Adsorption of the harmful hormone ethinyl estradiol inside hydrophobic cavities of CTA\textsuperscript{+} intercalated montmorillonite

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ABSTRACT

Hydrophobic cavities produced by cetyltrimethylammonium cation (CTA\textsuperscript{+}) exchanged and trapped in the interlayer space of montmorillonite were used to remove the harmful hormone contaminant ethinyl estradiol (EE2) from water. X-ray diffraction, thermogravimetry/derivative thermogravimetry, elemental analysis (carbon, hydrogen, nitrogen), Fourier transform infrared, scanning electron microscopy/energy dispersive spectroscopy, Brunauer–Emmett–Teller and contact angle analyses showed that the intercalation of 9, 16 and 34 wt% CTA\textsuperscript{+} in the montmorillonite resulted in the d\textsubscript{001} expansion from 1.37 to 1.58, 2.09 and 2.18 nm, respectively. EE2 adsorption experiments showed that the original clay montmorillonite does not remove EE2 from water whereas the intercalated composites showed high efficiency with adsorption capacities of 4.3, 8.8 and 7.3 mg g\textsuperscript{-1}/C\textsubscript{0} for M9CTA\textsuperscript{+}, M16CTA\textsuperscript{+} and M34CTA\textsuperscript{+}, respectively. Moreover, experiments with montmorillonite simply impregnated with cetyltrimethylammonium bromide showed that the intercalation of CTA\textsuperscript{+} to form the hydrophobic cavity is very important for the adsorption properties. Simple solvent extraction can be used to remove the adsorbed EE2 without significant loss of CTA\textsuperscript{+}, which allows the recovery and reuse of the adsorbent for at least five times.

Key words | cetyltrimethylammonium cation (CTA\textsuperscript{+}), clay, ethinyl estradiol, hormone, montmorillonite, organophilic

INTRODUCTION

Some emerging contaminants, synthetic and natural estrogens, called endocrine disruptors, even at extremely low concentrations, have the property of inducing adverse effects in different organisms in aquatic ecosystems. Some of the toxic effects are alteration of the endocrine system, reduction of sperm count, endometriosis, and an increased incidence of breast, testicular and prostate cancer (Feng et al. 2010; Silva et al. 2012; Han et al. 2013; Aftafa et al. 2014; Rovani et al. 2014; Ben Fredj et al. 2015).

Ethinyl estradiol (EE2), the main active component found in contraceptive pills and post-menopausal hormonal supplements used in treatments of humans and animals, is considered the most hazardous endocirionic disruptor (Feng et al. 2010; Larcher et al. 2012; Silva et al. 2012; Han et al. 2013). EE2 is commonly discharged in domestic sewages and some industrial wastewaters (Larcher et al. 2012; Silva et al. 2012; Oliveira et al. 2015). Current methods of wastewater treatment including biological processes are not efficient to remove this hormone (Carr et al. 2011; Han et al. 2012; Cai et al. 2013). For this reason, different approaches have been tested to remove hormones in conventional wastewater treatment plants, e.g. photochemical degradation, and advanced oxidation processes (Zhang et al. 2010; Nasuhoglu et al. 2012; Pan et al. 2014). Adsorption on activated carbon has been reported as one of the most promising ways to remove hormones and other endocrinious disruptors from water (Saha et al. 2010; Han et al. 2013). However, diffusion limitations apparently hinder the adsorption of EE2 on typical microporous activated carbons due to the relatively large diameter of its molecule (∼12 Å) (Purceno et al. 2012). It has also been suggested that the hydrophobic/hydrophilic balance is important for the efficient adsorption of EE2. The hydrophobic character favors the adsorption of the EE2 hormone. On the other hand, the hydrophilic property is important to promote a good interaction and interface with the aqueous medium to
allow the transfer of the contaminant molecule to the adsorbent.

In the present work, the clay montmorillonite was modified using a cationic surfactant, cetyltrimethylammonium (C16H33(CH3)3N+ = CTA+), to create hydrophobic cavities for the adsorption of the hormone EE2 from aqueous solution. Montmorillonite intercalated with different quaternary alkyl ammonium cations (Lin et al. 2011) has been used for the adsorption of different contaminants, such as the dyes acid orange 7 (Kranşan et al. 2014), direct red (Zohra et al. 2013; Jin et al. 2014), sulfamethoxazole (Lu et al. 2014), phenols (2,4,5-trichlorophenol) (Zaghouane-Boudiaf et al. 2014), bisphenol A (Wang et al. 2015) and pesticides (bentazon: Shirzad-Siboni et al. 2015)). However, no adsorption of hormone on organoclays was investigated in the literature.

The CTA+ trapped inside the clay structure by a cation exchange mechanism should produce hydrophobic cavities whereas the silanol clay surface groups (Figure 1) should produce a hydrophilic surface resulting in an amphiphilic material. This material can be easily dispersed in water whereas the interlayer C16 organized chains will interact and trap the hydrophobic EE2 molecules.

MATERIALS AND METHODS

Synthesis of adsorbents

The montmorillonite sample used in this study was obtained from northern Patagonia, Argentina, with chemical composition of: SiO2: 54.72%, Al2O3: 16.45%, Fe2O3: 4.52%, MgO: 3.12%, Na2O: 3.26%, TiO2: 0.80%, CaO: 0.26%, K2O: 0.08% and MnO: 0.03% (Roca Jalil et al. 2015) with a simplified formula \( [(\text{Si}_{7.99}\text{Al}_{0.01})_{IV}\text{(Al}_{2.72}\text{Mg}_{0.54}\text{Fe}_{0.50}\text{Ti}_{0.09}\text{Mn}_{0.003})_{VI}\text{O}_{20}(\text{OH})_{4}\text{M}_{0.90}] \). The measured cation exchange capacity (CEC) of the natural clay mineral is 0.89 meq g\(^{-1}\) of clay mineral, where its principal cation is Na\(^+\) (0.53 meq g\(^{-1}\) clay mineral) (Roca Jalil et al. 2015). The clay montmorillonite was dispersed in distilled water and stirred (350 rpm) at room temperature (r.t.), for 24 h to reach complete swell. Then, the swollen montmorillonite was treated with HCl (0.05 M) in order to destroy carbonates present in the clay particles, followed by an addition of oxygenated water (35% v/v) in order to eliminate organic contamination. The solid phase was then stirred in a 1 M sodium chloride solution. After saturation the solid was washed with distilled water to remove excess of NaCl, and then the Na-montmorillonite was dried in an oven at 60°C. The surfactant-modified montmorillonite was prepared in accordance with the following procedure: Na-montmorillonite (2.0 g) was dispersed in distilled water and stirred (350 rpm) for 12 h at r.t. to swell and to reach a homogeneous suspension. The suspension was treated with an adequate amount of cetyltrimethylammonium bromide suspension (CTAB in 100 mL distilled water), which was slowly added, under stirring. To obtain the four different types of adsorbents (montmorillonite clay (M), M9CTA\(^{+}\), M16CTA\(^{+}\) and M34CTA\(^{+}\)), the following weights of the CTAB were used: 0.5, 0.7 and 1.5 g, respectively. Each mixture was stirred for 24 h at r.t. and the resulting organomontmorillonite (M-CTA\(^{+}\)) was filtered, washed with deionized water until the conductivity was below 10 \( \mu \text{S cm}^{-1} \) and then dried in an oven at 60°C, for 12 h. The dry material was ground.

Characterization of materials

The samples of the synthesis were characterized by thermogravimetry/derivative thermogravimetry (TG/DTG) (TG
DTG Shimatzu-60H performed in air atmosphere, with a heating rate of 10°C min⁻¹ to 900°C. Adsorption–desorption isotherms of nitrogen at 77 K were analyzed using a volumetric sorption analyzer (Autosorb-1MP, Quantachrome Instruments Corp., Boynton Beach, FL, USA). The samples were degassed at 150°C for 12 h until the residual pressure was less than 0.5 Pa. X-ray diffraction (XRD) measurements were performed on a Shimadzu, XRD-7000 X-ray diffractometer equipped using Cu Kα radiation over the range 4–70° (2θ) and scan rate was of 4° (2θ) min⁻¹, preset time 0.75 (sec). Scanning electron microscope (SEM) images were obtained on a Quanta 200 FEG – FEI electron microscope. The contact angle was obtained using 50 mg pressed pellets with a 15μL water droplet. Fourier transform infrared spectroscopy (PerkinElmer GX FTIR) was carried out using diffuse reflectance.

Application – adsorption test

Adsorption isotherms were obtained at 25 ± 2°C, using 10 mg of the sorbent and 10 mL of different concentrations of EE2 (0, 5, 10, 15, 20 and 25 mg L⁻¹). Only the first experiments were carried out in triplicate (M16CTA⁺) and the majority were done in duplicate. The mixtures were kept under stirring of 180 rpm for 24 h. The determination of the equilibrium concentration was carried out in a UV-visible (UV-VIS) spectrophotometer (Biosystems, Model SP 2550 UV) (λ = 280 nm).

Experiments to study the interaction between the hormone (EE2) and the surfactant (CTAB) were carried out as follows. The montmorillonite (0.66 g) was impregnated with CTAB (0.34 g) dissolved in 20 mL of acetonitrile, and the system was kept under magnetic stirring for 24 h. The solvent was then evaporated and the solid dried in an oven for 12 h at 60°C, yielding the M34CTAB_imp material, which was subjected to an adsorption test with EE2 solution (20 ppm) for 24 h under stirring. After being filtered, the supernatant was analyzed by UV-VIS spectroscopy (λ = 280 nm).

Effect of pH

The effect of pH on the adsorption capacity of EE2 hormone was evaluated by adjusting a solution of 20 mg L⁻¹ EE2 with HCl solution or NaOH 1 mol L⁻¹, at pH values of 2, 6 and 10. The test was performed using 10 mg of adsorbent (M9CTA⁺ or M34CTA⁺) and 10 mL of EE2 solution. Dispersions of the adsorbent were stirred in a shaker for 60 min and the equilibrium concentration was determined from UV-VIS measurements at 280 nm).

Reuse

The reuse studies were carried out with the M34CTA⁺, 200 mg, placed in contact with 200 mL of 20 ppm EE2 for 24 h under 180 rpm stirring. The supernatant was analyzed by UV-VIS spectroscopy (280 nm). The remaining solution was removed and the adsorbent was washed with acetonitrile and centrifuged. Subsequently, 200 mL of a fresh solution of 20 ppm EE2 was added and the procedure repeated until saturation of the material.

RESULTS AND DISCUSSION

The clay-CTA⁺ intercalated composites were prepared with different CTA⁺ contents according to a well-known described intercalation process (He et al. 2004; Lin et al. 2011; Karaca et al. 2013; Gürses et al. 2014; Shirzad-Siboni et al. 2015). Three composites were prepared using different CTA⁺/M ratios according to the CEC of the montmorillonite (0.89 mmol g⁻¹) (Roca Jalil et al. 2013). After intercalation, extensive washing and drying, the obtained materials were analyzed by infrared (IR) absorption (Figure S1, Supplementary Material, available with the online version of your paper), TG and carbon, hydrogen and nitrogen elemental analyses, which suggested CTA⁺
contents of 9, 16 and 34 wt% (Figure S2, Table S1, Supplementary Material, available online).

The DTG (Figure S2, Supplementary Material, available online) and DTG curves for the pure montmorillonite showed a main weight loss (Figure 2), i.e. an endothermic peak of ca. 10% centered at ca. 90°C related to surface adsorbed water (Hu et al. 2015; Sun et al. 2013; Yu et al. 2014).

The DTG curve of the pure CTAB (Figure 2) suggests that the decomposition takes place as a small shoulder at 220°C followed by an intense peak at ca. 248°C. On the other hand, the modified organoclays M16CTA⁺ and M34CTA⁺ showed a peak near 250°C likely related to the decomposition of non-intercalated CTAB present in the sample and also a shoulder at higher temperature, ca. 300°C, likely related to the presence of intercalated CTA⁺.

This non-intercalated CTAB is likely related to molecules adsorbed on the clay surface by an interaction with surface –O²⁻ groups (e.g. Si–O²⁻ and Al–O²⁻) and also CTAB intercalated very near to the clay layer edges. Both the CTA on the external surface and that located in the edges should decompose at lower temperatures.

It is interesting to observe the presence of only one peak at 300°C for the sample M9CTA⁺ which suggests the presence of intercalated CTA⁺ only.

XRD analyses of the M/CTA⁺ composites (Figure 3) showed that when CTA⁺ is added to the clay at 9 wt% the d001 peak shifts to a significantly lower 2θ value indicating an interlamellar expansion with a slight reduction of the intensity and broadening of the peaks.

The interlamellar spacing gradually increased with the amount of CTAB used in the synthesis, i.e. from 1.37 nm (for pure montmorillonite) to 1.58, 2.09 and 2.18 nm for M9CTA⁺, M16CTA⁺ and M34CTA⁺, respectively. This result combined with IR (Figure S1, Supplementary Material) strongly suggests that CTA⁺ was intercalated in the interlamellar space of montmorillonite. A previous study showed that the lateral-monolayer arrangement in the organoclay gave a basal spacing of 1.3–1.5 nm and a spacing of 1.77–1.98 nm for the lateral-bilayer (Hu et al. 2015; Sun et al. 2013). Therefore, a reflection at 1.58, 2.09 and 2.18 nm for M9CTA⁺, M16CTA⁺ and M34CTA⁺ indicated the transition structure of surfactants from lateral-monolayer to lateral-bilayer within the clay interlayer, consistent with the results of He et al. (2004). The observed deviations of subsequent diffraction orders from integer values should be caused by the instrumental effect that stems from the Bragg-Brentano parafocusing method (Sun et al. 2015).

SEM images of the starting montmorillonite showed a morphology based on aggregated flakes. It is interesting to observe that the presence of CTA⁺ up to 34% did not seem to change significantly the morphology of the composites (Figure 4).

Energy dispersive spectroscopy (EDS) spectra (Figure S3, Supplementary Material, available online) of the M-CTA⁺ composites showed typical montmorillonite peaks of Si, O, Al, C, Fe and Mg (Kirman et al. 2014; Shirzad-Siboni et al. 2015). In the sample M34CTA⁺, the characteristic peaks of bromide were observed, suggesting excess surfactant CTAB. On the other hand, no Br signal was observed for the other samples, suggesting that all the CTA⁺ present in the clay lost Br⁻ due to the exchange process (Equation (1)):

\[
\text{CTA}^+\text{Br}^- + \text{Clay}^-\text{Na}^+ \rightarrow \text{Clay}^-\text{(CTA}^+)\text{Intercalated} + \text{NaBr}
\]

Considering the CEC of the montmorillonite used, i.e. 0.89 mmol g⁻¹ (Roca Jalil et al. 2015), the intercalated CTA⁺ used the CEC at approximately 38 and 75% for the composites M9CTA⁺ and M16CTA⁺. On the other hand, the sample M34CTA⁺ contains ca. 1.81 mmol CTA⁺ g⁻¹clay, which is twice the CEC. This result suggests that for the sample M34CTA⁺ part of the CTA⁺ is in excess and likely impregnated on the outer part of the clay. The specific surface area (Brunauer–Emmett–Teller) obtained for the montmorillonite natural clay (M) was 53 m²g⁻¹, which agrees with previous work (Roca Jalil et al. 2015). Upon intercalation of CTA⁺ in the clay, the surface area decreased to 9, 10 and 11 m²g⁻¹.
for the samples M9CTA\(^+\), M16CTA\(^+\) and M34CTA\(^+\), respectively (see nitrogen adsorption isotherms Figure S4, Supplementary Material, available online).

The contact angle of the samples M, M9CTA\(^+\), M16CTA\(^+\) and M34CTA\(^+\) were 0, 42, 60 and 62\(^o\), respectively (Figure 5), suggesting that the presence of CTA\(^+\) increases the hydrophilicity of the composites, which also agrees with previous work (Zhuang et al. 2015).

The removal of EE2 from aqueous solution was investigated for the different M/CTA\(^+\) composites prepared and the obtained adsorption isotherms are shown in Figure 6.

It can be observed that the pure clay montmorillonite did not adsorb EE2. On the other hand, the presence of 9\% CTA\(^+\) in the clay structure increased the adsorption capacity to reach a maximum adsorption capacity of approximately 4.3 mg\(_{EE2}\) g\(^{-1}\). As the CTA\(^+\) content increased to 16\% the EE2 adsorption further increased to ca. 8.8 mg\(_{EE2}\) g\(^{-1}\). It is interesting to observe that for the
sample M34CTA\(^+\) the EE2 adsorption decreases to 7.3 mg\(\text{EE2 g}^{-1}\). Considering that the pure clay does not adsorb EE2 and only the added CTA\(^+\) is responsible for the EE2 adsorption one can estimate the amount of EE2 adsorption per CTA\(^+\) present in the composite. For the composite 9% CTA\(^+\) a value of 46 mg\(\text{EE2 g}^{-1}\)CTA\(^+\). As the CTA\(^+\) increased to 16% the adsorption capacity a similar value of 52 mg\(\text{EE2 g}^{-1}\)CTA\(^+\) was obtained. On the other hand, when the CTA\(^+\) increased to 34% the EE2 adsorption strongly decreased to 20 mg\(\text{EE2 g}^{-1}\)CTA\(^+\). This result suggests that the excess of CTA\(^+\) hinders the EE2 adsorption by the composite. Although the reasons for this effect are not clear one can speculate that the CTA\(^+\) in the 9% and 16% composites are completely intercalated creating hydrophobic cavities accessible for the EE2 molecules. The hydrophobic EE2 molecules should interact with the hydrophobic C\(_{16}\) carbon chain of CTABr and trap the EE2 molecule in the clay interlayer space.

On the other hand, at 34% there is an excess of CTA\(^-\)Br\(^-\), which is located on the external surface of the composite. These CTAB molecules are likely blocking the hydrophobic cavities, which hinders the adsorption of the EE2 molecules (Figure 7).

Analysis of the isotherm data adjusted for the Langmuir and Freundlich models (see Supplementary Material, Table S2, available online) showed a better fitting to the Freundlich adsorption considering the formation of multilayers.

The EE2 adsorption obtained in this work can be compared with different values reported in the literature (Kumar & Mohan 2011, 2012; Teixeira et al. 2013; Rovani et al. 2014; Al-Khateeb et al. 2014). For this comparison, the adsorption capacity normalized by surface area, i.e. mg\(\text{EE2 m}^{-2}\), was used (Figure 8).

A higher adsorption capacity for M16CTA\(^+\), i.e. 0.88 mg\(\text{EE2 m}^{-2}\), can be observed, compared with other materials such as activated carbon, carbon nanotubes and carbon nanofiber composites.

The composite was tested in consecutive adsorption experiments, following a regeneration procedure. After the first use, the composite was washed with acetonitrile at room temperature to remove the EE2 adsorbed, dried in an oven at 60 °C for 1 h and used again for a new adsorption experiment with a fresh EE2 solution. The obtained results (Figure S6, Supplementary Material, available online) showed that simple solvent extraction can be used to remove the adsorbed EE2 without significant loss of CTA\(^+\), which allows the recovery and reuse of the adsorbent for at least five times. Probably, the intercalated ion-exchanged CTA\(^+\) is strongly anchored to the clay structure and cannot be solubilized by the acetonitrile.

The effect of the location of CTAB, i.e. intercalated or impregnated on the outer surface of the clay, was investigated. The CTAB was impregnated on the external clay surface using an organic solvent (acetonitrile) for impregnation. XRD analysis of this sample, M34CTABimpreg, (Supplementary Material, Figure S5, available online) showed a slight \(d_{001}\) increase to 1.9 nm suggesting that some CTA\(^+\) was intercalated in the clay. Adsorption experiments using CTAB impregnated on the clay surface (not intercalated) showed much lower adsorption capacity (Figure S7, available online), suggesting that the intercalation of the CTA\(^+\) in the interlayer space, to form the hydrophobic cavity, is important for the adsorption of the hormone EE2 (Figure 9).

Figure 10 shows the effect of pH on materials M9CTA\(^+\) and M34CTA\(^+\). It was observed that as the pH increased the adsorption also increased.
Figure 8 | Comparison of adsorption capacity of M16CTA$^+$ with literature data for different adsorbent materials used for ethinyl estradiol adsorption in aqueous solution (MWCN: multi-walled carbon nanotubes).

Figure 9 | EE2 adsorption on M16CTA$^+$ compared to simple CTAB impregnated on the clay surface.

Figure 10 | Effect of pH on M9CTA$^+$ and M34CTA$^+$ adsorption of EE2.
CONCLUSION

Montmorillonite intercalated with 9–34% of CTA+ produces amphiphilic composites with contact angles between 42 and 62° which can be easily dispersed in water but with a strong hydrophobic character to attract organic contaminant molecules. These composites can remove the hormone EE2 from contaminated water at an adsorption capacity of 8–9 mgEE2 g⁻¹. These results are discussed in terms of hydrophobic cavities generated in the interlamellar space of the clay, which can sorb the EE2 molecules. These materials can be regenerated by simple solvent extraction and reused without significant loss of adsorption capacity even after five reuses. A potential application of these adsorbents can be envisaged for the treatment of wastewaters produced in contraceptive pill industrial units, which might have relatively high concentrations of hormone contaminants.

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