Simultaneous elution of polycyclic aromatic hydrocarbons and heavy metals from contaminated soil by two amino acids derived from β-cyclodextrins

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Abstract
Two highly water-soluble amino acids, which derived from β-CDs, i.e., glutamic acid-β-cyclodextrin (GluCD) and ethylene-diamine-β-cyclodextrin (EDCD), were synthesized and were examined for their effect on solubilization of anthracene (ANT), complexation of cadmium (Cd²⁺), and elution removal of ANT and Cd²⁺ in soil. The results showed that GluCD and EDCD were powerful complexant for ANT and Cd²⁺. In the presence of 10 g/L GluCD and EDCD, the solubilization of ANT increased by 47.04 and 23.85 times compared to the control, respectively. GluCD resulted in approximately 90% complexation of Cd²⁺ while 70% complexation was observed for EDCD. Simultaneously, GluCD and EDCD could greatly enhance the elution removal of ANT and Cd²⁺. The addition of 10 g/L GluCD, 53.5% of ANT and 85.6% of Cd²⁺ were eluted, respectively. The ANT had a negligible effect on the Cd²⁺ removal due to different complexing sites of ANT and Cd²⁺, while Cd²⁺ enhanced the ANT removal under the addition of GluCD because Cd²⁺ neutralized the –COOH group of GluCD. Adversely, the removal of ANT was decreased with Cd²⁺ under the addition of EDCD, this was due to the fact that Cd²⁺ enhanced the polarity of EDCD molecule and inhibited the complexation between ANT and EDCD. The study suggested that GluCD could be preferred and be successfully applied to remediation of heavy metals or organic compounds in contaminated soil.

Key words: anthracene; cadmium; cyclodextrin; solubilization; elution; soil
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Introduction
Organic and inorganic contaminations such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals have occurred as a result of various anthropogenic activities (discharge of industrial waste, wood treatments, metallurgical operations, burning of fossil fuels, etc.). The accumulation of both PAHs and heavy metals in the environment not only results in potential environmental risk but also poses a serious threat to human health due to their toxic, mutagenic, and carcinogenic nature, and their long-lasting sorption by soils (Wang and Brusseau, 1995a; Brusseau et al., 1997). The remediation of PAHs and heavy metals contaminated sites is an important environmental issue. Various physical, chemical, biological, and their combined technologies have been developed to remediate PAHs and heavy metals contaminated soils (Menzie et al., 1992; Wagrowski and Hites, 1997; Fountain et al., 1991). Owing to the high cost and disruption of treatments, traditional remediation of contaminated soils such as ex-situ treatment and disposal has promote the development of in situ alternatives (Fountain et al., 1991; Martin and Ruby, 2003). Several methods relate to in situ remediation have been explored, including vitrification, soil washing/acid extraction, phytoremediation, soil flushing (Brusseau et al., 1997), and bioremediation (Lovley and Coates, 1997). Co-occurrence of PAHs and heavy metals usually complicate the cleanup of sites (Wang and Brusseau, 1995b). However, little efforts were conducted on simultaneous removal of heavy metals and PAHs from soils.

Several publications indicated the potential of surfactants for the removal of PAHs and heavy metals from soil (Mulligan et al., 2001; Park and Bielefeldt, 2003). As a commonly used surfactant, ethylene diaminetetraacetic acid (EDTA) was proved effective in removing heavy metals from soil (Sun et al., 2001). However, most of surfactants are toxic and generally interact strongly with soil (Wolf and Feijtel, 1998; Zhu et al., 2003). The high cost of EDTA also limited its application on a large scale. Given the limitation associated with classical extracting
agents, there is an interest in examining the ability of other materials to facilitate the elution of PAHs and heavy metals contaminants for applications in subsurface remediation. Cyclodextrins (CDs) are cyclic oligosaccharides, containing six, seven, or eight D-(+)-glucopyranose units (α-, β-, and γ-cyclodextrin) attached by α-1,4-linkage. CDs can trap the lipophilic organic compounds as guest in a cage-like meshwork, thereby enhancing its solubility and dissolution rate (Blanco et al., 1991). β-Cyclodextrin (β-CD) appears to be the best natural cyclodextrin due to its efficient organic compound complexation and availability in pure form (Cheng and Li, 2001). However, natural β-CD can be modified to improve the low aqueous solubility. Previous studies demonstrated the enhanced performance of β-CD derivatives, such as 2-hydroxypropyl-β-cyclodextrin (HPCD), methyl-β-cyclodextrin (MCD), and carboxymethyl-β-cyclodextrin (CMCD) on removal of PAHs and heavy metals from soils (Cheng and Li, 2001; Chatain et al., 2004). β-CD derivatives present several advantages over β-CD flushing agents such as high water solubility stability and complexing capacity (Chatain et al., 2004; Albers and Muller, 1992).

In the present study, two novel amino acid derived β-CDs were synthesized, and were examined for their capability for elution and complexation of PAHs and heavy metals in contaminated soil.

1 Materials and methods

1.1 Materials

β-CD was obtained from Chemical Reagent Factory of Shanghai (China) with a purity of 99%. The solubilities of natural CDs in water are different with the number of glucose units (6, 7, or 8, respectively for α-, β-, and γ-CD). β-CD is the cheapest in cost, but weak in water solubility (15.8 mmol/L). β-CD derivatives, such as glutamic acid-β-cyclodextrin (GluCD) and ethylenediamine-β-cyclodextrin (EDCD), containing amino groups and carboxyl groups are excellent in water-solubility. Anthracene (ANT) was obtained from Yunnan Chemical Reagent Factory of Shanghai, China (purity ≥ 99%). ANT was chosen in this study due to its various physicochemical properties and its wide spread presence in contaminated sites (Wang and Brusseau, 1995a; Wagrowski and Hites, 1997). The properties of ANT and β-CD are shown in Table 1. Cadmium (cadmium nitrate, purity ≥ 99%) was selected as representative of heavy metal. Tested soil was aseptically sampled from Jinyun Mt. (29°49′N, 106°22′E) located about 32 km northwest of Chongqing, China. The soil was classified by the Chinese soil taxonomy as yellow soil, which was dominant in southern China. The sampling site was forested and practically undisturbed by land-use activities. After collection, the soil sample was transported to the laboratory in cooler, and air-dried. Before use, the soil was crushed and sieved through a 2-mm sieve. The soil was characterized with respect to an organic carbon content of 1.66%, a pH of 6.25 (1 g soil to 1 mL solution), a cation exchange capacity (CEC) of 13 mmol/100 g, a Cd content of 0.26 mg/kg, and a ANT content of 2.23 µg/kg.

1.2 Methods

1.2.1 Synthesis of amino acid derived β-CD

Epichlorohydrin (10.2 g) was added dropwise to the solution of β-CD (8.1 g), potassium hydroxide (6.7 g) and sodium glutamine (16.9 g) or ethylene diamine (6.0 g) in 70 mL distilled water at 50°C. The mixture was then reacted at 60°C for 1 hr. The pH was adjusted to 5–6 using 10% (V/V) vitriol liquor after the solution was cooled to room temperature and then left to stand overnight. The mixture was dehydrated by using anhydrous ethanol (150 mL) and was pumped through a column packed with neutral alumina. Ethanol (60%, V/V) was used as flushing agent and was repeatedly passed through the column. The effluent was left to stand for 4–5 hr and supernatant was moved away. With the addition of enough anhydrous methanol to the residue, white GluCD or EDCD precipitate was obtained and the precipitate was then filtered, freeze-dried and stored for further use.

1.2.2 Solubilization of ANT by CDs

The generator column approach was used to determine the solubilization of ANT by CDs (Wang et al., 1993). The generator column used was a 40-cm long glass chromatography column packed with pre-washed quartz sands coated with excess ANT (0.05–0.1 g). The column was plugged with glass wool at both ends and contained a large pore-diameter fritted disc sealed at the outlet. Twenty-five milliliter of distilled water, or solutions containing different concentrations of CDs were passed through the column, and a fraction of the effluent was immediately analyzed for solute concentration. The remaining effluent was then repeatedly passed through the column so as to obtain a relatively constant effluent concentration. All experiments were carried out at room temperature (25°C). The concentration of ANT was measured by UV-Vis spectrophotometry (Shanghai Chemical Instrument Inc., China). The wavelengths used for UV detection were 250 nm for ANT. During the solubility measurement, 1 mL effluent was withdrawn and diluted with a methanol/water (1/1, V/V) solution in 10 mL volumetric flasks. The role

<table>
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<tr>
<th>Table 1</th>
<th>Physico-chemical properties of ANT and β-CD*</th>
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<tr>
<td></td>
<td>Molecular weight (g/mol)</td>
</tr>
<tr>
<td>ANT</td>
<td>178</td>
</tr>
<tr>
<td>β-CD</td>
<td>1135</td>
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</table>

ANT: anthracene; β-CD: β-cyclodextrin.

* Data were cited from Wang and Brusseau, 1995a; Brusseau et al., 1997.
of methanol is to inhibit the formation of CD-solute complexes (Wang and Brusseau, 1995a). The effects of CDs on the UV spectra of the ANT were negligible within the range of experiments.

1.2.4 Simultaneous elution experiments of ANT and the following (Dai et al., 1998):

\[ \text{ANT and Cd}^{2+} / \text{distilled water or solution containing either 10 g} \]

The initial concentrations of ANT and cadmium sorbed by the soils for each set of experiments were 4 and 101.2 mg/L. A 40-cm long glass chromatography column was packed with 60 g soil. The column was plugged with glass wool at both ends and contained a large pore-diameter fritted disc sealed at the outlet. After packing, the columns were slowly wetted from the bottom with electrolyte solution (0.01 mol/L CaCl₂). To prepare the contaminant-elution experiments, solutions containing ANT (0.05–0.07 mg/L), cadmium (100 mg/L), or a combination of both were pumped into the soil-packed column. To ensure uniform and complete contamination of the soil columns, the solution was continuously pumped into the columns until the effluent concentrations of both cadmium and ANT were equal to the influent concentrations. An atomic absorption spectrophotometer (WFX-810, Beijing Rayleigh Analytical Instrument Co., Ltd., China) was used to monitor the concentration of cadmium in the effluent. An atomic absorption spectrophotometer was continuously pumped into the columns until the effluent concentration of Cd was pumped into the soil-packed column. To ensure uniform and complete contamination of the soil columns, the solution was continuously pumped into the columns until the effluent concentrations of both cadmium and ANT were equal to the influent concentrations. An atomic absorption spectrophotometer (WFX-810, Beijing Rayleigh Analytical Instrument Co., Ltd., China) was used to monitor the concentration of cadmium in the effluent. An atomic absorption spectrophotometer was used to monitor the concentration of cadmium in the effluent fractions. The initial concentrations of ANT and cadmium sorbed by the soils for each set of experiments were 4 and 101.2 mg/kg soil, respectively. Immediately after contamination, distilled water or solution containing either 10 g/L GluCD or 10 g/L EDCD was pumped through the columns. The AN and Cd²⁺ removed by distilled water was a blank control group.

2 Results and discussion

2.1 Synthesis and characterization of amino acid derived β-CD

The synthesis of amino acid derived β-CD is shown as the following (Dai et al., 1998):

\[
\begin{align*}
\text{KOH} & \quad \text{CICH}_2\text{CH}_2\text{OH} + \text{NH}_3 + \text{R} \rightarrow \text{CICH}_2\text{CH}_2\text{NHR} \\
\text{KOH} & \quad \text{H}_2\text{C} = \text{CH}_2\text{NHR} \rightarrow \text{CICH}_2\text{CH}_2\text{NHR}
\end{align*}
\]

R is -\text{CH}_2\text{CH}_2\text{NH}_2 \text{ or } -\text{CH}_2\text{COOH}

Firstly, amino group is reacted with epichlorohydrin under basic condition to form 1-amino-2-hydroxyl-3-chloropropane. Secondly, 3-amino-1,2-epoxypropane is formed after the cyclization of 1-amino-2-hydroxyl-3-chloropropane. Thirdly, β-CDs is reacted with 3-amino-1,2-epoxypropane to form amino acids derived β-CDs. These reactions for each set of synthesis experiment were performed in aqueous solution, and the structures of the final products were determined through Fourier transform infrared (FT-IR-8400, Shimadzu, Japan) (FT-IR). Figure 1 shows that some different bands arose in the spectra of GluCD and EDCD. At 1719 cm⁻¹, a new peak appeared in the spectra of GluCD attributed to C=O stretching. In addition, an adsorption bound boosted up in the range of 1400–1500 cm⁻¹ for GluCD could be ascribed to N–H deformation and C=N stretching. These results supported the conclusion that the synthetic compound was GluCD. A distinct difference was observed between spectra of β-CD and EDCD. At about 3382 cm⁻¹, the EDCD presented a new strong adsorption which mainly ascribed to NH₂ stretching indicated the synthesis of EDCD.

2.2 Solubilization and complexation studies for ANT and Cd²⁺

The solubilization effects of β-CD, GluCD and EDCD on ANT are plotted in Fig. 2. The results showed that the apparent aqueous solubility of ANT was linearly increased with the concentrations of β-CD, GluCD and EDCD which might be ascribed to the formation of 1:1 inclusion complexes in solution (Wang and Brusseau, 1995b). The apparent aqueous solubility of ANT can be expressed by the following Eq. (2):

\[
S_t = S_0 (1 + K_S C_0)
\]

where, \(S_t\) is the aqueous-phase concentration of ANT with β-CD, GluCD or EDCD, \(S_0\) is the concentration of ANT without β-CD, GluCD or EDCD, \(C_0\) is the initial concentration of β-CD, GluCD or EDCD, and \(K_S\) is the stability constant of inclusion complexes for ANT with β-CD, GluCD or EDCD. In Eq. (2), it was assumed that the concentration of β-CD, GluCD or EDCD is not depleted to an appreciable extent by complexing with
ANT. The relative aqueous-phase concentrations ($S_t/S_0$) of ANT are plotted against the concentration of β-CD, GluCD or EDCD. The regression equations and correlation coefficients of the solubilization curves of ANT by β-CD, GluCD and EDCD are listed in Fig. 2. Figure 2 reveals that GluCD resulted in the most notable solubilization of ANT followed by EDCD and β-CD. The prerequisite for solutes fitting completely in the cavity of cyclodextrin and forming an inclusion complex is that the molecule must be of appropriate in shape and size (Wang et al., 1993). Table 1 shows that the molecule volume of the ANT was smaller than this cavity volume for β-CD. Therefore, they can enter into their respective cavities for β-CD, GluCD and EDCD and inclusion complexes. In addition, the water-soluble of the compounds and CDs pose a strong effect on the stability. As a result, the solubilization capability of β-CD for ANT was greater than that of naphthalene due to the weaker water-soluble of naphthalene than ANT (Wang and Brusseau, 1995a). In addition, the ANT was oriented coincident with the cavity axis when it extended through the β-CD cavity. The reason might be that the length of ANT was larger than the diameter and the depth of the β-CD cavity. However, the length of naphthalene is only slightly smaller than the diameter of the β-CD cavity, maximum contact with the β-CD cavity was obtained when the short axis of naphthalene was parallel to the axis of the cavity (Wang and Brusseau, 1995b). In this study, inclusion complexes of ANT with GluCD or EDCD might be formed when the ANT extended through the GluCD or EDCD cavity (Fig. 3). Higher water-solubility of GluCD and EDCD compared to the β-CD was based on: (1) the strong hydrophilic groups –COOH or –NH$_2$ in GluCD and EDCD; (2) the break of reticulated CD polymer formed by hydrogen bonding interactions. Other than –NH$_2$ group, hydrophilicity of GluCD was enhanced by the –COOH group which contributed to much stronger water-solubility of GluCD as compared to EDCD (Dai et al., 1998). In this study, the same results were obtained. Addition of 10 g/L GluCD resulted in 47.04 times increase in solubilization of ANT but only 23.85 times increase for EDCD at equal concentration.

The complexation results of Cd$^{2+}$ by GluCD or EDCD at various concentrations are shown in Table 2. GluCD and EDCD complexed approximately 90% and 70% of the Cd$^{2+}$, respectively and the complexation capacity increased with cyclodextrin concentrations. Previous study suggested that the functional groups of –COOH and –NH$_2$ in GluCD and EDCD played important roles in complexation (Wang and Brusseau, 1995b). For example, hexamethylene-diamine and EDTA, –NH$_2$ of EDCD combines with Cd$^{2+}$ to from four coordinate bonds, and GluCD-Cd$^{2+}$ complexation contains Cd$^{2+}$ attached by six coordinate bonds to –COOH and –NH$_2$ (Fig. 3). In this case, GluCD is comparable to fulvic acids and anionic surfactants in complexation capacity for heavy metals (Xue and Sigg, 1999; Doong et al., 1998).

### Table 2: Complexation of Cd$^{2+}$ by GluCD or EDCD at various concentrations (unit: mg/L)

<table>
<thead>
<tr>
<th>Amino acids derived β-CDs</th>
<th>Rate of free Cd$^{2+}$ to total Cd$^{2+}$</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>GluCD</td>
<td>0.98</td>
</tr>
<tr>
<td>EDCD</td>
<td>0.95</td>
</tr>
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</table>
As shown in Fig. 4 and Table 3, the effect of ANT on the removal of Cd$^{2+}$ by EDCD and GluCD was unnoticeable, because ANT and Cd$^{2+}$ were complexed at different locations. The most possible reason might be that the group of –NH$_2$ and –COOH in EDCD and GluCD were responsible for the complexation of Cd$^{2+}$ outside the cavity while ANT was complexed by inclusion in the cavity (Fig. 3). The result was well consistent with previous report (Brusseau et al., 1997). However, the presence of Cd$^{2+}$ posed a significant impact on the removal of ANT by EDCD and GluCD. Much more ANT was removed by GluCD in the presence of Cd$^{2+}$ compared to control sample. It is possible that the presence of Cd$^{2+}$ neutralizes the charge of –COOH and reduces the polarity of GluCD molecule, and thus enhance the partition of ANT to the interior of the GluCD. However, noticeable reduction in ANT removal by EDCD in the presence of Cd$^{2+}$ also indicated the higher polarity of EDCD molecule when Cd$^{2+}$ was complexed by EDCD (Fig. 3).

### 3 Conclusions

Two β-CDs derived amino acids were synthesized and were proved have high water solubility due to a large hydrophilic group. The results of the solubilization and elution experiments showed that EDCD and GluCD could significantly enhance the complexation and elution of anthracene and cadmium, which commonly considered as low-polarity organic and heavy metal contaminants. GluCD was found to be more effective than EDCD in eluting anthracene and cadmium from soil under the same concentration. The presence of anthracene in the system had a negligible effect on the removal of Cd$^{2+}$ by EDCD and GluCD due to different complex locations of EDCD and GluCD for ANT and Cd$^{2+}$. Amino acid derived β-CDs could increase or decrease the elution efficiency of Cd$^{2+}$ from soil which mainly contributed to the change of Cd$^{2+}$-CD complexation polarity. For GluCD, the cyclodextrin simultaneously remove anthracene and Cd$^{2+}$ from contaminated soil.

The mechanism of how the formation of complex of hydrophobe organic compound and heavy metal with cyclodextrin compounds affect the toxicity of hydrophobe organic compound and heavy metals need to be further investigated.

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### References


Chatain V, Hanna K, Brauer C, Bayard R, Germain P,


