



Adsorption and circular dichroism of tetracycline on sodium and calcium-montmorillonites

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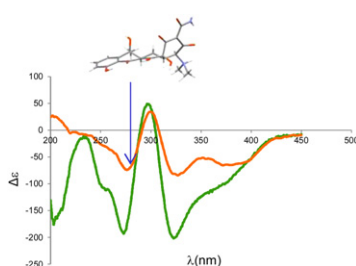
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HIGHLIGHTS

- Molecular changes of TC adsorbed on Na⁺ and Ca²⁺-montmorillonite were studied.
- Cation exchange and Ca-complexes occur when TC are adsorbed on Ca²⁺-montmorillonite.
- TC adsorption promotes the extended conformation of the molecule.

GRAPHICAL ABSTRACT



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ABSTRACT

The interaction of tetracycline (TC) with a Na⁺-montmorillonite and a Ca²⁺-montmorillonite in aqueous media was investigated using a batch technique complemented with X-ray diffraction (XRD) and circular dichroism (CD). The adsorption of TC decreases by increasing the pH in both cases, although Ca²⁺-montmorillonite is a more effective adsorbent than Na⁺-montmorillonite in the pH range 6–8.5. In both cases, TC locates in the interlayer spacing, increasing the d_{001} basal spacing from nearly 13.7 Å to nearly 22.0 Å. CD data of TC solutions indicates that increasing the pH induces structural changes from the twisted conformation to the extended conformation of the TC molecule. The presence of Ca²⁺ in the solution enhances this effect in the pH range 6–10.5. Adsorption on Na⁺-montmorillonite and Ca²⁺-montmorillonite significantly induces the adoption of the extended conformation of TC. Chelation of TC with Ca²⁺ ions in the interlayer is also demonstrated. Therefore, Ca²⁺ ions in the interlayer behave as active sites for TC adsorption on montmorillonite, leading to the formation of montmorillonite-Ca²⁺-TC complexes. The conformational changes that TC suffers upon adsorption may have important effects on the bioavailability and antimicrobial activity of the antibiotic.

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1. Introduction

Tetracyclines are antibiotics that show bacteriostatic activity toward gram-negative and gram-positive microorganisms [1], reason why they are widely used in medicine and veterinary. The so-called tetracycline (TC) is one of these products. The chemical properties of TC have been extensively studied [2–4]. It has different

acid groups in its chemical structure and can exist under different ionic species and conformations depending on the pH of the aqueous solution. The presence of such groups in the TC molecule generates potential sites of interaction with metal ions [4]. This interaction is important to understand and predict the bioavailability of TC, since in blood plasma this drug is transported as calcium complexes [3].

Clay minerals are widely used in pharmaceuticals, having a variety of applications ranging from carriers in sustained-release dosage forms to suspending agents, and giving rise to an increasing interest in the analysis of possible interactions of clay particles with

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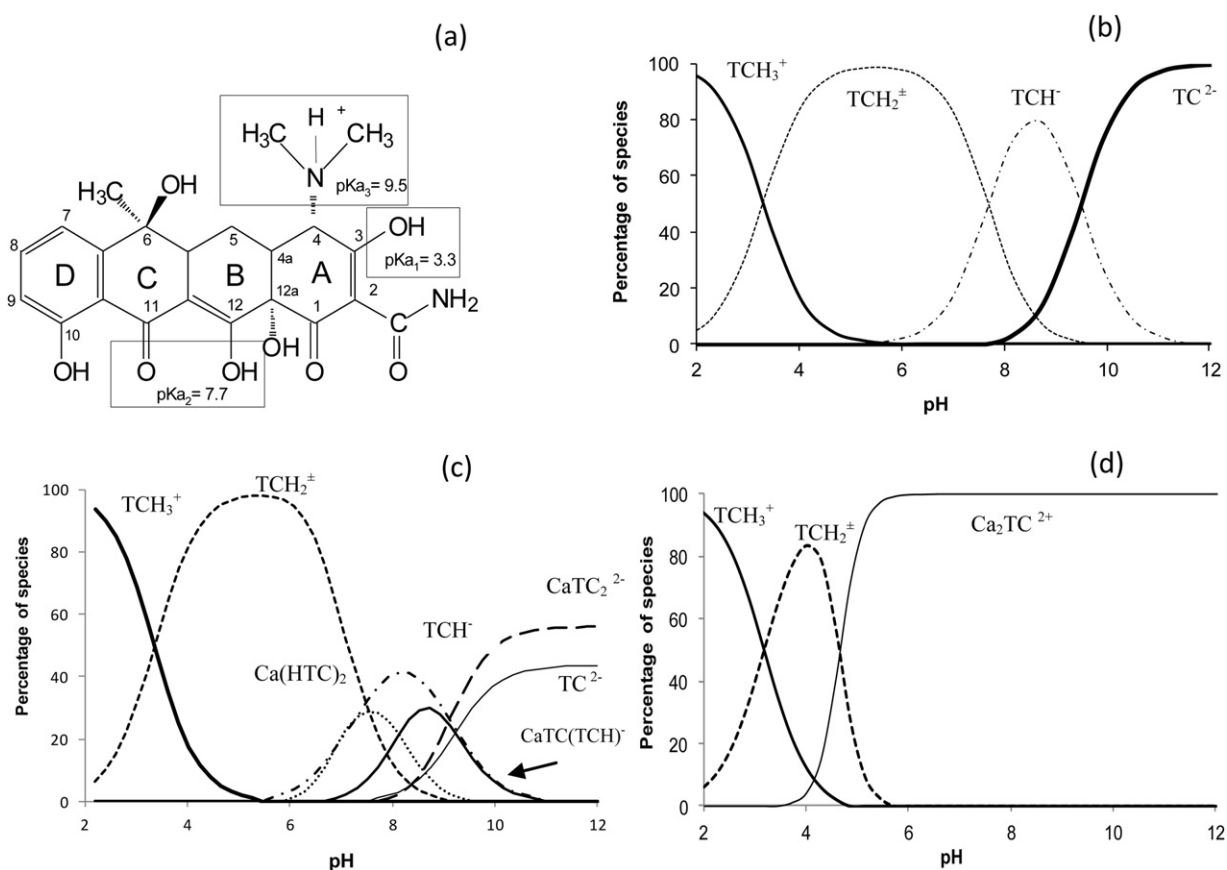


Fig. 1. (a) Structure of a fully protonated TC molecule, and distribution of TC species as a function of pH for the following Ca:TC molar ratios: (b) 0:1, (c) 1:1, (d) 200:1.

drugs. Montmorillonite is a clay mineral extensively used in pharmaceutical applications. It is found among the most important minerals for the human health care [5] due to its laminar structure with high surface area and its high cation exchange capacity, which make montmorillonite able to interact with many drugs affecting their bioavailability and influencing their activity [6].

Previous reports have analyzed the interaction between TC and clay particles by performing adsorption studies of TC on different clay minerals, including montmorillonite [7], hectorite and kaolinite [8], palygorskite [9], rectorite [10] and illite [11]. Li et al. [7] studied simultaneous desorption of metal cations and the uptake of H^+ accompanying TC adsorption and provided experimental evidences of cation exchange as the main adsorption mechanism by smectites in neutral to slightly acidic conditions. Adsorption experiments were also used to investigate the interactions between TC and a Patagonian montmorillonite in our laboratory. It has been found that the affinity of TC for the mineral depends of the pH, ionic strength [12] and the exchanging cation [13]. Electrostatic and non-electrostatic interactions between TC and clay minerals and the formation of Ca–TC complexes at the surface have been proposed to explain the adsorption behavior under different experimental conditions [13,14].

Spectroscopic and X-ray diffraction (XRD) techniques were also used to study the interaction between TC and clay minerals. From the analysis of IR spectra of TC adsorbed on sodium montmorillonite and in solution, it was established that cation exchange is the main adsorption mechanism at pH values where the cationic species of TC is dominant [15]. These authors also noted that adsorption of the zwitterion species is accompanied by proton uptake, being the fully protonated cation the resulting adsorbed species. Similar conclusions were informed by Kulshrestha et al.

[16] for adsorption of oxytetracycline on sodium montmorillonite. XRD studies of TC and montmorillonite showed that TC molecules are incorporated into the interlaminal space [17,18] and, according to Chang et al. [17], the expansion of this interlaminal space depends on the TC conformation.

Circular dichroism (CD) spectroscopy has been widely used to study the conformational properties of a wide range of optically active molecules, from small molecules to natural or synthetic macromolecules. CD is observed when optically active matter absorbs left- and right-hand circularly polarized light slightly differently. As CD is sensitive to conformational changes of chiral molecules, it is often used to elucidate the effects of different experimental conditions such pH and metal ions on these changes [19]. Earlier papers have presented conformational studies of TC and its derivatives both in the presence and absence of metal ions [20,21] and at different pH conditions [22]. Most of the studies were performed to molecules in solution, and there are only a few studies that address the conformational analysis of molecules at the surface of clay minerals. Naka et al. [23], for example, studied by CD the coadsorption of chiral and achiral metal complexes of $[Fe(2,2',2''\text{-terpyridyl})]^{2+}$ and $[Ni(1,10\text{-phenanthroline})_3]^{2+}$ on a saponite clay. Cai et al. [24], on the other hand, applied CD to investigate conformational changes of DNA molecules after interaction with the surface of a kaolinite clay. In our understanding, no CD conformational study of TC on montmorillonite have been published so far. Due to the extensive use of TC as an antibiotic and the potential use of minerals as excipients in pharmaceutical formulations, the study of TC conformations when it is adsorbed on montmorillonite results of significant importance.

The aim of this work is to study the interaction between TC and two montmorillonite samples having respectively Na^+ and Ca^{2+} as

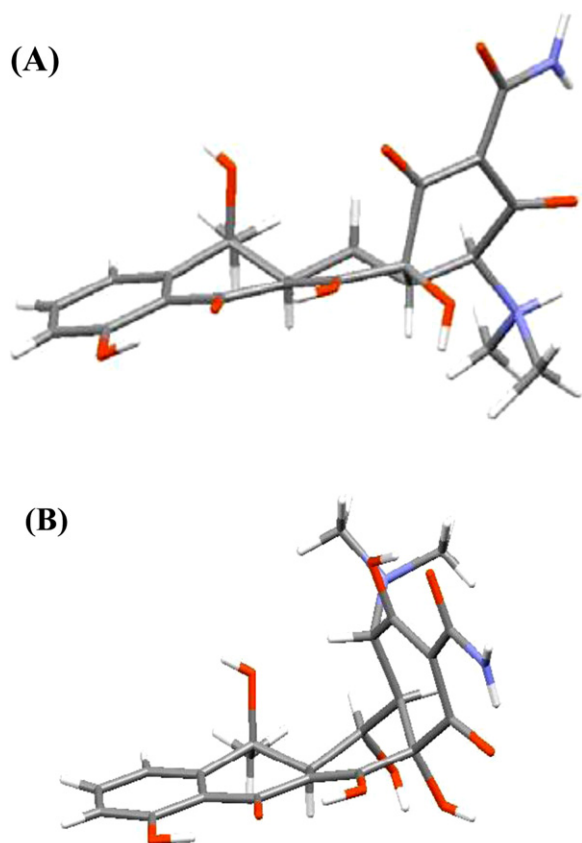


Fig. 2. TC conformations: extended (A) and twisted (B).

exchanging cations by combining batch adsorption experiments, XRD and CD, in order to evaluate possible structural and conformational changes induced by adsorption.

2. Materials and methods

2.1. Materials

The clay mineral used is a montmorillonite obtained from the Northern Patagonia, Argentina. The deposits are located close to the margin of the Colorado River, La Pampa province. The chemical analysis and physicochemical properties of the sample were previously reported [25,26]. The structural formula of the naturally occurring sample, calculated from the $M^{+}_{0.49} [(Al_{1.42} Fe^{3+}_{0.18}$

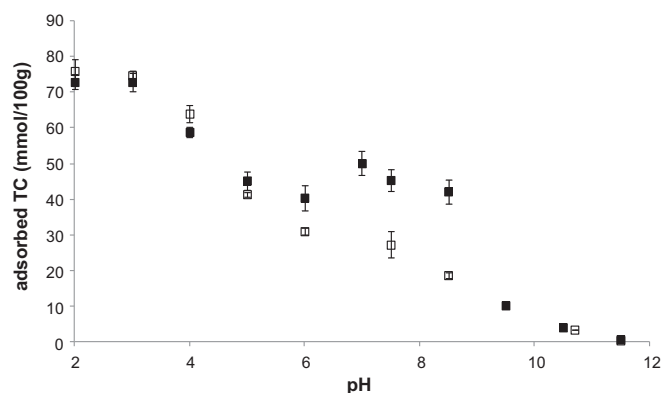


Fig. 3. Effect of pH on TC adsorption by Na⁺-montmorillonite (□) and Ca²⁺-montmorillonite (■) in 0.01 M NaCl.

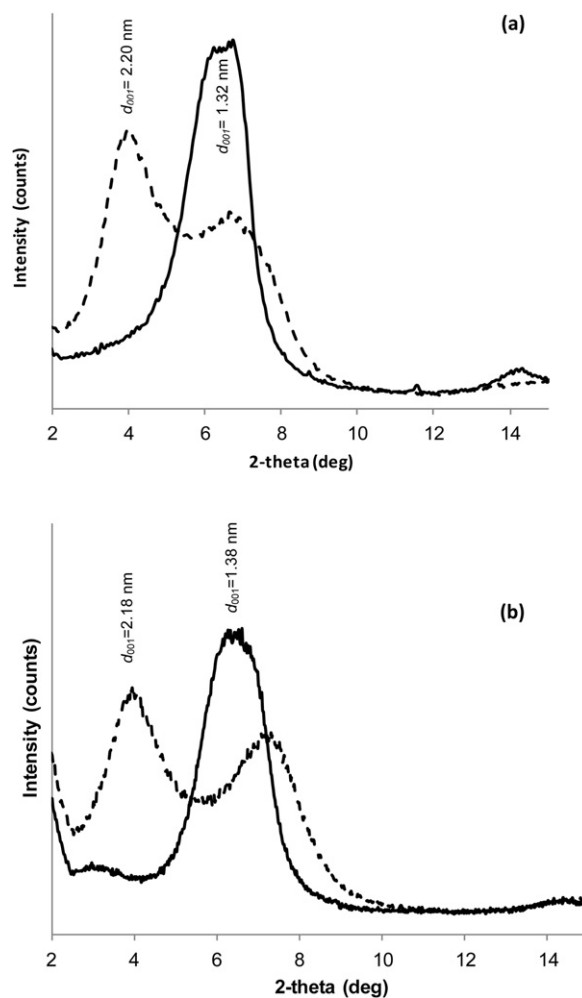


Fig. 4. XRD patterns of: (a) Na⁺-montmorillonite in the absence (solid line) and the presence (dashed line) of TC (37 mmol/100 g); (b) Ca²⁺-montmorillonite in the absence (solid line) and the presence (dashed line) of TC (62 mmol/100 g) at pH = 8 and 0.01 M NaCl in both cases.

$Mg_{0.41} (Si_{3.92} Al_{0.08}) O_{10} (OH)_2$) were M^{+} is predominantly Na⁺ (>90%) and is the cation that compensates for the structural negative electric charge of the mineral. It has a cation exchange capacity (CEC) of 104 meq/100 g as measured by the ammonium acetate method [26]. This sample will be called Na⁺-montmorillonite. A calcium montmorillonite (Ca²⁺-montmorillonite) sample was obtained by contacting 2 g of the Na⁺-montmorillonite with 50 mL of 1 N CaCl₂ and shaking during 24 h. The dispersion was then centrifuged at 8000 rpm during 30 min, the supernatant was removed and the solid was washed until negative chloride assay. Finally, the sample was dried at 40 °C, grounded and stored.

Tetracycline hydrochloride was obtained from PARAFARM (99%) and was used without further purification. TC stock solutions were prepared just before use to avoid degradation caused by oxygen and light [27]. NaOH and HCl solutions were used for pH adjustment, and NaCl was used for ionic strength control.

2.2. Adsorption studies

A batch equilibration method was used for the adsorption studies at constant TC concentration (7.6×10^{-4} M), constant ionic strength (0.01 M NaCl) and varying pH (2–11) with Na⁺- and Ca²⁺-montmorillonite. A full description of the methodology was previously reported [12]. Briefly, a series of 10 mL centrifuge tubes were filled with 3.8 mL of 0.01 M NaCl solution, 2.2 mL of a stock TC

solution (2.78×10^{-3} M) at the desired pH and 4 mL of a montmorillonite dispersion (900 mg/L). The pH of the resulting dispersion was readjusted if necessary by adding either NaOH or HCl solutions. The tubes were closed and shaken in darkness during 24 h and then were centrifuged at 8000 rpm for 30 min. No changes in the pH were detected after these procedures. The concentration of TC in the supernatant solution after adsorption was quantified using Uv–vis spectrophotometry. Since the position of the absorption bands of TC changes with pH, calibration curves were performed at each studied pH with TC standard solutions in the concentration range 0–33 mg/L. For example, at pH 3 the readings were performed at 356 nm, and at pH 10 at 386 nm. The amount of TC adsorbed was calculated from the difference between the initial and final concentrations. All adsorption experiments were performed in triplicate at 20 °C.

2.3. X-ray diffraction analysis

A 15 mg/mL dispersion of montmorillonite (either Na⁺-montmorillonite or Ca²⁺-montmorillonite) was prepared in 0.01 M NaCl at pH 8. Afterwards, this dispersion was deposited and oriented onto a glass slide and let dry under constant relative humidity conditions of 36%. For comparison, dispersions of Na⁺-montmorillonite with adsorbed TC (adsorbed amount 37 mmol/100 g) and Ca²⁺-montmorillonite with adsorbed TC (adsorbed amount 62 mmol/100 g) were prepared dispersing TC-montmorillonite adsorption complexes in 0.01 M NaCl (15 mg/mL). The dispersions were deposited on glass slides and let dry as described above.

XRD patterns were obtained using a RIGAKU Geigerflex X-ray diffractometer with CuK α radiation at 20 mA and 40 kV. Scans were recorded between 2° and 40° (2 θ) with a step size of 0.05° and scanning speed of 2° min⁻¹.

2.4. Spectroscopic and spectropolarimetric measurements

Uv–vis absorption spectra were registered with a SHIMADZU 260 spectrophotometer using a quartz cell with a 1 cm path length.

CD spectra were recorded at room temperature with a JASCO J-810 spectropolarimeter in the 200–450 nm range with band width of 3 nm. A cell with a 1 cm path length was used. Each measurement was the average of five repeated scans. CD spectra of TC solutions (3.5×10^{-5} M) were registered at varying pH (6–10.5) in absence and presence of calcium with a concentration ratio Ca:TC of 200:1.

CD spectra of TC adsorbed on montmorillonite were obtained as follows. A dispersion containing 900 mg/L of montmorillonite and 3.5×10^{-5} M TC was prepared in 0.01 M NaCl at pH 8. Under this condition the adsorption of TC on montmorillonite was 6.08 mmol/100 g in both cases (Na⁺-montmorillonite and Ca²⁺-montmorillonite), meaning that 79% of TC was adsorbed and 21% remained in solution. This concentration of TC and therefore this adsorbed amount were established according to the requirements of the CD measurements, where a concentration of chromophore with absorbance values between 0.4 and 0.8 must be used. Higher TC concentrations, aiming to obtain higher loadings, would lead to high photometric errors. The spectra were recorded as described above, using a 900 mg/L montmorillonite dispersion in 0.01 M NaCl at pH 8 as a blank. In this way, the observed CD spectra are only due to tetracycline, allowing to perform a rather direct analysis of its conformation in its adsorbed state.

3. Results and discussion

Fig. 1 shows a scheme of the fully protonated TC molecule together with the calculated distribution of TC species as a function

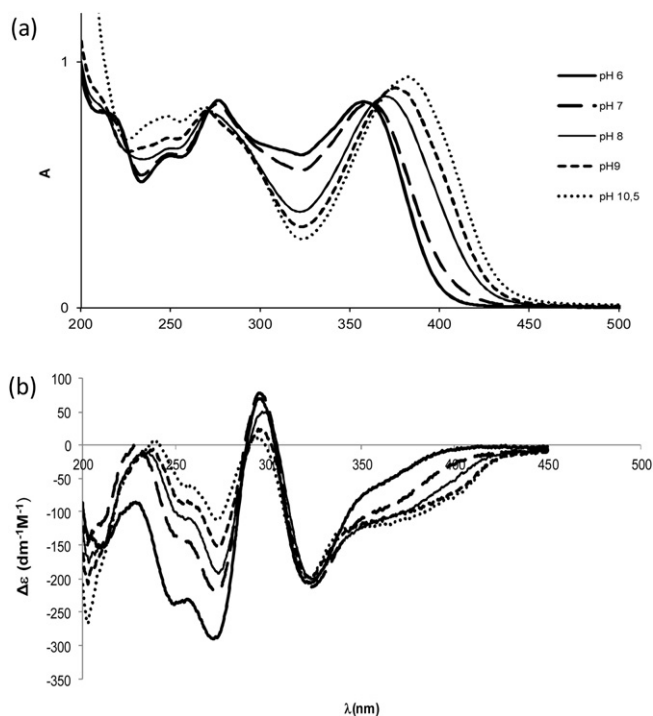


Fig. 5. (a) Uv–vis spectra and (b) CD spectra of TC aqueous solutions (3.5×10^{-5} M) at varying pH.

of pH at different Ca²⁺ concentrations. For calculations, the equilibrium constants for the formation of TC species were taken from Lambs et al. [21]; these constants are also listed in Parolo et al. [13]. Fig. 1a shows the three different groups of the molecule that can undergo protonation–deprotonation reactions depending on the pH of the aqueous solution [28]. The three pK_a values of the molecule, 3.3, 7.7, and 9.5, have been assigned to the –OH₃ group, the –OH₁₂ group, and the protonated nitrogen of the dimethylamino group, respectively [29]. These values lead to the formation of four different species with different degree of protonation, TCH₃⁺, TCH₂[±], TCH⁻, TC²⁻, whose distribution in absence of Ca²⁺ is shown in Fig. 1b. TCH₃⁺ predominates at pH 9.5.

The molecule of TC contains electron-donor groups and can form stable complexes with metal ions [21]. Therefore, additional species of TC appear in the presence of Ca²⁺, and the concentration of these species is dependent on the Ca:TC molar ratio. Fig. 1c shows the calculated distribution of TC species in solution for a Ca:TC ratio 1:1 (Ca²⁺ and TC concentrations: 7.60×10^{-4} M); Fig. 1d shows the species distribution for a Ca:TC ratio 200:1 (Ca²⁺ concentration: 1.52×10^{-2} M, TC concentration: 7.60×10^{-4} M). All equilibrium constants for calculations were also taken from Lambs et al. [21] and Parolo et al. [13]. For a Ca:TC ratio 1:1, the complexes Ca(TCH)₂, Ca(TC)(TCH)⁻, Ca(TC)₂²⁻ appear at significant concentrations at pH > 6; for a Ca:TC ratio 200:1, the Ca₂TC²⁺ complex dominates the speciation at pH > 4.7.

It is known that TC species in solution adopt different conformations depending on the state of protonation and/or complexation with metal ions [21]. There are two main conformations: the “extended conformation” and the “twisted conformation” (Fig. 2). The extended conformation, where the dimethylamino group lies below the plane defined by the BCD ring system, predominates in basic solutions in absence of metal ions. The twisted conformation, where the dimethylamino group lies above the BCD ring system in order to relieve the steric crowding between the protonated nitrogen (N₄) of the dimethylamino group and OH_{12a}, predominates in neutral or acid solutions [22]. The equilibrium between both

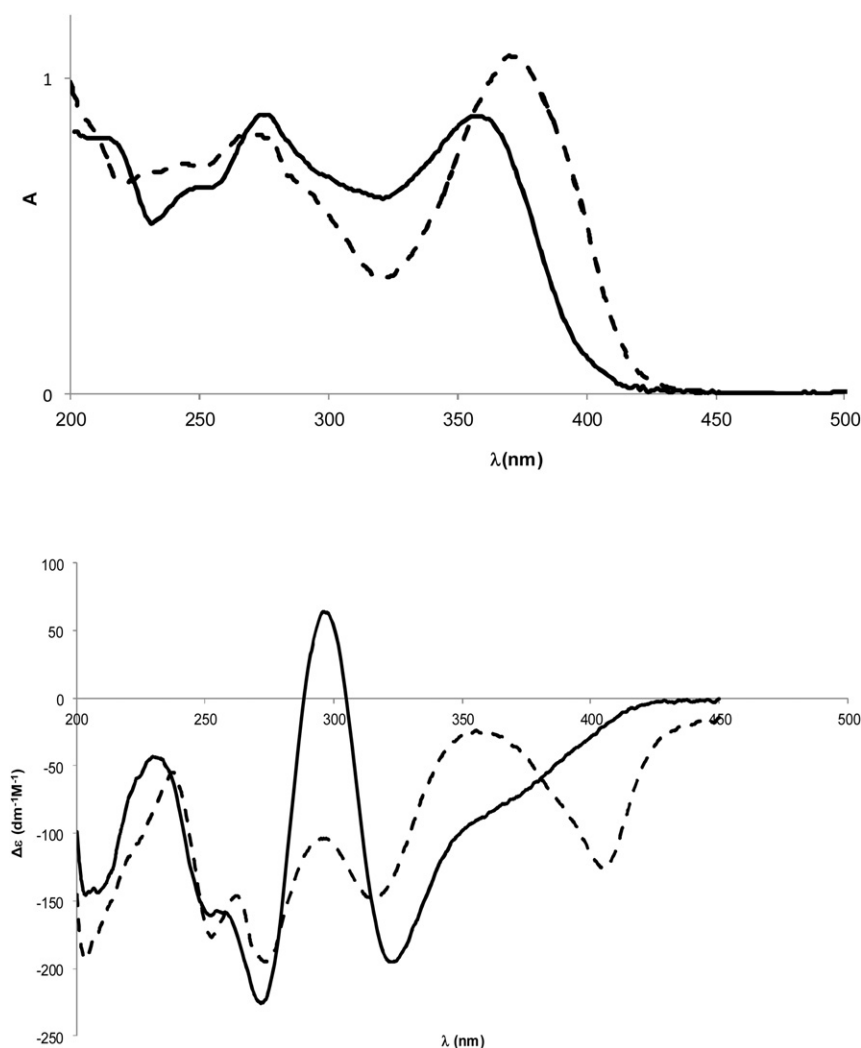


Fig. 6. (a) Uv–vis spectra of TC solutions (3.5×10^{-5} M) and (b) CD spectra of TC solutions (3.5×10^{-5} M) in absence (solid line) and in presence (dashed line) of Ca^{2+} (200:1 molar ratio) at pH 8.

conformers seems to play an important role in the pharmacokinetic properties of TC [2]. In the presence of metal ions, the adopted conformation depends on the type of ion. The binding to Mg^{2+} induces the twisted conformation whereas the binding to Ca^{2+} stabilizes the extended conformation [22]. According to Lambs et al. [21], there is chelation of one Ca^{2+} ion to the N4-O12a position and a second Ca^{2+} ion to the O12–O10 position, the chelation to N4–O12a being the responsible for the induction to the extended conformation.

Fig. 3 shows TC adsorption on Na^+ -montmorillonite and Ca^{2+} -montmorillonite at different pH. In both cases the adsorption is relatively high (around 75 mmol/100 g) at low pH, then decreases as pH increases and becomes negligible at pH higher than 10. At pH values ranging from 6 to 8.5 a higher TC retention on Ca^{2+} -montmorillonite is observed. Similar results were reported in the literature for the adsorption of TC on several clay minerals [8,30]. For the case of the natural montmorillonite the effects of pH were reported to be a consequence of the adsorption of TCH_3^+ , TCH_2^\pm and TCH^- species [12]. The cationic TCH_3^+ species interacts both electrostatically and non-electrostatically with the montmorillonite surface and adsorbs mainly by a cation exchange process. Even though the net charge of TCH_2^\pm and TCH^- is not positive, both species have the positively charged $-\text{NH}(\text{CH}_3)_2^+$ group that allows interaction with the negatively charged montmorillonite surface. Non-electrostatic interactions may be also present with these two species [12]. For the case of calcium montmorillonite, the same

kind of interactions was proposed together with an extra interaction, the formation of ternary montmorillonite- Ca^{2+} -TC complexes at the surface [13]. Cation exchange prevails at pH values below 5 and thus TC adsorption decreases by increasing calcium concentration; formation of montmorillonite- Ca^{2+} -TC complexes prevails from pH 5 to 9 leading to an increased TC adsorption by increasing calcium concentration [13].

Fig. 4 shows the XRD data of Na^+ -montmorillonite and Ca^{2+} -montmorillonite without and with adsorbed TC at pH 8 in 0.01 M NaCl. In the absence of TC, both solids show a pattern with only one broad 001 reflection that corresponds to basal spacings of 1.32 nm ($6.64^\circ 2\theta$) for Na^+ -montmorillonite and 1.38 nm ($6.44^\circ 2\theta$) for Ca^{2+} -montmorillonite. Ca^{2+} -montmorillonite has usually a basal spacing of around 1.50 nm, and in fact, a diffractogram (not shown) of this sample dispersed in water or in 0.01 M Ca^{2+} has a basal spacing of 1.54 nm [13]. However, since the data shown in Fig. 4 were obtained for Ca^{2+} -montmorillonite dispersed in 0.01 M NaCl, the presence of sodium ions makes that the basal spacing shifts to 1.38 nm. The observed spacing are the result of the stacking of montmorillonite layers with water molecules and inorganic counterions (Na^+ for Na^+ -montmorillonite and a mix of Na^+ and Ca^{2+} for Ca^{2+} -montmorillonite in 0.01 M NaCl) in the interlayer space and evidence the coexistence of two hydration states, with one and two intercalated water layers [31]. With adsorbed TC an extra 0 0 1 reflection appears in both cases, corresponding to basal spacings of

2.20 nm ($4.02^\circ 2\theta$) for Na^+ -montmorillonite and 2.18 nm ($4.07^\circ 2\theta$) for Ca^{2+} -montmorillonite. This extra 001 reflection is the result of the stacking of montmorillonite layers containing TC in the interlayer. The presence of two coexisting basal spacings in montmorillonite with adsorbed TC is usually found in the literature [7], and indicates an irregular stacking of montmorillonite layers [13]. Since the thickness of each 2:1 aluminosilicate layer in montmorillonite is about 0.96 nm [32], the interlayer spacing with intercalated TC results to be 1.24 nm for Na^+ -montmorillonite and 1.22 nm for Ca^{2+} -montmorillonite. According to the size of the TC molecule (length 1.29 nm, height 0.62 nm and width 0.75 nm for the twisted conformation) [33], these interlayer spacings are large enough to accommodate a monolayer of TC molecules together with some water molecules and counterions [12]. In summary, XRD data show that TC locates in the interlayer of both montmorillonite samples when adsorbed. This is so for the experimental conditions of Fig. 4, where adsorbed amounts were 37 mmol/100 g for Na^+ -montmorillonite and 62 mmol/100 g for Ca^{2+} -montmorillonite. It is believed that this kind of intercalation also occurs for an adsorbed amount of 6.08 mmol/100 g, which is the loading explored in CD experiments. Unfortunately, XRD is not sensitive enough to detect TC intercalation at such adsorption level, as shown by Chang et al. [17], who needed TC adsorptions of 25 and 34 mmol/100 g to detect intercalation in their SWy-2 and SAz-1 montmorillonite samples.

Fig. 5 shows the Uv–vis and the CD spectra of TC solutions at several pH values (6–10.5) in absence of Ca^{2+} . The effects of pH are already known [2] and are used here to recall the conformation changes that TC adopts. The Uv–vis spectra exhibits three main maxima at around 214, 271 and 368 nm (these given values are the exact values for the spectrum at pH 8). This light absorption is the result of the presence of two chromophoric systems which are separated from each other by the C12a–C4a bond: the tricarbonyl system of ring A that contributes to the 271 nm band, and the BCD ring system that contributes to all three absorption maxima [34]. The increase in the pH produces spectral changes, more notably in the band of the visible region that suffers a bathochromic shift from 358 to 383 nm by changing the pH from 6 to 10.5. This is the result of deprotonation reactions that lead to the formation of TCH^- and TC^{2-} species.

The CD spectra under the same conditions show that TC exhibits two main negative bands at 273 nm and 324 nm, and a positive band at 298 nm. The spectra are very similar to those previously reported by Mitscher et al. [35] and Lambs et al. [21]. The most evident effect of pH is observed in the band at 273 nm, corresponding to the 2-acetamide-1,3-dioxo function of ring A of the molecule. Increasing pH produces a decrease of CD signal intensity. The strong negative band at 273 nm at pH 6 is characteristic of the twisted conformation [21], and the decrease in the signal intensity as pH increases is indicative of a change from twisted to extended conformation [21,35]. TC adopts the extended conformation at high pH due to the deprotonation of the dimethylamino nitrogen (N4) that allows for hydrogen bonding between OH12a and N4 [22].

The Uv–vis and CD absorption spectra of TC at pH 8 and at two extreme situations of calcium concentration (absence and presence of calcium at a Ca:TC ratio 200:1) are shown in Fig. 6. The results exemplify the effects of Ca^{2+} on the spectrum of TC; very similar results were found at most of the other investigated pH (see below). The Uv–vis spectrum exhibits the three maxima at 214, 271 and 368 nm already mentioned. The presence of Ca^{2+} produces spectral changes that are very similar to those produced by increasing pH, with an important bathochromic shift of the long-wavelength absorption band from 368 nm to 373 nm. The CD spectra under the same conditions (Fig. 6b) show that the presence of Ca^{2+} produces a decrease in the intensity of the signal at 273 nm and a new and well evident negative signal, called Cotton effect, at 400 nm. This

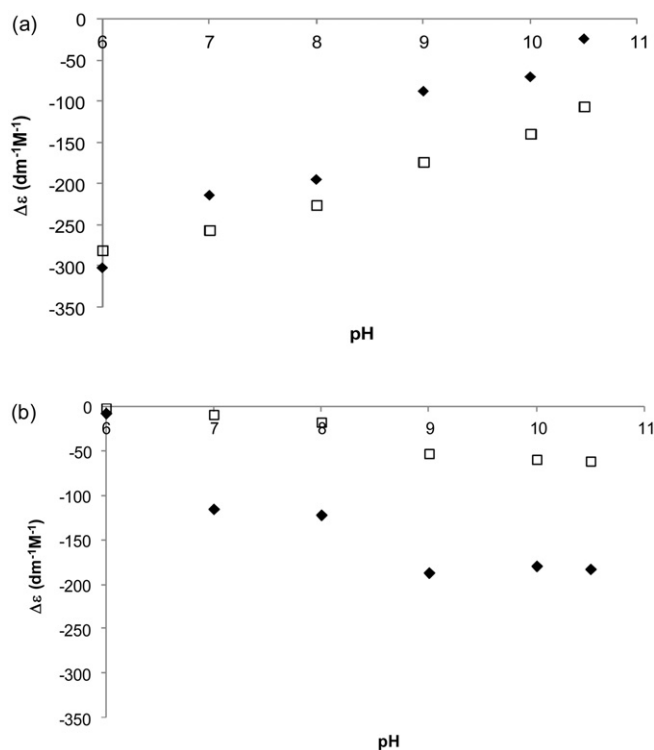


Fig. 7. (a) Effect of pH on $\Delta\epsilon$ of TC solutions (3.5×10^{-5} M) corresponding to the bands at 273 nm (A chromophore), and (b) at 400 nm (BCD chromophore) in the presence (◇) and the absence of Ca^{2+} (□).

Cotton effect in the visible region has been also reported by other authors [20–22,35].

Wessels et al. [22] conducted a relevant study about the binding of TC to Ca^{2+} at pH 8 through Uv–vis absorption, CD, fluorescence emission, and time-of-flight secondary-ion-mass spectroscopy (TOF–SIMS). Their conclusions are in agreement with the results shown above and can explain the effects of calcium on the Uv–vis and CD spectra of TC. They proposed that a 2:1 metal:ligand complex is formed at high Ca^{2+} concentration (Ca:TC $\geq 15.8:1$), which is in line with the presence of the $\text{Ca}_2\text{TC}^{2+}$ species that dominates the speciation (Fig. 1d). The BCD ring system, responsible for the absorption in the visible region, is the first chelation site. Due to this chelation, N4 of the quaternary amino group deprotonates and hence induces TC to adopt the extended conformation resulting in less negative values of $\Delta\epsilon$ at 273 nm (Fig. 6b). In the extended conformation, O12 and O1 are in the same plane and become available as a coordination site for the second calcium ion, leading to the occurrence of the negative Cotton effect at 400 nm (Fig. 6b). In a brief form, the decrease in the intensity of the signal at 273 nm is produced because chelation with Ca^{2+} promotes the extended conformation of TC, and the occurrence of the Cotton effect at 400 nm is the result of a chelation involving both the BCD and A ring systems.

The effects of Ca^{2+} on $\Delta\epsilon$ at 273 nm and 400 nm at different studied pH are resumed in Fig. 7, where $\Delta\epsilon$ values for TC solutions in the absence and the presence of Ca^{2+} (200:1 Ca:TC ratio) are compared. Except for the case at pH 6, where $\Delta\epsilon$ at 273 nm is slightly more negative, $\Delta\epsilon$ is always less negative in the presence of Ca^{2+} (Fig. 7a). This shows that chelation with Ca^{2+} enhances the effects of increasing pH in promoting the extended conformation. Fig. 7b, on the other hand, shows that the negative Cotton effect at 400 nm appears at pH 7 and higher pH, indicating that the chelation to the BCD and A ring systems takes place at all these pH values. This chelation does not seem to occur at pH 6. Therefore, according to data in Fig. 7, the types of binding of TC to Ca^{2+} , which were

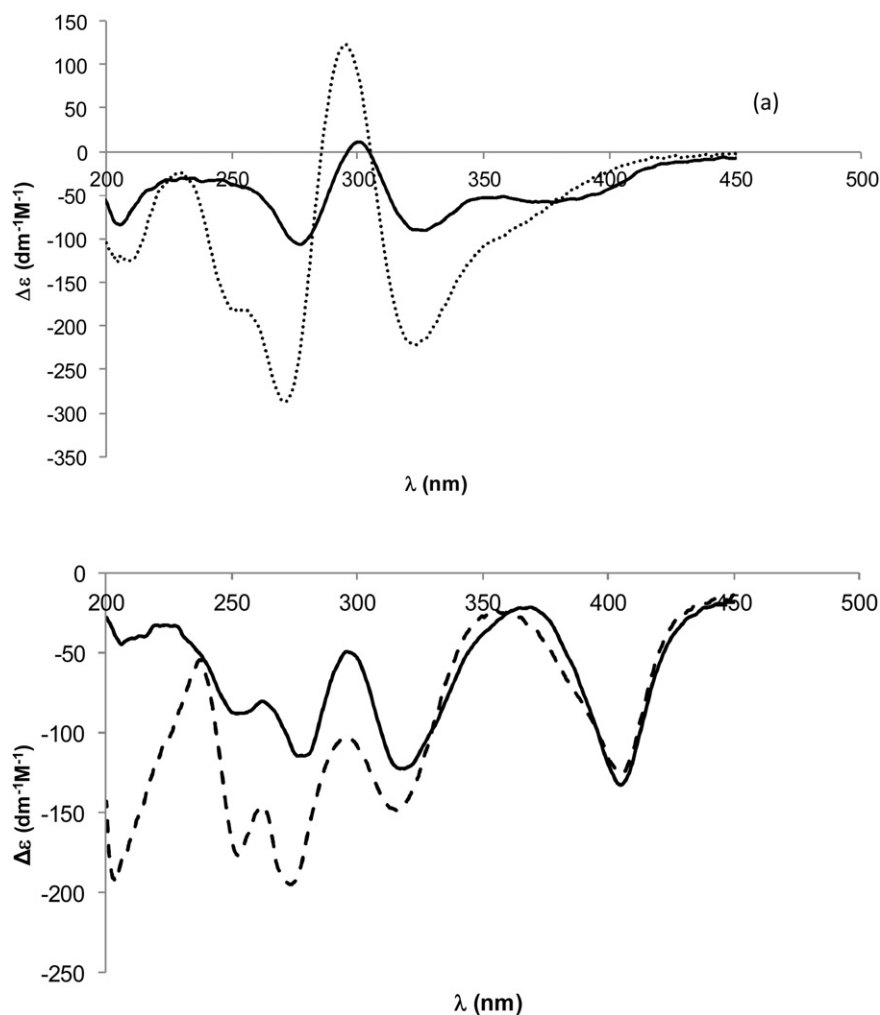


Fig. 8. CD spectra of 3.5×10^{-5} M TC at pH 8 for: (a) TC solution (dotted line) and TC-natural montmorillonite adsorption system (solid line); (b) TC solution with a Ca:TC molar ratio 200:1 (dashed line) and TC-calcium montmorillonite adsorption system (solid line).

deduced by Wessels et al. [22] from data at pH 8, occur in a rather ample range of pH (7 to 10.5).

Fig. 8 shows the effect of the presence of montmorillonite on the CD spectrum of TC at pH 8. Fig. 8a compares the spectra of TC solutions in the absence and the presence of Na^+ -montmorillonite, whereas Fig. 8b compares the spectra of TC solutions in the presence of Ca^{2+} (Ca:TC ratio 200:1) and in the presence of Ca^{2+} -montmorillonite (without extra calcium added). There is a substantial decrease in the negative Cotton effect at 273 nm when Na^+ -montmorillonite is present. The decrease is stronger than that produced by increasing the pH from 8 to 10.5 (Fig. 5) and much stronger than the produced by the presence of calcium at a Ca:TC ratio 200:1 (Fig. 6) at pH 8. Since 79 % of TC is adsorbed on the Na^+ -montmorillonite under the experimental conditions, the effects can be attributed to conformational changes of TC due to adsorption. Thus, adsorption is strongly promoting the adoption of the extended conformation, and this conformation prevails for TC molecules located between two 2:1 Na^+ -montmorillonite layers. It is not clear so far why adsorption is promoting the extended conformation. It is known that the Uv-vis spectrum of TC adsorbed on montmorillonite shows a bathochromic shift of the visible absorption band at 366 nm, indicating interaction of the BCD ring with the clay surface [12]. This interaction could induce TC to adopt the extended conformation, such as the interaction of the BCD ring with the first bonded calcium ion does.

Fig. 8b shows that in the presence of Ca^{2+} -montmorillonite the $\Delta\epsilon$ value at 273 nm is also rather low, indicating that TC prefers the extended conformation when it is located in the interlayer spacing. In this case, in addition, there is a strong Cotton effect at 400 nm, revealing the chelation of Ca^{2+} to the BCD and A ring systems. The effect is similar to that shown by the TC solution in a Ca:TC ratio 200:1. Under the experimental conditions of Fig. 8b, the Ca:TC ratio at the clay surface is 3.4:1, and thus the presence of ternary surface complexes of the type montmorillonite- Ca^{2+} -TC can be postulated. Although the exact interaction mode between the montmorillonite surface, calcium and TC cannot be unambiguously determined in this system, CD data gives some important information in this respect. It seems that TC is interacting with the montmorillonite surface through the BCD ring (as it occurs with Na^+ -montmorillonite) and with calcium through both the BCD and A ring systems. The first interaction probably facilitates the second interaction by bringing O12 and O1 to the same plane, groups that become available as a coordination site for calcium. The other coordination sites of this calcium ion in the interlayer would be surely occupied by interlayer water molecules or (better) some surface groups of the negatively charged montmorillonite surface.

Considering that montmorillonite is widely used as excipient in pharmaceuticals, and that antimicrobial activity and bioavailability of an antibiotic depend, among others, on its conformational equilibrium, the structural changes suffered by TC upon adsorption may have important implications on its pharmacological properties.

4. Conclusions

The adsorption of TC on Na⁺-montmorillonite and Ca²⁺-montmorillonite occurs in an ample range of pH. Although the adsorption vs. pH curves are rather similar in both cases, showing a decrease in TC adsorption as pH increases, Ca²⁺-montmorillonite is better adsorbent than Na⁺-montmorillonite in the pH range 6–8.5. The adsorption leads to intercalation of TC molecules between the 2:1 montmorillonite layers.

The spectropolarimetric results presented in this contribution provide evidence that adsorbed TC molecules adopts a more extended conformation than in solution under the same experimental conditions. The effects of adsorption are stronger than those produced by increasing the pH of the solution and much stronger than the produced by the presence of calcium at a Ca:TC ratio 200:1 at the same pH. The appearing of a new Cotton effect in the presence of Ca²⁺ as exchanging cation evidences chelation with this ion and the formation of ternary montmorillonite-Ca²⁺-TC complexes, being Ca²⁺ an active site for adsorption on Ca²⁺-montmorillonite.

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