions,[21] and has become the method of choice for characterizing all types of binding reactions. It is also the best method to study the binding thermodynamics of supramolecular systems.[22] The application of ITC technique to polymeric systems was reported recently and mainly focused on the study of polymer−surfactant interactions.[23−25] Yui et al.[26,27] employed ITC to study the inclusion complexation of two linear polymers with α-CD based molecular tube. Arnaud and Bouteiller[28] demonstrated that ITC is a powerful tool to characterize the self-association of supramolecular polymers. However, there is no report on the ITC study of the inclusion complexation between CDs and azo polymers.

In this study, the inclusion complexation behavior of α-CD and azo cellulose (AZO−HPMC) was investigated using ITC and UV−visible spectroscopy. The thermodynamic parameters, such as the enthalpy (ΔH), entropy (ΔS) change, Gibbs free energy (ΔG), equilibrium constant (K), and the inclusion stiochiometry (n) of the supramolecular complexes were determined. The effects of azobenzene content (DS_{azo}) as well as the configurational changes of the azobenzene groups on the inclusion complexation were also investigated. The thermody-

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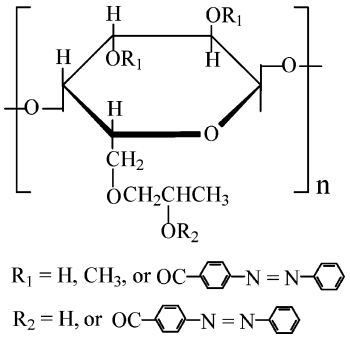

Figure 1. Chemical structure of AZO−HPMC.

namic and spectroscopic results of this study provide new insights and further understanding on the inclusion complexation between CDs and azo polymers.

Experimental Section

Materials. AZO−HPMC polymers with different degrees of azo-substitution (DS_{azo}) were prepared by esterification reaction of the hydroxyl groups on HPMC with the acid chloride groups on 4-phenylazobenzoyl chloride. The preparation and characterization of these polymers have been reported previously.[18,19] The structure of AZO−HPMC is illustrated in Figure 1. Aqueous solutions of AZO−HPMC were prepared using deionized water (Millipore Q, 18.2 MΩ·cm) and then cooled below 5 °C in a refrigerator. α-CD (98%) was obtained from Fluka and used as received.

Photoirradiation. Photoirradiation was carried out with a 500 W Hg ARC lamp (Oriel, model 68910) at room temperature in a dark room. UV and visible light were isolated by 370 and 410 nm interference filters, respectively. The exposure energy of irradiation was about 2 mW/cm^2. Dark-adapted samples were obtained by irradiating aqueous solutions of AZO−HPMC with visible light (λ = 410 nm) for 20 min and then kept in the dark for 72 h to ensure that all the azobenzene chromophores were in the trans state. UV-irradiated samples were obtained by irradiating aqueous solutions of AZO−HPMC at room temperature with UV light (λ = 370 nm) until the photostationary state was reached.

Isothermal Titration Calorimetry (ITC). The calorimetric experiments were carried out using the Microcal isothermal titration calorimeter (Northampton, MA). This power compensation differential instrument was previously described in detail by Wiseman et al.[29] It has a reference cell and a sample cell of approximately 1.35 mL, which are both insulated by an adiabatic shield. The titration was carried out by stepwise injections of 50 mM α-CD aqueous solution from a 250 μL injection syringe into the sample cell filled with water, 5 g/L HPMC, or AZO−HPMC aqueous solution, respectively. The syringe is tailor-made such that the tip acts as a blade-type stirrer to ensure an optimum mixing efficiency at 400 rpm. An injection schedule was automatically carried out using interactive software after setting up the number of injections, volume of each injection, and time between each injection. This arrangement minimizes the contributions from the heat of dilution of the polymer. All measurements were carried out at a constant temperature of 25.0 ± 0.02 °C. Calorimetric data were processed by the computer program Origin for ITC.

UV—Visible Spectroscopy. UV−Visible absorption measurements were performed on a Shimadzu UV−vis spectrophotometer (UV-2501PC) in a dark room at 25 °C.

Results and Discussion

Isothermal Titration Calorimetry. Figure 2 shows the isothermal titration calorimetric thermograms of the raw signals (cell feedback, CFB) obtained from titrating 50 mM α-CD aqueous solution into water, HPMC and AZO−HPMC (DS_{azo} = 0.018) aqueous solutions, respectively. Integration of the area of CFB by subtracting the

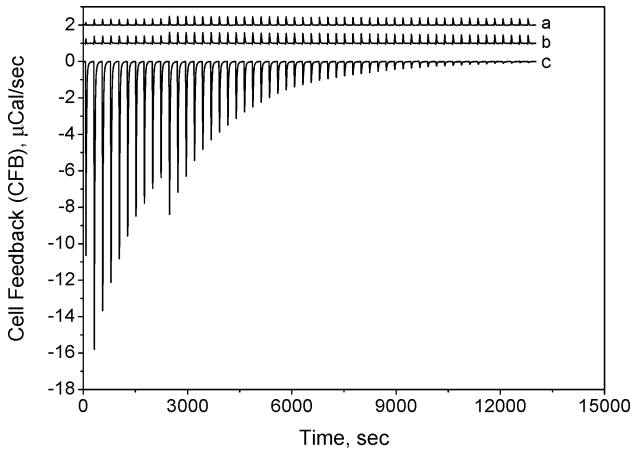

Figure 2. Cell feedback (CFB) vs time for titrating 50 mM α-CD aqueous solution into water (a), 5 g/L HPMC (b) ,and 5 g/L AZO−HPMC (DS_{azo} = 0.018) (c) aqueous solutions at 298 K. Curves a and b were shifted up by 2 and 1 μcal/s respectively for clarity.

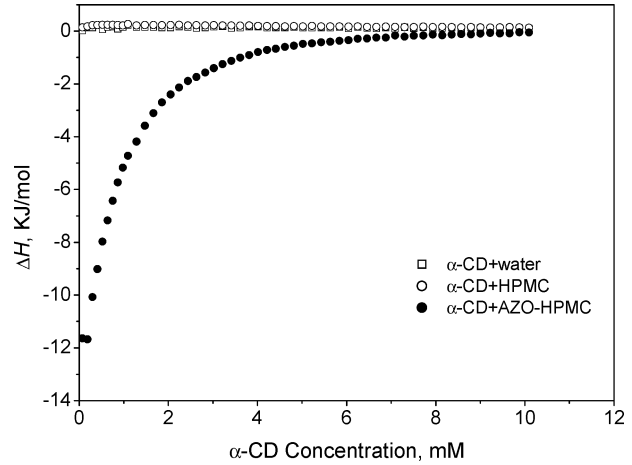

Figure 3. Differential enthalpic binding curves for titrating 50 mM α-CD aqueous solution into water, 5 g/L HPMC, and 5 g/L AZO−HPMC (DS_{azo} = 0.018) aqueous solutions at 298 K.

dilution heat of α-CD gives the differential enthalpic binding curves as shown in Figure 3. It can be seen that the titration of α-CD into HPMC solution is almost identical to dilution of α-CD. The heat changes are negligible compared with the binding heat of α-CD with AZO−HPMC. This implies that there is almost no interaction between α-CD and HPMC without azo-substitution or the interaction is very weak. However, the titration of α-CD into AZO−HPMC is completely different from that of titration α-CD into water and HPMC solution, suggesting that α-CD molecules interact strongly with the azobenzene groups in AZO−HPMC. This is attributable to the formation of inclusion complexes. As seen in Figure 3, the inclusion complexation process is exothermic, indicating that it is energetically favorable for the less polar azobenzene groups to be included into the cavity of α-CD. The complexation takes place instantly at the first injection of α-CD into the AZO−HPMC solution. Upon further addition of α-CD, the binding curve progressively merges with the dilution curve of α-CD. This is because the azobenzene groups in AZO−HPMC are gradually saturated with bound α-CD and eventually no binding site (azobenzene group) remains, which indicates that all the azobenzene groups are included into the cavity of α-CD.

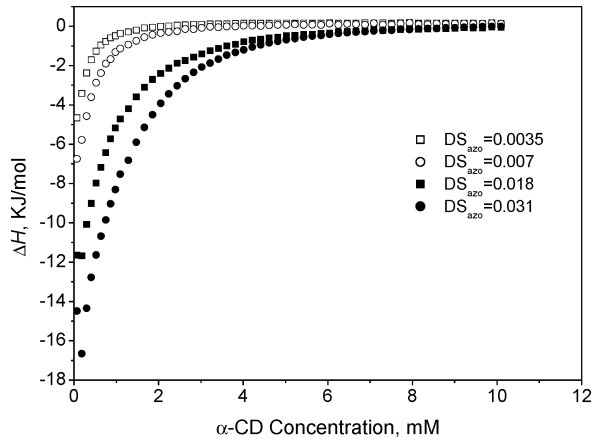

Figure 4. Differential enthalpic binding curves for titrating 50 mM α-CD into 5 g/L aqueous solutions of AZO−HPMC with different DS_{azo} at 298 K.

Figure 4 shows the differential enthalpic binding curves as a function of α-CD concentration obtained from titrating 50 mM α-CD into 5 g/L aqueous solutions of AZO−HPMC with different DS_{azo}. It can be seen that the binding regime in terms of α-CD concentration becomes broader with increasing DS_{azo}, this is because an AZO−HPMC with higher DS_{azo} provides more azobenzene binding sites for the complexation with α-CD. In addition, the slopes of the enthalpic binding profiles become less steep with increasing DS_{azo}.

In this study, the binding isotherms were evaluated by the nonlinear fitting with a one binding site model (see appendix), assuming that α-CD forms 1:1 inclusion complex with AZO−HPMC. In other words, each azobenzene group binds exactly with one α-CD molecule. The inclusion complexation process can be described by

$$\text{Azo} + \text{CD} \overset{K}{\rightleftharpoons} \text{Azo·CD} \tag{1}$$

where Azo and Azo·CD correspond to the azobenzene groups and the inclusion complexes formed between CD molecules and azobenzene groups, respectively. The equilibrium constant K is expressed as

$$K = \frac{[\text{Azo·CD}]}{[\text{Azo}][\text{CD}]} \tag{2}$$

where [Azo] and [CD] are the equilibrium concentrations of azobenzene groups and CD molecules respectively, [Azo·CD] is the concentration of the complexes.

The Gibbs free energy ΔG can be calculated using eq 3:

$$\Delta G = -RT \ln K \tag{3}$$

Consequently the entropy change ΔS can be determined from eq 4:

$$\Delta S = \frac{\Delta H - \Delta G}{T} \tag{4}$$

Figure 5 shows the nonlinear fitting of the enthalpy curves. It can be seen that the fitting curves (solid lines) show good agreement with the experimental data expressed by the open symbols, indicating that the fitting model is appropriate and the thermodynamic parameters derived from the fitting curves are reliable. The thermodynamic parameters extracted from the model fitting are summarized in Table 1. The stoichiometric number n is found to be essentially independent of DS_{azo} and is close to 1, indicating that each azobenzene group is encapsulated by one α-CD molecule if all the azobenzene groups are assumed to be identical. In other words, the 1:1 stoichiometry of the inclusion complexation is confirmed. By comparing the size of *trans*-azobenzene and the dimensions of α-CD cavity (Figure 6), one can expect that each α-CD molecule is able to encapsulate one azobenzene group, which is in agreement with the stoichiometric number $n = 1$ derived from the model fitting of ITC data. This is also consistent with the result obtained from spectroscopic measurements, which will be discussed later.

Table 1. Thermodynamic Parameters of the Inclusion Complexation of α-CD and AZO−HPMC in Aqueous Solutions at 298 K

DS_{azo}	n	K (mol^{-1}·L)	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (J/mol·K)
0.0035	1.12 ± 0.14	3421 ± 275	−21.07 ± 1.15	−20.17	−3.05
0.007	1.09 ± 0.15	2219 ± 96	−24.89 ± 0.55	−19.10	−19.46
0.018	1.17 ± 0.07	1104 ± 54	−33.61 ± 2.55	−17.36	−54.43
0.031	0.92 ± 0.05	1132 ± 74	−40.07 ± 3.48	−17.43	−76.00

As shown in Table 1, a large negative enthalpy change (ΔH) is observed in all systems studied. On the other hand, the entropy changes (ΔS) corresponding to the complexation are also negative, suggesting that the inclusion complexation is enthalpy driven and entropy opposed. Negative ΔH and ΔS values have been reported in most of the systems involving the inclusion complexation of CDs.[26,30−32] When α-CD molecules form inclusion complexes with the azobenzene groups in AZO−HPMC, both α-CD molecules and azobenzene groups lose their degree of freedom of motion and translational entropy, thus the system (AZO−HPMC/α-CD complex in aqueous medium) becomes less random, which contributes to the negative entropy change ($\Delta S < 0$). On the basis of the thermodynamic studies, some insights into the factors that contribute to the inclusion complexation can be obtained. In general, a variety of noncovalent forces such as hydrophobic interaction, van der Waals interaction, and electrostatic interaction as well as hydrogen bonding may be responsible for the inclusion complexation.[33] For AZO−HPMC/α-CD complexes, electrostatic interaction and hydrogen bonding can be excluded from the driving forces for the inclusion complexation since there is neither ionic species for electrostatic interaction nor sites for hydrogen bonding. Thus, the arguments focus on the hydrophobic interaction and van der Waals interaction. It is known that hydrophobic interaction is characterized by small positive value of ΔH and positive value of ΔS, while van der Waals interaction is characterized by negative ΔH.[34] The negative values of both ΔH and ΔS of the inclusion complexation of AZO−HPMC and α-CD are consistent with the characteristics of van der Waals interaction. Therefore, it is believed that van der Waals interaction is the dominant driving force for the inclusion complexation of AZO−HPMC and α-CD. However, the hydrophobic interaction cannot be ruled out, since the complexation may be driven by a combination of van der Waals interaction and hydrophobic interaction.

It is interesting to note that the equilibrium constant K decreases with increasing DS_{azo} and consequently the Gibbs free energy ΔG slightly increases ($-\Delta G$ decreases), as shown in Table 1, suggesting that an AZO−HPMC with higher DS_{azo} is less favored to form inclusion complexes. This is attributable to the H-aggregation

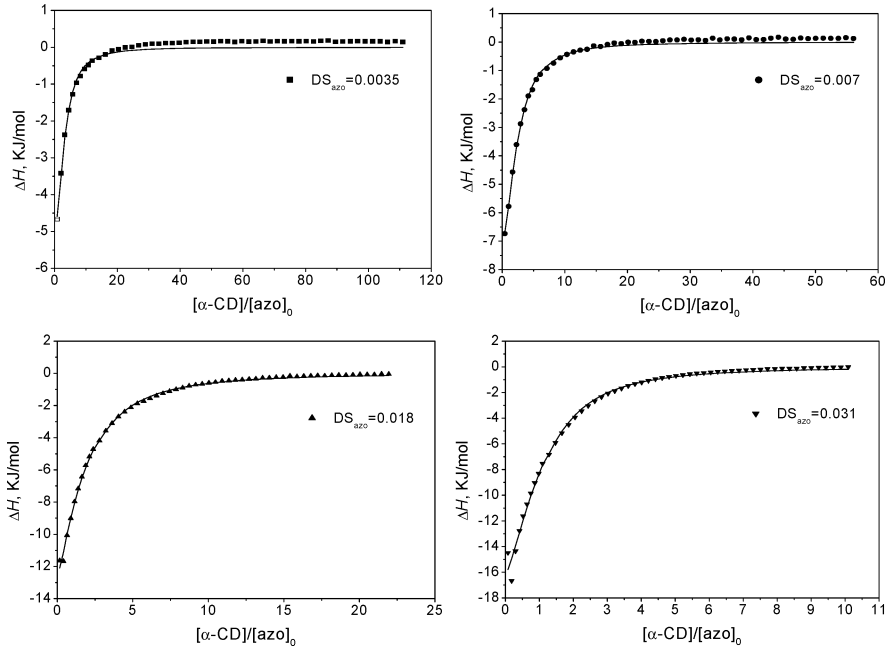

Figure 5. Schematic illustration of the dimensions of azobenzene groups and α-CD.

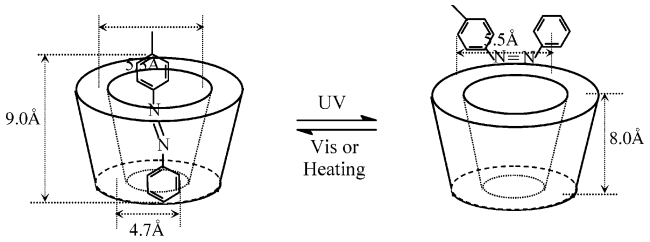

Figure 6. Nonlinear fitting curves of ΔH vs $[\alpha\text{-CD}]/[\text{Azo}]_0$.

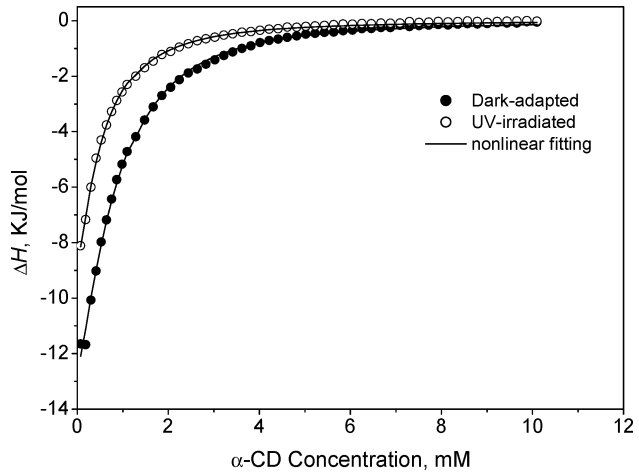

Figure 7. Differential enthalpic binding curves for titrating 50 mM α-CD into dark-adapted and UV-irradiated AZO−HPMC (DS_{azo} = 0.018) aqueous solutions (5 g/L) at 298 K.

between azobenzene groups in AZO−HPMC. H-aggregation in azobenzene amphiphiles and polymers has been proven by many researchers.[35,36] In the complexation process, the H-aggregates between azobenzene groups must be dissociated from each other to allow the α-CD molecules to bind with the azobenzene groups. With increasing DS_{azo}, the H-aggregation becomes stronger, and consequently the complexation becomes more difficult.

Figure 7 shows the differential enthalpic curves obtained from titrating 50 mM α-CD aqueous solution into dark-adapted and UV-irradiated AZO−HPMC (DS_{azo} = 0.018) aqueous solutions, respectively. It should be emphasized that the azobenzene groups in AZO−HPMC cannot completely convert to the cis state after sufficient irradiation with UV light, and a fraction of the azobenzene groups remains in the trans state. In other words, the UV-irradiated AZO−HPMC contains both *trans*- and *cis*-azobenzene groups, while all the azobenzene groups in dark-adapted AZO−HPMC are considered to be in trans state. Comparing the enthalpy curves shown in Figure 7, it is evident that the saturation of UV-irradiated AZO−HPMC takes place at a lower α-CD concentration, suggesting that less α-CD molecules are bound to the polymer compared with dark-adapted AZO−HPMC.

As shown in Figure 7, the nonlinear fitting curves (the solid lines) agree well with the experimental data. The thermodynamic parameters are summarized in Table 2. The values of equilibrium constant K, enthalpy change ΔH, Gibbs free energy ΔG, and entropy change ΔS for the inclusion complexation are essentially independent of UV irradiation. However, the stoichiometric number n is significantly reduced from 1.17 to 0.41 upon trans−cis isomerization of the azobenzene groups. In other words, if all the azobenzene groups in dark-adapted AZO−HPMC are bound with α-CD to form 1:1 inclusion complex, only approximately 41% of the total azobenzene groups in UV-irradiated AZO−HPMC are bound with α-CD. The percentage of unbound azobenzene sites (59%) is in good agreement with the photostationary cis fraction (59.3%) of AZO−HPMC (DS_{azo} = 0.018) at the same condition. This clearly indicates that cis azobenzene cannot form inclusion complex with α-CD, which is consistent with the results obtained from induced circular dichroism studies on azobenzene compounds.[13,14] However, due to the incomplete isomerization, the UV-irradiated AZO−HPMC still exhibits its ability to bind α-CD molecules because of the remaining *trans*-azobenzene groups.

UV—Vis Spectroscopy. Figure 8 shows the UV−vis absorption spectra of AZO−HPMC (DS_{azo} = 0.018, 0.4 g/L) in aqueous solutions with or without α-CD. In the absence of α-CD, the spectrum of AZO−HPMC

Table 2. Thermodynamic Parameters of the Inclusion Complexation of α-CD with Dark-Adapted and UV-Irradiated AZO−HPMC in Aqueous Solutions at 298 K

AZO−HPMC	n	K (mol^{-1}·L)	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (J/mol·K)
dark-adapted	1.17 ± 0.07	1104 ± 54	−33.61 ± 2.55	−17.36	−54.43
UV-irradiated	0.41 ± 0.05	1152 ± 41	−35.46 ± 3.71	−17.47	−60.37

shows a major absorption peak at 326 nm. Upon addition of α-CD, the spectra progressively shift to longer wavelengths (red shift). The maximum absorption wavelength (λ_{max}) increases with increasing α-CD concentration. On the other hand, the absorbance at 326 nm (the maximum absorption wavelength for AZO−HPMC in the absence of α-CD) decreases with increasing α-CD concentration.

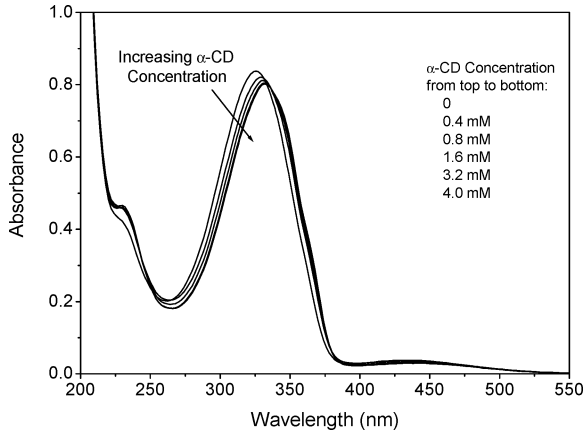

Figure 8. UV−vis absorption spectra for 0.4 g/L aqueous solutions of AZO−HPMC (DS_{azo} = 0.018) in the presence of different amounts of α-CD.

To quantify the spectral changes, the absorbance at 326 nm (A_{326}) and maximum absorption wavelength (λ_{max}) were plotted as a function of α-CD concentration in Figure 9. These spectra changes also indicate strong interactions between AZO−HPMC and α-CD. The red shift of the spectra is attributable to the less polar environment surrounding the azobenzene groups, which suggests that the azobenzene groups in AZO−HPMC are included into the hydrophobic cavity of α-CD. Similar observations have also been found in the interactions of low molecular weight azobenzene compounds with α- or β-CD.[11,12] In these reports, the red shift of the UV−vis spectrum was considered as clear evidence for the formation of inclusion complexes.

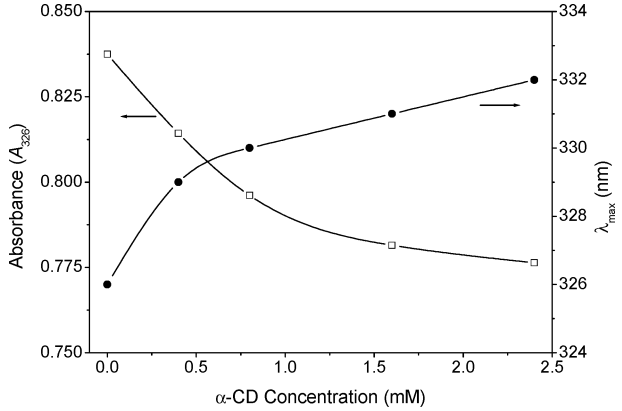

Figure 9. Absorbance at 326 nm (A_{326}) and maximum absorption wavelength (λ_{max}) as a function of α-CD concentration.

According to the Benesi−Hildebrand equation,[37] the equilibrium constant K can be related to the changes of absorbance ΔA in the presence of α-CD, as described in eq 5:

$$\frac{[CD]_0[Azo]_0}{\Delta A} = \frac{1}{K\Delta\epsilon} + \frac{[CD]_0}{\Delta\epsilon} \quad (5)$$

where $[Azo]_0$ and $[CD]_0$ are the initial (or total) concentrations of azobenzene groups and α-CD, respectively. $\Delta\epsilon$ is the difference in the molar extinction coefficients between the free and complexed azobenzene groups.

Figure 10 shows a plot of $[CD]_0[Azo]_0/\Delta A$ as a function of $[CD]_0$. It can be seen that the results fit to eq 5 fairly well. This confirms that the stoichiometry of the complex is 1:1. The slope yields $\Delta\epsilon$ = 22.21 M^{-1}·cm^{-1}. From the intercept of the plot, the equilibrium constant K for the inclusion complexation of AZO−HPMC with α-CD can be obtained (K = 1510 mol^{-1}·L). The Gibbs free energy ΔG was calculated to be −18.1 kJ·mol^{-1}. These values are comparable to the values obtained from ITC study, as shown in Table 1.

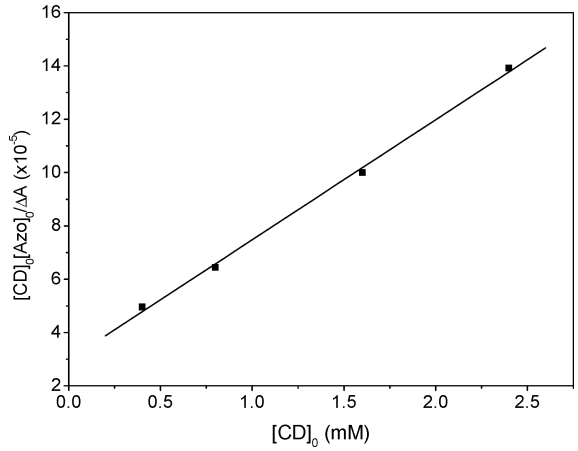

Figure 10. Plot of $[CD]_0[Azo]_0/\Delta A$ as a function of [α-CD] for the inclusion complexation between AZO−HPMC (DS_{azo} = 0.018) and α-CD.

Conclusions

Supramolecular complexes of azocellulose (AZO−HPMC) and α-CD were prepared and characterized using isothermal titration calorimetry (ITC) and UV−visible spectroscopy. A "one binding site" model was used to evaluate the ITC results of the inclusion complexation between α-CD and AZO−HPMC. The fitting curves show good agreement with the experimental data. The equilibrium constant K, enthalpy change ΔH, Gibbs free energy ΔG, entropy change ΔS, and the stoichiometric number n for the inclusion complexation were determined. α-CD forms stable inclusion complex with AZO−HPMC with the stoichiometry of 1:1. The inclusion complexation is exothermic. Both negative enthalpy and entropy changes are observed, suggesting that the inclusion complexation is enthalpy driven and entropy opposed. With increasing DS_{azo}, the equilibrium constant decreases, indicating

that AZO−HPMC with higher DS_{azo} is less favored to form inclusion complexes. The stoichiometric number n is independent of DS_{azo}, but significantly reduced upon trans−cis isomerization of the azobenzene groups, indicating that the *cis*-azobenzene groups cannot form inclusion complex with α-CD. A red-shift in the UV−vis absorption spectra is observed when α-CD is added to the aqueous solution of AZO−HPMC, which is a clear evidence for the formation of inclusion complexes. The spectroscopic data were analyzed using Benesi−Hildebrand equation. The results are comparable to the ITC studies.

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Appendix. One Binding Site Model

The ITC data were fitted with a one binding site model. The equilibrium constant K defined by eq 2 can be expressed as

$$K = \frac{[\text{Azo}\cdot\text{CD}]}{[\text{Azo}][\text{CD}]} = \frac{[\text{Azo}]_0\Theta}{[\text{Azo}]_0(1-\Theta)[\text{CD}]} = \frac{\Theta}{(1-\Theta)[\text{CD}]} \tag{A1}$$

$$\Theta = \frac{[\text{Azo}\cdot\text{CD}]}{[\text{Azo}]_0} \tag{A2}$$

where Θ is the fraction of azobenzene groups complexed with α-CD. According to the mass conservation, the equilibrium concentration of α-CD, [CD] can be expressed by

$$[\text{CD}] = [\text{CD}]_0 - [\text{Azo}]_0 n\Theta \tag{A3}$$

where n is the molar number of binding sites in 1 mol of azobenzene groups.

Combining eqs A1 and A3 gives

$$\Theta^2 - \Theta\left[1 + \frac{[\text{CD}]_0}{n[\text{Azo}]_0} + \frac{1}{nK[\text{Azo}]_0}\right] + \frac{[\text{CD}]_0}{n[\text{Azo}]_0} = 0 \tag{A4}$$

The total heat Q of the solution is given as

$$Q = [\text{Azo}]_0\Theta n V_0 \Delta H \tag{A5}$$

where ΔH is the enthalpy change of the complexation, V_0 is the total volume of the solution. Solving the quadratic equation (eq A4) for Θ and then substituting it into eq A5 gives

$$Q = \frac{n[\text{Azo}]_0\Delta H V_0}{2} \times \left[1 + \frac{[\text{CD}]_0}{n[\text{Azo}]_0} + \frac{1}{nK[\text{Azo}]_0} - \sqrt{\left(1 + \frac{[\text{CD}]_0}{n[\text{Azo}]_0} + \frac{1}{nK[\text{Azo}]_0}\right)^2 - \frac{4[\text{CD}]_0}{n[\text{Azo}]_0}}\right] \tag{A6}$$

After correcting the displaced volume, the calculated heat effect for the ith injection is

$$\Delta Q_i = Q_i + \frac{dV_i}{V_0}\left[\frac{Q_i + Q_{i-1}}{2}\right] - Q_{i-1} \tag{A7}$$

The fitting process of the experimental data is described as follows:

(1) Calculate ΔQ_i for each injection and compare these values with the measured heat for each corresponding injection.

(2) Improve the initial values of n, K, and ΔH by the standard Marquardt method.

(3) Repeat the above procedures until no further significant improvement in the fitting can be achieved.

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