MethodsX 10 (2023) 102109

Contents lists available at ScienceDirect

MethodsX

journal homepage: www.elsevier.com/locate/mex

Using a batch test to derive sorption data of fluoroquinolone antibiotics in humic acids

Joel Fabregat-Palau[a,b], Anna Rigol[a,c,*], Zhiqiang Yu[b], Miquel Vidal[a]

[a] *Department of Chemical Engineering and Analytical Chemistry, Faculty of Chemistry, Universitat de Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain*

[b] *State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Wushan, Guangdong 510640, People's Republic of China*

[c] *Institut de Recerca de l'Aigua (IdRA), Universitat de Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain*

ARTICLE INFO

Method name:
Batch experiments to assess the effect of varying factors on sorption

Keywords:
OECD guidelines
Batch test
Fluoroquinolones
Humic acids
Sorption
Desorption
Analogy
K_d

ABSTRACT

Fluoroquinolone antibiotics (FQs) are of concern due to their disrupting effects on environmental bacterial communities. Evaluating FQ sorption by soil components is important to understand their interaction with soils and to address their environmental (bio)availability. However, data in soil organic components, especially humic acids, are scarce. Batch experiments following OECD guidelines are suitable for testing the sorption of pollutants in solid matrices. Here, we applied this methodology, with specific changes in the experimental setup, to derive sorption data and to identify the factors affecting sorption of four common FQs in seven humic acids with contrasting properties. The effect of shaking time, pH, calcium concentration in solution and dissolved organic carbon (DOC) content on the quantification of the solid-liquid distribution coefficient (K_d) of norfloxacin in three reference humic acids was evaluated. Sorption reversibility and sorption analogy of four FQs were additionally assessed in these three reference materials, whereas the effect of initial norfloxacin concentration was evaluated in the overall set of seven humic acids. Sorption was fast, strong, non-linear, irreversible and affected by changes in the pH and calcium levels in solution. The bell-shaped sorption trend at varying pH values confirmed the key role of FQ speciation in sorption and the high K_d values indicated a positive effect of soil organic matter components on FQ sorption in bulk soils at environmentally relevant pH values.

- Relevant factors affecting sorption of pollutants in environmental matrices must be considered to derive K_d values with low variability and high representativeness.
- In this article we modify the experimental conditions of standard batch tests to identify the factors that affect the sorption of FQs in humic acids.
- The methodological approach followed can be extrapolated to the evaluation of other combinations of pollutant and environmental matrix.

---

[*] Corresponding author.
E-mail address: annarigol@ub.edu (A. Rigol).
Social media: (A. Rigol)

https://doi.org/10.1016/j.mex.2023.102109
Received 2 January 2023; Accepted 3 March 2023

Specifications table

Subject area:	Environmental Science
More specific subject area:	*Sorption/desorption process*
Name of your method:	*Batch experiments to assess the effect of varying factors on sorption*
Name and reference of original method:	*OECD Guidelines for the Testing of Chemicals: Adsorption – Desorption Using a Batch Equilibrium Method*
Resource availability:	*N.A.*

Scope and objectives

Fluoroquinolone antibiotics (FQs) are synthetic chemicals used to treat infections caused by both Gram-positive and Gram-negative bacteria. They have been widely used in the last decades in both human and veterinary medicine [1]. As they are excreted by animals due to their partial uptake, high levels of FQs have been reported in animal manure such as pig slurry and chicken droppings [2]. The application of these organic materials as a soil amendment is a common agricultural practice to increase soil fertility, but it can lead to detectable FQ levels in soils that may disrupt bacterial communities and promote the generation of antibiotic resistance [3], thus diminishing the effectiveness of antibiotics to treat infections [4]. Assessing FQ sorption by soil particles helps to address their environmental (bio)availability [2]. However, while it has been observed that adding humic acid (HA) to soils as an organic surrogate increases FQ sorption [5], other studies have reported higher sorption by soils without organic matter [6], highlighting the need to better understand the role of soil organic matter in FQ sorption. A quantitative parameter describing the sorption/desorption process that is used in environmental models is the solid-liquid distribution coefficient (K_d). However, K_d values for FQs in HAs may be highly affected by methodological conditions [7]. Hence, the generation of new K_d (FQ) values under varying experimental conditions in pure soil organic components is still required to better understand the overall contribution of the organic phase to FQ sorption in bulk soils.

Batch experiments following the guidelines of the Organisation for Economic Co-operation and Development (OECD) are suitable for testing the sorption process of pollutants in solid matrices and to derive related K_d values [8]. Specific modifications can be made to this methodology to evaluate the influence of different factors on the sorption process. Several commercial FQs differ only slightly in their chemical structure, and their sorption analogy in soil organic phases is currently unclear. Accordingly, here we applied modifications to the batch methodology proposed by the OECD to derive sorption data evaluating the effect of varying shaking time, pH, calcium and DOC contents. In addition, desorption experiments were also carried out with three reference humic acids and norfloxacin (NOR) as FQ-representative. The sorption analogy of NOR, ciprofloxacin (CIP), enrofloxacin (ENR) and ofloxacin (OFL) was also assessed in these three materials, whereas the effect of initial NOR concentration was also tested in larger set of seven humic acids. Overall, these modifications of the OECD protocol allowed a better ranking of the effects of these factors on the overall sorption process of FQs in humic acids and an estimation of their significance in inducing K_d (FQ) variability.

Reagents and materials

NOR, CIP, ENR and OFL, all with a purity of ≥ 95%, were purchased from Sigma-Aldrich (Germany). Their main physicochemical properties are detailed elsewhere [9]. FQs, as ionizable pollutants, have pK_{a1} and pK_{a2} values of around 6 and 8, respectively. Then, they may be present in the environment as different species. As illustrated in Fig. 1 for NOR, at $pH < pK_{a1}$, both the piperazine

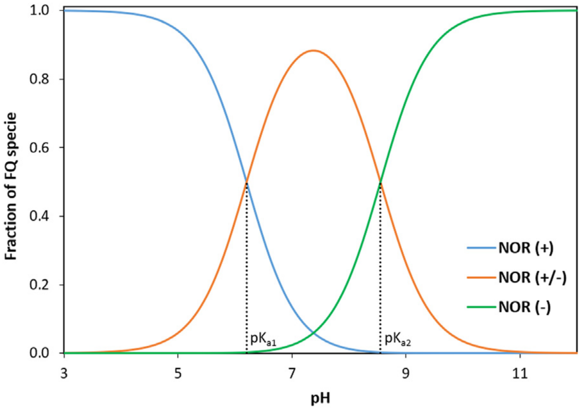

Fig. 1. NOR speciation diagram at environmentally relevant pH values. $pK_{a1} = 6.20$ and $pK_{a2} = 8.55$ [10].

Table 1
Main physicochemical properties of the HA samples.

	LHA	ESHA	PPHA	SRHA	FHA	AHA	JHA
% C	63.8[a]	59.5[a]	56.4[a]	54.6[a]	45.9[b]	42.2[b]	39.2[b]
% O	31.3[a]	32.2[a]	37.3[a]	40.0[a]	49.8[b]	53.4[b]	56.3[b]
Molar C/O ratio	2.72	2.47	2.01	1.82	1.23	1.05	0.93
Aromatic C (%)[c]	58[a]	41[a]	47[a]	35[a]	N.R.	N.R.	N.R.
Carboxyl (meq g C^{-1})	7.46[a]	8.28[a]	9.01[a]	9.59[a]	N.R.	N.R.	N.R.
Phenolic (meq g C^{-1})	2.31[a]	1.87[a]	1.91[a]	4.24[a]	N.R.	N.R.	N.R.
DOC (mg C L^{-1})	3.7	6.2	7.5	70	13	23	18
$SUVA_{254}$ (L mg^{-1} m^{-1})	5.0	3.0	2.8	1.5	2.1	1.8	1.2

[a] Data extracted from the IHSS official webpage.

[b] data obtained from Rigol et al. [11];

[c] Referred to total carbon; N.R.: not reported.

and the carboxyl groups are protonated and, thus, the cationic species are predominant. Anionic species are abundant at pH > pK_{a2}, when both the piperazine and the carboxyl groups are deprotonated. At pH values between pK_{a1} and pK_{a2}, the carboxyl group is deprotonated and the piperazine group is protonated, being the zwitterion the predominant species [6].

Milli-Q double deionized water (18.2 MΩ cm) was obtained from a water purification system (USF Purelab Plus, Spain). A 1000 mg L^{-1} FQ stock solution was prepared in Milli-Q water (pH 4.0) acidified by using HCl (37%, Panreac, Spain), with the working solutions prepared by appropriate dilution. Extra pure (≥ 99%) calcium chloride dihydrate, sodium azide, potassium bromide and sodium hydroxide were supplied by Merck (Germany). HPLC-grade acetonitrile (ACN), formic acid and triethanolamine were purchased from Panreac (Spain).

Pahokee Peat (PPHA, 1S103H), Leonardite (LHA, 1S104H), Elliot Soil (ESHA, 4S102H) and Suwannee River (SRHA, 3S101H) humic acids (HAs) were purchased from the International Humic Substances Society (https://humic-substances.org/). Janssen (JHA, 12.086.58), Aldrich (AHA, H1,675-2, sodium salt) and Fluka (FHA, 53680) HAs were purchased from Janssen Chimica (Belgium), Sigma-Aldrich (Germany) and Fluka Chemika (Switzerland), respectively. Their main physicochemical properties are summarized in Table 1. The carbon content of the seven HAs ranged from 39 to 64%, while the aromatic carbon contents ranged from 35 to 58% for the HAs with available ^{13}C-NMR data. The C/O molar ratio, which has been previously used as a proxy for the material aromaticity of carbon-rich samples [12], ranged from 0.9 to 2.7.

The DOC content, determined in a 0.2 g L^{-1} suspension containing 0.01 M $CaCl_2$ at pH 5.0 (Analytik Jena Multi N/C), ranged from 4 to 70 mg C L^{-1}, with the SRHA having the highest DOC content. Specific ultra-violet absorption at 254 nm ($SUVA_{254}$), a parameter related to DOC aromaticity, was acquired using a UV-visible spectrometer (Varian Cary 100) and a 1-cm optical cell and calculated as detailed elsewhere [13]. This ranged from 1.2 to 5.0. FTIR spectra (Thermo Nicolet 5700) were recorded by diluting dried (40 °C, 48 hours) HA samples with solid KBr and are depicted in Fig. 2.

The FTIR spectra of all samples agreed with those previously reported for humic acids. Peaks at 1600 cm^{-1}, attributed to the C = C stretching bands in aromatic rings, confirmed the presence of an aromatic domain in the HA structure. Both the broad band at 3400 cm^{-1}, attributed to the O – H stretching bands of alcohol, phenol and carboxyl groups, and the band at 1400 cm^{-1}, attributed to the O – H bending of carboxyl groups confirmed the presence of protonated functionalities. The bands at 2850 – 2960 cm^{-1} were attributed to the C – H stretching of CH_2 and CH_3 alkyl groups [14].

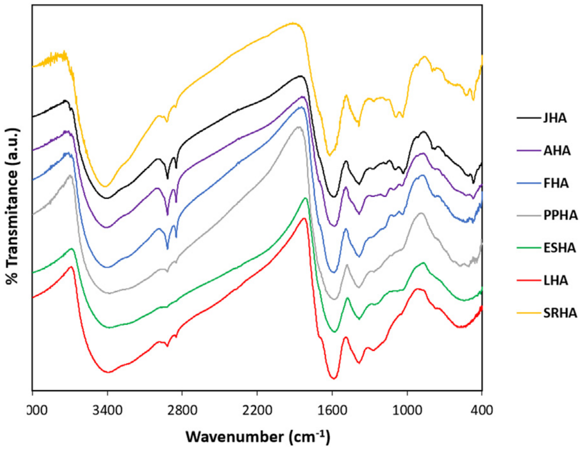

Fig. 2. Solid-state FTIR spectra of the seven humic acid samples.

General description of the batch methodology used to quantify sorption

Batch sorption experiments were conducted according to the OECD guidelines [8]. Although experiments performed in bulk soils commonly use solid loadings in the gram range (to achieve soil suspensions of around 10 – 100 g L^{-1}) and spiking concentrations of FQs of around hundreds of mg L^{-1} [5,15,16], the conditions of the batch tests have to be adapted to the HAs due to their expected strong sorption affinity for FQs as well as the cost of the HA samples. HA suspensions of around 0.01 – 0.20 g L^{-1} have been reported to be suitable for testing the sorption of FQs through batch experiments [7,17]. Due to both the lower solid suspensions and the high sorption affinity, the initial FQ concentration has to be carefully selected to derive sorption data under environmentally relevant conditions, where HA samples are not expected to reach a complete saturation of their sorption sites and the FQ concentrations in solution after equilibrium are high enough to be determined.

Briefly, 6 mg of the HA sample were weighed in a 50-mL polypropylene centrifuge tube. Then, 30 mL of an aqueous solution of 10 mM $CaCl_2$ containing 0.1% NaN_3 (to avoid microbial degradation), adjusted to pH 5.0 using 0.5 M HCl, were added to achieve an HA suspension of 0.20 g L^{-1}. The tubes were then placed in an end-over-end shaker at 30 rpm and at room temperature (25 ± 1 °C) to achieve a pre-equilibrium state. After this, a certain amount of the FQ working solution was added to achieve a given initial FQ concentration. The tubes were shaken end-over-end at 30 rpm and at room temperature (25 ± 1 °C) while wrapped in aluminium foil to avoid photodegradation. At selected times, the tubes were taken and centrifuged for 15 min at 4°C and 7,800 *g* (AJ2-HS, Beckman Coulter, USA). The supernatants were collected using a plastic pipette, filtered through 0.45-μm nylon filters, and stored in 20-mL polypropylene vials at 4°C until analysis. Control samples were run in parallel to account for any FQ losses during the experimental setup, and blank samples (without FQ spikes) were also prepared to ensure that no FQs were already present in the HAs. All experiments were run in triplicate, and the relative standard deviation (%RSD) of the replicates were < 20% in all cases. Modifications of this general procedure to assess the effects of different factors affecting sorption are further detailed in each section.

Determination of FQ concentrations by HPLC-FLD

An Agilent 1200 chromatograph was used for the FQ determination in the supernatants, using a ZORBAX SB-C_{18} (5 µm, 4.6 × 250 mm) separation column and an isocratic 85:15 0.1% formic acid (adjusted to pH 3.0 using triethanolamine):ACN mobile phase. The presence of organic buffers such as oxalic acid was avoided in the aqueous phase by the high calcium content tested in some scenarios, which can promote the precipitation of calcium oxalate salts. Methanol was also avoided as an organic mobile phase given its ability to form methyl FQ esters under acid conditions [18]. The flow rate and injection volume were set at 1 mL min^{-1} and 10 µL, respectively. NOR, CIP, ENR and OFL detection was carried out using a fluorescence detector (FLD) with an excitation/emission wavelength of 280/447, 284/449, 284/467 and 298/499 nm, respectively. The limit of quantification (LOQ), determined as a signal-to-noise ratio of 10:1, was 0.01 mg L^{-1} for all four FQs.

External FQ calibration curves were constructed using seven calibration standards with FQ concentrations ranging from 0.1 to 5 mg L^{-1}. Calibration standards were prepared for each FQ using appropriate dilutions of the FQ stock solutions containing 0.1% NaN_3 and the same calcium concentration as that of the tested samples, and adjusted to pH 5.0. The Pearson coefficients of the calibration curves were higher than 0.99 in all cases. Control experiments run at an FQ concentration of 0.2 mg L^{-1} and at varying extreme pH values (i.e., pH 3 and pH 11) ensured an accurate determination (error bias ± 10%) of the FQ in the supernatant. Ten consecutive injections of a 0.2-mg L^{-1} NOR aqueous standard showed a 3.8% RSD, whereas 10 injections of 0.2-mg L^{-1} NOR aqueous standards on 10 different days showed a 6.1% RSD. Similar values were obtained for CIP, ENR and OFL.

Effect of shaking time on FQ sorption

To test the effect of time on K_d (FQ) quantification, NOR was selected as the FQ representative, whereas LHA, PPHA and ESHA were selected as the HA representatives. NOR was spiked at a concentration of 10 mg L^{-1}. The tubes were shaken for time periods varying from 0 to 48 hours, with the NOR concentrations sorbed by the solid phase monitored during this time interval. The sorbed NOR concentration in the solid phase (C_S, mg kg^{-1}) at each timepoint was calculated as the difference between the initial spiked NOR concentration (C_{in}, mg L^{-1}) and the NOR concentration determined in the corresponding supernatant (C_{eq}, mg L^{-1}) by HPLC-FLD, using Eq. 1 after ensuring null losses in the experimental setup:

$$C_S = \frac{(C_{in} - C_{eq}) \cdot V}{m} \tag{1}$$

where V (L) is the total volume of contact solution and m (kg) the dry mass of the HA.

The kinetic results are summarized in Fig. 3, which reveal that NOR was rapidly sorbed by all the HAs before the uptake slowed down until equilibrium was reached.

The kinetic results indicated that from 6 hours onwards, the sorption equilibrium between NOR and the HA samples was achieved. The experiments were run for 48 hours. To obtain quantitative information from the kinetic process, the kinetic data were fitted to the pseudo-second order model (PSOM) equation (Eq. 2) using Matlab (cftoolbox, Matlab® R2009a (MathWorks Inc., USA)). The PSOM equation has been previously applied successfully to describe the sorption kinetic uptake of contaminants in solid matrices [19]:

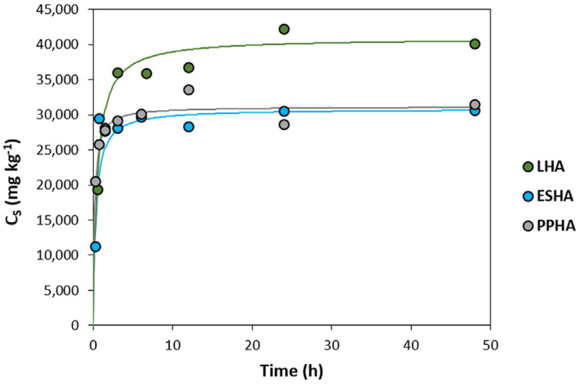

Fig. 3. Sorption kinetics of NOR in the LHA, ESHA and PPHA samples. Solid lines indicate the PSOM fitting.

$$\frac{t}{C_{S,t}} = \frac{1}{K_2\, C_{S,eq}^2} + \frac{t}{C_{S,eq}} = \frac{1}{V_0} + \frac{t}{C_{S,eq}} \tag{2}$$

where $C_{S,t}$ (mg kg^{-1}) is the sorbed concentration at time t (h), K_2 (kg mg^{-1} h^{-1}) the PSOM rate constant, and $C_{S,eq}$ the sorbed amount at equilibrium (mg kg^{-1}). The initial PSOM rate constant, which describes the pollutant uptake kinetic rate (V_0, mg kg^{-1} h^{-1}), can be further calculated as the product of K_2 and $C_{S,eq}{}^2$ [19]. The fitting parameters of the kinetic data to the PSOM equation are summarized in Table 2. The Pearson coefficients of the resulting PSOM fittings rose to 0.999.

Table 2
The PSOM fitting parameters of NOR in PPHA, ESHA and LHA.

Humic acid	$C_{S,eq}$ (mg kg^{-1})	K_2 ($\cdot 10^{-3}$) (kg mg^{-1} h^{-1})	V_o (mg kg^{-1} h^{-1})	r^2
LHA	40,916	0.049	81,565	0.998
ESHA	30,611	0.113	105,551	0.999
PPHA	31,156	0.184	178,730	0.997

Effect of initial FQ concentration on sorption

The effect of the initial FQ concentration on sorption, using NOR as the FQ representative, was evaluated in the seven HAs by deriving the respective sorption isotherms, which are defined as the plot of the sorbed FQ concentrations in the solid phase (C_S, mg kg^{-1}) against the remaining FQ concentration in the liquid phase (C_{eq}, mg L^{-1}). The initial NOR concentrations tested ranged from 1 to 10 mg L^{-1}. The resulting sorbed concentrations were calculated using Eq. 1.

The solid-liquid distribution coefficient (K_d, L kg^{-1}) is defined as the ratio of the sorbed concentration of the pollutant by the solid phase to the pollutant concentration in the liquid phase under equilibrium conditions (Eq. 3), resulting in the slope of the sorption isotherm:

$$K_d = \frac{C_S}{C_{eq}} \tag{3}$$

In contrast to the linear isotherms, where K_d values are constant regardless of the initial FQ concentrations [12,15], the derived K_d values for non-linear isotherms usually decrease with increasing C_{in}. The sorption isotherms for NOR in the seven HAs are plotted in Fig. 4. Several sorption parameters were derived for the interpretation of the data, as shown in Table 3. The isotherms were fitted to the Freundlich model (see Eq. 4) using Matlab (cftoolbox, Matlab® R2009a (MathWorks Inc., USA)). The Freundlich fitting has been widely applied to describe the sorption of different pollutants on heterogeneous surfaces containing sorption sites with different affinities [20]:

$$C_S = K_F\,\left(C_{eq}\right)^N \tag{4}$$

where K_F ((mg kg^{-1})/(mg L^{-1})^N) and N (dimensionless) are the fitted Freundlich parameters representative of the sorption affinity and the heterogeneity of the sorption sites available on the sorbent surface, respectively. A value of $N < 1$ is indicative of the presence of heterogeneous sorption sites with different affinities for the sorbate, with the sites showing higher affinity being the first to be involved in the sorption process [20].

For all the isotherms, values of $N < 1$ were observed when considering the overall C_{in} range, indicating sorption site heterogeneity. This suggests that at low loading concentrations, FQs were sorbed at sorption sites with a high affinity. When higher concentrations

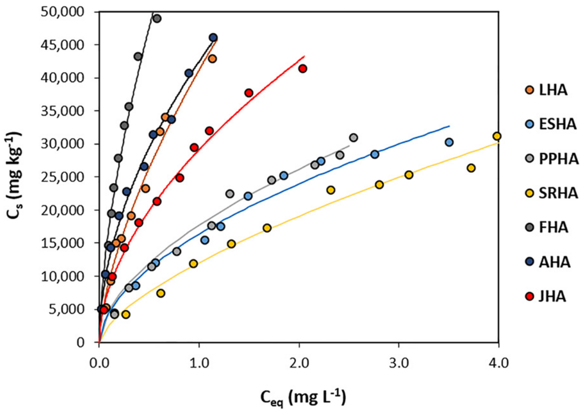

Fig. 4. Sorption isotherms for NOR in the seven HA samples. Dashed lines indicate the Freundlich fitting.

Table 3
Sorption parameters of NOR in the seven HA samples. 95% confidence intervals of the fitted parameters are also given.

HA	K_F[a]	N	r^2	K_d range (L kg^{-1})	K_d linear (L kg^{-1})	%S
LHA	41,200 ± 3,240	0.64 ± 0.10	0.98	88,300 – 37,800	72,900 ± 6,740	93
ESHA	16,400 ± 1,530	0.55 ± 0.11	0.96	28,900 – 8,650	22,300 ± 3,300	83
PPHA	17,000 ± 990	0.63 ± 0.08	0.99	27,900 – 11,800	23,500 ± 4,620	84
SRHA	12,100 ± 1,360	0.66 ± 0.10	0.98	16,100 – 7,080	12,600 ± 1,550	70
FHA	73,000 ± 8,300	0.62 ± 0.02	0.97	209,000 – 84,700	158,000 ± 19,900	97
AHA	42,400 ± 1,690	0.52 ± 0.05	0.99	195,000 – 40,500	137,000 ± 36,700	97
JHA	29,300 ± 880	0.53 ± 0.05	0.99	98,200 – 20,300	62,400 ± 18,800	93

[a] K_F units are (mg kg^{-1})/(mg L^{-1})^N

were tested, the saturation of the sorption sites with a high affinity led to sorption at sites with a lower affinity. The presence of the different sorption sites involved may indicate different interaction mechanisms between NOR and HAs.

K_d values at the different C_{in} tested were calculated with Eq. 3, yielding a range of K_d values for each HA. As a closer look at the isotherms revealed a linear pattern at low C_{in} values (1 – 3 mg L^{-1}), with $r^2 > 0.93$, $K_{d,linear}$ values, which are more representative of environmental scenarios, were determined from the slope of the isotherm in the linear range. Similarly, sorption percentages (%S) were calculated using Eq. 5 for the experimental points falling within the linear range of the sorption isotherm (C_{in} values ranging 1 – 3 mg L^{-1}) and are reported as the mean value.

$$\%S = \frac{\left(C_{in} - C_{eq}\right)}{C_{eq}} \cdot 100 \tag{5}$$

The K_d values for NOR derived within the linear range of the sorption isotherm correlated well with the derived K_F values ($r^2 = 0.88$; $p < 0.01$) and varied within one order of magnitude depending on the physicochemical properties of the HA (from 12,600 to 158,000 L kg^{-1}). By contrast, the K_d ranges showed variations up to a maximum of five-fold depending on the C_{in}, indicating that the origin of the HA had a higher effect on K_d variability than the C_{in} tested.

Additionally, K_{OC} (NOR) values were also derived, by normalizing the K_d (NOR) values to the organic carbon (OC) content for each HA (K_{OC}, L kg OC^{-1}) according to Eq. 6:

$$K_{OC} = \frac{K_d}{f_{OC}} \tag{6}$$

where f_{OC} is the fraction of OC (kg OC kg^{-1}).

K_{OC} values ranged 23,100 – 344,200 L kg OC^{-1} and agreed with those reported in the literature for sarafloxacin and OFL (33,100 – 155,000 L kg OC^{-1}) at pH 5 – 6 [21,22]. The derivation of K_{OC} values has allowed to reduce K_d variability for some hydrophobic pollutants [23]. However, the variability that still persists in K_{OC} (NOR) values indicate that not only the amount of OC present in the HA matters, but also their functionalities, given the hydrophilic nature of FQs.

Overall, the high K_d values derived for all the HAs indicated a high sorption affinity of FQs for soil organic matter fractions at a slightly acidic pH, which is also in line with the high (≥ 70%) sorption percentages obtained.

Effect of initial FQ concentration on desorption

The effect of the initial FQ concentration on desorption pattern was also evaluated by deriving desorption isotherms. To run desorption experiments, the respective solid residues from the sorption isotherms were incubated at 40°C for 72 hours to simulate soil aging, before adding 30 mL of a fresh aqueous solution containing 10 mM $CaCl_2$ and 0.1% NaN_3 adjusted to pH 5.0, without a further NOR spike. Only the HA sorption residues for LHA, PPHA and ESHA were chosen to evaluate NOR desorption. The tubes were shaken for 48 hours, which was expected to be enough based on previous findings [7].

The concentration remaining in the solid phase after running the desorption experiments ($C_{S,des}$, mg kg^{-1}) was calculated as detailed in Eq. 7:

$$C_{S,des} = C_{in,des} - \frac{C_{eq,des} \cdot V}{m} \tag{7}$$

where $C_{eq,des}$ (mg L^{-1}) is the FQ concentration in the aqueous phase quantified by HPLC-FLD after the desorption experiments and $C_{in,des}$ (mg kg^{-1}) the initial FQ concentration in the solid after the sorption step. Thus, $C_{in,des}$ is C_S corrected by the amount of FQ present in the residual volume of the solution (V_{res}, L^{-1}) and incorporated into the solid after drying the residues from the sorption experiments (Eq. 8):

$$C_{in,des} = C_S + \frac{C_{eq} \cdot V_{res}}{m} \tag{8}$$

The desorption isotherms for NOR in the selected materials are displayed in Fig. 5. Similar to the sorption results, several desorption parameters were derived for the interpretation of the data, as summarized in Table 4.

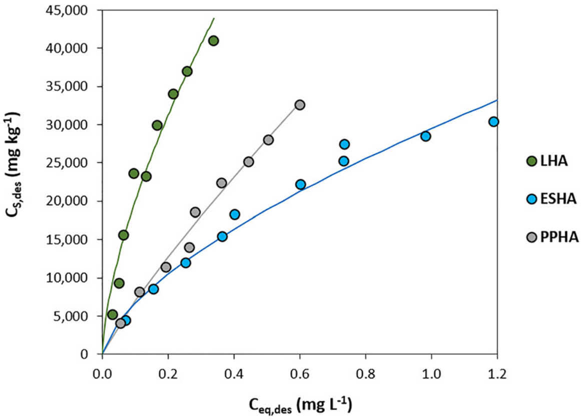

Fig. 5. NOR desorption isotherms in LHA, ESHA and PPHA. Dashed lines indicate the Freundlich fitting.

Table 4
Desorption parameters for NOR in LHA, ESHA and PPHA. 95% confidence intervals of the fitted parameters are also given.

HA	K_F[a]	N	r^2	$K_{d,des}$ range (L kg^{-1})	$K_{d,des}$ linear (L kg^{-1})	%D	H
LHA	87,900 ± 2,500	0.64 ± 0.17	0.95	251,000 – 121,000	215,000 ± 60,400	2.7	2.9
ESHA	29,500 ± 2,910	0.64 ± 0.17	0.94	63,900 – 25,600	50,300 ± 8,500	9.3	2.3
PPHA	51,200 ± 5,640	0.87 ± 0.12	0.99	74,200 – 53,200	63,300 ± 11,900	7.3	2.7

[a] K_F units are (mg kg^{-1})/(mg L^{-1})^N

Given that the overall desorption isotherms showed a non-linear pattern, they were also fitted to the Freundlich model (Eq. 4). Furthermore, $K_{d,des}$ values at the different C_{in} tested were calculated using Eq. 9, producing a range of $K_{d,des}$ for each HA. In the desorption tests, the isotherms also revealed a linear pattern ($r^2 > 0.94$) at low FQ concentrations (C_{in} = 1 – 3 mg L^{-1}). Linear desorption solid-liquid distribution coefficients ($K_{d,des}$) were determined from the slope of the isotherm in the linear range. Additionally, desorption percentages (%D) were calculated using Eq. 10 for the experimental points falling within the linear desorption isotherm range, with the mean values reported. The hysteresis coefficient (H), which indicates the degree of sorption irreversibility, was calculated with Eq. 11.

$$K_{d,des} = \frac{C_{S,des}}{C_{eq,des}} \tag{9}$$

$$\%D = \frac{C_{eq,des} \cdot V}{C_{in,des} \cdot m} \cdot 100 \tag{10}$$

$$H = \frac{K_{d,des}}{K_d} \tag{11}$$

The $K_{d,des}$ values derived within the linear isotherm range for the three different HAs ranged from 50,300 to 215,000 L kg^{-1} and were higher than the respective sorption K_d values in all cases. The $K_{d,des}$ ranges derived for individual experimental points varied around two-fold, and the desorption percentages were relatively low ($\leq$ 9.3%). The hysteresis coefficients were higher than two in all the HA materials tested, suggesting strong sorption irreversibility at a low initial FQ concentration. The desorption isotherm patterns also suggested that the fraction of FQ sorbed at low-affinity sites were also more likely to be remobilized to the liquid phase, which was observed at increasing FQ concentrations.

Effect of calcium concentration and DOC content on FQ sorption

FQ sorption in soils is affected by the cationic content of the aqueous solution, and besides, they are capable of interacting with certain solubilized DOC species [24,25]. Therefore, it is of interest to derive sorption parameters under certain conditions involving both calcium and DOC. The DOC content in soils typically ranges from 15 to 40 mg C L^{-1}, although it can be higher in organic soils. The sum of water-soluble cations (Na, K, Mg and Ca) in soils with contrasting physicochemical properties typically ranges from 1 to 10 meq L^{-1} [15,23].

The effect of the initial calcium concentration in the aqueous phase on NOR sorption was evaluated in LHA, PPHA and ESHA. The DOC content under these different calcium conditions was also simultaneously monitored (Analytik Jena Multi N/C). The calcium concentrations of the aqueous phase were set to 0 (Milli-Q water, referred to as the sample Ca scenario, being < 0.005 mM in all cases), 5, 10 or 20 mM $CaCl_2$, all of them adjusted to pH 5.0 with HCl. With the exception of the experiments performed in Milli-Q water, all the aqueous solutions contained 0.1% NaN_3. NOR was spiked at a concentration of 2 mg L^{-1}. The sorbed concentrations were calculated using Eq. 1, while the K_d values were calculated with Eq. 3.

The results of these experiments are depicted in Fig. 6, while the specific K_d values under the different conditions are reported in Table S1 of the Supplementary Material. NOR is mainly present in its cationic form at pH 5.0. Thus, calcium cations may compete with NOR for the available sorption sites present in the HA. NOR sorption was reduced in all the HA samples by an increase in the calcium concentration from 0 to 5 mM, with the K_d values decreasing between three- and six-fold in the presence of calcium. The reduction of the observed K_d at increasing calcium concentrations from 5 to 20 mM was similar for the three tested HAs, with the decrease for ESHA being slightly greater at a calcium concentration of 20 mM.

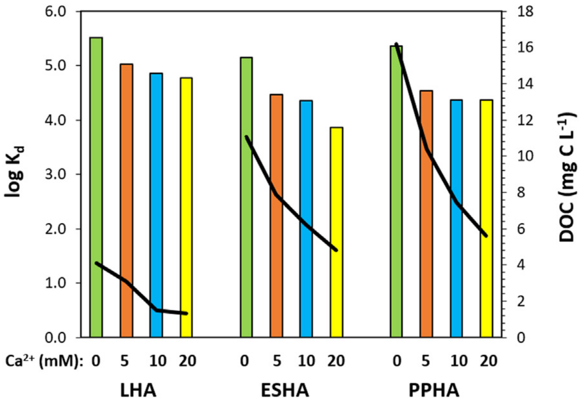

Fig. 6. Effect of calcium concentration (bars) and DOC content (solid lines) on NOR sorption by LHA, ESHA and PPHA.

The increase in the calcium concentration (and thus in the ionic strength) of the contact solution reduced the DOC content, likely through an associated decrease in organic compound solubility and an increase in organic matter flocculation. The resulting DOC contents were dependent on the HA characteristics and were relatively low (a maximum DOC content of 17 mg C L^{-1} was found in the sample Ca scenarios). The higher sorption observed when the DOC contents were maximal suggested that DOC did not have a significant effect on K_d (NOR) under the experimental conditions tested. Overall, the calcium concentration was the main factor inhibiting NOR sorption from 0 to 5 mM Ca, with a minor additional effect for higher calcium concentrations regardless of the DOC content.

Effect of pH on FQ sorption

The effect of pH on NOR sorption by LHA, PPHA and ESHA was also evaluated. Aqueous solutions containing 10 mM $CaCl_2$ and 0.1% NaN_3 were adjusted, using HCl or NaOH depending on the acid/base properties of the HA, to a pH value from 3 to 11 after the pre-equilibrium state and prior to the NOR spike. NOR was spiked at a concentration of 2 mg L^{-1}, and the pH after equilibrium was monitored. The sorbed concentrations were calculated using Eq. 1, while the K_d values were derived with Eq. 3. The log K_d vs. pH relationship for NOR in LHA, ESHA and PPHA at a pH ranging from 3 to 11 is shown in Fig. 7. The specific K_d values in the different tested conditions are reported in Table S1.

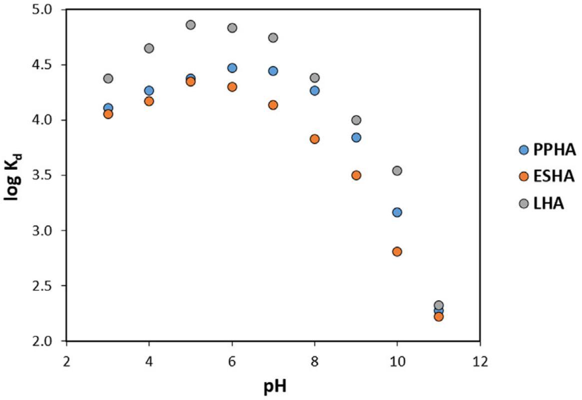

Fig. 7. Sorption of NOR by HAs at varying pH values.

A bell-shaped distribution of the log K_d (NOR) values with an increasing pH was observed, which agrees with the changes in the NOR species at varying pH values (see Fig. 1). NOR sorption was the highest at pH ∼ 5, indicating that the sorption of positively-charged FQ species was favoured. A further increase in pH led to a decrease in NOR sorption, likely due to the electrostatic repulsions between the negatively-charged functional groups present on the HA surface and the NOR zwitterion. The decrease in sorption was also observed at pH > pK_{a2}, in which NOR existed mainly in its negative form, leading to log K_d values of around 2.3. In fact, under basic pH conditions, the role of the HA characteristics diminished, with the K_d (NOR) values approaching to each other in the three HA samples tested. To determine the contribution of each FQ species in sorption, a pH-partitioning model was applied according to Septian et al. [26]. The experimental points of log K_d (NOR) *vs* pH were fitted to Eq. 12 using Matlab (cftoolbox, Matlab® R2009a (MathWorks Inc., USA)) for each HA sample:

$$\log K_d = \log K_d\left(FQ^+\right)\, f\,\left(FQ^+\right) + \log K_d\left(FQ^\pm\right)\, f\,\left(FQ^\pm\right) + \log K_d(FQ^-)\, f\,(FQ^-) \tag{12}$$

where f (FQ) is the fraction of the respective FQ species that can be expressed as a function of pH, and log K_d values normalized to the cationic (log K_d (FQ^+)), zwitterion (log K_d ($FQ^\pm$)) and anionic (log K_d (FQ^-)) fluoroquinolone species can be obtained from the fitting. Results of the pH-partitioning model are reported in Table 5. Log K_d (FQ^+) and log K_d ($FQ^\pm$) values in each HA sample were comparable and higher than the respective log K_d (FQ^-), suggesting a lower sorption affinity of the negatively charged FQ species. The relatively low fitting goodness of the pH-partitioning model, also observed for CIP in clay minerals [26], illustrates the difficulty of this model to consider potential repulsions between FQs and sorbent surface charge at extreme pH values, where the fraction of a single FQ species is predominant (f ≈ 1) but the surface charge of the HA is still affected by pH changes.

Table 5
pH-partitioning model fitting parameters. 95% confidence intervals are reported.

Humic Acid	log K_d (FQ^+)	log K_d ($FQ^\pm$)	log K_d (FQ^-)	r^2
LHA	4.63 ± 0.59	4.92 ± 0.88	3.04 ± 0.67	0.80
ESHA	4.20 ± 0.38	4.28 ± 0.56	2.63 ± 0.43	0.90
PPHA	4.24 ± 0.48	4.71 ± 0.71	2.86 ± 0.55	0.84

K_d (NOR) data in each HA sample was additionally fitted to second-order polynomial equations, resulting in fitting goodness of around 0.98 (see Table 6). This approach allowed to calculate the pH values for which sorption was maximized for each HA. The calculated maximum sorption occurred at pH ~ 5.5 for the three tested HA samples, for which NOR was mainly under cationic form.

Table 6
Second-order model fitting parameters. 95% confidence intervals are reported.

Humic Acid	Fitting results of the second-order equation	r^2	pH of maximum sorption
LHA	$\log K_d = -0.08\ (\pm 0.02)\ pH^2 + 0.95\ (\pm 0.25)\ pH + 2.23\ (\pm 0.80)$	0.98	5.6
ESHA	$\log K_d = -0.06\ (\pm 0.01)\ pH^2 + 0.67\ (\pm 0.10)\ pH + 2.57\ (\pm 0.33)$	0.99	5.2
PPHA	$\log K_d = -0.08\ (\pm 0.02)\ pH^2 + 0.89\ (\pm 0.24)\ pH + 2.00\ (\pm 0.78)$	0.98	5.7

Individual K_d values within the whole pH range varied around 150-fold for ESHA and PPHA and 300-fold for LHA, highlighting the significant role of pH in FQ sorption by HAs. Overall, these pH-dependent sorption trends and the derived K_d values highlighted the significant role of FQ speciation in the sorption process, which has to be considered when designing FQ sorption tests.

Comparison of the sorption behavior of different FQs

After investigating a number of experimental factors potentially affecting FQ sorption, the FQ sorption analogy in LHA, PPHA and ESHA was also examined. The different FQs were spiked at a concentration of 2 mg L^{-1}. The sorbed concentrations were calculated using Eq. 1, while the K_d values were derived with Eq. 3.

The K_d (FQ) values obtained for all four FQs in the three HAs are summarized in Fig. 8. Specific K_d (FQ) values can be found in Table S1. All the K_d (FQ) values were high. A general trend in the K_d (FQ) values was observed among the four FQs as follows: NOR > CIP > ENR > OFL, which was inversely related to the molecular weight and/or the log K_{OW} of the FQs [27]. The K_d values between NOR (highest sorption) and OFL (lowest sorption) varied by around three-fold for LHA and PPHA and by five-fold for ESHA. Two-way ANOVA with replicates run at $\alpha = 0.05$ revealed that both the HA material and the FQ significantly affected K_d (FQ) values.

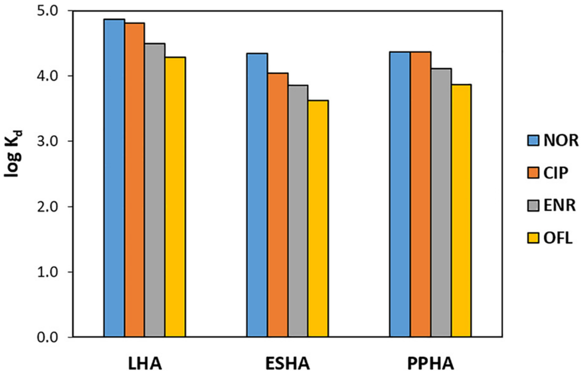

Fig. 8. Log K_d (FQ) values of FQs in LHA, ESHA and PPHA.

Conclusions

The outputs of this study confirm the need to apply modifications to standard batch tests in order to evaluate the different factors affecting sorption and to assess their impact on K_d variability. As FQ sorption by HAs is affected by multiple parameters (e.g., pH, ionic strength and the initial FQ concentration), sorption data derived for FQs in HAs in the literature may contain high variability. Shaking time and DOC content had a low impact on K_d (FQ) variability, whereas pH, calcium concentration and the initial FQ concentration highly influenced K_d (FQ) variability. The effects of these factors have to be considered when deriving FQ sorption data in HAs with reduced variability and high environmental representativeness.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Joel Fabregat-Palau: Conceptualization, Methodology, Formal analysis, Validation, Data curation, Visualization, Writing – original draft. **Anna Rigol:** Conceptualization, Methodology, Resources, Visualization, Supervision, Writing – review & editing, Project administration, Funding acquisition. **Zhiqiang Yu:** Conceptualization, Methodology, Resources, Supervision, Funding acquisition. **Miquel Vidal:** Conceptualization, Methodology, Resources, Visualization, Supervision, Writing – review & editing, Project administration, Funding acquisition.

Data availability

Data will be made available on request.

Acknowledgments

This work was carried out in a Generalitat de Catalunya Research Group (2021 SGR 01342) and has received funding from the Ministerio de Ciencia e Innovación de España (PID2020-114551RB-I00). Joel Fabregat-Palau acknowledges funding from the

Europe Aid ‘SEW-REAP’ project [ECRIP ICI+/2014/348–010] of the European Union. The authors acknowledge Dr. Laura Rodríguez (Universitat de Barcelona) for the discussion of the FTIR data, and the Centres Científics i Tecnològics of the Universitat de Barcelona (CCiTUB) personnel that provided guidance during the HPLC analyses.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2023.102109.

References

[1] M.I. Andersson, A.P. McGowan, Development of the quinolones, J. Antimicrob. Chemother. 51 (90001) (2003) 1–11, doi:10.1093/jac/dkg212.

[2] L. Riaz, T. Mahmood, A. Khalid, A. Rashid, M.B. Ahmed Siddique, A. Kamal, M.S. Coyne, Fluoroquinolones (FQs) in the environment: A review on their abundance, sorption and toxicity in soil, Chemosphere 191 (2018) 704–720, doi:10.1016/j.chemosphere.2017.10.092.

[3] L.S. Redgrave, S.B. Sutton, M.A. Webber, L.J.V. Piddock, Fluoroquinolone resistance: Mechanisms, impact on bacteria, and role in evolutionary success, Trends in Microbiol 22 (8) (2014) 438–445, doi:10.1016/j.tim.2014.04.007.

[4] WHOGlobal Priority list of Antibiotic-Resistance Bacteria to Guide Research, Discovery, and Development of New Antibiotics, World Health Organization, Geneva, 2017 https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed Accessed 10 December 2022.

[5] M. Teixidó, J. Medeiros, J. Beltrán, M.D. Prat, M. Granados, Sorption of enrofloxacin and ciprofloxacin in agricultural soils: Effect of organic matter, Adsorpt. Sci. Technol. 32 (2–3) (2014) 153–163, doi:10.1260/0263-6174.32.2-3.153.

[6] D. Wu, H. Li, S. Liao, X. Sun, H. Peng, D. Zhang, B. Pan, Co-sorption of ofloxacin and Cu(II) in soils before and after organic matter removal, Sci. Total Environ. 481 (1) (2014) 209–216, doi:10.1016/j.scitotenv.2014.02.041.

[7] M.J. Martínez-Mejía, I. Sato, S. Rath, Sorption mechanism of enrofloxacin on humic acids extracted from Brazilian soils, Environ. Sci. Pollut. Res. 24 (19) (2017) 15995–16006, doi:10.1007/s11356-017-9210-3.

[8] OECD, OECD 106 Adsorption - Desorption Using a Batch Equilibrium Method, OECD Guideline for the Testing of Chemicals (2000) 1–44, doi:10.1787/9789264069602-en.

[9] X. Van Doorslaer, J. Dewulf, H. Van Langenhove, K. Demeestere, Fluoroquinolone antibiotics: An emerging class of environmental micropollutants, Sci. Total Environ. 500–501 (2014) 250–269, doi:10.1016/j.scitotenv.2014.08.075.

[10] A. Rusu, G. Tóth, L. Szocs, J. Kökösi, M. Kraszni, Á. Gyéresi, B. Noszál, Triprotic site-specific acid-base equilibria and related properties of fluoroquinolone antibacterials, J. Pharm. Biomed. Anal. 66 (2012) 50–57, doi:10.1016/j.jpba.2012.02.024.

[11] A. Rigol, J.F. López-Sánchez, G. Rauret, Capillary zone electrophoresis of humic acids, J. Chromatogr. A 664 (1994) 301–305, doi:10.1016/0021-9673(94)87021-7.

[12] J. Fabregat-Palau, M. Vidal, A. Rigol, Examining sorption of perfluoroalkyl substances (PFAS) in biochars and other carbon-rich materials, Chemosphere 302 (2022) 134733, doi:10.1016/j.chemosphere.2022.134733.

[13] J.L. Weishaar, G.R. Aiken, B.A. Bergamaschi, M.S. Fram, R. Fujii, K. Mopper, Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon, Environ. Sci. Technol. 37 (20) (2003) 4702–4708, doi:10.1021/es030360x.

[14] F.J. Rodriguez, L.A. Núñez, Characterization of aquatic humic substances, Water Environ. J. 25 (2011) 163–170, doi:10.1111/j.1747-6593.2009.00205.x.

[15] J. Fabregat-Palau, Z. Yu, X. Zeng, M. Vidal, A. Rigol, Deriving parametric and probabilistic K_d values for fluoroquinolones in soils, Sci. Total Environ. Available online (2022) 160266, doi:10.1016/j.scitotenv.2022.160266.

[16] L.M. Peruchi, A.H. Fostier, S. Rath, Sorption of norfloxacin in soils: Analytical method, kinetics and Freundlich isotherms, Chemosphere 119 (2015) 310–317, doi:10.1016/j.chemosphere.2014.06.008.

[17] L. Aristilde, G. Sposito, Complexes of the antimicrobial ciprofloxacin with soil, peat, and aquatic humic substances, Environ. Toxicol. Chem. 32 (7) (2013) 1467–1478, doi:10.1002/etc.2214.

[18] B. De Witte, J. Dewulf, K. Demeestere, M. De Ruyck, H. Van Langenhove, Critical points in the analysis of ciprofloxacin by high-performance liquid chromatography, J. Chromatogr. A 1140 (1–2) (2007) 126–130, doi:10.1016/j.chroma.2006.11.076.

[19] Y.S. Ho, G. McKay, Sorption of dye from aqueous solution by peat, Chem. Eng. J. 70 (2) (1998) 115–124, doi:10.1016/S1385-8947(98)00076-X.

[20] G. Limousin, J.-P. Gaudet, L. Charlet, S. Szenknect, V. Barthès, M. Krimissa, Sorption isotherms: A review on physical bases, modeling and measurement, Appl. Geochem. 22 (2007) 249–275, doi:10.1016/j.apgeochem.2006.09.010.

[21] H-C.H. Lützhøft, W.H.J. Vaes, A.P. Freidig, B. Halling-Sørensen, J.L.M. Hermens, Influence of pH and other modifying factors on the distribution behavior of 4-quinolone and humic acids studied by “Negligible-Depletion” SPME-HPLC, Environ. Sci. Thecnol. 34 (2000) 4989–4994, doi:10.1021/es000917y.

[22] L. Wang, N. Liang, H. Li, Y. Yang, D. Zhang, S. Liao, B. Pan, Quantifying the dynamic fluorescence quenching of phenanthrene and ofloxacin by dissolved humic acids, Environ. Pollut. 196 (2015) 379–385, doi:10.1016/j.envpol.2014.10.029.

[23] J. Fabregat-Palau, M. Vidal, A. Rigol, Modelling the sorption behaviour of perfluoroalkyl carboxylates and perfluoroalkane sulfonates in soils, Sci. Total Environ. 801 (2021) 149343, doi:10.1016/j.scitotenv.2021.149343.

[24] N. Carmosini, L.S. Lee, Ciprofloxacin sorption by dissolved organic carbon from reference and bio-waste materials, Chemosphere 77 (6) (2009) 813–820, doi:10.1016/j.chemosphere.2009.08.003.

[25] X. Kong, S. Feng, X. Zhang, Y. Li, Effects of bile salts and divalent cations on the adsorption of norfloxacin by agricultural soils, J. Environ. Sci. 26 (2014) 846–854, doi:10.1016/S1001-0742(13)60480-5.

[26] A. Septian, S. Oh, W.S. Shin, Sorption of antibiotics onto montmorillonite and kaolinite: competition modelling, Environ. Technol. (2018) 1–14, doi:10.1080/09593330.2018.1459870.

[27] D.L. Ross, S.K. Elkinton, C.M. Riley, Physicochemical properties of the fluoroquinolone antimicrobials. III 1-Octanol/water partition coefficients and their relationships to structure, Int. J. Pharm. 88 (1992) 379–389, doi:10.1016/0378-5173(92)90336-Z.