EVALUATING THE SORPTION OF THE ANTIMICROBIAL DANOFLOXACION IN SOILS, CLAYS AND HUMIC ACIDS

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ABSTRACT

Large-scale animal production has been responsible for the introduction of fluoroquinolones in the environment and the occurrence of adverse effects in non-target organisms. Under this scenario, this study evaluated the sorption of danofloxacin (DAN) in a Brazilian sandy (N1) and clay soil (S2) and in their isolated humic acids (HAs) and clays. The drug presented higher sorption on S2 with Freundlich coefficient pH-dependent ranging from 85 to 676 $\mu g^{1-1/n}$ $(mL)^{1/n}$ g^{-1} on soils. Distribution coefficients (Kd) showed the order: humic acids > clays > soils for N1 and S2. The Kd of N1 HA (less humificated and richer in oxygenated groups) was higher than S2 HA, but S2 clay (with higher content of oxides of Fe and Al) sorbed more DAN than N1 clay. The DRX data pointed a sorption process restricted to the external surfaces of kaolinite. After the sorption of DAN, the FTIR of HAs presented a reduction of signals of COO^-, $C=O$ and OH^- groups, suggesting the contribution of ionic interactions and hydrogen bonding for sorption. The cation exchange mechanism was supported by the dependence of the ionic strength and nature of the exchangeable cation for the sorption of DAN. Also, the FTIR of sorbed DAN on S2 clay indicated the contribution of Fe and Al oxides in additional interactions with the molecule.

Keywords: Brazilian soils, clays, danofloxacin, humic acids, sorption

1. INTRODUCTION

Dispersion of veterinary antimicrobials is a matter of global concern, as they lead to contamination of soils, sediments and water bodies, the bioaccumulation and adverse effects in non-target organisms, the appearance of resistant bacteria and genes and the transfer of pharmaceuticals inter species through the food web, as previously ascertained (Blanco et al., 2017; Carvalho and Santos, 2016; Kuppusamy et al., 2018; Pan and Chu, 2017).

Antiparasitics, antimicrobials, hormones, antifungals, anti-inflammatory, among others, are commonly employed in food producing animals. Of these, the antimicrobials are the pharmaceuticals most widely used in the livestock industry (Baynes et al., 2016), including the animoglycosides, β-lactams, ionophores, peptides, cephalosporines, fluoroquinolones (FQs), lincosamides, macrolides, sulphonamides, tetracyclines and trimethoprim (Kuppusamy et al., 2018).

The widespread use of FQs has resulted in their detection in different environmental matrices, with concentrations of µg L^{-1} in wastewater, ng L^{-1} to µg L^{-1} in freshwater, µg kg^{-1} in soils and µg kg^{-1} to g kg^{-1} in animal manure (Riaz et al., 2018). Regarding the uncertainties about the effects of the veterinary drugs (VDs) on environment and human health, the World Health Organization, the World Organization for Animal Health and the FDA classified FQs and other antimicrobials as critically important (FAI, 2019).

FQs are a synthetic antimicrobial family derived from second generation of quinolones. They contain a fluorine atom in position 6 and one piperazinyl group at position 7, which are responsible for the antimicrobial activity and lower toxicity than first generation drugs (Doorslaer et al., 2014).

Danofloxacin (DAN) is an FQ for husbandry use and marketed in Brazil by Zoetis with the generic name Advocin® (SINDAN, 2019). DAN is active against gram-negative bacteria, gram-positive and mycoplasma due to its capacity to inhibit DNA gyrase. The drug exhibits excellent activity against respiratory pathogens of cattle, pigs and poultry, demonstrating therapeutic efficacy in the treatment of natural and induced respiratory infections in these species (Chen et al., 1997; Pfizer, 2018).

FQs are molecules of amphoteric nature and can exist in different speciation forms, according to its pka values. Thus, the sorption of these pharmaceuticals is pH-dependent and influenced by ionic strength of the media, by the soil cation exchange capacity and texture (Doorslaer et al., 2014). In addition, they display a low leaching potential and a high affinity for sludge, soils and sediments, due to the formation of ion-ion or ion-dipole interactions with soils (Doorslaer et al., 2014, Riaz et al., 2018).

Computational and spectroscopic investigations, employed to elucidate the sorption of fluoroquinolones in soils, have indicated that these drugs are able to substitute or to complex with cations associated with negative sorption sites (Gu et al., 2015; Nowara et al., 1997). Thus, the soil organic phase acts as sorbent for these VDs, as the presence of COOH/OH moieties makes complexation possible, mediated by cations, with polar groups of the molecules (Mackay and Vasudevan, 2012).

Previous studies have investigated the sorption of FQs on humic acids (HAs) and clay minerals, pointing these materials to be suitable for the remediation of environmental matrices. In addition, the use of the soil mineral fraction as an adsorbent for pollutants is feasible because of their low cost, high surface area, high porosity and abundance (Rivagli et al., 2014; Zhao et al., 2019).

FQs have similar structures and their adsorption behavior is expected to show a general trend for different drugs. However, the groups within a peculiar molecule and the matrix composition may influence the interactions of these VDs and soils or their organic and inorganic phases.

Our literature review indicates that there have been few studies dealing with sorption of DAN in soils so far. However, these studies did not ascertain the factors of influence on the retention of this drug nor how the interaction with soils and their organic and inorganic phases occurs. Based on these facts, the aim of this study is to evaluate the sorption coefficients of DAN in two Brazilian subtropical soils and their clay and HA fractions. In addition, to investigate the factors responsible for the drug retention.

2. MATERIAL AND METHODS

2.1 Reagents, solvents and standard solutions

DAN 99.8% (CAS registry No. 112398-08-0) was purchased from Fluka (EUA) and their stock solution (1000 µg mL^{-1}) was prepared with formic acid and methanol of chromatographic grade purchased from Merck (Germany). Formic acid was purchased from Synth (Brazil). Ethylenediaminetetraacetic acid (EDTA) was purchased from Aldrich (EUA). Aqueous solutions were prepared with ultrapure water from Milli-Q system (Millipore, EUA). Boric acid (Sigma-Aldrich, EUA), phosphoric acid (Synth, Brazil), acetic acid (Synth, Brazil), hydrochloric acid (Synth, Brazil) and sodium azide (Sigma-Aldrich, EUA) were used to prepare Britton-Robinson Buffer (BR). Sodium pyrophosphate, 30% hydrogen peroxide, sodium hexametaphosphate and sodium carbonate were purchased from Synth (Brazil).

2.2 Sampling sites, sample collection and soil characterization

To investigate the sorption of DAN, we selected two Brazilian subtropical soils with distinct texture, organic matter and cation exchange capacity (CEC) content. The soils were collected in different regions of São Paulo State, Brazil. The sampling site, the soils characterization, and the sampling procedure of sandy soil N1 and clay soil S2 are described in Dionisio and Rath (2016). According to the U.S. Soil Taxonomy, N1 and S2 correspond to entisol quartzipsamment and oxisol, respectively.

2.3 Extraction of humic acid and clay

The HAs were extracted from soils N1 and S2 adopting the protocol proposed by the International Humic Substance Society. A molecular weight cutoff of 1000 MW (Spectra/Por®1, USA) was employed for HA dialysis. The purified HAs were stored at ambient temperature until use.

The oxidation of soil organic matter was performed according to the procedure of the Agronomic Institute of Campinas (Camargo, 1986). Then, the steps of dispersion and separation of mineral factions were executed as proposed by International Soil Reference and Information Centre (ISRIC, 2002).

Initially, 200 mL of 0.1 mol L^{-1} sodium pyrophosphate and 50 mL of hydrogen peroxide 30% were added to samples of 10 g of soil. The mixture was kept at rest overnight and heated at 40 °C for 8 hours the following day. Then, the temperature was increased to 80 °C until next dryness. The samples were washed and centrifuged until measured conductivity was below 0.4 mS/cm. Then, 20 g of treated soils were transferred to a 1 L polythene bottle receiving 20 mL of dispersing agent (sodium hexametaphosphate 4% + sodium carbonate 1%). The volume was completed to 400 mL and the flask was stirred overnight. The suspension was passed through a 50 μm sieve, washed and transferred to a graduated cylinder. The volume was completed to 1 L and the recipe was kept at rest for 5.5 hours. The clay fraction was collected according to Stockes' Law. The suctioned liquid was evaporated and dried overnight at 105 °C.

2.4 Characterization of humic acid and clay fractions

Fourier transform infrared spectroscopy (FTIR): The FTIR spectra were obtained on an Agilent Cary 630 FTIR (USA) with the KBr technique at 400 – 4000 cm^{-1} with resolution of 4 cm^{-1} and sixty-four scans.

X-ray diffraction (XRD): XRD spectra of samples were produced using the powder method on a Shimadzu XRD7000 diffractometer (Japan) using Cu Kα irradiation at 40 kV/30 mA and 3 to 65º 2Ө. The scanning rate was 2 º /min with step size of 0.02 º. Occurrence of the possible minerals was identified by comparing the intensity of the signals, the calculated d-spacing values from the Bragg's Law and the data from the American Mineralogist Crystal Structure Database. The software Match! Phase Identification from Powder Diffraction (version 1.11k) was employed to confirm the results.

Elemental composition: The HAs were analyzed on a Perkin Elmer 2400 CHNS (USA) elemental analyzer. Relative quantities of C, H and N were measured directly with the analyzer, and the data were recalculated on an ash-free basis. The oxygen contribution was estimated as the difference between the summed C, H and N concentrations and 100%.

2.5 Studies conditions and sample processing

All assays were performed in falcon tubes protected from light at 25 °C employing a horizontal shaker (Marconi, Brazil). In all experiments the soil/solution mixtures were pre-equilibrated for 24 hours before the addition of DAN. For the quantification of DAN, the soil/solution/drug mixtures were centrifuged at 4186 *g* for 15 minutes and an aliquot of 2.00 mL was collected and transferred to another flask to receive approximately 10 mg of EDTA followed by sonication for 10 minutes. Finally, the solutions were filtered (0.22 μm) and free DAN was determined by the chromatographic method 2.6.

2.6 HPLC analysis

Quantification of DAN was performed with an Agilent 1200 liquid chromatography (Agilent Tech., EUA) equipped with a fluorescence detector, an X Bridge™ Shield RP 18 (4.6 × 200 mm, 5 μm) column and an X Bridge™ Shield RP 18 (4.6 × 20 mm, 5 μm) guard column. The mobile phase used a mixture of methanol and formic acid 0.1% (29:71, v/v). The flow rate was 1.0 mL min^{-1} and the injection volume was 10 μL. The column temperature was 30 °C and the detection was performed using the wavelengths of excitation and emission of 245 and 280 nm, respectively.

2.7 Preliminary studies

The preliminary studies comprised of: a) the evaluation of stability of DAN in solution, b) the optimization of the optimum soil/solution ratio and, c) the determination of sorption equilibration time.

The stability test was performed in five solutions of 50.0 mg L^{-1} DAN in 40 mmol L^{-1} BR buffer at pH 5.0, 7.5 and 10 at 25 °C. The samples were kept in falcon tubes protected from light at 25 °C and sampled for 6 days following the step 2.6.

For selection of soil/solution ratio 1 mg of DAN was added to triplicate soil mixtures of 0.5, 1.0, 2.0 and 5.0 g (previously equilibrated for 24 hours with 40 mL of 40 mmol L^{-1} BR buffer at pH 5.0) and kept under agitation for 24 hours before the chromatographic quantification.

To evaluate the sorption kinetics, free DAN (at initial concentration of 25 mg L^{-1}) was determined in soil/40 mmol L^{-1} BR buffer mixtures (1:80, w/v; pH 5.0) after the equilibration period of 96 hours (n = 2). Kinetic data were fitted to pseudo-first-order, pseudo-second-order (PSO) and Elovich models.

2.8 Sorption on soils, humic acids and clays

To determine the distribution coefficient (Kd) on soils, clays and Has, the DAN, initially added at 2.5 mg g^{-1}, was quantified after the equilibrium of 24 hours of mixtures 1:80 (w/v, n = 3) prepared in 0.01 mol L^{-1} $CaCl_2$. The assays were performed in triplicate and the samples were dried at 65 °C before the XRD analysis.

2.9 Sorption and desorption isotherms

Sorption and desorption experiments were performed using the batch equilibrium method in agreement with the parallel method described in the OECD 106 Guide (OECD, 2000). Initially, DAN (0.5 – 2.5 mg g^{-1}) was added to soil mixtures 1:80 (w/v, n = 3) of 40 mmol L^{-1} BR buffer (pH 5.0, 7.5 and 10) and 0.01 mol L^{-1} $CaCl_2$ following agitation for 24 hours. Then, the aqueous phase was processed, analyzed and replaced by a new one. Desorbed DAN was determined in solution after 72 h of equilibration.

2.10 Influence of ionic strength and cations on sorption of danofloxacin

The influence of ionic strength on sorption was evaluated measuring the concentration of free DAN in soil mixtures, 1:80 (w/v) prepared in 0.005, 0.01, 0.05 and 0.1 mol L^{-1} $CaCl_2$, after the equilibration period of 24 hours. To investigate the sorption in the presence of different cations, the initial aqueous phase was replaced by magnesium (0.01 mol L^{-1}), potassium (0.02 mol L^{-1}) and sodium chloride (0.02 mol L^{-1}). All experiments were performed in triplicate.

3. RESULTS AND DISCUSSION

3.1 Humic acid and clay characterization

The mineral and organic phases of two Brazilian soils with distinct textural classes were isolated to investigate the sorption of DAN. The characterization of clays was performed by FTIR and XRD. The constitution of HAs was investigated by FTIR and elemental analysis.

The bands observed in the spectra of clays and HAs are shown in Fig. 1, and their possible assignments are presented in Table 1.
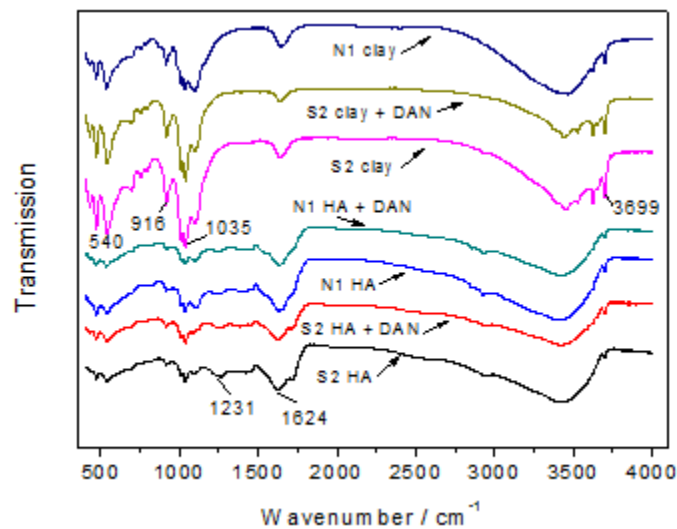
Fig. 1: Fourier Transform Infrared spectra of N1 and S2 clay/humic acid before and after sorption of danofloxacin.

Clay		Humic acid	
Band (cm^{-1})	Assignments	Band (cm^{-1})	Assignments
3699	Al--O-H stretching	3699	OH axial deformation of kaolinite
3622	Al--O-H stretching (inter-octahedral)	3404	OH deformation of phenolic group; aliphatic OH; H$_2$O; amine
3449	H-O-H stretching	2920	C-H asymmetric axial deformation of methyl and methylene
1035	Si-O-Si; Si-O stretching	1624	Asymmetric axial deformation of COO$^-$ covalently linked to metal; amide; ketone or quinone
916	Al--O-H angular deformation	1231	C-OH axial deformation of carbonic and phenolic groups
797	O-H translacional deformation	1037	Si-O axial deformation of kaolinite;
696	Si-O angular deformation	473	Si-O deformation of kaolinite
540	Si-O-Al angular deformation		
473	Si-O; Si-O-Fe stretching		

Table 1: Infrared bands of clays/humic acids and their possible assignments.[1]

(1) References: Bergaya, 2006; Madejová, 2003; Nakamoto, 2009; Russel and Wilson, 1987; Stevenson, 1994.

The spectra of clays are similar, showing the same minerals in the samples and the presence of characteristics bands ranging in intensity. The appearance of signals around 3699, 3622, 3449, 1035, 916, 797, 696, 540 and 473 cm^{-1} indicates kaolinite in clays, confirmed by the presence of

a broad band at 3449 cm^{-1} that is attributed to the presence of water of hydration in these minerals. Vibrations observed at 916 cm^{-1} indicate the presence of hematite. Bands observed at 473, 540, 1035, 3622 and 3699 cm^{-1}, referent to the Al-OH, Si-O-Al and Si-O-Fe vibrations, are more intense for S2 clay, pointing that this material is richer in oxides of iron and aluminum.

The infrared spectra profile of HAs was also similar, showing characteristics peaks and the presence of some residual kaolinite and gibbsite, common impurities of the extraction process (Russel, 1987). In 2920 cm^{-1} the N1 AH presented a slightly more pronounced peak, indicating a more aliphatic composition, rich in substances less humificated such as carbohydrates (Stevenson, 1994). On the other hand, the more humificated S2 HA displayed a greater amount of aromatic structures, which is pointed by the more intense signal in 1231 cm^{-1}, referent to the C-OH axial deformation of carbonic and phenolic groups.

XRD technique was employed to investigate the mineralogical composition of the materials as the species present a diffractometric pattern, characteristic for each crystalline substance and equivalent to a digital print. Fig. 2 presents the spectra of isolated clays. According to the calculated d values of diffraction peaks it was possible to identify the presence of kaolinite and hematite on booth clays, corroborating with the information pointed through the FTIR results.
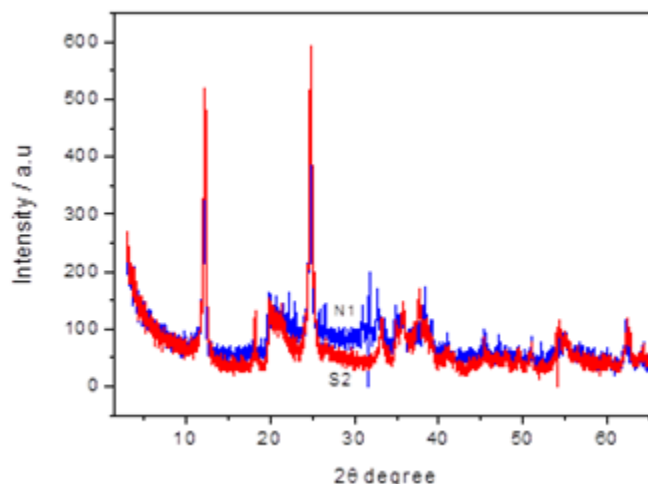

Fig. 2: X-ray diffraction spectra of N1 and S2 clays.

The elemental analysis indicated respectively 32.96; 4.07; 2.83 and 60.14% of carbon, hydrogen, nitrogen and oxygen for N1 HA. For S2 HA the results were 34.54; 3.79; 2.34 and 59.33%, respectively. The C/H ratio was calculated once this parameter is associated with the aromaticity and humification degree of HAs (Stevenson, 1994). Therefore, the lower C/H ratio of N1 HA (8.09) confirmed a greater number of aliphatic groups and a less humificated material in

comparison with S2 HA (C/H = 10.18), corroborating with the FTIR information. Also, the O/C ratio of the HAs indicated a higher presence of oxygenated groups in N1 HA (O/C = 1,82) in comparison with S2 HA (O/C = 1,71), which is pointed by the more intense band in 1624 cm^{-1}, referent to the asymmetric axial deformation of COO$^-$ and C=O groups (Fig. 1).

3.2 Experimental conditions and preliminary studies

Adsorption of DAN on test vessel was negligible in preliminary studies and all assays were performed in falcon tubes. The quantification of sorbed DAN was carried out through the difference between its initial and final amount present in solution. The addition of EDTA, followed by a sonication step prior to the chromatographic analysis, was necessary to inhibit the formation of drug-cation-soluble organic matter complexes in solution. Peruchi et al. (2015) demonstrated that these high molar mass complexes have less affinity for the octadecyl stationary phase and elute at the beginning of the chromatogram, causing a decrease on norfloxacin signal and a strong matrix effect. Thus, the sample preparation step 2.5 was included previously to the drug chromatographic determination in all experiments.

Due to the amphoteric behavior of DAN, sorption coefficients of the VD were also evaluated in distinct pH values. The pHs 5.0, 7.5 and 10 were selected because the molecule was 94%, 89% and 96% cationic, zwitterionic and anionic, respectively (pka$_1$: 6.22 and pka$_2$: 9.43).

As the OECD 106 guidelines do not establish a protocol of how the pH can be controlled, several buffers like chloroacetate, acetate, MES, PIPES and MOPS were reported for sorption studies of ionizable VDs in soils (Carrasquillo et al., 2008; Figueroa-Diva et al., 2010; Figueroa and Mackay, 2005; Gu and Karthikeyan, 2005; Vasudevan et al., 2009). Sorption studies were initially performed in 0.01 mol L^{-1} CaCl$_2$ with pH adjusted through the addition of HCl or NaOH, which resulted in a significant change of the initial values at the end of the experiments. Therefore, the BR buffer was chosen for the continuation of the studies, as it enables the preparation of buffers over a wide pH range (2 < pH > 12) with the same reagents. Thus, it was possible to evaluate the sorption of ionizable forms of DAN at different pH values without changing the chemical composition of the medium.

According to the stability test (data not shown), DAN was stable for six days in 40 mmol L^{-1} BR buffer at three selected pH values, as no significant difference (p = 0.05) was observed between the initial and final mean concentration of the drug in solution.

Evaluation of an appropriate soil-to-solution ratio was performed at 1:8, 1:20, 1:40 and 1:80 (w/v). The condition 1:80 was chosen for subsequent experiments since the sorption of DAN was above 50%, which is in agreement with OECD 106 recommendations (OECD, 2000).

The plots of sorbed DAN versus time (data not shown) indicated that the period of 1440 minutes (24 h) was sufficient to reach the apparent sorption equilibrium. Therefore, this time was adopted for sorption experiments. According to the data, a rapid sorption was observed during the first 480 minutes, followed by a slower sorption process. The experimental results were best fitted to the PSO model (r > 0.99), suggesting a chemisorption process proportional to the solute concentration and to the number of sites not occupied in the sorbent (Ho and Mackay, 1998). According to this approach, the PSO rate constant (k) indicated a faster sorption of DAN in S2 in comparison to N1 soil (k of 7.99×10^{-5} and 1.12×10^{-5} g μg^{-1} min^{-1}, respectively). The equilibrium sorption capacity (q_e) pointed to a higher sorption on S2 in contrast with N1 (q_e of 1819 and 1111 μg g^{-1}, respectively).

3.3 Sorption on soils, humic acids and clays

The Kd values of sorption of DAN in 0.01 mol L^{-1} $CaCl_2$ on soils, clays and HAs are indicated in Fig. 3. These studies were performed in the natural soil pH close to 5, where the molecule was up to 90% in cationic form.

According to these data, the antimicrobial presented Kd from 111 – 3378 mL g^{-1} for N1 and 427 – 2381 mL g^{-1} for S2, showing the tendency: sorption on HA > clay > soil in both cases. Except for the HAs, the retention of DAN was higher for S2 clay and soil.

As expected, a superior Kd for DAN on soil S2 was confirmed, since its presents a clay texture, a higher cation exchange capacity (CEC), a higher organic matter content and, thus, a great amount of possible sorption sites in comparison with the sandy soil N1 (see physicochemical properties, Dionísio and Rath, 2016). Therefore, the behavior observed for DAN is consistent with previous studies that indicated a favored sorption of ciprofloxacin (CIP), norfloxacin (NOR) and enrofloxacin (ENR) on soil S2 to the detriment of N1 (Rath et al., 2019).
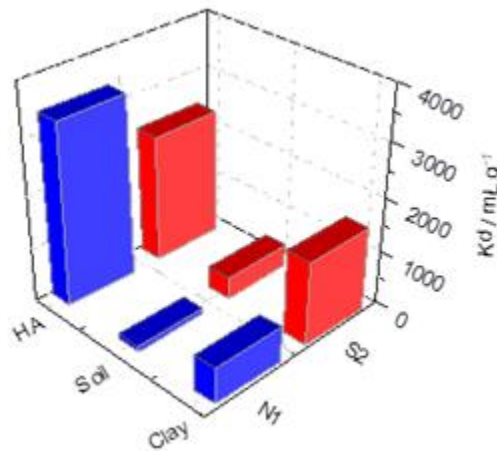
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Fig. 3: Distribution coefficient (Kd) of danofloxacin on humic acids, soils and clays (n = 3).

The results pointed in Fig. 3 indicate that the type of soil, the soil fraction and the constitution of the clays and HAs influenced the sorption of DAN. The sorption on HAs was about 2 to 9% higher in comparison with the clays and 15 – 68% greater in relation to the original soil. Also, the Kd of DAN on N1 HA is 42% greater in relation to S2 HA.

So, our data corroborate with Martínez-Mejía et al. (2017) which observed a sorption of ENR onto the humic acid about 10 to 30 - fold higher than onto the soil from which they were extracted. Furthermore, Teixidó et al., (2014) also ascertained that the presence of humic acids increased the sorption magnitude of ENR and CIP on soils.

Due to their composition, the humic substances play an important role on the sorption of VDs, as they act as a sorbent for these substances. This soil organic phase is pH dependent and composed of carbon and hydrogen with large non-polar domains with COOH/OH ligand groups. Thus, theoretically, they are able to link with FQs in acid media via: a) ionic interaction between the negatively charged moieties of the HA and the positively charged nitrogen at piperazinyl group of FQ; b) hydrogen bonding between the oxygens of carbonyl or carboxylic group of FQ and hydroxyl group of HA; c) cation bridging, mediated by positive ions, involving the oxygens of the carbonyl or carboxylic group of FQ molecule and hydroxyl groups of the HAs and; d) interaction of the ternary complex drug-ion-drug formed in solution and negatively charged moieties of the HAs (Cuprys et al., 2018).

As observed, FTIR spectra and C/H ratio pointed that N1 HA is a less humificated material, possessing a greater number of aliphatic groups, a major content of low molecular weight humic substances and a higher content of oxygenated groups (like COO^- and $C=O$ moieties), which act as possible sorption sites and enables a greater interaction with DAN species and, thus, an

increase on sorption of the VD in comparison with S2 HA. These results corroborate with Martínez-Mejía et al. (2017), which indicated a smaller Kd for ENR in more aromatic, condensed and recalcitrant HA structure.

According to Fig. 1 we observed a reduction on peak intensity of FTIR of HAs in the presence of DAN in 1624 cm^{-1} (asymmetric axial deformation of COO^{-}, C=O) and 3000 – 3600 cm^{-1} (OH deformation of phenolic group; aliphatic OH; H$_2$O), which according to Cuprys et al., (2018) may suggests: a) the formation of ionic interactions between carboxylic groups of HAs and DAN^{+} and/or DAN^{+}--Ca^{2+} moieties and; b) the formation of hydrogen bonding or cation bridging involving DAN species and OH groups located at HAs.

The hydration of kaolinite surface takes to the formation of Al-OH-Al, Al-OH and silanol (Si-OH) groups with pH-dependent ionization. Thus, even with point of zero charge close to pH 3 and the dissociation of silanol group in pH < 2, these clays present an excess of negative charge in a wide range of soil pH (Melo and Alleoni, 2009), favoring the sorption of cationic DAN and resulting in higher Kd values in comparison with the natural soils, as verified in Fig. 3.

The XRD allowed us to investigate the sorption of DAN on clays. As expected, the sorption was restricted to the external surfaces of kaolinite, due to its nonexpanding property. Except by a reduction on signal intensity of kaolinite and hematite, the spectra of sorbed DAN on clays (data not shown) did not present a displacement of the initial signals, confirming no intercalation of VD molecules into the interlayers of the mineral, which is consistent with results presented for ENR (Wan et al., 2013).

The sorption on S2 clay was 2.4 times higher in comparison with N1 clay. As observed, the FTIR data (Fig. 1) indicated a higher content of hematite and oxides of iron and aluminum in S2 isolated clay and a reduction of signals referent to Al-OH, Si-O-Al and Si-O-Fe vibrations was observed after the sorption of DAN. Considering the highly reactive surface of iron and aluminum oxides and their capacity to sorb FQs, it is plausible to presume the contribution of these minerals in additional interactions with DAN, which could reduce the concentration of the free molecule in solution and increase the Kd on S2 soil and clay. This assumption is supported by the studies which demonstrated the formation of a surface monodentate complex between Al oxides and COO^{-}--CIP group and keto oxygen and one oxygen from COO^{-}, involved in the formation of a six-membered ring with Fe oxides (Gu and Karthikeyan, 2005).

3.4 Sorption and desorption isotherms

The sorption and desorption isotherms were constructed according to the soil/solution ratio and apparent equilibrium time established by the preliminary studies. The isotherms (Fig. 4) exhibited an adequate fit to the linear form of Freundlich equation, with r > 0.92 in all cases.
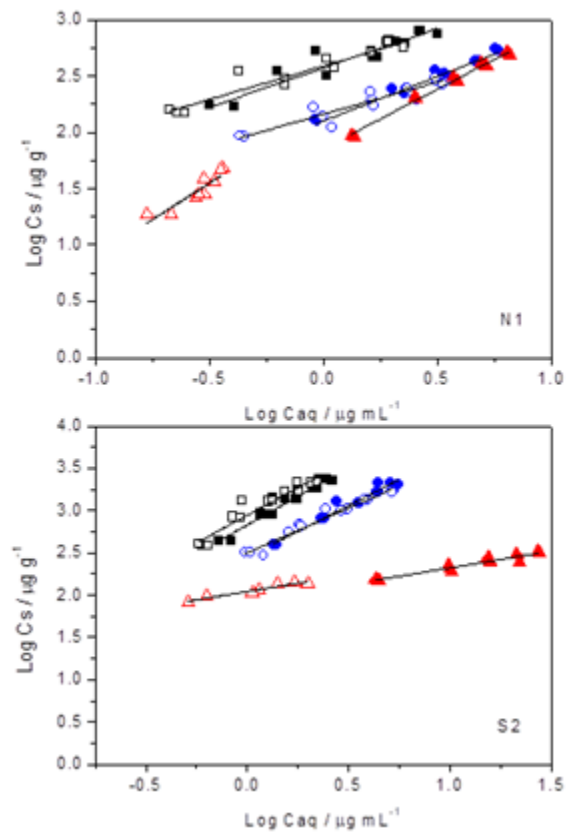
Fig. 4: Sorption (full symbols) and desorption (empty symbols) isotherms for danofloxacin on soils N1 and S2 at pH 5 (square), 7.5 (circle) and 10 (triangle).

N1 and S2 presented Freundlich coefficient (KF) for sorption of 371, 126, 76 / 676, 280 and 85 $\mu g^{1-1/n}$ $(mL)^{1/n}$ g^{-1}, with regression constant (1/n) of 0.697, 0.790, 1.050 / 1.357, 1.226 and 0.395 for pH 5; 7.5 and 10, respectively. These results confirm the higher retention capacity of the VD on S2 soil and indicated an increase of sorption capacity with the decrease of pH as observed for CIP (Jalil et al., 2015; Li et al., 2011; Vasudevan et al., 2009), ENR (Yan et al., 2012), levofloxacin (Liu et al., 2015) and NOR (Jie et al., 2009) in other soils and clay minerals.

The influence of pH on the sorption of DAN in N1 and S2 soils was recently ascertained in a work of our research group. Employing BR buffer, the Kd of the VD was assessed in pH 2 – 11, indicating maxima sorption of the cationic DAN in pH 5 and a reduction of the Kdvalues in basic media (Rath et al., 2019). This finding agrees with the behavior observed for other FQs and suggests the cation exchange as the main sorption mechanism of DAN on evaluated soils and the contribution of secondary interactions, like cation bridging and hydrogen bonding, in basic media (Carrasquillo et al., 2008; Nowara et al., 1997).

The desorption step presented KF values of 387, 144, 131 / 882, 317 and 110 $\mu g^{1-1/n}$ $(mL)^{1/n}$ g^{-1}), with 1/n of 0.594, 0.573, 1.305 / 1.309, 1.053 and 0.392, for N1 and S2 in pH 5, 7.5 and 10, respectively, which indicated an unfavored desorption process, whereas desorption KF > sorption KF.

Our KFs of DAN are lower in comparison with the values of 2280 – 3000 $\mu g^{1-1/n}$ $(mL)^{1/n}$ g^{-1}, presented by Pfizer (Pfizer 2018), for three soils varying in pH (5.6 – 7.6), organic carbon content (0.59 – 3.1%) and with a considerably superior CEC in contrast with our soils (125 – 294 versus 19.3 and 66 meq kg^{-1} for N1 and S2, respectively). However, it is important to observe an increase of sorption of FQs in soils with higher CEC (Carrasquilo et al.,; 2008; Conckle et al., 2010; Vasudevan et al., 2009; Figueroa-Diva et al., 2010).

Sorption isotherms in 0.01 mol L^{-1} $CaCl_2$ (data not shown) presented r > 0.95, with KF of 217 and 630$\mu g^{1-1/n}$ $(mL)^{1/n}$ g^{-1} and 1/n of 0.5306 and 0.5855 for N1 and S2 soils, respectively. It should be noted that all experiments were assessed under uncontrolled pH and, thus, the drug was predominantly positively charged due to natural soil pH close to 5. The results do not indicate an interference of BR buffer in pH = 5 for soil S2 (KF = 676 $\mu g^{1-1/n}$ $(mL)^{1/n}$ g^{-1}). However, the KF of N1 decreased to 371 (BR buffer, pH = 5) from 217 $\mu g^{1-1/n}$ $(mL)^{1/n}$ g^{-1} in calcium chloride. This behavior may be attributed to the appearance of a competition, between the protonated DAN and Ca^{2+} ions for the negative sorption sites, in cooperation with the lower CEC of this soil.

3.5 Influence of ionic strength and cations on sorption of danofloxacin

Some studies have demonstrated a significant influence of cationic species on the sorption of FQs in soils and clays (Chen et al, 2013; Jie et al, 2009; Li et al., 2019; Yan et al., 2012; Zhang et al., 2012). Thus, the ionic strength (I) of $CaCl_2$ and the effect of other cations in solution was checked for DAN on N1 and S2.

As shown in Fig. 5, the increase of Ca^{2+} (from 0.005 to 0.1 mol L^{-1}) resulted in a reduction of the Kd values of N1 and S2 from 151 to 35 and 638 to 123 mL g^{-1}, respectively. These results agree with the behavior observed for other FQs, as some other studies have pointed an inhibition of sorption of these VDs, following the increase of I of cations in solution phase (Chen et al., 2013; Yan et al., 2012).
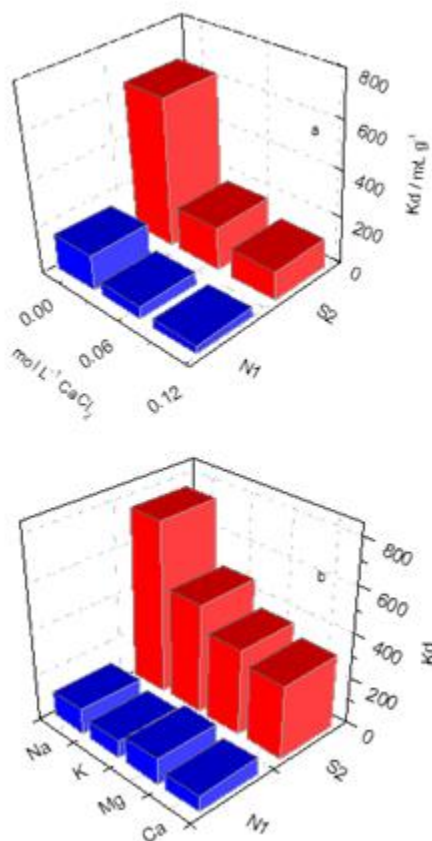
Fig. 5: Distribution coefficients (Kd) of danofloxacin on soils N1 and S2 in 0.005, 0.05 and 0.1 mol L^{-1} CaCl$_2$ solutions (a) and in the presence of mono and divalent cations (b).

On the other hand, some works have indicated an enhanced sorption of FQs, due to a higher binding affinity and the formation of a ternary VD--sorption sites complex with metal ions acting as mediators. Cu^{2+} and Zn^{2+} lead to an increase of sorbed ENR (Grauer-Bacart et al., 2015) and CIP presented enhanced sorption following the order: $Cu^{2+} > Pb^{2+} > Cd^{2+} > Ca^{2+}$ (Tan et al., 2014).

According to the Fig 5, soil S2 was the most affected by the nature of the exchangeable cation, presenting Kd values following the order: $Na^+ > K^+ > Mg^{2+} > Ca^{2+}$. For N1, the Kd values decreased in the sequence: $Na^+ > Mg^{2+} > K^+ \approx Ca^{2+}$. Also, the replacement of divalent cation Ca^{2+} for monovalent ion Na^+ in solution increased the Kd of S2 in 135%.

These results, especially for S2, indicate that divalent cations competed with DAN for sorption sites located at humic substances and external surfaces of kaolinite. In opposition, monovalent cations facilitate the sorption rather than compete with it.

Nevertheless, the different effect of cations in solution observed in N1 was ever reported for NOR in distinct soils. Kong et al. (2014) pointed that the enhancement or reduction on sorption efficiency depends not only the metal ion type in solution, but also by the soil properties, such as their CEC.

4. CONCLUSIONS

The studies performed in this work evaluated the sorption of the antimicrobial DAN in two Brazilian sandy and clay soils. The experiments indicated that the sorption of this VD was influenced by several factors such as the textural class of soils, their physicochemical properties, by the pH, the I and nature of the exchangeable cation in solution.

Isolated clays and HAs demonstrated to play an important role for the sorption of DAN and retained more drug in comparison with the natural soils, while the HAs was most effective for molecule immobilization.

The results are consistent with previous studies performed with other FQs and indicate the contribution of ionic interactions, like cation exchange and cation bridging, hydrogen bonding and the participation of Al and Fe oxides for the retention of DAN on evaluated soils, HAs and clays.

The degree of humification of HAs and the constitution of clays showed to influence the retention of DAN and thus, these components of soils can affect the drug bioavailability in the environment.

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