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Amoxicillin Trihydrate Characterization and investigative adsorption using a Brazilian montmorillonite

Bruna de Lemos Novo[1,2], Fernanda Arruda Nogueira Gomes da Silva[3],
Luiz Carlos Bertolino[2], Lidia Yokoyama[1]

[1]Universidade Federal do Rio de Janeiro, Escola de Química. Avenida Athos da Silveira Ramos, 149, Bloco E, sala 206, Cidade Universitária, Rio de Janeiro, RJ, Brasil.

[2]Ministério da Ciência, Tecnologia e Inovações, Centro de Tecnologia Mineral. Avenida Pedro Calmon, 900, Cidade Universitária, Rio de Janeiro, RJ, Brasil.

[3]Universidade Federal do Rio de Janeiro, Instituto de Química. Avenida Athos da Silveira Ramos, 149, Bloco A, sala 630, Cidade Universitária, Rio de Janeiro, RJ, Brasil.

e-mail: brunalemosnovo@eq.ufrj.br, fnogueira@iq.ufrj.br, lcbertino@cetem.gov.br, lidia@eq.ufrj.br

ABSTRACT

The objective of this work was to investigate the adsorptive potential of a previously benefited and characterized brazilian montmorillonite sample, regarding amoxicillin trihydrate removal, a zwitterionic species, present in aqueous solutions. 500 mg capsule of antibiotic was properly characterized by XRD, FTIR, SEM/EDS and surface charge measurement through Zeta potential. Amoxicillin aqueous solution and supernatants from adsorption essays were characterized by Molecular Absorption Spectrophotometry in Ultraviolet-Visible Region (UV/VIS), from 200 to 400 nm. Three investigative essays were performed, varying montmorillonite mass (0.050, 0.525 and 1.000 g) and contact time between substances (10, 95 and 180 min). Amoxicillin sample is crystalline, morphologically prismatic, essentially composed of carbon, nitrogen, sulfur, hydrogen and oxygen. Its zwitterionic character was confirmed by Zeta potential, presenting positive (pH 2 to 4), relatively neutral (pH 4 to 6), and negative (above pH 6) surface charges. Test results indicated that the highest removals (21.4 and 41.8 %) were for the largest clay mineral masses (0.525 and 1.000 g, respectively), with pHs close to amoxicillin pka$_1$ (2.68). Larger clay mineral masses have more adsorbent sites, which, associated with components opposite charges in desired pH range, promote a greater interaction. Thus, brazilian montmorillonite can promote amoxicillin removal from aqueous solutions.

Keywords: Brazilian montmorillonite; Amoxicillin trihydrate; Adsorption; Investigation.

1. INTRODUCTION

Antibiotics are the most active chemotherapeutics among drugs; they exert their therapeutic effect by antagonizing the growth of bacteria [1]. Due to its variety of applications, both in human and veterinary medicine, it has been the most prescribed drug class worldwide. Data obtained by World Health Organization indicate that from 2016 to 2018, for every 1000 inhabitants, overall consumption of antibiotics ranged from approximately 4 to 64 daily doses. In Brazil, this consumption rate is 22.75, the highest among American countries with available data, with penicillin and beta-lactam antibactericides, being the most consumed antibiotics [2].

Nevertheless, with the intensification of consumption, problems related to its improper or illicit disposal in water bodies also arise, causing damage to the environment, fauna, and flora [3–8]. Conventional physical-chemical processes of water treatment (coagulation, flocculation, decanting, filtration, disinfection, chlorination and fluoridation) cannot promote the removal or degradation of these medicinal products, mainly due to their recalcitrance and low concentrations with which they are found an aquatic environment. Thus, several studies have been conducted about removing these contaminants from aqueous effluents [9–13].

Advanced technologies such as Advanced Oxidative Processes (AOPs) and adsorption by activated carbon has been pointed out as the most efficient processes regarding these pollutants removal [3,14–21]. However, although efficient, these processes present limitations with their cost, making their large-scale application unfeasible. Therefore, it emerges as an economically viable alternative, clay minerals use as adsorbents [14,22–28].

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Clay minerals are a diverse group of hydrous layer aluminosilicates that constitute the greater part of phyllosilicate family. Their small size and large ratio of surface area to volume give clay minerals a set of unique properties, including high cation exchange capacities, catalytic properties, and plastic behavior when moist [29]. They are classified based on similarities in chemical composition and crystalline structure, which can be divided into groups such as 1:1 type layer (kaolinite), 1:1 type tube (halloysite), 2:1 type layer (montmorillonite, smectite, vermiculite), the regular mixed layer type (chlorite group) and 2:1 type layer-chain type (attapulgite, sepiolite) [30]. Among clay minerals, montmorillonite is highlighted because it consists of the most abundant mineral present in bentonite rocks. Due to its high surface area, mesoporosity and negative surface charge, [31] it has been studied as a drug adsorbent, [13,32–34] mainly in the removal of antibiotics such as amoxicillin [14,22,23,27].

Amoxicillin is a broad-spectrum antibacterial compound with a molecular structure consisting of a lactam ring group), which has been widely used for food-producing animals and human beings [35]. It is a zwitterionic species, presenting both anionic and cationic functional groups. This is due to ionization of carboxylic acid and amine functional groups present in its chemical structure, generating the ionic species COO^- and NH^{3+}, in addition to an ionizable phenolic structure, consequence of its three dissociation constants ($pka_1 = 2.68$, $pka_2 = 7.49$ and $pka_3 = 9.63$) [11,36,37]. Thus, total electrical load, due to groups ionization, is pH dependent.

According to Homsirikamol et al. [37], amoxicillin is positive up to $pH = pKa_1$; charged both positively and negatively (zwitterion species) at $pKa_1 < pH < pKa_2$ and negative at $pH > pKa_2$. Such information is useful concerning this antibiotic removal by adsorptive process, since bentonite surface negative charge − due to its isomorphous substitution of Al^{3+} for Si^{4+} in tetrahedral layer and Mg^{2+} for Al^{3+} in octahedral sheet −, can attract amoxicillin molecule which has a positive charge from protonation of amine groups [14], through an electrostatic interaction, as described by de Oliveira et al. [19] as being the main driving force for pharmaceuticals adsorption.

Thus, the objective of this work was to characterize and evaluate the best conditions for amoxicillin trihydrate removal in aqueous solution using a previously ore-dressed and characterized montmorillonite from Cubati and Pedra Lavrada region – Paraíba, Brazil, [31] through investigative batch adsorption tests by Molecular Absorption Spectrophotometry in Ultraviolet-Visible Region (UV/VIS). Brazilian smectites are widely used in local commerce for craft purposes, ensuring the livelihood of the regional population. Most of them usually presents impurities, requiring specific treatments to make them industrially attractive, allowing them a more noble application, as proposed in this work. As far as the authors are concerned, this is the first study to evaluate amoxicillin adsorptive behavior with a clay mineral by UV/VIS technique.

2. MATERIALS AND METHODS

2.1. Amoxicillin characterization

The contents of 500 mg amoxicillin trihydrate capsule were macerated in gral and pistil up to granulometry below 100 µm (checked by same opening sieve) and then characterized by X-Ray Diffraction (XRD), Infrared spectroscopy with Fourier transform (FTIR), Scanning Electron Microscope coupled with Energy Dispersive Spectroscopy (SEM/EDS), and surface charge measurements by means of Zeta potential, as described in detail below, according to the procedure adopted in each laboratory responsible for the analysis.

X-ray diffractogram was obtained using the powder method on Bruker D4 Endeavor diffractometer with CoKα radiation ($\lambda = 1.78897$ Å), at Technological Characterization Sector laboratory of CETEM, operated with 40 kV and 40 mA, goniometer speed of 0.01 (2θ) per step with time 1 second count. The analysis interval was from 4 to 80 (2θ), with a detector LynxEye position. Qualitative spectrum interpretations were performed by comparison standards contained in the PDF4+ database with Bruker Diffrac EVA software. The interplanar distance conversion from 2θ (degrees) to d (Å) was carried out.

Infrared spectroscopy with Fourier transform (FTIR) was performed on Nicolet 6700 FT-IR spectrophotometer, with records from 4000 to 400 cm^{-1} (medium infrared), 4 cm^{-1} resolution, in KBr tablets, at Instruments and Research Laboratory of UFRJ Institute of Chemistry.

Scanning Electron Microscope (SEM) analyses were performed in Fei Quanta 400 equipment, at Technological Characterization Sector laboratory of CETEM. Samples were covered with carbon in Sputter Coater BAL-TEC equipment SCD 005/CEA 035 model and analyzed in high vacuum in secondary electron modules, backscattered electrons and Energy Dispersive Spectroscopy (EDS) for qualitative determination of chemical elements.

Determination of surface charge through Zeta potential was performed in Zetasizer Nano ZS equipment, at CETEM Surface and Nanometric Analysis Laboratory. 10 mL of potassium chloride electrolyte (KCl) 10^{-3} mol L^{-1} were added to approximately 0.02 g of amoxicillin trihydrate sample. pH adjustment was done manually using hydrochloric acid solution (HCl) 1.0 mol L^{-1}, in order to obtain pH equivalent to 1.5. For pH automatic adjustment in the equipment, 0.1 and 0.5 mol L^{-1} of KOH and 0.1 mol L^{-1} of HCl solutions were used, to promote an initially acidic assay towards alkaline (pH of 2 to 12).

2.2. Preparation and characterization of amoxicillin aqueous solution

Amoxicillin aqueous solution was prepared using 500 mg capsule of standard amoxicillin trihydrate provided by the National Institute of Health Quality Control of Oswaldo Cruz Foundation (INCQS-FIOCRUZ). Sample preparation followed the procedures described by Brazilian Pharmacopoeia [38], obtaining a solution-stock concentration equal to 500 mg L^{-1}. In addition to the official preparation, named Pharmacopoeia Amoxicillin (PA) manually antibiotic dissolution was also tested for comparative purposes, named Manual Amoxicillin (MA), dissolving capsule contents in 400 mL of deionized water, and subsequently transferred to a 1.0 L volumetric balloon.

Analytical method used to determine amoxicillin in an aqueous solution was Molecular Absorption Spectrophotometry in Ultraviolet-Visible Region (UV/VIS). UV/VIS spectra were analyzed between the range of 200 to 400 nm wavelength, at intervals of 2 nm, with quartz bucket of 10 mm, in Thermo Scientific™ GENESYS 150 UV-Visible Spectrophotometer. According to Brazilian Pharmacopoeia [38], characteristic band of amoxicillin occurs at a wavelength of 272 nm, which was evaluated in all generated spectra. For antibiotic studied, preparation form of synthetic amoxicillin solution; conditioning form (at room temperature or refrigerated at 4ºC), time required for its degradation (1, 4, 12, 14 and 23 days) at different conditionings forms and different concentrations (10, 50 and 100 mg L^{-1}) were evaluated.

2.3. Adsorption investigative trials

Adsorption tests were performed using Brazilian montmorillonite previously ore dressed and characterized [31]. Amoxicillin adsorption investigation essays followed a 2^2 experimental planning, with triplicates at the central point. For each variable, maximum, medium and minimum values were stipulated to determine the best design conditions (being 0.050, 0.525 and 1.000 g of montmorillonite mass and 10, 95 and 180 min of contact time, respectively), with a total of 19 experiments (since in essay 3 the lowest clay mineral mass was not used, see explanation in Section 3.2 – essay 3). In addition, different concentrations of HCl were used to adjust solutions pH, according to experimental observation of the test. All experimental conditions are presented in Table 1.

Tests were performed using 50 mL Falcon Tubes, where 5 or 10 mL of 500 mg L^{-1} stock solution were arranged (depending on the final concentration desired, see Table 1) previously prepared with the respective montmorillonite masses. pH adjustment was performed with HCl 1 mol L^{-1} or 6 mol L^{-1} disposable pipette drip under 40 mL of deionized water, and after adjustment, the final volume obtained was 50 mL. Preparation time for all trials was approximately 10 min for each sample.

Prepared solutions were submitted to mechanical agitation in NT 714 Refrigerated Floor Shaker Incubator of Nova Técnica, at 27°C, with rotation of 150 r.p.m. by the pre-established times and centrifugated in eppendorf centrifuge 5810 equipment, at 27ºC, with rotation of 4000 r.p.m. for 5 minutes, at CETEM Bioprocess Laboratory. Then, all samples were filtered on 80 g qualitative filter paper at glass funnel for further supernatant analysis in UV/VIS equipment.

After bands and absorbances at 272 nm wavelengths analysis, it was possible to calculate the removal percentage of the drug. This calculation is represented in Equation 1.

$$\text{Removal (\%)} = \frac{\text{Initial}_{\text{abs}} - \text{Final}_{\text{abs}}}{\text{Initial}_{\text{abs}}} \quad (1)$$

Where $\text{Initial}_{\text{abs}}$ is the absorbance, in 272 nm, before adsorption and $\text{Final}_{\text{abs}}$ is the absorbance, in 272 nm, after adsorption.

Table 1: Experimental conditions of adsorption investigative essays**.**

TRIALS	MASSES (g)	AMOXICILLIN CONCENTRATION (mg L^{-1})	pH ADJUSTMENT	CONTACT TIME (min)
1	0.050/0.525/1.000	100	HCl 6 mol L^{-1}	10/95/180
2	0.050/0.525/1.000	50	HCl 1 mol L^{-1}	10/95/180
3	0.525/1.000	50	HCl 1 mol L^{-1}	10/95/180

3. RESULTS AND DISCUSSION

3.1. Amoxicillin characterization

Figure 1 presents XRD (a), FTIR (b), UV/VIS specters (c) and Zeta potential curve (d) of encapsulated amoxicillin trihydrate sample. Diffractogram indicated that this antibiotic is crystalline, showing characteristic peaks of the studied drug [39].

Infrared spectrum of amoxicillin trihydrate sample presented bands between 3525 and 557 cm^{-1}. According to Min et al. [40], two small bands at 3525 and 3461 cm^{-1} refer to crystallisation water (-OH stretch) and amide (-NH stretching), respectively, followed by bands at 3178 and 3039 cm^{-1}, characteristic of phenol (-OH stretching) and aromatic (-CH stretching), respectively. Prominent characteristic bands between 1800 and 1650 cm^{-1} (1776 and 1687 cm^{-1}) indicate carbonyl (-C=O) in beta-lactam ring and amide, respectively. 1581 cm^{-1} band is attributed to carboxylate asymmetric stretching. According to Zha et al. [22], bands in 1484 and 1120 cm^{-1} are attributed to N-C stretches of primary amine and O-C, respectively. Multiple bands in the region of 1150 and 950 cm^{-1} represented typical in-plane CH bending vibrations of aromatic compounds. No more information about the other bands was found in literature.

By UV/VIS specters, amoxicillin trihydrate sample has two characteristic bands: one at 228 nm and the other at 272 nm, [12,16,17,38] being the last one used to amoxicillin characterization [38]. By specters analysis, preparation form does not interfere in antibiotic characterization. Therefore, manual preparation (MA) of amoxicillin trihydrate sample was used in adsorption tests.

It is possible to observe by Zeta potential curve (mV) as a function of pH range (2 to 12) that amoxicillin trihydrate presents three different charges: positive between approximately pH 2 and 4, relatively neutral between pH 4 and 6 and negative between pH 6 and 12. These results are in accordance with Homsirikamol et al. [37], indicating that this variation in surface charges is related to ionizable species present in amoxicillin

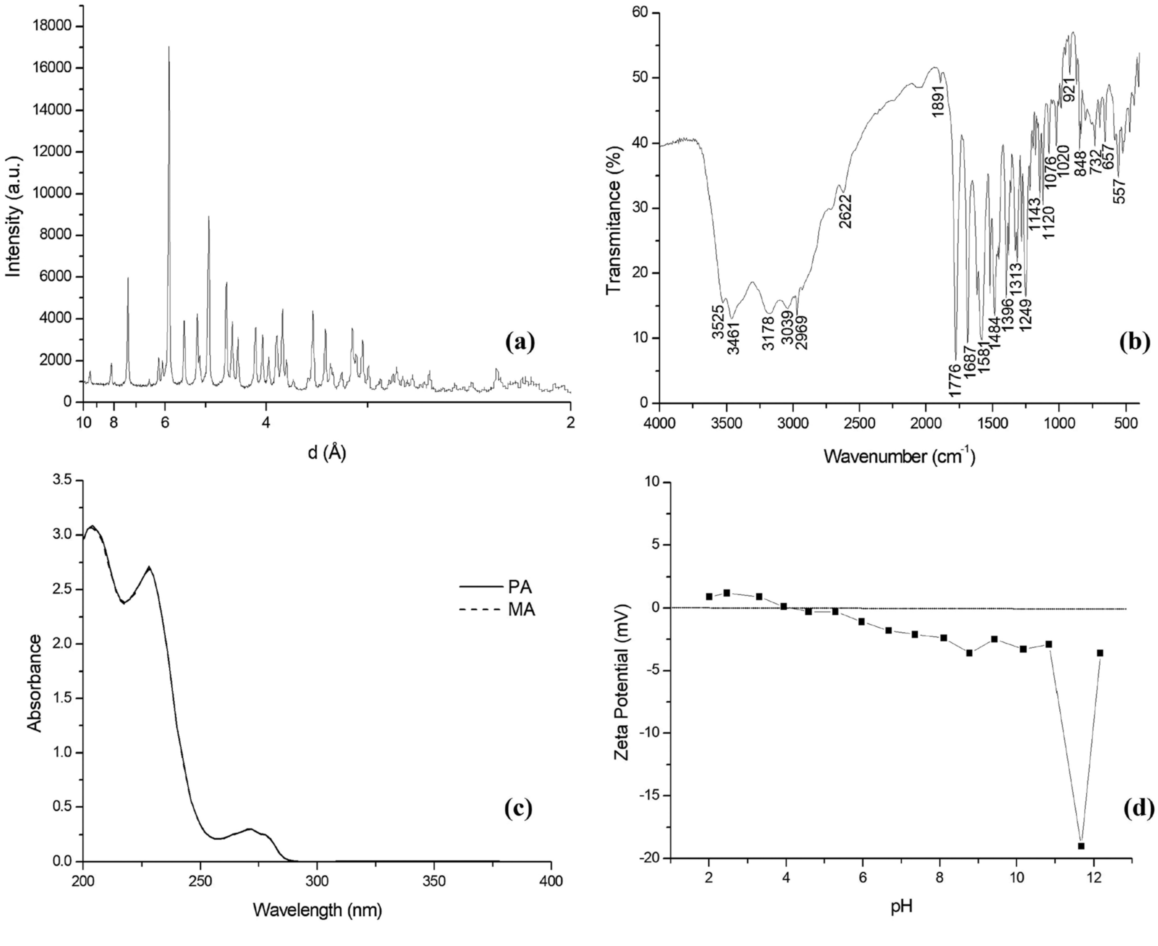

Figure 1: XRD (a); FTIR (b); UV/VIS specters (c) and Surface charge measurement (Zeta potential) (d) of amoxicillin trihydrate sample.

structure. Thus, positive surface charge is due to NH_3^+ species majority presence, followed by NH_3^+ and COO^- species simultaneous presence in neutral range (zwitterionic region) and COO^- increase and $C_6H_5O^-$ species with solution alkalization. These results allow us to understand more about the pH range in which we should act in adsorptive processes with montmorillonite as an adsorbent.

Montmorillonite sample presents negative surface charge in practically the entire pH range studied [31]. Therefore, the favorable range for adsorption of this drug is between pH 2 and 4, in which amoxicillin is positively charged and montmorillonite negatively charged, since cation exchange and eletrostatic interaction seems to be the main adsorption mechanisms between clay minerals and antibiotics [19,25,33]. According to Putra et al. [14], protonation for both functional groups of bentonite and amoxicillin, at low pH values, increases clay minerals adsorption capacity. Thus, acidification of the medium becomes necessary to achieve the highest removal efficiency.

Amoxicillin trihydrate sample Scanning Electron Microscopy image, Figure 2 (a), presented agglomerated crystals with a prismatic aspect, which is in accordance with what was observed by Feng et al. [41]. EDS, as shown in Figure 2 (b), presented its main constituent elements: silicon (Si), carbon (C), nitrogen (N) and oxygen (O), elements that are present in its chemical structure. The presence of magnesium (Mg) is related to magnesium stearate excipient, used as a lubricant in capsule production.

To assess the best form of packaging, samples were evaluated at both room temperature and refrigerated at approximately 4°C after 24 h of preparation. It was observed that there is no significant difference in packaging. However, it is known that the drug has a time of degradation, and this time has also been studied. Amoxicillin behavior packed at room temperature and under refrigeration was evaluated for 4, 12, 14 and 23 days. Absorbances in 272 nm increased over time, indicating drug degradation. Moreover, according to Table 2, it is observed that, when packed at room temperature, amoxicillin degrades more rapidly when compared to its packaging under refrigeration. This fact is due to substance intrinsic photodegradation [42]. Therefore, the best use conditions for amoxicillin solution are under refrigeration for up to 14 days.

In addition to previous characterizations, it was necessary to study the antibiotic of interest in different concentrations to evaluate how it interferes in absorbance. Thus, drug solutions of 10, 50 and 100 mg L^{-1} were prepared and submitted to UV/VIS analysis. According to Table 3, there is an increase in absorbance with

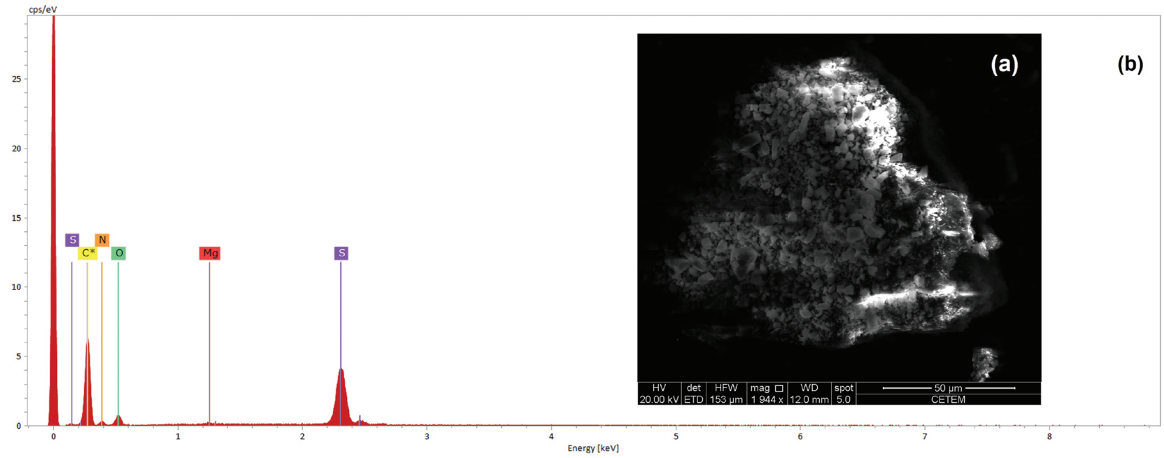

Figure 2: SEM (a) and EDS (b) of amoxicillin trihydrate sample.

Table 2: Absorbances at 272 nm of 100 mg L^{-1} amoxicillin samples, at room temperature and under refrigeration over 1, 4, 12, 14 and 23 days.

	ABSORBANCES AT 272 nm	
TIME (DAYS)	ROOM TEMPERATURE	UNDER REFRIGERATION
1	0.304	0.299
4	0.311	0.309
12	0.345	0.306
14	0.348	0.300
23	0.477	1.568

Table 3: Absorbances at 272 nm of amoxicillin samples at concentrations of 100, 50 and 10 mg L^{-1}.

SAMPLE	ABSORBANCE AT 272 nm
100 mg L^{-1} Amox	0.330
50 mg L^{-1} Amox	0.186
10 mg L^{-1} Amox	0.067

increased concentration. Thus, concentrations of 50 and 100 mg L^{-1} were chosen, since 10 mg L^{-1} presented a low absorbance value in the wavelength of interest, limiting effects observation caused by an interaction between drug and clay mineral.

3.2. Adsorption investigative trials

Adsorption investigative essays aimed to better understand the interaction, via adsorptive process, between montmorillonite and amoxicillin. Several trials were carried out, as described in Table 1, and the main results for each one of them are presented below. Abbreviations "A", "MWA" and "MWW" refers to amoxicillin, montmorillonite with amoxicillin and montmorillonite with water, respectively.

3.2.1. Essay 1

Figure 3 shows essay 1 experiments specters (Table 1). Adsorption essays absorbances showed higher values when compared to amoxicillin pattern sample (0.323), presenting an opposite behavior to that expected in terms of contaminants removal by UV/VIS monitoring reported in literature [43,44]. This fact suggests that there was the formation of some species, originated from the contact between montmorillonite and amoxicillin, causing an absorbance increase in drug characteristic wavelength.

Except for experiments with 0.05 g of montmorillonite, all essays remained with pH below 3.0, which would not be a problem since they remain below pH 4, where there is amoxicillin cationic species predominance, favoring adsorption. During the experiment, bubbles were observed both during pH adjustment and supernatant even after centrifugation and filtration. This is due to dolomite presence in clay minerals, as described by Novo et al. [31], which consists of calcium and magnesium carbonate. When acidify with HCl, there is the formation of magnesium and calcium chloride ($MgCl_2$ and $CaCl_2$, respectively), carbon dioxide (CO_2) and water (H_2O), as described in Equation 2.

$$CaMg(CO_3)_2\,(s) + 4\,HCl\,(aq) \rightarrow MgCl_2\,(aq) + CaCl_2\,(aq) + 2\,CO_2\,(aq/g) + 2\,H_2O \qquad (2)$$

Thus, with acidification, dolomite decarbonation occurs, forming carbon dioxide and water, which explains the presence of observed bubbles. Latter two react forming carbonic acid (H_2CO_3), promoting an even greater decrease in medium pH. This fact explains, therefore, pH value falls of most experiments. On the other

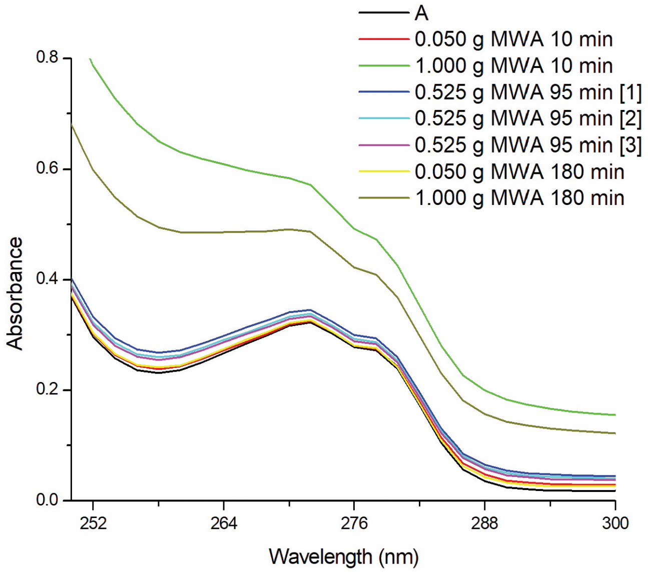

Figure 3: UV/VIS specters of supernatants at 272 nm using 100 mg L^{-1} amoxicillin solution and 0.050, 0.525 and 1.000 g of montmorillonite, under different contact times (10, 95 and 180 min), with pH below 4.0.

hand, experiments with 0.05 g did not present this behavior, probably due to the lower mass of clay mineral used, which has a smaller amount of dolomite and is not so affected by acidification.

As supernatants absorbances after adsorption were higher than standard amoxicillin solution absorbance, it was not possible to calculate drug removal percentage. Therefore, in order to investigate and understand this effect, another essay was carried out with less concentrated HCl (1 mol L^{-1}) for pH adjustment and with 50 mg L^{-1} amoxicillin solution, in order to observe whether a lower drug concentration would present a different spectrum in terms of adsorption. Furthermore, it was investigated whether contact time or montmorillonite solution had significant absorbances at 272 nm, aiming to eliminate possible causes of absorbance increase.

3.2.2. Essay 2

In order to investigate whether contact time or montmorillonite masses influences on amoxicillin absorbance at 272 nm, experiments were performed with amoxicillin trihydrate solution under 10, 95 and 180 min, Figure 4 (a), and with 0.050, 0.525 and 1.000 g of montmorillonite in water, Figure 4 (b), with pH adjusted to less than 4.0. It is possible to observe, in Figure 4 (a), that stirring time does not interfere in absorbance obtained at 272 nm, since generated curves are similar. By Figure 4 (b) it is possible to observe that clay mineral absorbance at 272 nm (0.035 average) had a low value compared to amoxicillin solution (0.160 average). Therefore, there is no interference in amoxicillin measurement at this wavelength. In this way, both contact time and clay mineral presence are discarded as responsible for absorbances increase after adsorption.

Figure 5 shows the spectra obtained from essay 2 experiments (Table 1). As observed in essay 1 (Figure 3), absorbances after adsorption process were higher than amoxicillin standard absorbance, reinforcing that there is

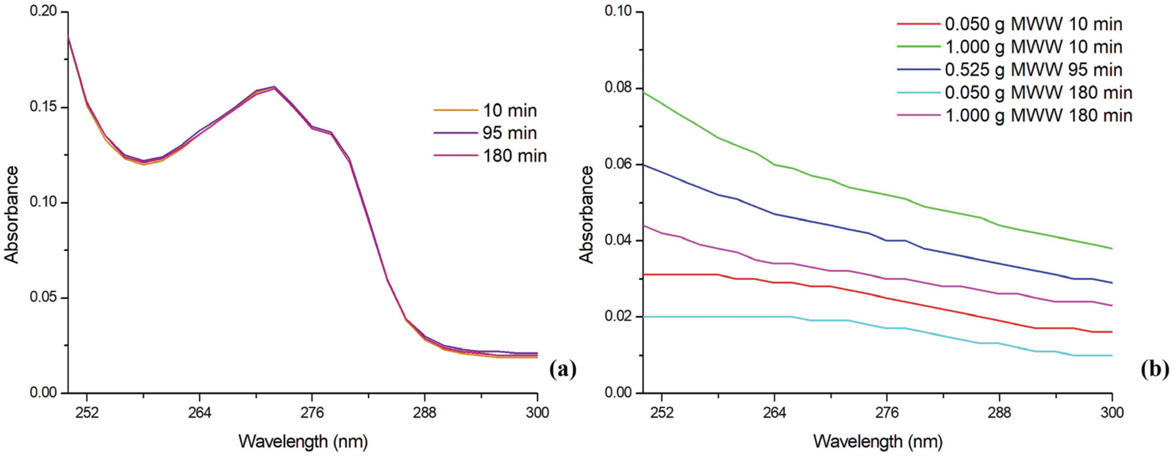

Figure 4: UV/VIS specters at 272 nm of 50 mg L^{-1} amoxicillin solution, under 10, 95 and 180 min of contact time (a) and of 0.050, 0.525 and 1.000 g of montmorillonite with water under 10, 95 and 180 min of contact time (b), all experiments with pH below 4.0.

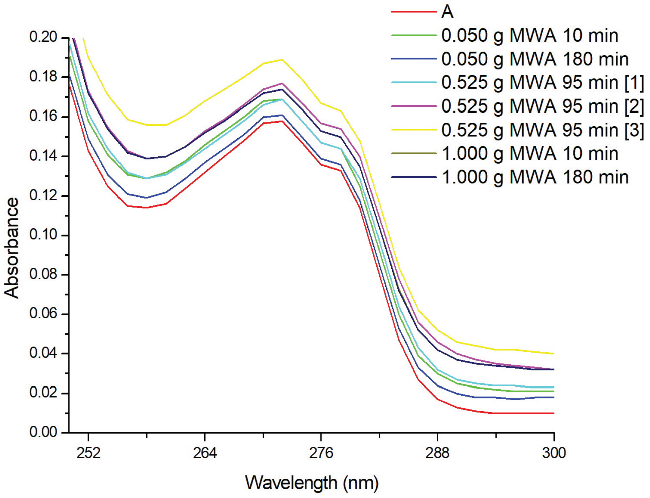

Figure 5: UV/VIS specters of supernatants at 272 nm using 50 mg L^{-1} amoxicillin solution and 0.050, 0.525 and 1.000 g of montmorillonite, under 10, 95 and 180 min of contact time, with pH below 4.0.

some interaction between montmorillonite and amoxicillin capable of forming an intermediate substance, and it is not possible to assess contaminant removal percentage by clay mineral. In addition, due to carbonated mineral presence, pH control became quite difficult, presenting disparities in central points absorbances, not making it possible to have an exact knowledge of what was happening during the process.

Since there were signs of formation of an intermediate substance, it was decided to measure absorbance after amoxicillin and montmorillonite mixture, subject only to preparation time (approximately 10 min), i.e., after system preparation for contact time, at essay 3.

3.2.3. Essay 3

In essay 3, same amoxicillin and HCl solutions concentrations (50 mg L^{-1} and 1 mol L^{-1}, respectively) from essay 2 were used. However, only 0.525 and 1.000 g of montmorillonite were used under preparation time and compared with samples under 10, 95 and 180 min of contact time from essay 2, since 0.050 g of clay mineral consisted of a very small mass to verify the effects. Figure 6 presents UV/VIS specters of the comparison between standard amoxicillin with samples under preparation time.

It was observed that there was a significant increase in absorbance in the initial minutes of contact at 272 nm of samples containing adsorbent and adsorbate. Therefore, this fact confirms the formation of some intermediate species, due to first contact between montmorillonite and amoxicillin. Similar behavior was observed by Hrioua et al. [45], by synthesizing complexes containing Cu (II), Zn (II) and Fe (III) with amoxicillin, aiming to improve antibacterial activity against *Escherichia. coli* bacteria. Thus, it is believed that, in addition to an electrostatic interaction due to constituents different surface charges, a chemical interaction may occur, such as the formation of a complex similar to that tested by [45], since montmorillonite has metals in its constitution (Mg, Fe and Al) that can interact with amoxicillin ionizable sites and can form metal-antibiotic complexes. More studies on this type of interaction should be carried out.

Besides that, it was observed that an increase in clay mineral mass causes an increase in absorbance. Logically, biggest clay mineral masses provides a greater number of particles that are available to interact with antibiotic, promoting more intense interactions, which is reflected in absorbance reading. Furthermore, the amount of dolomite can also influence and tests considering its previous removal must be done.

Figure 7 (a) presents the experiment performed with the comparison between 0.525 g of montmorillonite in contact with 50 mg L^{-1} amoxicillin standard solution, under 95 min of contact time with a sample under the same conditions, at preparation time. By spectrum analysis, samples submitted to contact time presented lower absorbances than the sample under preparation time (0.215), allowing amoxicillin percentage removal calculation by Equation 1.

Removals percentages obtained were 21.4, 17.7 and 12.1%, for experiments [1], [2] and [3], respectively, which presented absorbances values of 0.169, 0.177 and 0.189 respectively. Disparities in pH values were observed (2.7, 1.5 and 1.0 for central points [1], [2] and [3], respectively), which may be the main reason for

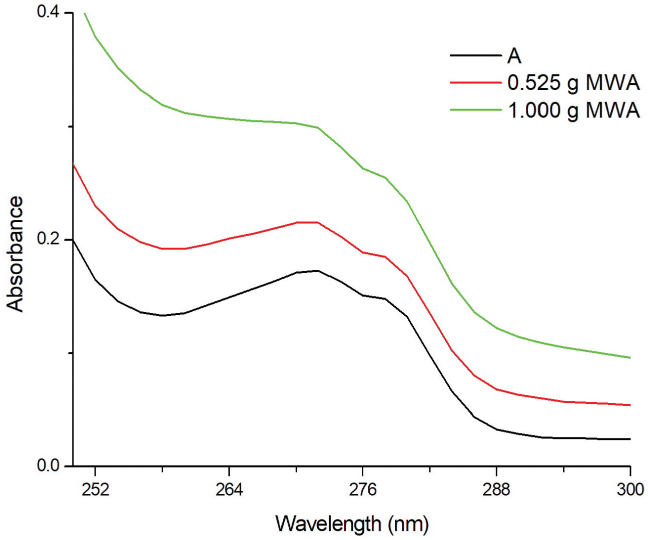

Figure 6: UV/VIS specters of supernatants at 272 nm using 50 mg L^{-1} amoxicillin solution in comparison with 0.525 and 1.000 g of montmorillonite with amoxicillin under preparation time, with pH below 4.0.

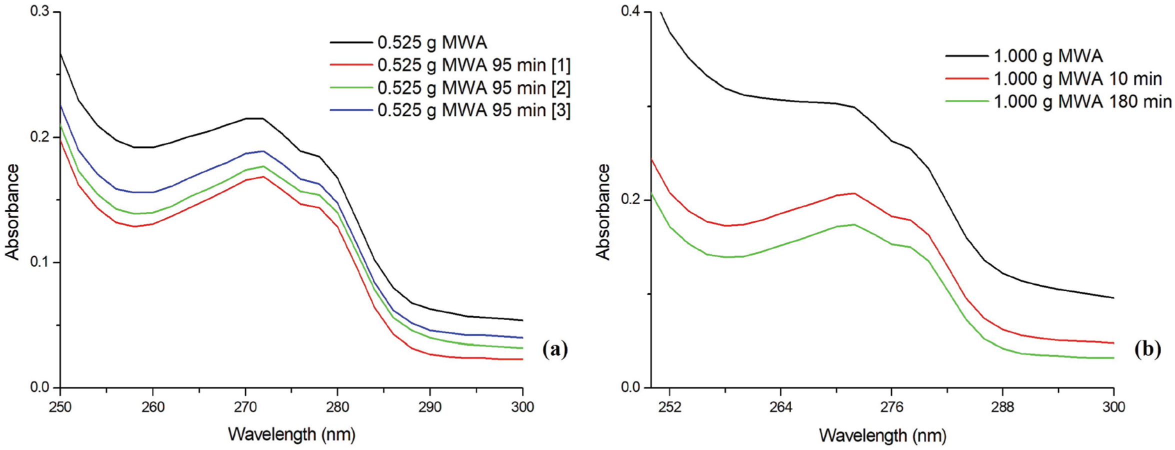

Figure 7: UV/VIS spectrum of supernatants at 272 nm using 50 mg L^{-1} amoxicillin solution in comparison with 0.525 g of montmorillonite with amoxicillin under preparation time (a) and with 1.000 g of montmorillonite with amoxicillin under preparation time (b), all experiments with pH below 4.0.

divergences in amoxicillin removal percentages. Central point [1] presented the highest removal (21.4%), the lowest absorbance (0.169) and the highest pH value (2.7), when compared to other values obtained. Therefore, for these results, it is noted that higher pHs promote lower absorbances and, consequently, greater removals.

Same behavior was found to the experiment performed with 1.000 g of montmorillonite in contact with 50 mg L^{-1} amoxicillin standard solution, under 10 and 180 min of contact time in comparison with the sample at preparation time under the same conditions, as shown in Figure 7 (b). According to Equation 1, 30.8 and 41.8% of amoxicillin removal were obtained for samples with a contact time of 10 min (pH 1.4, absorbance value of 0.207) and 180 min (pH 2.5, absorbance value of 0.174), respectively. Highest removal percentage was also for sample with higher pH (2.5) among those evaluated.

Analyzing the best results regarding drug removal, it is observed that the two best results (21.4% and 41.8%, at pH 2.7 and 2.5, respectively) were those that were close to amoxicillin pka_1 (2.68), in which there is the major presence of cationic species. Thus, the results obtained corroborate with literature [14,25,34], making pH control (especially above 2.0 and below 3.0) necessary.

In addition, it was observed that the increase in clay mineral mass (0.525 to 1.000 g) promoted greater removals (21.4 and 41.8%) and that, according to what had been discussed and presented in previous trials, longer stirring times do not promote a significant gain to the studied system, indicating that adsorption kinetics can be fast.

4. CONCLUSIONS

Regarding amoxicillin characterization, it was possible to verify that it is crystalline, featuring prismatic morphology, essentially composed by carbon, nitrogen, sulfur, hydrogen and oxygen, presenting both positive and negative surface charge in pH range studied, confirming its zwitterionic character. Moreover, by means of spectrophotometry in UV/VIS region, in a wavelength range of 200 to 400 nm, it presents absorbances at 228 and 272 nm. The latter is used to monitor changes that occurred when placed in contact with clay mineral.

Adsorption investigative trials indicated that when montmorillonite working sample was used, supernatants spectra presented absorbances higher than antibiotic standard solution spectrum, indicating an intermediate species supposed formation from contact between adsorbate and adsorbent. This fact was confirmed when tests were performed under preparation time (approximately 10 min), which showed, in the first moments, absorbance superior to standard amoxicillin absorbance. Adsorptive process observation and calculation was only possible, therefore, when tests under contact time (10, 95 and 180 min) were compared with system under preparation time, in which samples submitted to contact presented lower absorbances, indicating drug possible removal.

pH control and clay mineral masses showed themselves important variables for adsorptive process effectiveness, since higher removal percentages (21.4 and 41.8%) were for experiments with pH close to amoxicillin pka_1 (2.68) (2.7 and 2.5, respectively) and for largest montmorillonite masses (0.525 and 1.000 g). According to literature, the predominant adsorption mechanism between clay minerals and drugs is cation exchange, resulting from electrostatic interactions between amoxicillin positive charge and montmorillonite negative in this pH range. However, due to constituents structural complexity, chemical interactions may occur, since complexes between metals and amoxicillin can be formed, explaining absorbances increase and should, therefore, be investigated.

In any case, further studies should be carried out with decarbonated clay mineral samples, in order to verify system behavior in the absence of dolomite, since its presence seems to interfere not only on pH control, but also in absorbances reading. Thus, adsorptive potential of brazilian montmorillonite was confirmed, since with proper treatment, it can promote amoxicillin removal from aqueous solutions.

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