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Removal of sulfonamide antibiotics from water: Evidence of adsorption into an organophilic zeolite Y by its structural modifications

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ABSTRACT

Sulfonamide antibiotics are persistent pollutants of aquatic bodies, known to induce high levels of bacterial resistance. We investigated the adsorption of sulfadiazine, sulfamethazine, and sulfachloropyridazine sulfonamides into a highly dealuminated faujasite zeolite (Y) with cage window sizes comparable to sulfonamide dimensions. At maximal solubility the antibiotics were almost completely (>90%) and quickly (t < 1 min) removed from the water by zeolite. The maximal amount of sulfonamides adsorbed was 18–26% DW of dry zeolite weight, as evidenced by thermogravimetric analyses and accounted for about one antibiotic molecule per zeolitic cage. The presence of this organic inside the cage was revealed by unit cell parameter variations and structural deformations obtained by X-ray structure analyses carried out using the Rietveld method on exhausted zeolite. The most evident deformation effects were the lowering of the *Fd*-3*m* real symmetry in the parent zeolite to *Fd*-3 and the remarkable deformations which occurred in the 12-membered ring cage window after sulfadiazine or sulfachloropyridazine adsorption. After sulfamethazine adsorption, zeolite deformation caused a lowering in symmetry up to the monoclinic *P*2/*m* space group. The effective and irreversible adsorption of sulfonamides into organophylic Y zeolite makes this cheap and environmentally friendly material a suitable candidate for removing sulfonamides from water.

1. Introduction

In recent years, the occurrence and fate of pharmaceutically active compounds in the aquatic environment has been recognized as one of the emerging issues in environmental chemistry [1]. The occurrence of antibiotics in hospital effluents and waste or surface waters may present two kinds of risks. Firstly, after supply, antibiotics select for resistant bacteria in the treated individuals themselves. Secondly, the presence of antibiotics in streams, lakes and water supplies encourages the growth of resistant bacteria in humans and wildlife [2]. Growing resistance means that what were once effective and cheap antibiotics may become unsuitable for treating infections.

Sulfonamide antibiotics comprise a class of synthetic sulfanilamide derivatives, widely used for the treatment of bacterial, protozoal and fungal infections in human therapy, livestock production and aquaculture [3]. They act as competitive inhibitors of p-aminobenzoate in folate biosynthesis. Sulfonamides are known to induce high levels of resistance using a bypass mechanism—the metabolic step which is inhibited by the antibiotic may be replaced (bypassed) by an alternative metabolic step [4]. They are sufficiently stable in manure to maintain significant residual activity until field application [5]. In liquid manure, N-aniline acetyl sulfonamides conjugates can be cleaved back to parent compounds [6]. They may reach the soil through the faeces of treated grazing livestock and/or the spreading of manure as fertilizer on agricultural soils where they may persist in an unmetabolized form for months [7]. In non-acidic soils, sulfonamides may exist mainly in anionic form due to the pK_a value of the sulfonamide group (pK_a 5.0–7.5 [8,9]) and therefore be potentially highly mobile and thus, pollute water bodies [10]. In fact, the presence of a net negative charge on soil surfaces makes this environmental compartment ineffective in the retention of negative compounds. The adsorption of sulfonamide antibiotics in soil organic matter is reported to be a time-dependent process [11], while on clay components it exhibits pronounced acidic pH dependence [12]. Sulfonamides may also directly reach water bodies through hospital and fish farming wastewaters [1] bypassing soil filtering and depuration activity. Furthermore, in municipal sewage treatment plants, sulfonamides may not be effectively eliminated owing to their anionic character. In fact, pollutant biodegradation is largely achieved by sorption on activated sludge, which is partly mediated through hydrophobic

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interactions [13]. Approximately 20 years after industrial aquaculture began, evidence has emerged on the transfer of antibiotic resistance between aquatic bacteria and human pathogens [14]. A number of important studies indicate that the bacterial flora in the environment surrounding aquaculture sites contain an increased number of antibiotic-resistant bacteria [15].

Despite the need to clean up wastewaters which have been highly polluted with sulfonamide antibiotics, no sorbents with specific adsorption potential and favourable kinetics have been identified to date. The aim of this study is to verify the efficiency of sorbents such as zeolitic materials in removing sulfonamide antibiotics from water bodies.

Zeolites are crystalline alumino-silicates, characterized by three-dimensional networks containing channels and cavities whose dimensions are comparable with small organic molecules. Such networks of well-defined micropores may act as adsorption and reaction sites whose selectivity and activity can be modulated by acting on their structure and chemical composition. The three-dimensional framework, consisting of nanometre-sized channels and cages, imparts high porosity and a large surface area to these materials. One of their defining features is that the shape of their internal pore structure can strongly affect their adsorption selectivity with respect to host molecules. The most fundamental consideration regarding the adsorption of chemical species by zeolites is molecular sieving. The pores, or rather the active sites within the pores, exclusively process molecules that fit them, so that species with a kinetic diameter which makes them too large to pass are effectively "sieved".

Zeolites are commonly used in areas as diverse as laundry detergents, gas separation, oil refining and the petrochemical industries, agriculture, wastewater and sewage treatment. The ability of zeolites with a low Si/Al ratio to remove cations by ionic exchange has been largely demonstrated and utilized in water treatment plants which produce drinking water [16]. On the contrary, zeolites characterized by a high Si/Al ratio are hydrophobic and organophilic materials widely used in adsorption-related applications. To date, studies and applications on organic pollutant adsorption in these microporous materials from aqueous media are scarce [17–19]. Moreover, their purpose is the remediation of non-polar hydrocarbons pollutants which are frequently found in refineries and gasoline station groundwater [20] and not of negatively ionisable and polar compounds, such as sulfonamide antibiotics.

In this study, a Y faujasite hydrophobic zeolite is presented due to its ability to specifically adsorb high amounts of sulfonamide antibiotics inside its cavities with very favourable kinetics. These findings were supported by X-ray analyses which demonstrated the accommodation of antibiotic molecules inside the zeolite cage.

2. Experimental

2.1. Materials

Sulfadiazine (4-amino-2-N-pyrimidinyl-benzene sulfonamide, SD), sulfamethazine (4-amino-N-(4,6-dimethyl-2-pyrimidinyl) benzene sulfonamide, SM), and sulfachloropyridazine (4-amino-N-(6-Cl-3-pyridazinyl)benzene sulfonamide, SC) were purchased as analytical standards by Dr. Ehrenstorfer GmbH (Germany) with a purity of 98.7%, 99.5%, and 98.0%, respectively. These antimicrobial agents have been chosen because of their widespread consumption and predominant occurrence in water bodies and soils [21–23]. The chemical structures of sulfonamides are shown in Table 1. Stock solutions of antibiotics at maximal solubility were prepared by adding SD, SM and SC antibiotics to distilled water in amounts exceeding those required to saturate the solution. The suspensions were sonicated for 15 min, shaken at 50 °C for 30 min and, after cooling at room temperature, filtered through 0.45 μm Durapore® membrane filters to eliminate the undissolved solute from the solutions. The solubility of the antibiotics, measured by means of high performance liquid chromatography (HPLC), was 71.9 ± 4.8, 135.8 ± 3.9 and, 173.6 ± 7.7 μM for SD, SM and SC, respectively.

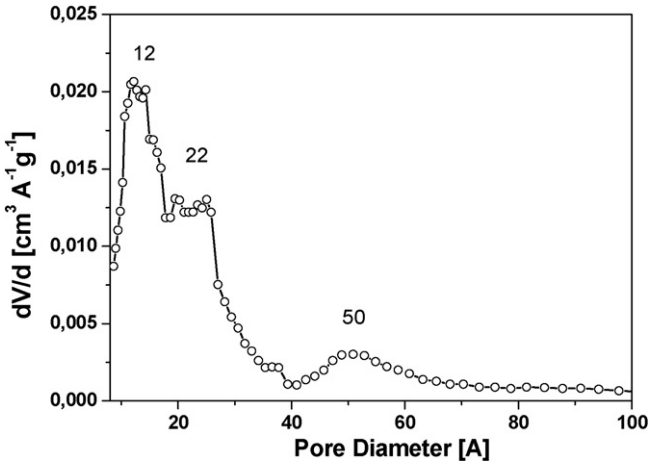


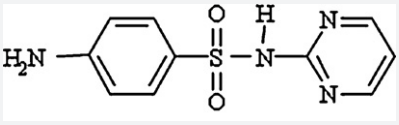
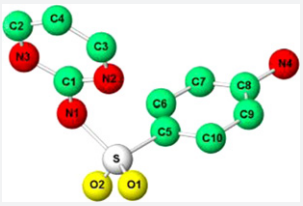
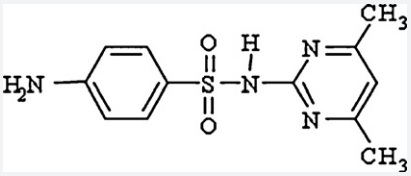
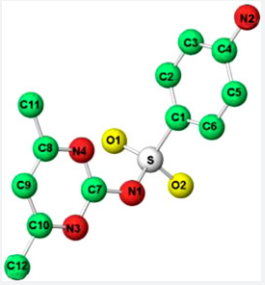
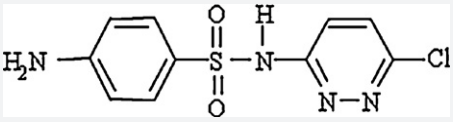
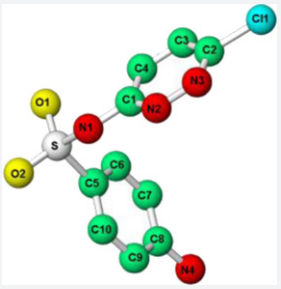
Fig. 1. Pore size distribution of zeolite Y determined by nitrogen adsorption isotherm at −196 °C using a cylindrical pore NLDFT method in the desorption branch.

"Y" type faujausite zeolite powder (code HSZ-390HUA) with a 200 SiO_2/Al_2O_3 (mol/mol) ratio, a 7.0 Å × 7.1 Å dimension and a 12-membered ring window diameter was purchased in its protonated form from the Tosoh Corporation (Japan). The specific surface area was measured by means of nitrogen adsorption at liquid nitrogen temperature (−196 °C) in the pressure range 5×10^{-6} to 760 torr (1 Torr = 133.33 Pa) using an Autosorb-1-MP (Quantachrome Instruments) (Fig. S-1 in Supporting Information). Prior to adsorption, the samples were outgassed for 1 h at 90 °C, 1 h at 130 °C, and finally 16 h at 300 °C under high vacuum conditions (final pressure 1×10^{-9} Torr). The specific zeolite Y area, determined by the Brunauer–Emmett–Teller approach and using 0.01 as the value of maximum relative pressure, was 853 $m^2\ g^{-1}$ (Table 2). The pore size distribution was calculated by applying the cylindrical pore NLDFT method in the desorption branch (Fig. 1). A total specific surface area of ca. 850 $m^2\ g^{-1}$, most of which related to the presence of structural micropores (616 $m^2\ g^{-1}$), was determined (Table 2). As expected, zeolite Y shows structural 12 Å micropores whose related volume is 0.21 $cm^3\ g^{-1}$. The presence of 22 Å (related volume = 0.12 $cm^3\ g^{-1}$) and 50 Å pores (related volume = 0.08 $cm^3\ g^{-1}$) were also found.

2.2. Adsorption screening

A preliminary study of the adsorption properties of zeolite Y from distilled water towards a mixture of sulfonamide antibiotics was conducted. The adsorption screening was performed at room temperature and 65 °C, by adding the zeolite to a solution containing SD, SM, and SC (ca. 40 μM each, prepared by diluting antibiotics stock solutions) with a zeolite:antibiotic solution ratio of 1 mg:2 mL in polyallomer centrifuge tubes (Nalgene, NY, USA). Suspensions were shaken for 24 h and then centrifuged at 20,000 × *g* for 15 min. Finally, an aliquot of the supernatant was withdrawn and analyzed by HPLC. The amount of antibiotics adsorbed by zeolite was calculated by the difference between the initial and final concentrations. A control was run in the absence of zeolites in order to check the stability of the antibiotics and the adsorption properties of the centrifuge tube. No decrease in sulfonamides concentration was recorded after 24 h.

Table 1
Structures and chemical characteristics of sulfonamide antibiotics under investigation.

Structure	Chemical name (abbreviation)	Molecular weight ($g\,mol^{-1}$)	pK_a	3D structure[a]	Distance (Å)
	Sulfadiazine (SD)	250.3	6.4 [8]		C2–O1 (5.12) O1–N4 (7.38)
	Sulfamethazine (SM)	278.3	7.5 [8]		C12–N2 (10.50) C11–O2 (6.72)
	Sulfachloropyridazine (SC)	284.7	5.5 [9]		Cl1–O2 (8.11) O1–N4 (6.50)

[a] References for 3D structures of sulfadiazine, sulfamethazine and sulfacloropyridazine are [28–30], respectively.

The effect of dissolved organic matter (DOM) on the adsorption screening of zeolite Y towards sulfonamides was also investigated at room temperature. DOM was extracted from the first 10 cm of a forest soil sample (Lythic Ustorthents, sand:silt:clay = 40:44:16, pH 5.2) from the north-east of Italy (Imola, BO). The soil was chosen because of its high organic carbon content (total organic carbon 32 $g\,kg^{-1}$). The suspension formed by a 1:4 soil:distilled water ratio was shaken in polypropylene copolymer centrifuge tubes on a horizontal shaker for 24 h at RT. After centrifugation at 15,000 × *g* to remove the heaviest particulates, the DOM solution (pH 5.8, electrical conductivity = 0.16 mS) was used without any additional purification in order to verify if the presence of inorganic and organic molecules with different dimensions from those of sulfonamides could interfere with their adsorption into zeolite Y. The freeze–drying of the DOM solution gave 240 $mg\,L^{-1}$ as a residue. The nature and dimensional characterization of the DOM contained in the soil solution was not investigated since this went beyond the scope of this work.

2.3. Adsorption kinetics

With the aim of evaluating the time needed for antibiotics to reach the adsorption equilibrium in zeolites, adsorption kinetics for SD, SM, or SC on Y were followed over time. Zeolite Y was added to sulfonamide solutions (ca. 20 μM each) with a zeolite:antibiotic aqueous solution ratio of 1 mg:2 mL. Samples were shaken at room temperature and at different times, the supernatants were separated from the solid phase by centrifugation and directly analyzed by HPLC. Adsorption kinetics was conducted in triplicate.

2.4. Adsorption isotherms

SD, SM, and SC sulfonamides adsorption isotherms from distilled water were performed on zeolite Y in batches at room temperature with a zeolite:antibiotic solution ratio of 1 mg:2 mL. Owing to the low solubility of sulfonamides, zeolite Y was exposed to a number of adsorption cycles in the presence of a sulfonamide solu-

Table 2
Main textural properties of zeolite Y.

S_{BET}[a] ($m^2\,g^{-1}$)	S_{microp}[b] ($m^2\,g^{-1}$)	V_P[c] ($cm^3\,g^{-1}$)	V_{microp}[b] (12 Å) ($cm^3\,g^{-1}$)	V_{mesop}[d] (22 Å) ($cm^3\,g^{-1}$)	V_{mesop}[d] (50 Å) ($cm^3\,g^{-1}$)
852	616	0.62	0.21	0.12	0.08

[a] Brunauer–Emmet–Teller specific surface area.
[b] Micropore surface area and volume by *t*-plot method.
[c] Total pore volume.
[d] Volume of mesopores.

tion at maximal solubility. At each adsorption cycle, the suspension was shaken for 30 min and then centrifuged. The supernatant was removed and analyzed by HPLC, and replaced by fresh sulfonamide solution. Subsequent adsorption cycles were performed until the zeolite Y reached its maximal adsorption capacity (exhausted zeolite). Adsorption experiments were conducted in triplicate.

The antibiotic concentration in aqueous phase at equilibrium was expressed as C_e (μM) while the amount of antibiotics adsorbed in zeolite was calculated by the difference between the initial and final (C_e) concentrations and expressed as C_s (μmol g^{-1} of adsorbent).

The antibiotic concentration used in these experiments (maximal solubility for each sulfonamide) is ca. 1 order of magnitude higher than those measured in effluents from fish feedings [24] and ca. 3 orders higher than their occurrence in natural waters [3]. This procedure was considered opportune for obtaining the maximal loadings of zeolite in the shortest time in order to subsequently perform thermogravimetric and diffractometric analyses upon air-drying.

In a separate adsorption experiment, the exhausted zeolite suspension with the remaining antibiotic solution after the last adsorption cycle was used to test sulfa drugs desorption as described below.

2.5. Desorption isotherms

Desorption isotherms was carried out on exhausted zeolite by following this dilution technique. One-half volume of supernatant containing the antibiotic was removed and substituted with distilled water. The system was then shaken for 24 h and centrifuged. Then, a second half-volume of the diluted supernatant was replaced by an equal volume of distilled water and analyzed. The dilution step was repeated several times, until no analytes could be detected in the solution. The chemical concentration after each desorption step (C_e) was determined using HPLC, and the amount which remained adsorbed in the zeolite (C_s) was calculated by the difference. Desorption experiments were conducted in triplicate.

2.6. Chromatographic analyses

The concentration of the three sulfonamides was determined by HPLC-UV. The system was assembled with a Jasco 880-PU Intelligent pump, a Jasco AS-2055 plus Intelligent Sampler, a Jasco 875-UV Intelligent UV–vis detector at 224 nm, Borwin v 1.2160 chromatography software, a Jones Chromatography model 7971 column heater, and a 4.60 mm × 150 mm Synergi 4 μm Hydro-RP 80A analytical column (Phenomenex, USA). The analytical column was kept at 35 °C and eluted with acetonitrile:water (23:77 by volume, pH 2.7 for H_3PO_4) eluant at 1 mL min^{-1} flow. Under these chromatographic conditions, the retention times for SD, SM, and SC were 3.0, 4.0, and 6.0 min, respectively. All solvents were HPLC grade.

2.7. Thermogravimetric analyses

Thermogravimetric analyses were carried out using a TGDTA92 instrument (SETARAM, France). About 20 mg of pure antibiotic, Y zeolite, or exhausted zeolite were weighed into an aluminium crucible and heated continuously from 30 to 700 °C at a heating rate of 10 °C min^{-1} under airflow of 8 L h^{-1}. Calcinated kaolinite was used as a reference material. The furnace was calibrated using an indium transition temperature. The weight losses were referred to the weight of the air-dried sample.

2.8. Diffractometric analyses

A powder pattern of the as-synthesized Y sample was measured on a Bruker D8 Advance Diffractometer equipped with a Sol-X detector, using Cu $K\alpha_1,\alpha_2$ radiation in the 3–110° 2θ range and a counting time of 12 s $step^{-1}$. Rietveld structure refinement was then performed using the GSAS package [25] with EXPGUI interface [26]. X-ray diffraction (XRD) patterns were then collected on our zeolite Y after sulfadiazine, sulfamethazine and sulfachloropyridazine adsorption respectively, using the same experimental conditions as the parent Y zeolite.

In all Rietveld structure refinements, the Bragg peak profile was modelled using a pseudo-Voigt function with 0.01% cut-off peak intensity. The background curve was fitted using a Chebyschev polynomial with 16 variable coefficients. The 2θ-zero shift was accurately refined into the data set pattern. The scale factor and unit-cell parameters were allowed to vary for all the histograms. The refined structural parameters for each data histogram were the following: fractional coordinates and isotropic displacement factors for all atoms (one for each tetrahedral sites and framework oxygen atoms), and occupancy factors for extraframework ions. Occupancy factors and isotropic displacement factors were varied in alternate cycles. Soft constraints were imposed on tetrahedral cations and coordinated framework oxygen atom distances during the first stages of the refinements, and left free in the last cycles. The positions of extraframework sites were determined using Fourier and Difference Fourier maps.

3. Results and discussion

Faujasite Y (200 SiO_2/Al_2O_3 ratio) was the zeolite tested in this adsorption study, on the basis of its pore dimension and high hydrophobicity. Faujasite crystallizes in the cubic space group *Fd*-3*m*, with a lattice constant ranging from about 24.2 to 25.1 Å, depending on the framework aluminium concentration, cations, and state of hydration [27]. The pore structure is characterized by approximately 12 Å diameter cages, which are linked through access windows which are 7.0 Å × 7.1 Å in diameter and are composed of rings of twelve linked tetrahedra (12-membered rings). These cages and windows permit quite large molecules to enter, making this structure potentially useful in the adsorption of the sulfonamide antibiotics (sulfa drugs) under study.

Crystal structures for the three sulfa drugs, sulfadiazine [28], sulfamethazine [29], and sulfachloropyridazine [30], were crystallographically characterized using the single crystal XRD method. The molecular structure of these antibiotics is not linear, but the benzene and heteroaromatic ring form a distorted "V" configuration, with a dihedral angle of 76.0°, 75.5°, 83.0° in sulfadiazine, sulfamethazine, and sulfachloropyridazine, respectively. The molecular dimension of sulfonamides is given in Table 1. For all three molecules, at least one dimension is lower than the zeolite window diameter, thus making their diffusion through the cage window possible.

3.1. Adsorption screening

The adsorption screening of a mixture of the three antibiotics in zeolite Y gave positive results as shown in Fig. 2. All three sulfonamides were removed from the water to the same extent by zeolite Y (>90% of the ca. 40 μM initial concentration) revealing no temperature effect in the range between room temperature and 65 °C. These results indicate a non-competitive effect among the antibiotics for the zeolite adsorption sites. The dimensional and physical–chemical differences among the three sulfonamides structures (Table 1) are too small to differently influence their adsorption to zeolite sites. The absence of a temperature effect could be ascribed to covalent bonding. In our case, the formation of covalent bonds between the host and guest is excluded by stoichiometric antibiotic recovery when the zeolite is treated with an aqueous acetonitrile solution (50:50 by volume). Therefore, the

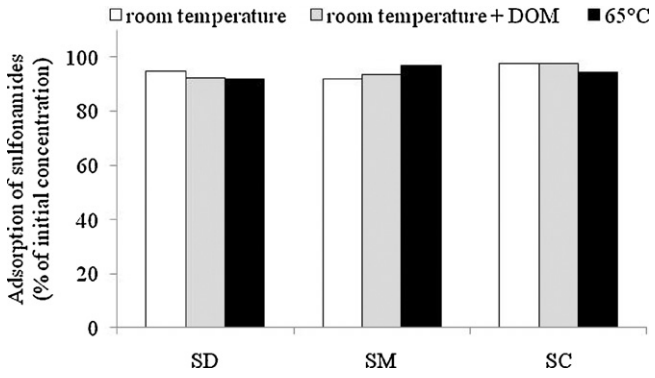


Fig. 2. Adsorption screening of a solution containing a mixture of sulfadiazine (SD), sulfamethazine (SM), and sulfachloropyridazine (SC) (40 μM each) in zeolite Y at different temperatures and in the presence of dissolved organic matter from forest soil (zeolite:antibiotic solution ratio of 1 mg:2 mL, mean of three replicates, standard deviation <4%).

absence of temperature effects on adsorption can be explained by assuming increased diffusivity of antibiotics molecules inside zeolite pores and cages which counterbalance the decreased extent of adsorption at the highest temperatures.

As far as the occurrence of ionic binding between zeolite and sulfonamides is concerned, both neutral and ionic forms of sulfonamides should be considered. Sulfa drugs are present in aqueous solution as a mixture of cationic, neutral and anionic molecules with the most abundant species as a function of their acidity along with the medium pH. In our experiment, before adsorption, antibiotic solutions were left in equilibrium with atmospheric CO_2, thus reaching a slightly lower pH than 6 (approximately 5.8). At this pH value, along with the neutral species, all three sulfonamides present their anionic form, which is the most abundant in sulfachloropyridazine (pK_a 5.5, Table 1) and which is less abundant in sulfadiazine (pK_a 6.4, Table 1) and sulfamethazine (pK_a 7.5, Table 1). Despite the presence of the anionic form for all the sulfa drugs when adsorption occurs, the adsorption of these anions has been ruled out as the zeolite which is used is not an anion exchanger. In fact, the high silica zeolite used does not have the positive charges that are involved in the stabilization of negative species.

Concerning the possibility for sulfonamides to be adsorbed in their protonated form, it should be taken into account that aromatic heterocyclic nitrogen is more basic than anilinic nitrogen. In fact, despite the more basic character of the amino group nitrogen (N in sp^3 hybridization) compared with that of aromatic heterocyclic nitrogen (N in sp^2 hybridization), the conjugation of lone pair electrons of aniline nitrogen with benzene ring carbons makes these electrons less available for protonation. The heterocyclic nitrogen k_b of sulfa drugs is too low ($pK_b > 12$; [31]) since protonation occurs. Nevertheless, sulfonamides could be protonated by the exchangeable protons (Brønsted sites) that are present on the zeolite surfaces and subsequently interact as cations. In our case, this mechanism of protonation followed by the binding of sulfa drugs under their cationic form cannot be explained by the low isomorphic substitutions of Al for Si in the zeolite being studied (200 SiO_2/Al_2O_3 ratio). In fact, the adsorption of one drug molecule per zeolite cage on average (vide infra) cannot be supported by the presence of approximately one exchangeable proton per eight cages. Therefore, the adsorption mechanism proposed is of a hydrophobic type because of its reversibility in organic solvents. In fact, if ionic interactions did occur, they ought not to have been weakened by interactions with an organic solvent which is less polar than water.

An adsorption screening of sulfonamides into zeolite Y from water containing organic matter which could interfere with antibiotic adsorption was performed in order to evaluate the performance of our sorbent under real conditions. The adsorption screening from water containing DOM from a forest soil, which is highly rich in organic matter (TOC 32 g kg^{-1}), showed an unmodified adsorption performance in comparison with those in distilled water (Fig. 2). This finding seems promising for zeolite Y field application.

3.2. Adsorption kinetics

The adsorption kinetics of the isolated sulfonamides in the zeolite Y was very favourable, and their removal from water was complete in less the 1 min (see Fig. S-2 in supporting information section). Therefore, adsorption in the subsequent experiments was performed with a contact time of 30 min to permit the process to reach an equilibrium state.

3.3. Adsorption/desorption isotherms

As the adsorption kinetics was favourable, an important question to be answered concerns the maximal zeolite adsorption capacity. For this reason, adsorption cycles were performed on zeolites for each single antibiotic due to the low solubility of the sulfa drugs. The maximal adsorption capacity of antibiotics in zeolite Y measured using HPLC was in the order of SD < SM < SC and equalled 15%, 21%, and 26% of the air-dried zeolite weight, respectively (see Fig. S-3 in supporting information section). The differences in the maximal adsorbed amount can only to some extent be attributed to the different antibiotic molar weight (see Table 1). In fact, the expression of the maximal adsorption capacity for zeolite Y in terms of antibiotics on a molar basis gives 583, 777, and 983 μmol g^{-1} zeolite for SD, SM, and SC, respectively.

In order to define the adsorption isotherms, the amount of antibiotic which remained in the solution at the equilibrium point, after each adsorption cycle, was reported as a function of the antibiotic as adsorbed in zeolite Y (Fig. 3). The adsorption isotherm for all sulfonamides clearly shows a two-stage trend, the first one is represented by the isotherm segment at low drug concentration (ranging from 0 to ca. 500 μmol g^{-1} zeolite along C_s axis) and the second one is described by the isotherm segment at a higher concentration. In the absence of degradation products, as in this case, these features could describe the different affinity of sulfonamides for zeolite adsorption sites. This different affinity was hypothesized as occurring due to interaction with zeolite pores of different dimensions: higher in micropores and lower in larger pores. It is likely that when the sites at higher affinity (micropores) are completely filled with antibiotics, adsorption continues on sites at lower affinity (pores with dimensions higher than 20 Å). However the migration of sulfa

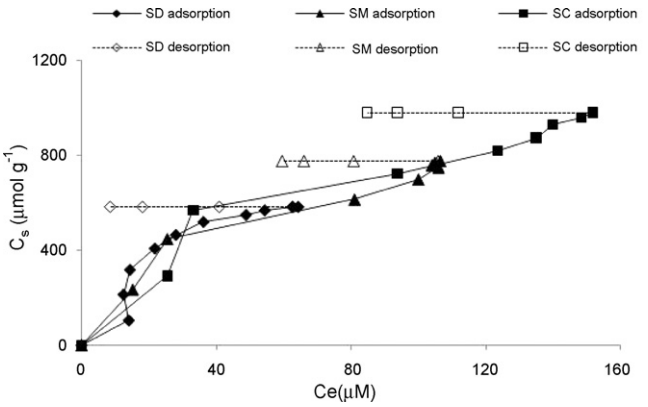


Fig. 3. Adsorption (black marks) and desorption (white marks) isotherms of sulfadiazine (SD), sulfamethazine (SM), or sulfachloropyridazine (SC) sulfonamide antibiotics by zeolite Y.

drugs inside hydrophobic zeolite pores continued till pores were completely filled. In fact, considering the number of cages in 1 g zeolite (4.2×10^{20}) and the number of sulfonamide molecules adsorbed in the same amount (4.0×10^{20}, 4.3×10^{20} and 5.3×10^{20} for SD, SM and SC, respectively), the presence of about one molecule for each zeolite cage can be calculated.

The reversibility of the adsorption process was evaluated by performing desorption experiments on exhausted zeolites by diluting the antibiotic concentration at the equilibrium point. As reported in Fig. 3, the desorption process did not have any significant effect on the release of the antibiotics from the zeolite. In fact, in the case of complete adsorption reversibility, the desorption isotherm derived from data points obtained at each desorption step should overlap the adsorption curve. On the contrary, according to our data, the desorption curve is almost parallel to the *x*-axis, revealing the tendency of sulfa drugs to remain adsorbed in zeolite Y. These findings are indicative of an irreversible adsorption process.

The amount of antibiotic adsorbed on exhausted zeolite as evaluated by means of thermogravimetric analyses accounted for 15.9%, 20.2%, and 24.6% of SD, SM, and SC, respectively (Fig. 4, left). These results are consistent with those measured using HPLC and the difference between the two techniques can be considered acceptable since the variation is $\leq$5%. Such a high extent of adsorption for organics is usually displaced for organic pollutants by other sorbents, for instance, activated carbon [32]. However, if compared to these, zeolites are usually preferable due to their more favourable adsorption kinetics along with their lower sensitivity to natural organic matter, which is always present in natural environments [32].

For all antibiotics, the non-equivalence of the peak pattern in the derivative thermogram for the adsorbed antibiotic (Fig. 4, right) compared to its bare form is more indicative of different types of interaction than a simple precipitation of antibiotics on a crystallite surface. In fact, if this were the case, the thermogram of antibiotics adsorbed in the zeolite should appear similar to that found in pure antibiotics.

3.4. Diffractometric analyses

Diffractometric analyses were performed on pure and exhausted zeolites in order to reveal whether structural modification occurred on the zeolitic framework as a consequence of incoming sulfonamide antibiotics. Rietveld structure refinement on zeolite Y (Table 3) was performed in the space group *Fd*-3*m* starting from the crystallographic data reported for protonated hydrophilic zeolite Y [33]. Difference Fourier maps revealed the presence of water molecules (16 molecules u.f.) which occupy two extraframework sites inside the supercage. Refinement parameters are reported in Table 3, while atomic coordinates, occupancies and temperature factors are shown in the supporting information section (Table S-1).

After adsorption, the incorporation of sulfa drugs inside the Y cavities was assessed using the following.

3.4.1. Diffraction patterns analysis

After sulfadiazine or sulfachloropyridazine adsorption, Rietveld structure refinement clearly indicated a lowering in real *Fd*-3*m* symmetry to *Fd*-3 in the parent zeolite Y. After sulfamethazine adsorption, the presence of reflections which are forbidden in the cubic system indicated a lowering in symmetry up to monoclinic *P*2/*m* space group, as shown by the diffraction pattern reported in Fig. 5. Y-SM unit cell parameters (Table 3) were obtained using the DICVOL program [34], and refined with LeBail fit, using the multi-dataset capabilities of the GSAS software suite. As will be discussed later, these lowerings in symmetry can be explained as a consequence of distortions in the 12-ring channel caused by the encapsulation of sulfonamides inside organophylic zeolite Y cages.

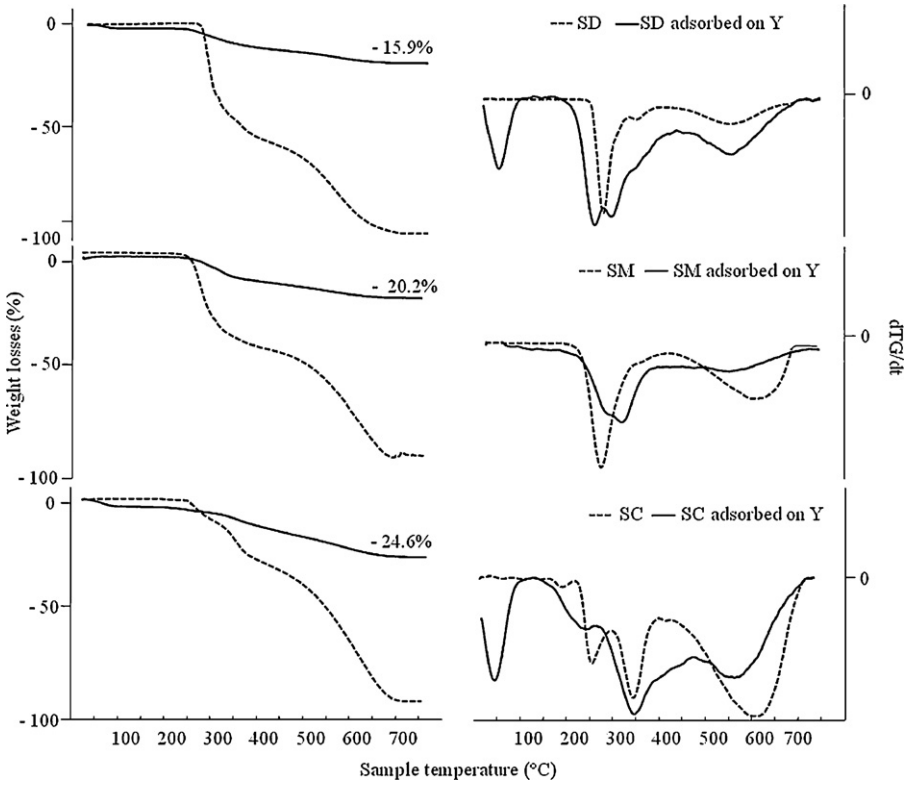


Fig. 4. Thermogravimetry (left) and derivative thermogravimetry (right) in dry air atmosphere of sulfadiazine (SD), sulfamethazine (SM), or sulfachloropyridazine (SC) pure and adsorbed into zeolite Y at maximal adsorption capacity.

Table 3
Lattice parameters and refinement details for Y zeolite before (Y) and after adsorption of sulfadiazine (Y-SD), sulfachloropyridazine (Y-SM), and sulfachloropyridazine (Y-SC).

Space group	Y *Fd-3m*	Y-SD *Fd-3*	Y-SC *Fd-3*	Y-SM *P2/m*
$A = b = c$ (Å)	24.259(4)	24.270(1)	24.266(1)	a = 14.656(1) b = 24.285(1) c = 9.918(1)
V (Å)^3	14277.1(4)	14296.2(4)	14288.1(4)	3384.4(4)
$\alpha = \beta = \gamma$ (°)	90	90	90	$\alpha = \gamma$ = 90 β = 106.6(1)
Refined pattern 2θ range (°)	3.3–110	3.3–120	3.3–110	3.3–110
R_{wp} (%)	12.8	12.7	10.0	11.2
R_p (%)	12.5	9.84	7.76	8.10
R_F^2 (%)	9.95	7.17	7.27	–
No. of contributing reflections	982	1870	1704	–
N_{obs}	5340	5844	5344	5299
N_{var}	40	59	56	32

λ = 1.5417(1) Å. $R_p = \Sigma[Y_{io} - Y_{ic}]/\Sigma Y_{io}$; $R_{wp} = [\sum w_i(Y_{io} - Y_{ic})^2 / \sum w_i {Y_{io}}^2]^{0.5}$; $R_F^2 = \sum |F_o^2 - F_c^2| / \sum |F_o^2|$. Estimated standard deviations in parentheses refer to the last digit.

3.4.1.1. Variations in unit cell parameters. As reported in Table 3, sulfadiazine or sulfachloropyridazine incorporation causes an increase in all cell parameters, thus indicating noticeable structural rearrangements. This result was also confirmed by a comparison between powder patterns collected before and after sulfa drugs adsorption on Y (Fig. S-4). In all the 2θ range which was investigated, strong differences in both the position (which depend on cell parameters values) and intensity (which depend on atomic parameters, such as positional coordinates x, y, z, isotropic temperature factor, etc.) diffraction peaks were detected. After sulfamethazine adsorption, unit cell parameters took on metrically monoclinic values, therefore the complexity of the system prevented Rietveld structure refinement.

3.4.2. Fourier maps analysis

The observed and difference Fourier maps generated using GSAS, revealed the presence of a number of extraframework ions inside the supercages, which can be attributed to encapsulated organic molecules. All localized atoms from antibiotics were at a distance of more than 3.3 Å from the framework oxygen atoms, indicating that they are only weakly bonded to the framework. The largest peak in the difference Fourier map was attributed to the sulfur atom, which occupied a site with similar fractional coordinates in both Y-SC and Y-SD. The atoms around sulfur are arranged in a distorted tetrahedral configuration, similar in both the Y-SC and Y-SD samples. Reasonable values were obtained for S–O1, S–O2, S–N, and S–C bond distances, which were set free during all stages of refinements. The positions occupied by atoms in pyridazine and benzene rings appear strongly disordered. These findings could be interpreted as being due to real static disorder or to dynamic disorder. For these reasons, the complete geometry of sulfonamide molecules inside the zeolitic cage was not achieved.

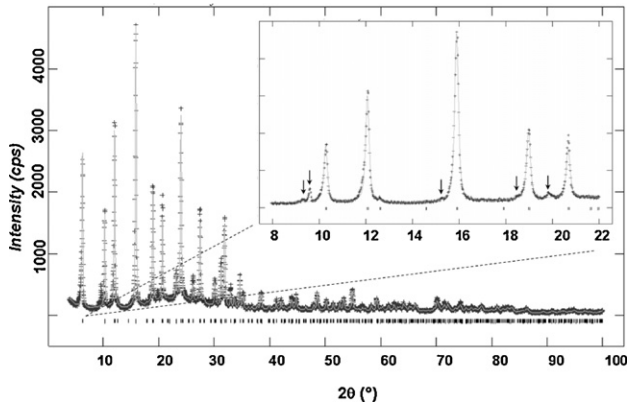
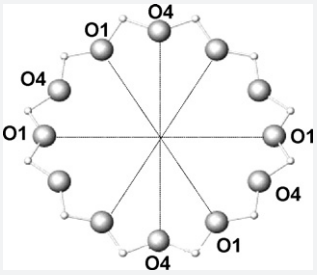


Fig. 5. Observed powder diffraction patterns of zeolite Y after sulfamethazine adsorption. The arrows reported in the patter indicate the reflections which are forbidden in the cubic system.

Table 4
Free diameter (Å) of the twelve-membered ring viewed normal to [001], assuming an oxygen ionic radius equal to 1.35 Å.

	Sample	O1–O1	O4–O4
	Y	7.00(1)	7.11(1)
	Y-SD	7.15(1)	7.12(1)
	Y-SC	7.22(1)	7.08(1)

3.4.3. Framework distortions

Rietveld structure refinements revealed that the presence of encapsulated antibiotic molecules caused remarkable distortion in the twelve-membered rings (see Table 4 for details). As can be clearly seen, the ring shape became more circular (in the presence of sulfadiazine) or more elliptical (in the presence of sulfachloropyridazine) when compared with that found in the parent zeolite Y. The most remarkable change appears related to the O1–O1 oxygen atoms distance, which increases as a consequence of adsorption and as a function of sulfonamide dimensions.

In conclusion, very favourable adsorption kinetics along with effective and highly irreversible adsorption for sulfonamide antibiotics in zeolite Y pores make this cheap and environmental friendly material a tool with interesting applications for the removal of sulfonamide antibiotics from water bodies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2010.01.066.

References

[1] B. Halling-Sorensen, S. Nors Nielsen, P.F. Lanzky, F. Ingerslev, H.C. Holten Lutzhoft, S.E. Jorgensen, Occurrence, fate and effects of pharmaceutical substances in the environment—a review, Chemosphere 36 (1998) 357–393.
[2] B. Hileman, Troubled waters: EPA, USGS try to quantify prevalence, risks of compounds from drugs, personal care products, Chem. Eng. News 79 (2001) 31–33.
[3] A.K. Sarmah, M.T. Meyer, A.B. Boxall, A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment, Chemosphere 65 (2006) 725–759.
[4] J. Acar, B. Rostel, Antimicrobial resistance: an overview, Rev. Sci. Tech. 20 (2001) 797–810.
[5] L. Migliore, C. Civitareale, G. Brambilla, G.D.D. Delupis, Toxicity of several important agricultural antibiotics in Artemia, Water Res. 31 (1995) 1801–1806.
[6] K.V. Berger, B. Petersen, H. Bünung-Pfaue, X. Persistenz von Gülle, Arzneistoffen in der nahrungskette, Arch. Lebensmittelhyg 37 (1986) 85–108.
[7] G. Hamscher, H. Pawelzick, H. Höper, H. Nau, Different behavior of tetracyclines and sulfonamides in sandy soils after repeated fertilization with liquid manure, Environ. Toxicol. Chem. 24 (2005) 861–868.
[8] Z. Qiang, C. Adams, Potentiometric determination of acid dissociation constants (pK_a) for human and veterinary antibiotics, Water Res. 38 (2004) 2874–2890.
[9] Y. Ishihama, M. Nakamura, T. Miwa, T. Kaijma, N. Asakawa, A rapid method for pK_a determination of drugs using pressure-assisted capillary electrophoresis with photodiode array detection in drug discovery, J. Pharm. Sci. 91 (2002) 933–942.
[10] M. Burkhardt, C. Stamm, C. Waul, H. Singer, S. Müller, Surface runoff and transport of sulfonamide antibiotics and tracers on manured grassland, J. Environ. Qual. 34 (2005) 1363–1371.
[11] M. Kahle, C. Stamm, Sorption of the veterinary antimicrobial sulfathiazole to organic materials of different origin, Environ. Sci. Technol. 41 (2007) 132–138.
[12] M. Kahle, C. Stamm, Time and pH-dependent sorption of the veterinary antimicrobial sulfathiazole to clay minerals and ferrihydrite, Chemosphere 68 (2007) 1224–1231.
[13] T.A. Ternes, Occurrence of drugs in German sewage treatment plants and rivers, Water Res. 32 (1998) 3245–3260.
[14] F.C. Cabello, Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment, Environ. Microbiol. 8 (2006) 1137–1144.
[15] C.D. Miranda, R. Zemelman, Antimicrobial multiresistance in bacteria isolated from freshwater Chilean salmon farms, Sci. Total Environ. 293 (2002) 207–218.
[16] C. Colella, Natural zeolites and environment, in: J. Čejka, H. van Bekkum, A. Corma, F. Schueth (Eds.), Introduction to Zeolite Science and Practice. 3rd Revised Edition, Studies in Surface Science and Catalysis, no. 168, Elsevier, Amsterdam, 2007, pp. 999–1035.
[17] H.-T. Shu, D. Li, A.A. Scala, Y.H. Ma, Adsorption of small organic pollutants from aqueous streams by aluminosilicate-based microporous materials, Sep. Purif. Technol. 11 (1997) 27–36.
[18] C.Y. Chang, W.T. Tsai, C.H. Ing, C.H. Chang, Adsorption of polyethylene glycol (PEG) from aqueous solution onto hydrophobic zeolite, J. Colloid Interface Sci. 260 (2003) 273–279.
[19] K.R. Franklin, R.R. Hunt, C.D. Williams, Sorption of butan-l-ol from aqueous solution by silica molecular sieves, Zeolites 8 (1988) 432–435.
[20] R. Vignola, U. Cova, F. Fabiani, G. Grillo, M. Molinari, R. Sbardellati, R. Sisto, Remediation of hydrocarbon contaminants in groundwater using specific zeolites in full-scale pump&treat and demonstrative permeable barrier tests, in: A. Gedeon, P. Massiani, F. Babonneau (Eds.), Zeolites and Related Materials: Trends, Targets and Challenges, proceedings of the 4. International FEZA Conference, Paris, France, 2-6 September 2008, Stud. Surf. Sci. Catal., vol. 174A, Amsterdam, Elsevier, 2008, pp. 573–576.
[21] F.R. Ungemach, Figures on quantities of antibacterials used for different purposes in the EU countries and interpretation, Acta Vet. Scand. Suppl. 93 (2000) 89–98.
[22] M.E. Lindsey, M. Meyer, E.M. Thurman, Analysis of trace levels of sulfonamide and tetracycline antimicrobials in groundwater and surface water using solid-phase extraction and liquid chromatography/mass spectrometry, Anal. Chem. 73 (2001) 4640–4646.
[23] A.B.A. Boxall, P.A. Blackwell, R. Cavallo, P. Kay, J. Tolls, The sorption and transport of sulphonamide antibiotic in soil systems, Toxicol. Lett. 131 (2002) 19–28.
[24] Judicious Antimicrobial Use of US Aquaculture: Principles And Practices, National Aquacultur Association, 2003, pp. 1–5.
[25] A.C. Larson, R.B. Von Dreele, General Structure Analysis System "GSAS", Los Alamos National Laboratory Report LAUR 86-748, 1994.
[26] B.H. Toby, EXPGUI, a graphical user interface for GSAS, J. Appl. Crystallogr. 34 (2001) 210–213.
[27] R. Szostak, Secondary synthesis methods, in: H. Van Bekkum, E.M. Flanigen, P.A. Jacobs, J.C. Jansen (Eds.), Introduction to Zeolite Science and Practice, 2nd Completely Revised and Expanded Edition, Stud. Surf. Sci. Catal., vol. 137, Amsterdam, Elsevier, 2001, pp. 261–272.
[28] H.S. Shin, G.S. Ihn, H.S. Kim, C.H. Koo, The crystal and molecular structure of sulfadiazine, J. Korean Chem. Soc. 18 (1974) 329–340.
[29] A.K. Basak, S.K. Mazumdar, Structure of sulphamethazine [4-Amino-N-(4,6-dimethyl-2-pyrimidinyl)benzenesulphonamide], $C_{12}H_{14}N_4O_2S$, Acta Cryst. C39 (1983) 492–494.
[30] Y.-S. Tan, Z.-F. Chen, H. Liang, Y. Zhang, Sulfachloropyridazine, Acta Cryst. E61 (2005) o1842–o1844.
[31] http://www.medicinescomplete.com/mc/clarke/current/index.htm.
[32] R. Di Detlef, U. Knappe, A. Rossner, S. Snyder, C. Strickland, Alternative Adsorbents For The Removal of Polar Organic Contaminants, American Water Works Association, 2007.
[33] M. Czjzek, H. Jobic, A.N. Fitch, T. Vogt, Direct determination of proton positions in D-Y and H-Y zeolite samples by neutron powder diffraction, J. Phys. Chem. 96 (1992) 1535–1540.
[34] A. Boultif, D. Louër, Powder pattern indexing with the dichotomy method, J. Appl. Cryst. 37 (2004) 724–731.