ORIGINAL ARTICLE

A solid-phase extraction approach for the identification of pharmaceutical–sludge adsorption mechanisms

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Abstract It is important to understand the adsorption mechanism of chemicals and active pharmaceutical ingredients (API) on sewage sludge since wastewater treatment plants are the last barrier before the release of these compounds to the environment. Adsorption models were developed considering mostly hydrophobic API–sludge interaction. They have poor predictive ability, especially with ionisable compounds. This work proposes a solid-phase extraction (SPE) approach to estimate rapidly the API–sludge interaction. Sludge-filled SPE cartridges could not be percolated with API spiked mobile phases so different powders were tested as SPE sludge supports. Polytetrafluoroethylene (PTFE) was selected and tested at different PTFE/sludge ratios under eight different adsorption conditions with three API ionisable compounds. The PTFE/sludge mixtures with 50% or less sludge could be used in SPE mode for API sorption studies with methanol/water liquid phases. The results gave insights into API–sludge interactions. It was found that π–π, hydrogen-bonding and charge–charge interactions were as important as hydrophobicity in the adsorption mechanism of charged APIs on sludge.

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1. Introduction

The fate and effects of active pharmaceutical ingredients (API) in the environment have raised the need for a risk assessment and safety evaluation. The requirement for environmental risk assessment was first triggered by pesticides and herbicides sprayed in fields and the environment [1] and then considered for humans active pharmaceutical ingredients (API) that could be released into the environment. Their molecular form or metabolites could affect living organisms, including humans even at very low concentrations [2].

Municipal and hospital wastewater treatment plants are important sources of release of pharmaceuticals into the environment

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[3,4]. Wastewater treatment plants are the last barriers before the pharmaceuticals are released into the aquatic environment. Some pharmaceuticals are not biodegraded by the wastewater treatment plant processes [5]. In this case, the API partitioning between the residual sludge and aqueous phase, K_d (the partition coefficient) is a key indicator to determine the fate of these pharmaceuticals in the environment. K_d is defined as the ratio of the concentration of the pharmaceutical in soil or sludge over its concentration in the aqueous phase:

$$K_d = [API]_{sludge}/[API]_{aq} \qquad (1)$$

K_d measurement is time and resource consuming and existing soil models fail to provide accurate prediction for the partitioning into sewage sludge. Various soil models based on the organic carbon partitioning theory have been developed for different types of chemicals but not specifically for APIs or for ionisable compounds, with the largest model training set including 52 compounds [6]. A sludge model has been developed based on 10 hydrophobic compounds measured in sludge but none of them are APIs [7]. Sewage sludge is a complex matrix mainly made of organic matter and nutrients from residual solids produced during wastewater treatment [8]. Little is known about binding mechanisms occurring in sewage sludge.

The main assumption is that hydrophobic interactions are the key mechanism of interaction occurring in sludge. Most soil models are based on the organic carbon partition coefficient, K_{OC} and are converted to K_d via the fraction of organic carbon, f_{OC} in the sludge [9]:

$$K_d = K_{OC}*f_{OC} \qquad (2)$$

The existing interaction models are mainly based on soil models. These models might be suitable for neutral organic compounds where hydrophobic interactions predominate. However, they fail to give reliable predictions for ionisable compounds. Many pharmaceuticals are ionisable compounds and therefore hydrophobicity may not be the only mechanism of interaction, hence the theoretical models become limited. The understanding of binding mechanisms in the sewage sludge matrix needs to be expanded [10–13]. More robust and accurate models for K_d predictions involving hydrophobic as well as more polar dipole, π–π and charge–charge (Coulomb) interactions must be built to predict the partitioning of APIs with sewage sludge, sediment and soil.

Solid-phase extraction (SPE) is a commercially available technique that uses a number of stationary phase chemistries to extract analytes from a wide variety of different liquid matrices [14].

The main advantage of SPE is its ease of use. SPE is not time consuming and generally requires only small volumes of extraction solvents. All interaction mechanisms can be used and combined offering possible mixed modes of interaction to favour the extraction of one class of compounds or another [15]. The selection of the stationary phase is associated with the desired class of compounds, hence the mechanism of interaction must be known [16].
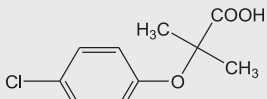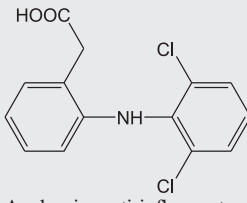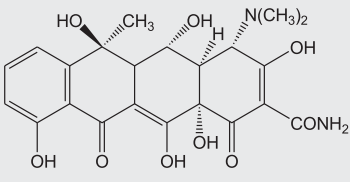
This work used the SPE technique to gain insights into the mechanism of interaction between APIs and sewage sludge. Three ionisable pharmaceuticals: clofibric acid, diclofenac and oxytetracycline, covering the range of low [17,18], medium [19] and high [20] K_d values, respectively, were selected for the study. The behaviour of these three pharmaceuticals was tested on commercially available SPE cartridges to order to obtain insights into the possible interaction mechanisms in the sludge. Sewage sludge could not be used directly as an SPE stationary phase as the aqueous eluent phase could not percolate through any wet sludge sample. To overcome this issue, various sludge–SPE packing mixtures were used as the stationary phase. Four SPE packing materials were tested as possible candidates. Bare silica was chosen for its well known packing properties in HPLC [16]. Silicon carbide was evaluated as it is used in many different environmental applications [20–22]. Polytetrafluoroethylene (PTFE or Teflon®) was selected for its very low polarity and non-adsorptive properties. Lastly, polyether ether ketone (PEEK) was chosen for its chemical stability, being commonly used as plastic connecting tubing in liquid chromatography.

2. Materials and methods

2.1. Chemicals and solvents

Clofibric acid (98.6%), diclofenac (99%) and oxytetracycline (97%) were all purchased from Sigma Aldrich (Gillingham. Dorset, UK) and were chosen for their acidic or zwitterionic character and their widespread and long term use explaining their frequent presence in the environment. The physico-chemical properties of the APIs are listed in Table 1. As seen by the pK_a values, clofibric acid and diclofenac are in a molecular form at low pH, such as pH 2 (condition 8) and in a negatively charged (carboxylate anion) form at intermediate and high pHs, such as pH 7.2 (condition 7). Oxytetracycline is a bulky compound always bearing charges: at low pH its tertiary amine group is positively charged. At pH 4.5, the isoelectric

Table 1 Physico-chemical properties of the three active pharmaceutical ingredients used as test solutes.

Compound	Clofibric acid	Diclofenac	Oxytetracycline
Structure			
Pharmaceutical class	Lipid regulator	Analgesic, anti-inflammatory	Antibacterial, antibiotic
MW	215	296	460
Log K_{ow}[a]	2.7	4.1	1.6
pK_a	3.0	4.15	3.3, 7.3, 9.1
Log K_d	1.5 [16]	1.5–2.7 [16,17]	3.5 [18]

[a]Predicted by ACDLab program.

point, it is a zwitterion (positive amine plus negative phenol) and at higher pH values, it bears a globally negative charge (Table 1).

Salts for buffers preparation such as potassium dihydrogen orthophosphate, dipotassium hydrogen phosphate and sodium phosphate were all purchased from Fisher Scientific (Loughborough, Leicestershire, UK).

Acetonitrile and methanol were obtained from Sigma Aldrich UK. Water was purified by reversed osmosis on an Elga Purelab Option-Q system (Elga LabWater, Marlow, UK).

2.2. SPE cartridges

2.2.1. Commercial phases
The commercially available SPE cartridges were purchased from Sigma Aldrich UK as part of a method development pack including Supelco C_{18}, C_8, CN, Phenyl, Diol, NH_2, SAX, SCX, WCX and Si cartridges. The main types of interaction for each phase are shown in Table 2. Five cartridges corresponding to the four types of interaction defined in Table 2 were fully tested. They included C_8, Phenyl, SAX, SCX and bare Si cartridges. All adsorption measurements were performed in triplicate with buffered spiked mobile phases.

2.2.2. Hand packed phases
The SPE packing materials were silica (100 μm average particle diameter), silicon carbide (200 μm), PTFE (200 μm) and PEEK (chunks of solids) were all purchased from Sigma Aldrich UK. The PEEK material was ground with a pestle and mortar before being sieved through a 500 μm grid.

The activated sewage sludge was collected from the activation tank in the nitrification zone at Totnes sewage treatment plant (Devon, UK) which treats domestic wastewaters. It was freeze-dried and ground before passed through the 500 μm sieve.

Empty SPE cartridges and frits were purchased from Sigma Aldrich UK.

2.2.2.1. Packing information.
The four packing materials and freeze-dried sludge were analysed to obtain their physico-chemical properties (Table 3). They were also observed by a scanning electron microscope (SEM) NeoScope JCM 5000 SEM (Jeol, France) (Fig. 1). The specific surface areas were obtained using the Brunauer, Emmett and Teller method with a Sorptomatic 1990 series (Thermo Scientific, France) [23]. Thermogravimetry analyses (TGA) were performed using a TGA 92-12 (Setaram, France) and particle size distribution was assessed on a Mastersizer 2000 laser diffractometer (Malvern, France).

2.2.2.2. Packing process.
A 500 mg aliquot of packing material was weighed and packed into empty SPE cartridges to determine their sorption properties. The sludge/packing experiments were performed working with three different ratios: 20% sludge/80% packing, 50% sludge/50% packing and 80% sludge/20% packing w/w. After careful mixing of the sludge and packing materials, 500 mg of the mixture was packed into a SPE cartridge for sorption experiments. Attempt to use 100% sludge (no added packing material) was unsuccessful (data not shown). All adsorption measurements were performed in triplicate.

2.3. Elution process

Eight different elution conditions were tested to assess the impact of solvent ratio, organic modifier and pH level on the sorption of the three APIs (Table 4). Five elutions were run with 0%, 20%, 50%, 80% and 100% (v/v) methanol in water to test the whole range of polarity from the most polar, pure water, to the least polar, pure methanol. Methanol was replaced by acetonitrile at 20% ratio for a sixth elution to assess if the nature of the solvent could impact the sorption. Acetonitrile, unlike methanol is an aprotic solvent. Another two elutions were added to study the effect of pH, with an acidic and a basic elution. The two pH conditions, pH 2 and pH 7.2, were chosen to match the classical elution mode for the two ion-exchange SPE commercial phases being far enough from the solute pK_a values to insure reproducibility. Also basic pHs (pH > 9) are not possible with silica whose silanol groups, Si–OH, ionise at pHs above 9 producing silica dissolution in silicate anions. In a classic SPE experiment, the cartridges are conditioned with a solvent to activate the sorbent phase, then the sample is loaded onto the cartridge similarly to our experimental protocol. The difference with a classical use of SPE was that the 'wash' step was considered as elution 1, and the typical 'elution' step as elution 2.

In a typical experiment the SPE cartridge was prepared with 500 mg of the selected stationary phase (SPE packing material alone, a mixture sludge/packing material or sludge) and hand packed. Then the cartridge was wetted by 2 mL of methanol or conditioning solvent. A 100 μL aliquot of a 50 mg/L solution of the three APIs was loaded onto each SPE cartridge and the appropriate solvent was next passed through the SPE cartridge. Next the APIs were desorbed from the cartridge using the two eluents listed in Table 4. A 2 mL aliquot of the first eluent, with opposite polarity compared to the conditioning solvent, was first passed through the cartridge collecting the eluting phase. This elution 1 step was followed by a second elution step with the same solvent as the conditioning solvent. A 2 mL aliquot of eluent 2 was used to ensure a maximum desorption from the studied sorbent phase. The pooled eluting solvent phases were added into HPLC vials and loaded onto the HPLC system for analysis without further treatment. The elution process for the pH study was slightly modified (Table 4, experiments 7 and 8).

Table 2 Types of interactions for the SPE stationary phases.

Type of interaction	Van der Waals (Hydrophobic)	π–π	H-bonding	Coulomb forces[a]
SPE phases	C_{18}	CN	Si	NH_2
	C_8	NH_2	Diol	SCX
	Phenyl	Phenyl	NH_2	WCX
	CN			SAX

[a]NH_2 is a weak anion exchanger at low pHs in its $-NH_3^+$ form. SCX and WCX are strong and weak cation exchangers, respectively, and SAX is a strong anion exchanger.

Table 3 Physico-chemical data of the SPE stationary phase powders and sludge.

Sample	Aspect	Thermogravimetry[a]	Particle size[b] (μm)	Surface area (m²/g)	Porosity[c]
Silica	White hard powder	Stable	2–100 **35**	420	0.8 cm³/g 7.5 nm
Silicon carbide	Green powder	Stable	3–110 **62**	2.9	Non porous
Polytetrafluoroethylene	White soft powder	Decompose around 500 °C	80–700 **250**	2.6	Non porous
Polyether ether ketone	Beige powder	Decompose around 500 °C	4–1000 **330**	80	0.19 cm³/g 9.5 nm
Sludge	Black powder	5% weight loss at 100 °C, 76% weight loss between 100 and 300 °C	1–2000 **480**	2.5	0.03 cm³/g 53 nm

[a]Sample weight loss upon heating under nitrogen circulation.
[b]Minimum and maximum particle size and **mean value** (see Fig. 1 for particle shape).
[c]Mesoporosity, pore thinner than 2 nm were not assessed. Pore volume (cm³/g) and mean diameter (nm) are listed.
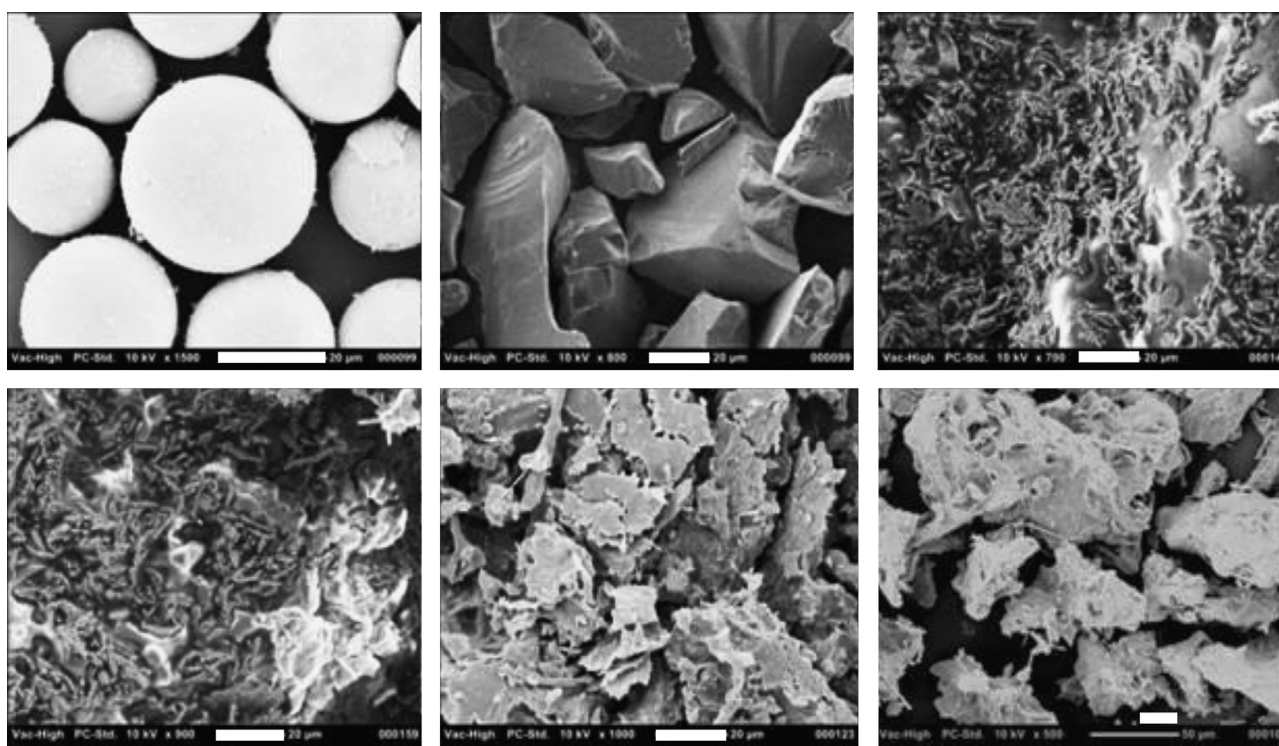

Fig. 1 SEM photographs of the SPE possible packing materials tested. From left to right: Top: spherical silica, coarse silicon carbide, PTFE; bottom: PEEK, two different magnifications of the freeze dried sludge. White bars are 20 μm.

2.4. Determination of recoveries sorbed

An Agilent 1100 HPLC system was used to determine the desorbed amount of API collected after SPE experiments. The pump was a double piston constant flow model; the UV detector had a 200–350 nm wavelength working range. The column was a Gemini C_{18} (Phenomenex, Macclesfield, UK) 150 mm × 4 mm with 3 μm particles working at 30 °C. All analyses were performed with a flow rate of 0.7 mL/min, 50–140 kg/cm² (5–14 MPa) pressure drop and injection volume of 20 μL. The pharmaceuticals were separated using a 16 min acetonitrile/buffer gradient elution scheme. The analysis started with a 90% (v/v) 0.01 M pH 3 phosphate buffer mobile phase/10% acetonitrile (v/v) for 1 min followed by acetonitrile linear increase from 10% to 70% (v/v) in 10 min. The 70% (v/v) composition was held constant for 2 min

Table 4 Composition of the mobile phases used for experimental conditions tested for each SPE material and sludge/PTFE mixture.

Experiment	Conditioning % (v/v)	Elution 1 % (v/v)	Elution 2 % (v/v)
1	Pure methanol	Pure water	Pure methanol
2	Methanol 80	Methanol 20	Methanol 80
3	Methanol 50	Methanol 50	Methanol 50
4	Methanol 20	Methanol 80	Methanol 20
5	Pure water	Pure methanol	Pure water
6	Acetonitrile 80	Acetonitrile 20	Acetonitrile 80
7	Pure methanol	Phosphate buffer (pH 7.2)	Phosphate buffer (pH 2) 20/methanol 80
8	Pure methanol	Phosphate buffer (pH 2)	Phosphate buffer (pH 10) 50/methanol 50

before returning to the initial solvent composition over a minute period, which was then held constant for another 2 min. The detection wavelength was set up at 220 nm with detection limit for clofibric acid, diclofenac and oxytetracycline of respectively 0.1, 0.03 and 0.1 mg/L. Quantification was done using an external standard containing the three pharmaceuticals at known concentration. Chromatograms were analysed using the Laura software version 4.0.2.75 (LabLogic, UK). Calibration was performed using a series of six standards of concentration ranging from 0.1 to 500 mg/L.

As the two successive SPE elutions were performed using different solvents, it was not possible to determine an overall K_d, as a K_d is solvent dependent. However, the percentage sorbed, $\%_{sorbed}$, onto the phase was calculated according to

$$\%_{sorbed} = 100 - \%_{recovered\ 1} - \%_{recovered\ 2} \tag{3}$$

in which

$\%_{sorbed}$ is the percentage of API remaining sorbed after the desorption procedure,

$\%_{recovered\ 1}$ is the API recovered percentage after methanol desorption 1, and

$\%_{recovered\ 2}$ is the API recovered percentage after acetonitrile or solvent 2 desorption.

2.5. Data comparison

The $\%_{sorbed}$ values were directly compared between the $\%_{sorbed}$ obtained with the commercial SPE phases and the ones obtained with hand-packed cartridges to identify trends in sorption depending on the different mechanisms of interaction.

3. Results and discussion

3.1. Physicochemical properties of packing materials

3.1.1. Thermal and chemical stability

Silica and silicon carbide were stable across the 10–800 °C thermogravimetric temperature range while the PTFE and PEEK degraded above 500 °C. The collected sludge presented about 5% weight loss around 100 °C due to water content and a subsequent 76% weight loss at temperatures between 110 and 300 °C attributed to volatile organic matter. All the packing materials were found to be thermally stable and therefore no special storage or packing condition was needed.

PTFE and silicon carbide are chemically very stable. PEEK should not be used with chlorinated solvents and some ketones. It is stable with water, methanol and acetonitrile. Silica will dissolve in polar solutions with pH higher than 9. The solvent compositions used for sludge extraction will ensure total inertness from the supporting packing material.

3.1.2. Particle shape and size distribution

Scanning electron microscopy (SEM) gave a good view of the particle shapes, surface topography and particle size distribution (Fig. 1). The silica sample was made of very spherical particles but with a significant polydispersity. The average silica particle diameter was 35 μm with particles as small as 2 μm and others as big as 100 μm (Table 3). The second inorganic material was the silicon carbide sample. The SEM pictures show coarse irregular particles with a wide polydispersity (mean particle diameter 62 μm). The PTFE and PEEK organic materials had the same polymeric microstructure but larger aggregates were observed for PEEK. The sludge sample was heterogeneous and non spherical with aggregated particles (Fig. 1). The particle shape characteristics gave useful information on the ability to be packed. Silica being the most spherical and homogeneous was the easiest to pack while the heterogeneous sludge proved to be difficult to pack on its own.

3.1.3. Surface area and porosity

The surface area was determined by nitrogen monolayer adsorption at 77 K (−196 °C) using the BET equation [23] and could only assess mesopores (2–50 nm). Silica had the largest surface area with 420 m²/g. Such a large surface area was due to the internal surface of pores, not to the external particle surface. Silica also had the larger pore volume (0.8 cm³/g) (Table 3). The other inorganic material, silicon carbide (SiC), was non-porous with a surface area of 2.9 m²/g, more than two orders of magnitude lower than that of silica. Similarly, with the two organic materials: PEEK was porous with a large (80 m²/g) surface area and PTFE was not porous with a 2.6 m²/g surface area, similar to SiC. The collected freeze dried sludge was also tested, returning a surface area of 2.5 m²/g corresponding to a very low porosity (Table 3). The sludge was the most heterogeneous sample with particle size ranging from 1 to 2000 μm (mean 480 μm). The knowledge of the surface area gives information about the likelihood of interactions happening; the larger the surface area, the better chance of interaction between a solute and the material. PTFE, SiC and sludge were in the same surface area range as one another but lower than for silica and PEEK.

3.2. SPE material chemical reactivity

The first experiment was to characterise the adsorption of the SPE material by itself. The support SPE material should adsorb as little as possible of the pharmaceutical so that, when working with sludge/material mixtures, the API adsorbed was only affected by the sludge. A small amount of adsorption could be accepted since

it could be taken into account to correct the final adsorption measurements. The three pharmaceuticals, especially oxytetracycline, adsorbed strongly (more than 60%) to silica, SiC and PEEK under all solvent conditions. Total (100%) sorption was observed with 80% and 100% water rich conditions. For the scope of this study, these three adsorbent candidates were considered as not meeting the criterion of being chemically inert. The general chemically inert nature of PEEK has not been questioned as it is used for HPLC tubing applications. However, the PEEK polymer extruded to form HPLC tubing is thermally treated to deactivate it. Clofibric acid and diclofenac slightly adsorbed (under 28% worst case) to PTFE in experiment 4 (i.e. 80% methanol in water, Table 4). PTFE is the only material selected as inert-enough possible SPE packing powder aid in sludge pharmaceutical adsorption studies.

3.3.   A comparison of hand-packed PTFE-sludge with commercial SPE cartridges

Wet sludge was not compatible with SPE elution as very little aqueous phase could go through a SPE cartridge filled with sludge. With 80% sludge content (20% PTFE) the elution was possible but very slow and the adsorption results showed an unacceptably large variability and a lack of reproducibility (a relative standard deviation of 170% was obtained in the worst case). It was possible to elute the aqueous phase through SPE cartridges containing a mixture of equal weight of PTFE and sludge. The percentage adsorption under the eight experimentally tested elution conditions is presented in Fig. 2B for the 50/50 sludge–PTFE mixture. Better precision was achieved for this ratio of sludge with RSD values lower than 20% (the higher %RSD was 15.1% for oxytetracycline with elution 2 as the recoveries for the triplicate measurements were 0.834, 1.119 and 1.071 µg). Fig. 2A also shows the results obtained with 20/80 sludge–PTFE. The oxytetracycline adsorption was higher with 20/80 sludge–PTFE (Fig. 2A) than with 50/50 sludge–PTFE (Fig. 2B). Also, the oxytetracycline overall adsorption was much less dependent on the elution conditions being between 70% and 90% for the eight tested elution conditions in Table 4 (Fig. 2A). Adsorption for oxytetracycline was expected to be high on hydrophobic, phenyl and cation

exchange phases since this compound had aromatic rings and amine functional groups. Similarly, diclofenac showed an acceptable variability with the 20/80 sludge–PTFE mixture. It adsorbed between 5% and 20%, except for experiment 8 which enhanced the sorption. Clofibric acid sorption was consistently low under all conditions, as expected for this low sorptive relatively small molecule. The two sludge-PTFE ratios were correlated with K_d values listed in Table 1 when methanol/water mobile phases (conditions 2, 3 and 4) were used for adsorption (Fig. 2)

Fig. 3 summarises the adsorption results for the hydrophobic C_8 and phenyl SPE adsorbents (Fig. 3A and B respectively) and for the SAX anion and SCX cation exchanger SPE adsorbents (Fig. 3C and D respectively) under the eight adsorption conditions for the three APIs selected in Table 4. Similarities can be observed between the adsorption results obtained with 20/80 sludge–PTFE, Fig. 2A) and the C_8 and phenyl SPE sorbent (Fig. 3A and B). Since the C_8 SPE sorbent mainly interacts with solutes through hydrophobic Van der Waals interactions and the phenyl SPE sorbent through hydrophobic and π–π interactions, these results suggest that hydrophobic interactions play an important role in solute sorption mechanisms for the three APIs.

However, the hydrophobic interaction mechanism is not the sole mechanism responsible for the sorption of these APIs to sewage sludge. At 50/50 sludge–PTFE (Fig. 2B), it was clear that the increased adsorption of clofibric acid and diclofenac was due to non-hydrophobic mechanisms. Observing the phenyl SPE adsorption (Fig. 3B), it seems that π–π interactions are significant since the SPE phase and all the selected pharmaceuticals had at least one aromatic ring. However, the percentage obtained with the SCX cation exchanger SPE phase (Fig. 3C) is comparable to the phenyl SPE results (Fig. 3B). It suggests that charge–charge Coulomb interactions induced by the SCX cation exchanger SPE phase can be as effective as hydrophobic and π–π interactions.

The strong clofibric acid and diclofenac adsorption obtained with SAX anion exchanger (Fig. 3D) was not observed with sludge containing PTFE (Fig. 2). This suggests that anion exchange was not part of the sludge API adsorption mechanism, at least at low and neutral pHs. Interestingly, the sorption behaviour of the three pharmaceuticals on the silica SPE (Fig. 4) was rather similar to
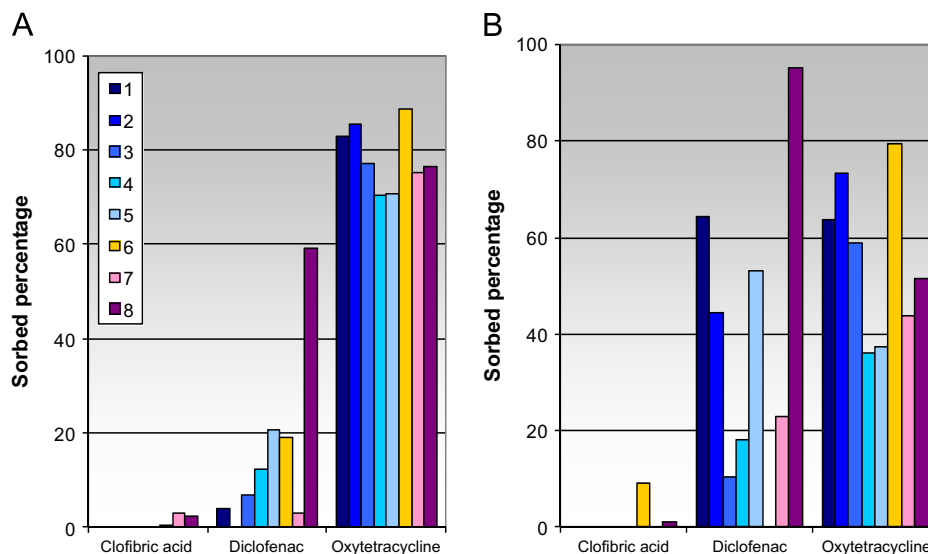

Fig. 2   Bar charts comparing the adsorbed percentage of the three test APIs under eight experimental adsorption conditions shown in Table 4 on PTFE–sludge 80/20 (left chart) and 50/50 (right chart) mixtures. (A) 80% PTFE–20% sludge and (B) 50% PTFE–50% sludge.
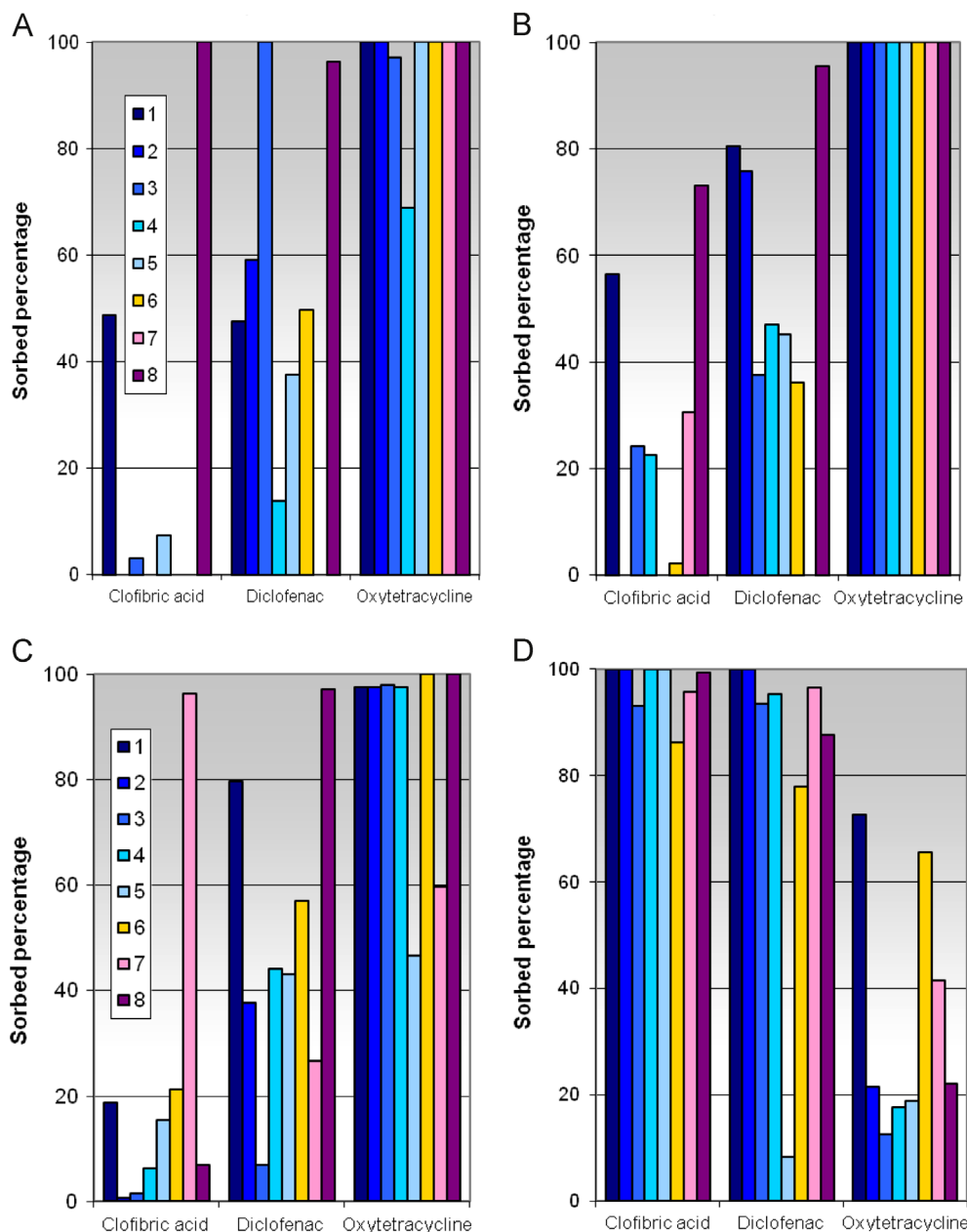
Fig. 3 Bar charts comparing the adsorbed percentage of the three test APIs under eight experimental adsorption conditions shown in Table 4 on four different SPE materials. (A) C_8 octylbonded silica, (B) phenyl-bonded silica, (C) SCX cation-exchanger, and (D) SAX anion-exchanger.

their behaviour on the phenyl and SCX SPE phases. The silanol groups of the silica surface can ionise at pH values higher than 7 giving negatively charged sites able to exchange cations [24] which explain the similarity with the SCX SPE phase at higher pH. For lower pHs, the main interaction with silica surface is hydrogen bonding with molecular silanols, which excludes hydrophobic interaction. The API adsorption results with silica (Fig. 4) highlighted that the pharmaceuticals could also adsorb via hydrogen bonding. All three tested pharmaceuticals had hydroxyl or carboxyl function groups as well as accessible nitrogen or oxygen electronegative atoms which made them sensitive to hydrogen bonding.

Adsorption studies were also performed with a NH_2 SPE material with weak anion exchanger properties (data not shown). The three APIs were almost 100% adsorbed on this SPE material under all eight different elution conditions. The comparison between the

sludge phase and commercial SPE phases suggested that hydrophobicity was an important factor but not the only one in the mechanism of API–sludge sorption. More complex and combined interactions including ion-exchange, π–π and H-bonding as well as hydrophobic interactions should be considered to understand the API–sludge sorption mechanism. The K_{OC} theory, which only considers hydrophobic interactions, may not accurately model API–sludge adsorption. This may be why it failed to give good predictions for pharmaceutical adsorption with sludge matrix.

4. Conclusion

SPE experiments were conducted to investigate the nature of the interaction between sewage sludge and ionisable pharmaceuticals.

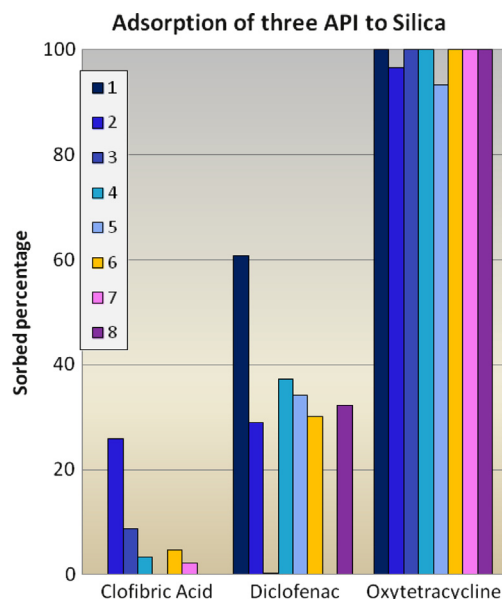## Adsorption of three API to Silica

Fig. 4 Bar charts comparing the adsorbed percentage of the three test APIs under eight experimental adsorption conditions shown in Table 4 bare silica SPE material.

The physicochemical nature of wet sludge precluded its direct use as a SPE sorbent. The mobile phase could not easily percolate through pure wet sludge. Mixing sludge with a selection of inert solid materials permitted APIs in spiked mobile phase to percolate through SPE phases. Testing different candidate materials for their chemical inertness showed that the most appropriate material was PTFE. Using sludge–PTFE mixtures as sorbent in SPE cartridges, it was possible to rapidly obtain the amount of sorbed API. A screening method for estimating the K_d value was developed provided that the correct packing material, sludge ratio and solvent were used. The existing K_{OC} theory, which assumes only hydrophobic binding, might not be directly applicable for the sewage sludge matrix since sludge–API interaction mechanism involves the hydrophobic interactions but also other interactions such as π–π, cation-exchange and hydrogen bonding interactions that can be of comparable or higher magnitude than Van der Waals interactions. These multiple interactions must be taken into account for developing new models with valuable predictive capability.

References

[1] G.M. Williams, R. Kroes, I.C. Munro, Safety evaluation and risk assessement of the herbicide Roundup® and its active ingredient, glyphosate, for humans, Regul. Toxicol. Pharmacol. 31 (2000) 117–165.

[2] S.D. Richardson, T.A. Ternes, Water analysis: emerging contaminants and current issues, Anal. Chem. 83 (2011) 4614–4648.

[3] A. Nikolaou, S. Meric, D. Fatta, Occurrence patterns of pharmaceuticals in water and wastewater environments, Anal. Bioanal. Chem. 387 (2007) 1225–1234.

[4] D.M. Whitacre, S.C. Monteiro, A.B.A. Boxall, Occurrence and fate of human pharmaceuticals in the environment, Rev. Environ. Contam. Toxicol. 202 (2010) 53–154.

[5] S.C. Monteiro, A.B.A. Boxall, Occurrence and fate of human pharmaceuticals in the environment, in: D.M. Whitacre (Ed.), Reviews of Environmental Contamination and Toxicology, vol. 202, Springer, New York, 2010, pp. 53–154.

[6] A. Sabljic, H. Gusten, H. Verhaar, et al., QSAR modelling of soil sorption. Improvements and systematics of Log Koc vs. Log Kow correlations, Chemosphere 31 (1995) 4489–4514.

[7] R.A. Dobbs, L. Wang, R. Govind, Sorption of toxic organic compounds on wastewater solids: correlation with fundamental properties, Environ. Sci. Technol. 23 (1989) 1092–1097.

[8] T.A. Ternes, A. Joss, Human Pharmaceuticals, Hormones and Fragrances, IWA Publishing, London, 2006.

[9] Anonymous, OECD Guidelines for the Testing of Chemicals. Test No. 121: Estimation of the Adsorption Coefficient (K_{oc}) on Soil and on Sewage Sludge Using High Performance Liquid Chromatography (HPLC), OECD Publishing, Brussels, 2001.

[10] T.L ter Laak, M.K. Durjava, J. Struijs, et al., Solid phase dosing and sampling technique to determine partition coefficients of hydrophobic chemicals in complex matrixes, Environ. Sci. Technol. 39 (2005) 3736–3742.

[11] A. Franco, S. Trapp, Estimation of the soil–water partition coefficient normalized to organic carbon for ionizable organic chemicals, Environ. Toxicol. Chem. 27 (2008) 1995–2004.

[12] M. Carballa, G. Fink, F. Omil, et al., Determination of the solid–water distribution coefficient (K_d) for pharmaceuticals, estrogens and musk fragrances in digested sludge, Water Res. 42 (2008) 287–295.

[13] V.L. Cunningham, Special characteristics of pharmaceuticals related to environmental fate, in: K. Kümmerer (Ed.), Pharmaceuticals in the Environment, Springer, Berlin, 2008, pp. 23–34.

[14] M.E. Thurmann, M.S. Mills, Solid-Phase Extraction: Principles and Practice, Chemical Analysis, vol. 147, Wiley, New York, 1998.

[15] A. Zwir-Ferenc, M. Biziuk, Solid phase extraction technique—trends, opportunities and applications, Pol. J. Environ. Stud. 15 (2006) 677–690.

[16] M.C. Hennion, Solid-phase extraction: method development, sorbents, and coupling with liquid chromatography, J. Chromatogr. A 856 (1999) 3–54.

[17] L. Barron, J. Havel, M. Purcell, et al., Predicting sorption of pharmaceuticals and personal care products onto soil and digested sludge using artificial neural networks, Analyst 134 (2009) 663–670.

[18] T. Urase, T. Kikuta, Separate estimation of adsorption and degradation of pharmaceutical substances and estrogens in the activated sludge process, Water Res. 39 (7) (2005) 1289–1300.

[19] T.A. Ternes, N. Herrmann, M. Bonerz, et al., A rapid method to measure the solid–water distribution coefficient (Kd) for pharmaceuticals and musk fragrances in sewage sludge, Water Res. 38 (19) (2004) 4075–4084.

[20] F. Stuer-Lauridsen, M. Birkved, L.P. Hansen, et al., Environmental risk assessment of human pharmaceuticals in Denmark after normal therapeutic use, Chemosphere 40 (7) (2000) 783–793.

[21] P.S. Bäuerlein, J.E. Mansell, T.L. ter Laak, et al., Sorption behavior of charged and neutral polar organic compounds on solid phase extraction materials: which functional group governs sorption?, Environ. Sci. Technol. 46 (2) (2011) 954–961.

[22] S. Droge, K.-U. Goss, Effect of sodium and calcium cations on the ion-exchange affinity of organic cations for soil organic matter, Environ. Sci. Technol. 46 (11) (2012) 5894–5901.

[23] S. Brunauer, P.H. Emmett, E. Teller, Adsorption of gases in multimolecular layers, J. Am. Chem. Soc. 60 (2) (1938) 309–319.

[24] A. Petruczynik1, M. Waksmundzka-Hajnos, M.L. Hajnos, The effect of chromatographic conditions on the separation of selected alkaloids in RP-HPTLC, J. Chromatogr. Sci. 43 (2005) 183–194.