

Evidence for the Existence of Ternary Complexes of Iron, Cyclodextrin, and Hydrophobic Guests in Aqueous Solution

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Carboxymethyl- β -cyclodextrin (CMCD) has been used to enhance Fenton ($\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{HO}\cdot + \text{OH}^-$) degradation of hydrophobic organic pollutants in aqueous systems. It has been proposed that the enhancement was due to the formation of a pollutant/CMCD/ Fe^{2+} ternary complex which enabled the degradation catalyst, Fe^{2+} , to be closer to the target pollutant molecule so as to favor the degradation reaction. To investigate this theory, aqueous solutions containing CMCD, Fe^{2+} , and anthracene or 2-naphthol were studied using fluorescence and NMR techniques. The fluorescence quenching results showed that in aqueous solution (pH > 3) anthracene or 2-naphthol was quenched more efficiently by Fe^{2+} in the presence of CMCD. When the less efficient quenchers Cd^{2+} or Ca^{2+} were added to the anthracene/CMCD/ Fe^{2+} solution, the fluorescence was restored by displacing Fe^{2+} from its binding site near the pollutant. NMR spectra showed evidence of formation of a 2-naphthol/CMCD binary complex. Peak broadening of 2-naphthol when Fe^{2+} was added to the 2-naphthol/CMCD solution indicated binding of Fe^{2+} to the binary complex. Peak sharpening via iron displacement resulted from addition of Cd^{2+} or Ca^{2+} to the 2-naphthol/CMCD/ Fe^{2+} solutions, further confirming the binding of Fe^{2+} to form the ternary complex. These results indicate that the pollutant/CMCD/ Fe^{2+} ternary complex is formed in aqueous solution.

Introduction

Cyclodextrins are cyclic oligosaccharides that contain six, seven, or eight glucose units. The truncated hydrophobic cavity inside the molecule enables it to encapsulate appropriately sized organic molecules.^{1–4} Because of this property, cyclodextrins have been widely used as vessels for reaction, a model system of enzymes, and solubilizers of water-insoluble species.^{1,5,6} The formation of host–guest binary complexes with natural or modified cyclodextrins has been extensively studied.^{7–10}

Recent studies on Fenton degradation of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) showed that the efficiency of degradation was enhanced by addition of carboxymethyl- β -cyclodextrin (CMCD).¹¹ It was proposed that the enhancement was a result of formation of a pollutant/CMCD/ Fe^{2+} ternary complex. In the Fenton reaction Fe^{2+} catalyzes the decomposition of hydrogen peroxide to produce hydroxyl radical, which can then attack and degrade pollutant molecules. In the presence of CMCD, a pollutant/CMCD binary complex is often formed due to the encapsulating ability of the cyclodextrin. At the same time, the carboxyl groups in CMCD molecules are able to bind Fe^{2+} . In such a ternary complex, pollutant molecules are brought closer to the catalyst and become an easier target for degradation by hydroxyl radical. Mass spectrometric evidence for such ternary complexes has been presented,¹² but those experiments did not probe the interaction directly in aqueous solution.

The present study focused on the formation of the pollutant/CMCD/ Fe^{2+} ternary complex in aqueous solution. Two model PAH molecules were used in this study: anthracene and 2-naphthol. The encapsulation of the selected PAH molecules by β -CD has previously been proven and studied.^{13–16} To

prepare CMCD the secondary hydroxyl groups of β -CD are partly substituted by carboxymethyl groups, though the size and the shape of the cavity likely remains the same. Consequently, CMCD is expected to retain the ability to bind with the two PAH molecules studied here.

Experimental Section

Anthracene (99+%) and $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$ (99.99%) were obtained from Aldrich. Ferrous sulfate heptahydrate and calcium chloride were obtained from J. T. Baker. Carboxymethyl- β -cyclodextrin (average degree of substitution = 3) was obtained from Cerestar, 2-naphthol (99+%) was obtained from EM Science, and potassium bromide (99%) was obtained from Mallinckrodt. All reagents were used as received. Purified water for the preparation of aqueous solutions was obtained from a Barnstead NanopureUV water treatment system.

To prepare aqueous anthracene solutions, a more concentrated anthracene stock solution was first prepared in hexane. A small aliquot of this stock solution was transferred into a clean dry volumetric flask, and the solvent was evaporated. Water (pH adjusted with H_2SO_4) was then used to fill the flask to the mark. The solute was dissolved by low-energy (60 W) sonication in a bath sonicator (Branson 1510) for about a half-hour. The final concentration of aqueous anthracene was 0.1 μM . Naphthol aqueous solutions were prepared by dissolving solid 2-naphthol in pH-adjusted water or deuterated water. A more concentrated stock solution was prepared first; then it was diluted to concentrations in the range of 10 μM to 5 mM. To prepare samples containing cyclodextrins, solid CMCD was added to the aqueous anthracene or 2-naphthol solutions, making the final CMCD concentration 2–10 mM. These samples were shaken on an orbital shaker (VWR) for 1–5 h before analysis in order to allow the CD and PAH to equilibrate.

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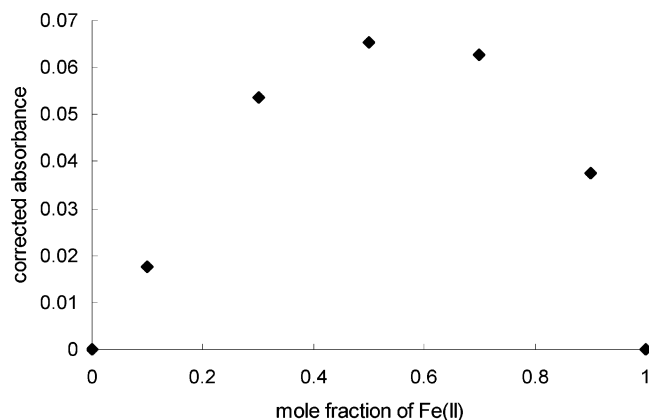


Figure 1. Corrected absorbance at 250 nm vs mole fraction of iron in mixtures with CMCD. The total moles of iron + cyclodextrin was kept constant. Solution pH was 3.1.

UV spectra were recorded with a Cary 500 UV–vis–near-IR spectrophotometer. The stoichiometry of iron/cyclodextrin complexes was determined from spectra of iron–cyclodextrin mixtures using the continuous variation method.¹⁷

Fluorescence spectra were recorded with a Photon Technology International QM-1 fluorometer. Aliquots (3 mL) of aqueous anthracene or 2-naphthol solutions were placed in a quartz cuvette and were subsequently modified by consecutive additions of 5 or 10 μL aliquots of FeSO₄(aq) or KBr(aq). The sample was stirred by a magnetic stirring bar throughout the whole period of measurement. In total 30–60 μL of quencher solution was added for each experiment. Compared to the 3 mL sample volume, these additions caused negligible decreases in the fluorescence due to dilution. To evaluate the possible interference of O₂, which is also a fluorescence quencher, some quenching experiments were carried out under low O₂ conditions. Sample preparation and transfer were undertaken under a nitrogen stream or in an argon box. The samples were sealed using rubber stoppers and kept under nitrogen atmosphere prior to measurement. During quenching experiments, FeSO₄ or KBr was injected into a septum-sealed quartz cuvette using a syringe. In the Cd²⁺ or Ca²⁺ substitution experiments, aliquots (10–30 μL) of concentrated Cd²⁺ or Ca²⁺ stock solutions were added to each sample.

¹H NMR spectra were recorded using Varian Unity 400 and 500 spectrometers operating at 400 or 500 MHz. D₂O was used as the solvent, and trace H₂O served as an internal standard. In NMR titration experiments, aliquots (10–20 μL) of concentrated

CMCD (7.5–100 mM) were added to aqueous 2-naphthol samples which were then analyzed by NMR following each addition.

Results and Discussion

(A) Binding Stoichiometry. Binding stoichiometry between CMCD and Fe²⁺ in aqueous solution (pH = 3.1) was measured by the continuous variation method.¹⁷ The iron/cyclodextrin complex exhibits absorbance at 250 nm; at this wavelength the individual species have only a negligible absorbance at the concentrations used. Therefore, the maximum corrected absorbance at this wavelength should occur when the greatest amount of complex is formed. The results are presented in Figure 1. The corrected absorbance at 250 nm reached a maximum at approximately 0.5 mole fraction of Fe²⁺, which indicates that a 1:1 binding stoichiometry is predominant for the CMCD/Fe²⁺ complex.

(B) Fluorescence Quenching by Fe²⁺, Br[−], and Eu³⁺. Fe²⁺ is a good fluorescence quencher for the two PAH molecules used in this study. Figure 2 shows the Stern–Volmer plot for anthracene fluorescence quenching by Fe²⁺ and Br[−] in the presence or absence of CMCD at pH = 3.1 (to avoid the precipitation of iron, all quenching experiments were carried out under acidic conditions). As can be seen in Figure 2a, the quenching efficiency of Fe²⁺ is significantly higher in the presence of CMCD, and the Stern–Volmer plot deviated significantly from linearity with CMCD present. In the case of Br[−] (Figure 2b), the quenching efficiency decreased in the presence of CMCD, and linear Stern–Volmer plots were obtained ($R^2 = 0.991$ with CMCD and $R^2 = 0.999$ without CMCD). The linear relationship indicates that only one quenching mechanism is involved.¹⁸ Since the negative charge on Br[−] makes it unlikely to bind with either anthracene or cyclodextrin, Br[−] quenching is considered to occur via a collisional mechanism. Less efficient quenching by Br[−] in the presence of CMCD is the result of a protecting effect by CMCD. Because of electrostatic repulsion between the bromide anion and the partially negative carboxyl groups on CMCD, the bromide ion is unlikely to have close contact with the CMCD or any species included within the hydrophobic cavity. Therefore, collisional quenching is decreased. A number of studies have shown that ionic species could not quench the fluorescence of aromatic compounds which were encapsulated into micelles having the same charge as the quencher.^{19–22} On the contrary, the positive charge of Fe²⁺ and the metal coordinating ability of carboxylic acid groups makes it likely that iron can bind to the rim of

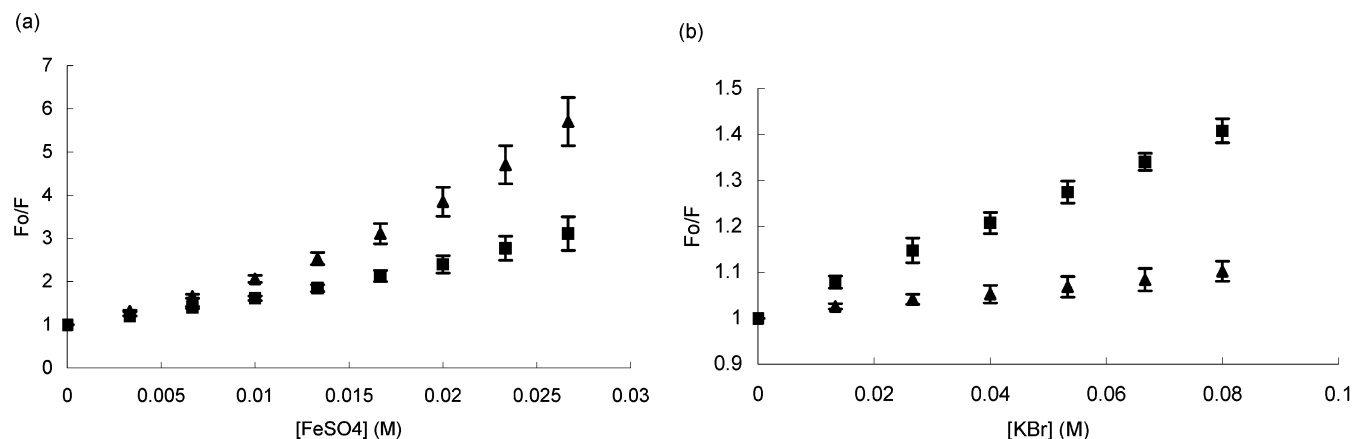


Figure 2. Stern–Volmer plot of anthracene (0.1 μM) quenched by (a) Fe²⁺ and (b) Br[−] in aqueous solution (pH = 3.1) in the presence (▲) and absence (■) of CMCD (2 mM). F is the fluorescence intensity with quencher, and F_0 is the fluorescence intensity without quencher.

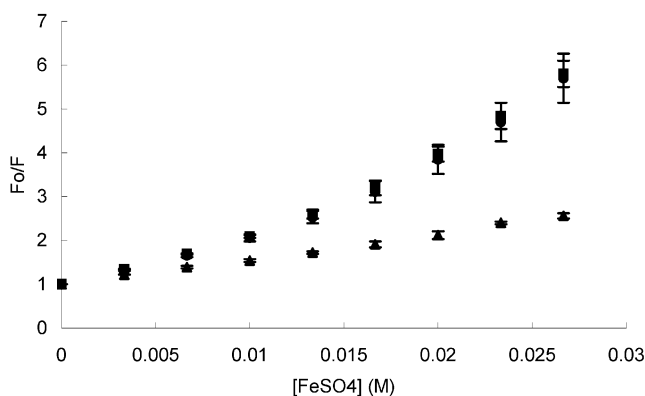


Figure 3. Stern–Volmer plot of anthracene (0.1 μM) quenched by Fe^{2+} in pH = 1.2 (▲), 3.1 (●), and 4.1 (■) solutions in the presence of CMCD (2 mM).

CMCD. Such binding would lead to a higher local concentration of Fe^{2+} near the included anthracene and therefore result in a higher quenching efficiency. Furthermore, the nonlinear result for Fe^{2+} quenching in the presence of CMCD is an indication of mixed collisional and static quenching mechanisms; the static mechanisms are only likely in the case of ternary pollutant/CMCD/ Fe^{2+} complexes. The quenching experiments were repeated with 2-naphthol, and similar behavior was observed. As an additional comparison, iron quenching of anthracene was carried out using underivatized β -cyclodextrin. For these experiments, there was no significant enhancement in Fe^{2+} quenching of anthracene. Since underivatized β -cyclodextrin lacks acid functional groups, it is expected to have much weaker binding with iron than the CMCD. Consequently, β -cyclodextrin is less likely to bring iron closer to anthracene.

Molecular oxygen quenches almost all known fluorophores.¹⁸ To test its interference, additional anthracene quenching experiments were carried out under low O_2 concentrations. The results were the same as those under air-equilibrated conditions, indicating that oxygen effects were not significant in these studies.

Acidity of the solution affected the quenching efficiency of Fe^{2+} in the presence of CMCD. Figure 3 shows anthracene fluorescence quenching in pH = 1.9, 3.1, and 4.1 solutions. At pH = 1.9 the quenching was reduced compared to higher pH systems. This effect was likely caused by protonation of the CMCD carboxyl groups, which makes them less likely to bind iron. The pK_a of CMCD is assumed to be near 3.75 based on the previously reported value of a closely related carboxylated cyclodextrin.²³ The pH = 1.9 system yielded a linear Stern–Volmer plot ($R^2 = 0.995$), indicating that only a single quenching mechanism (most likely collisional) was occurring. The same pH effect was also observed in 2-naphthol fluorescence quenching studies. These pH-dependent results further support the conclusion that the acid functional groups of CMCD aid in its ability to coordinate iron.

Europium(III) was also used as a fluorescence quencher in the present work. Similar to the results for iron, anthracene quenching by Eu^{3+} was significantly enhanced in the presence of CMCD. The effect for europium was more pronounced than for iron. These results are consistent with the formation of a ternary pollutant/CMCD/ Eu^{3+} complex analogous to the iron-containing complex.

(C) Ca^{2+} and Cd^{2+} Substitution of Fe^{2+} . For the fluorophores used in this study, Ca^{2+} and Cd^{2+} are less efficient fluorescence quenchers than Fe^{2+} . When Ca^{2+} or Cd^{2+} was added to an anthracene + CMCD + Fe^{2+} solution, the

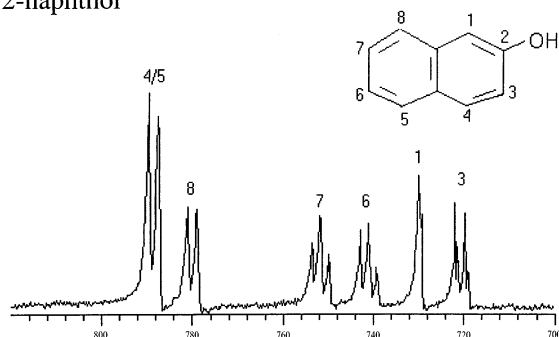
fluorescence was restored by a small but statistically significant amount. In the absence of CMCD (anthracene + Fe^{2+} solution), addition of Ca^{2+} or Cd^{2+} resulted in greater quenching. Without CMCD, the addition of Ca^{2+} or Cd^{2+} increased the total concentration of quenchers, which resulted in greater quenching of anthracene fluorescence. In the presence of CMCD, Ca^{2+} or Cd^{2+} competed with Fe^{2+} for the binding sites on CMCD. Consequently, some of the bound Fe^{2+} was displaced by Ca^{2+} or Cd^{2+} . Since Ca^{2+} and Cd^{2+} are poorer quenchers than iron, displacement of the iron resulted in an increase in fluorescence. These effects were small, so a statistical test for Ca^{2+} substitution was used to confirm that there was a significant difference. Results of a t -test yielded $t_{\text{calc}} = 5.9$ ($n_1 = n_2 = 3$; $t_{\text{table}} = 5.598$ at 99.5% confidence and 4 degrees of freedom), indicating that the fluorescence results for the anthracene + CMCD + Fe^{2+} solution before and after addition of Ca^{2+} were statistically different. To ensure the validity of these results, the intensity of fluorescence at two separate wavelengths (378 nm, 400 nm) were compared, and both indicated statistical differences. (At 378 nm, $\Delta F = -2900 \pm 1400$ without CMCD and $\Delta F = +2700 \pm 800$ with CMCD; at 400 nm, $\Delta F = -4379 \pm 2069$ without CMCD and $\Delta F = +1646 \pm 937$ with CMCD; ΔF is the fluorescence difference in the presence and absence of Ca^{2+} .) These results further indicate that Fe^{2+} binds in a ternary pollutant/CMCD/ Fe^{2+} complex and also indicate that iron binding to the complex is reversible.

(D) NMR Spectra. ^1H NMR spectra were recorded for aqueous solutions of 2-naphthol, 2-naphthol + CMCD, 2-naphthol + Fe^{2+} , and 2-naphthol + CMCD + Fe^{2+} (Figure 4). A COSY 2D NMR experiment was also carried out to assign the peaks of 2-naphthol as shown in Figure 4a. From an NMR titration experiment, it was found that upon addition of CMCD to a 2-naphthol solution, the hydrogens at positions 3, 6, and 7 shifted toward high field while hydrogens at positions 1, 4, 5, and 8 shifted toward low field. These shifts indicate that the 2-naphthol molecules were binding with CMCD. A binding constant of $224 \pm 10 \text{ M}^{-1}$ was calculated from these experiments. CMCD protons also exhibited a shift in the presence of 2-naphthol (data not shown). However, due to the complex NMR spectrum of CMCD, which is a mixture with different numbers of carboxymethyl substitutions on each molecule, it is difficult to determine which CMCD protons were involved in interactions with 2-naphthol.

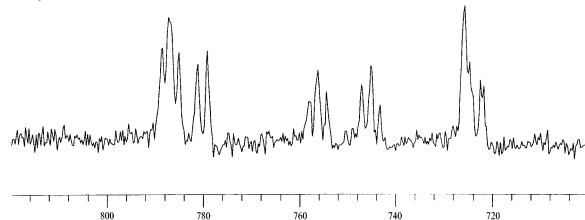
Fe^{2+} is paramagnetic when it coordinates with water molecules. As such it increases the relaxation rate of nuclei in close proximity to it ($R_2 \propto r^{-6}$, where R_2 is the transverse relaxation rate and r is the distance between the electron and ^1H spin^{24,25}), which results in peak broadening.^{26,27} In addition, the magnetic field associated with Fe^{2+} ions contributes to the local field around nearby nuclei, causing a change in the chemical shift of these nuclei.^{27–30} In aqueous solutions of 10 mM Fe^{2+} , 2-naphthol peaks were not broadened and did not show changes in chemical shift (Figure 4c). The apparent peak shift in this spectrum is an artifact caused by a shift in the water signal used for calibration. The shift in the water signal was caused by interaction with the Fe^{2+} present in solution. Note that all the peaks in Figure 4c are shifted to the same extent due to an offset in the reference peak. The lack of broadening of 2-naphthol peaks indicates that Fe^{2+} is not close enough to the 2-naphthol molecules to induce such a change. In other words, the iron and 2-naphthol do not exhibit noticeable interactions.

In the presence of CMCD, 2-naphthol peaks were significantly broadened when Fe^{2+} was also present (Figure 4d). Although there were no significant chemical shift changes, the

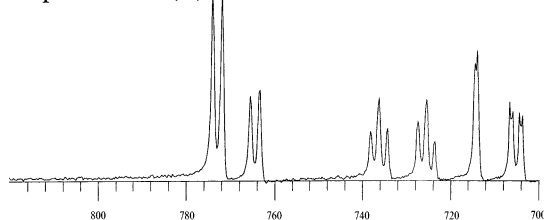
a: 2-naphthol



b: 2-naphthol + CMCD



c: 2-naphthol + Fe(II)



d: 2-naphthol + CMCD + Fe(II)

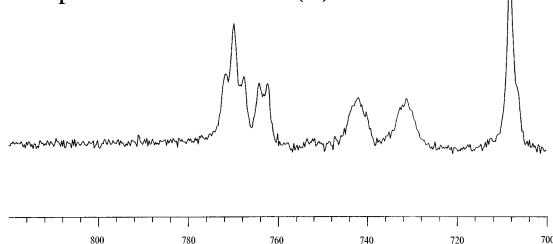


Figure 4. ¹H NMR spectra of (a) 2 mM 2-naphthol, (b) 0.5 mM 2-naphthol + 5 mM CMCD, (c) 5 mM 2-naphthol + 10 mM Fe²⁺, and (d) 2 mM 2-naphthol + 10 mM CMCD + 10 mM Fe²⁺.

observed broadening in peak width suggests that iron and 2-naphthol are in close proximity in this system.

Two diamagnetic cations, Ca²⁺ and Cd²⁺, were used to displace Fe²⁺ from CMCD binding sites. Both of these ions have been proven to bind with CMCD by the fluorescence experiments discussed above, and binding of Cd²⁺ was further proven by ¹¹³Cd NMR measurements (data not shown). The narrowing of 2-naphthol peaks was observed upon addition of Cd²⁺ or Ca²⁺ to the 2-naphthol + CMCD + Fe²⁺ solution. Figure 5 shows the spectrum of 2-naphthol in the presence of CMCD, Fe²⁺, and Cd²⁺. Compared to Figure 4d (2-naphthol + CMCD + Fe²⁺), the data in Figure 5 (2-naphthol + CMCD + Fe²⁺ + Cd²⁺) exhibit considerably less peak broadening. These data clearly indicate that a ternary 2-naphthol/CMCD/Fe²⁺ complex must exist in solution. Furthermore, this complex must exist in an arrangement that allows direct interaction of iron and 2-naphthol.

As additional control experiments, NMR spectra were recorded for the ternary systems using Ca²⁺ or Cd²⁺ instead of Fe²⁺ as the binding cation. Because Ca²⁺ and Cd²⁺ are

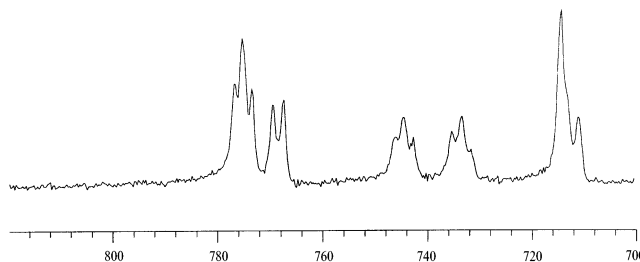


Figure 5. ¹H NMR spectrum of 2 mM 2-naphthol + 10 mM CMCD + 10 mM Fe²⁺ + 90 mM Cd²⁺.

diamagnetic, they cannot cause NMR peak broadening due to paramagnetic effects. The NMR spectra of 2-naphthol + CMCD + Ca²⁺ and 2-naphthol + CMCD + Cd²⁺ (not shown) did not exhibit any observable peak broadening or shift in 2-naphthol peak positions. The peak broadening observed with Fe²⁺ is therefore believed to be due to an interaction between the paramagnetic iron and the naphthol molecule. Since this interaction was only observed in the presence of CMCD, the CMCD must play a substantial role in mediating the iron–naphthol interaction.

Conclusions

Formation of pollutant/CMCD/iron ternary complexes in aqueous solution was confirmed by fluorescence and NMR studies. The significant difference in interaction between Fe²⁺ and 2-naphthol in the presence and absence of CMCD indicates that the two species are brought closer together by simultaneous binding to CMCD. The results support the theory that enhanced degradation of pollutant with addition of CMCD is due to the closer location of degradation catalyst to the target molecules. In addition to enhancement of Fenton degradation of pollutants, ternary host/cyclodextrin/metal complexes may have a broad range of other applications, possibly including sensors, drug delivery, and catalytic systems. Obtaining a fundamental understanding of the structure and binding in such complexes is essential to their effective utilization.

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