Stability and removal of atorvastatin, rosuvastatin and simvastatin from wastewater

Saleh Sulaiman, Mustafa Khamis, Shlomo Nir, Filomena Lelario, Laura Scrano, Sabino Aurelio Bufo, Gennaro Mecca & Rafik Karaman

Taylor & Francis
Taylor & Francis Group

Stability and removal of atorvastatin, rosuvastatin and simvastatin from wastewater

Saleh Sulaiman[a,b], Mustafa Khamis[c,d], Shlomo Nir[e], Filomena Lelario[a], Laura Scrano[f],
Sabino Aurelio Bufo[a], Gennaro Mecca[g] and Rafik Karaman[a,b*]

[a]*Department of Science, University of Basilicata, 85100 Potenza, Italy;* [b]*Department of Bioorganic Chemistry, Faculty of Pharmacy, Al-Quds University, Jerusalem 20002, Palestine;* [c]*Department of Chemistry and Chemical Technology, Faculty of Science and Technology, Al-Quds University, Jerusalem 20002, Palestine;* [d]*Department of Chemistry, Biology and Environmental Sciences, American University of Sharjah, Sharjah, UAE;* [e]*Department of Soil and Water Sciences, Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem, Rehovot 76100, Israel;* [f] *Department of Mediterranean Culture, University of Basilicata, 75100 Matera, Italy;* [g]*Exo Research Organization, Potenza, Italy*

Atorvastatin (ATO), rosuvastatin (RST) and simvastatin (SIM) are commonly used drugs that belong to the statin family (lowering human blood cholesterol levels) and have been detected as contaminants in natural waters. Stability and removal of ATO, RST and SIM from spiked wastewater produced at the Al-Quds University campus were investigated. All three statins were found to undergo degradation in wastewater (activated sludge). The degradation reactions of the three drugs in wastewater at room temperature follow first-order kinetics with rate constants of $2.2 \times 10^{-7}\,\text{s}^{-1}$ (ATO), $1.8 \times 10^{-7}\,\text{s}^{-1}$ (RST) and $1.8 \times 10^{-6}\,\text{s}^{-1}$ (SIM), which are larger than those obtained in pure water under the same conditions, $1.9 \times 10^{-8}\,\text{s}^{-1}$ (ATO), $2.2 \times 10^{-8}\,\text{s}^{-1}$ (RST) and $6.2 \times 10^{-7}\,\text{s}^{-1}$ (SIM). Degradation products were identified by LC-MS and LC/MS/MS. The overall performance of the wastewater treatment plant (WWTP) installed in the Al-Quds University campus towards the removal of these drugs was assessed showing that more than 90% of spiked ATO, RST and SIM were removed. In order to evaluate the efficiency of alternative removal methods to replace ultra-filtration membranes, adsorption isotherms for the three statins were investigated using both activated carbon and clay–micelle complex as adsorbents. The batch adsorption isotherms for the three statins were found to fit the Langmuir equation, with a larger number of adsorption sites and binding affinity for micelle–clay composite compared with activated carbon and filtration experiments of the three statins and their corresponding metabolites demonstrated a more efficient removal by micelle–clay filters.

Keywords: statins; wastewater treatment; stability in sludge; HF-membranes; activated carbon; micelle–clay complex

Nomenclature

CMC   critical micelle concentration
IRMPD infrared multiphoton dissociation
MC   micelle–clay complex
RO   reverse osmosis

1. Introduction

Widespread development in watershed recharge areas increases the likelihood of contamination in surface water and groundwater resources by wastewater effluents.[1] These effluents are contaminated with variety of organic wastewater compounds such as excreted hormones and pharmaceuticals, detergent components, and disposed household and personal care products. These contaminants attracted special concern because of their ability to interfere with the function of natural hormones in both aquatic organisms and humans.[2–5] Many of these compounds have been widely documented in surface waters receiving discharge from WWTPs.[6–12]

In recent years, the environmental occurrence of pharmaceutically active compounds (PhACs), human and veterinary medication, has been a source of growing concern.[13] It has been shown that they can adversely affect both aquatic and non-aquatic organisms and thus the ecosystem.[13] In most cases current water and wastewater treatment systems do not completely remove PhACs.[14–17]

Statins are a group of pharmaceuticals used for lowering cholesterol levels in the blood. They are commonly applied for the reduction of cardiovascular-related morbidity and mortality in patients with or at risk of coronary heart diseases. In the long and complex biosynthesis of cholesterol in humans, the first and rate-determining step is the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase which catalyses the conversion of HMG-CoA to mevalonic acid.[18,19] Clinical studies have demonstrated that statins are potent and competitive inhibitors of HMG-CoA reductase for cholesterol synthesis, thereby limiting the hepatic production of low-density lipoprotein
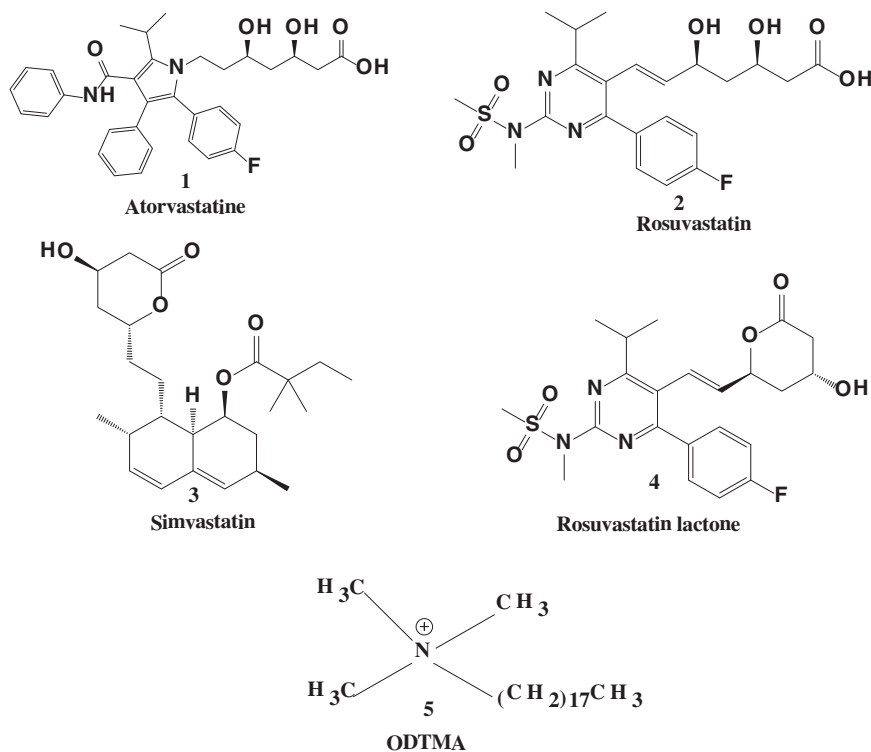

Figure 1.   Chemical structures of ATO (**1**), RST (**2**), SIM (**3**) and Rosuvastatin lactone (RSTL) (**4**), ODTMA ion (**5**).

(LDL).[20] The fully synthetic atorvastatin (ATO), rosu-vastatin (RST) and simvastatin (SIM) are presently the three most popular, second-generation HMG-CoA reduc-tase inhibitors in the statin family.[18]

ATO (structure **1**, Figure 1), [(3R,5R)-7-[2-(4-fluoro-phenyl)-4-(phenylcarbamoyl)-5-isopropyl-3-phenyl-pyrrol-1-yl]-3,5-dihydroxy-heptanoic acid, is one of the most prescribed drugs.[20] Recently, it has been observed that ATO, like other statins, can be efficient against Alzheimer's disease.[20] Less than 5% of a dose of ATO is recovered in urine following oral administra-tion. The presence of ATO in sewage effluents and sur-face waters has been observed in concentration levels of $\mu g\, L^{-1}$.[21]

Pharmacokinetic studies have shown that, after oral administration, ATO was rapidly metabolized into two active hydroxy metabolites (2-hydroxy ATO and 4-hydroxy ATO) and three inactive lactone metabolites. [22,23] However, the lactones were unstable as they hydrolysed readily to their original acid forms. In con-trast, RST was found not to be extensively metabolized, as 77% of it was excreted unchanged.[24] Approximately 90% of the non-metabolized RST was recovered in faeces, with the remaining 10% in urine. Two minor metabolites, the RST-5S-lactone (RSTL) and the N-desmethyl RST, were identified by LC-NMR and LC–MS/MS in the same study.[24]

ATO was found to undergo a self-sensitized photo oxygenation by sunlight in water.[20,25] The main

photoproducts, isolated by chromatographic techniques, were identified by spectroscopic means. They present a lactam ring arising from an oxidation of pyrrole ring and an alkyl/aryl shift. A mechanism involving sin-glet oxygen addition and an epoxide intermediate was suggested.[25]

Rosuvastatin (RST), bis [(E)-7-[4-(4-fluoro-phenyl)-6-isopropyl-2-[methyl (methylsulfonyl) amino] pyrimidin-5-yl] (3R, 5S)-3,5-dihydroxyhept-6-enoic acid] (RST, structure **2,** Figure 1), decreases the production of LDL cholesterol by blocking the action of the enzyme HMG-CoA reductase in the liver which is responsible for its production. This decreases the amount of cholesterol in the liver cells, which causes them to take up LDL choles-terol from the blood. The decreased cholesterol produc-tion and increased removal of LDL cholesterol from the blood ultimately results in lowered blood cholesterol levels.[26]

In the pharmacokinetic study of RST, RSTL (struc-ture **4**, Figure 1) was identified as one of the minor metabolites.[24]

SIM, is (+) (1S,3R,7S,8S,8αR)-1,2,3,7,8,8α-hexa-hydro-3,7-dimethyl-8-[2-[(2R,4R)-tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl]-1-naphthyl-2,2-dimethyl butanoate (SIM, structure **3**, Figure 1), is derived synthetically from a fermentation product of *Aspergillus terreus*.[27] After oral ingestion, SIM, which is an inactive lactone, is hydrolysed to the corresponding β-hydroxyacid form. The latter is a potent inhibitor of HMG-CoA reductase,

an essential enzyme involved in the in vivo synthesis of cholesterol.[28,29]

The goals of this study were (i) to explore the efficiency of the integrated advanced wastewater treatment technology towards the removal of ATO, RST and SIM and their metabolites from spiked wastewater samples; (ii) to evaluate the degradation kinetics of the above-mentioned compounds in water and sludge, and identify the biodegradation products and (iii) to determine the ability of the micelle–clay composite and activated carbon towards removal of these drugs by adsorption from aqueous solutions. Furthermore, adsorption kinetics and adsorption isotherms are evaluated and fitted to Langmuir equations. The micelle–clay composite which was used in this study is positively charged, has a large surface area and includes large hydrophobic domains. Micelle–clay composites have already been proven useful in the removal of about 20 neutral and anionic pollutants.[30]

2. Experimental

2.1. Materials and methods

2.1.1. Materials

All purchased chemicals were of analytical grade. The clay used was Wyoming Na-montmorillonite SWY-2 clay; obtained from the Source Clays Registry (Clay Mineral Society, Colombia, MO). Quartz sand (grain size 0.8– 1.2 mm) was obtained from Negev industrial minerals (Israel). Octadecyltrimethylammonium (ODTMA, structure **5** in Figure 1) bromide was obtained from Sigma-Aldrich. Pure ATO, RST and SIM (compounds **1**, **2** and **3** in Figure 1, respectively) were obtained from Birzeit Pharmaceutical Company (Palestine) as standard products with 99% purity, and were used all as received. Fine powder-activated charcoal (FAC) with particle size $\leq 60 \,\mu m$, and granular-activated charcoal (GAC) with particle size $\leq 700 \,\mu m$ were obtained from Sigma (Sigma Chemical Company, USA). The powder was used for batch adsorption experiments, whereas the granules were used in column experiments. Magnesium sulphate anhydrous, acetonitrile as well as methanol and water for analysis (HPLC grade) were purchased from Sigma-Aldrich (Munich, Germany). High-purity diethyl ether ($> 99\%$) was purchased from Biolab (Israel).

For sample enrichment and purification, SPE 1 g C-18 6 mL disposable cartridges (Waters, Milford, MA, USA) were used.

Equipment: Samples were shaken using Big Bill (Banstaed/ Themolyne, USA). The disappearance of ATO, RST and SIM was determined by using a high-pressure liquid chromatography system model 2695 HPLC (Waters, USA), equipped with a Waters 2996 Photodiode array. Data acquisition and control were carried out

using Empower™ software (Waters, USA). Analytes were separated on a 4.6 mm × 150 mm C18 XBridge® column (5 μm particle size) used in conjunction with a 4.6 mm, 20 μm, XBridge® C18 guard column.

HPLC conditions: mixture of 1% H_3PO_4:acetonitrile (1:1; v/v) as the mobile phase; flow rate of 1.5 mL min^{-1}; UV detection at a wavelength of 238 nm. Acrodisc® syringe filters with GHP membrane (hydrophilic polypropylene 0.45 μm porosity) from Waters were always used for all analytical filtration requirements [26] (BP-2007). The identification of ATO, RST and SIM degradation products was performed using a liquid chromatography system coupled to a hybrid linear quadrupole ion trap (LTQ) − Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer (Thermo Fisher Scientific, Bremen, Germany).

Analyses were performed by using the same column in a isocratic mode with two solutions, solution A: water with formic acid (0.1% v/v) and solution B: acetonitrile (100% v/v) [35:65] at ambient temperature at a flow rate of 1.0 mL min^{-1}, which was split 4:1 after the analytical column to allow 200 μl min^{-1} to enter the ESI source. Negative and positive ion ESI-MS was used for the detection of compounds of interest. Mass spectrometric conditions were optimized by direct infusion of standard solutions. The instrument was tuned to facilitate the ionization process and to achieve the highest sensitivity. The MS detector was tuned whenever the solvent flow rate conditions were changed, and the electrospray voltage, heated capillary temperature and voltage, tube lens voltage, sheath gas flow rate and auxiliary gas flow rate were optimized until the ion transmission was maximized. Full-scan experiments were performed in the ICR trapping cell in the range m/z 50–1000. Mass-to-charge ratio signals (m/z) were acquired as profile data at a resolution of 100,000 (full width at half maximum) at m/z 400. Data acquisition and analyses were accomplished using the Xcalibur software package (version 2.0 SR1 Thermo Electron). The simplest method to identify analytes by eXtracted Ion Chromatograms (XICs) was used. XIC collects ion intensities falling within a given mass-to-charge-ratio window and appears to be the method of choice for mass analysis leading to very simple chromatograms. The reduction of interferences in the XICs significantly facilitates the identification of putative metabolites. Data were collected in full MS scan mode and processed post-acquisition, to reconstruct the elution profile for the ions of interest, with a given m/z value and tolerance. The chromatographic raw data were imported, elaborated and plotted by SigmaPlot 10.0 (Systat Software, Inc., London, UK).

The advanced WWTP employed in this study is located at Al-Quds University − Palestine and was described in detail elsewhere.[31] Normally, the effluent from this plant is recycled for the irrigation of plants cropped in the fields of the University campus.

2.2. Methods

2.2.1. Characterization of wastewater used

The chemical and biological quality of wastewater before and after treatment was characterized according to American Public Health Association procedures [32,33] by performing measurements listed in Table S1 (see Supplementary data).

2.2.2. Efficiency of WWTP for ATO, RST and SIM removal

The efficiency of different treatment units was determined by spiking separately the secondary effluent with $1.0 \, \text{mg} \, L^{-1}$ of ATO, RST and SIM in the activated sludge reservoir (1000 L). Samples were collected from different locations of the WWTP as depicted in Figure S1, Supplementary data. SPE-C18 disposable cartridges were used to pre-concentrate 10 mL of each sample by adsorption of analytes. A part (20 μL) of the methanolic solution eluted from the SPE cartridge was injected into the HPLC, and analysed using the same conditions for the determination of ATO, RST and SIM. Recovery tests were performed using triplicate solutions of the three substances, and values ranging from 98% to 102% were obtained.

2.2.3. Stability of ATO, RST and SIM

Stability studies of ATO, RST and SIM were performed using $100 \, \text{mg} \, L^{-1}$ solutions in pure water, or activated sludge taken from the secondary treatment stage of the WWTP installed at Al-Quds University. At specific time intervals (0 to 35 days) samples were collected from the solutions (maintained under continuous orbital shaking), filtered and analysed by HPLC. The degradation by-products of ATO, RST and SIM were quantified using liquid chromatography/Fourier-transform ion cyclotron resonance/mass spectrometry.

2.2.4. Micelle–clay complex preparation

The ODMTA micelle–clay complex was prepared by mixing a smectitic clay mineral (montmorillonite) with the cationic surfactant ODTMA (as bromide salt) at multiples of $10 \, \text{g} \, L^{-1}$ (clay) and 12 mM (ODTMA, **5** in Figure 1) as described elsewhere.[31] The obtained complex, which has net positive charge and includes hydrophobic regions, is capable of efficiently binding neutral and negatively charged organic molecules.[34–37]

2.2.5. Batch adsorption experiments

Batch adsorption experiments were carried out on ATO, RST and SIM at different concentrations. Experiments were performed in 250 mL Erlenmeyer flasks containing 200 mg of either micelle–clay complex or FAC. Hundred millilitres of each drug solutions having known initial concentration were then introduced into each flask. The flasks were shaken in an oscillating shaker for 3 h at room temperature, and then 2.0 mL portions were filtered using 0.45 μm filters. Equilibrium concentrations of ATO, RST and SIM were then obtained by HPLC, using the conditions reported above. The retention times of ATO, RST and SIM were 6.6, 3.7 and 3.2 min, respectively.

2.2.6. Column filtration experiments

Column filtering experiments were performed using 50/1 (w/w) mixtures of quartz sand and either ODTMA–clay complex, or GAC, 20 cm layered in borosilicate columns of 25 cm length and 5 cm internal diameter. Each column contained 13 g of complex, or GAC. The bottom of the column was covered with 3 cm layer of quartz sand. Quartz sand was thoroughly washed by distilled water and dried at 105°C for 24 h before its use. Solutions in pure water (1 L each) containing different ATO, RST and SIM concentrations (0.01, 1, 10 and $100 \, \text{mg} \, L^{-1}$) were passed through either micelle–clay or GAC columns (one column for each solution). In all cases the flow rate was $2.0 \, \text{mL} \, \text{min}^{-1}$. Eluted fractions were collected in all column experiments and analysed.

All experiments reported were performed in three replicates and average values and standard deviations were calculated.

3. Results and discussion

3.1. Calibration curves

Linearity of the proposed analytical method was verified by analysing standard solutions in the range of 0.1–$100 \, \text{mg} \, L^{-1}$ for ATO, RST and SIM in pure water. The calibration curves were obtained using the HPLC method with the calculated regression coefficient ranging from 0.9998 to 0.9999. The reproducibility of triplicate subsequent injections ranged from 98.4% to 99.6%, depending on the sample concentration and type of analyte. The reproducibility of morning/evening injections on the basis of 6 h elapsed time ranged from 97.5% to 98.0%, and was also affected by the concentration and type of analyte. Correction coefficients were used for experimental samples.

Calibration curves and reproducibility trials were repeated preparing new calibration solutions by using wastewater taken from the activated sludge reservoir of Al-Quds WWTP. Results suffered a minor accuracy due to the variability of recovery percentages. Anyway, the determination coefficients of calibration curves were 0.9997 for ATO, 0.9995 for RST and 0.9999 for SIM. The limit of detection, based on a signal/noise of 3, was $0.03 \, \text{mg} \, L^{-1}$ for ATO and RST, and $0.02 \, \text{mg} \, L^{-1}$ for SIM. The limit of quantifications, based on a signal/noise of 10, was 0.08,

Table 1. Removal of ATO, RST and SIM from wastewater by different treatment units in Al-Quds WWTP; average values of three replicates.

Sample description		Sampling site as in Figure S1	Concentration of ATO, RST & SIM mg L^{-1}			Removal %		
			Means ± S.D.	Means ± S.D.	Means ± S.D.	ATO	RST	SIM
The initial concentration of drug in storage tank (after addition of drug)		1	1.0 ± 0.17	0.92 ± 0.04	0.97 ± 0.11			
UF-HF	Influent	2	0.85 ± 0.02	0.78 ± 0.03	0.72 ± 0.06			
	Brine produced	3	0.51 ± 0.03	0.397 ± 0.02	0.32 ± 0.02			
	Effluent	4	0.13 ± 0.004	0.24 ± 0.001	0.19 ± 0.004	84.6	69.2	73.6
UF-SW	Brine	5	0.12 ± 0.02	0.1 ± 0.02	0.097 ± 0.007			
	Effluent	6	b.l.d.	b.l.d.	0.067 ± 0.02	≈ 100.0	≈ 100.0	90.7
GAC	Effluent	7	—	—	b.l.d.	—	—	≈ 100.0

Note: b.l.d. = below the limit of detection.

0.08 and 0.06 mg L^{-1} for ATO, RST and SIM, respectively.

3.2. Wastewater characteristics

Table S2 (see Supplementary data) summarizes the chemical, physical and biological characteristics of wastewater sampled from the activated sludge reservoir of Al-Quds WWTP. Table S2 reveals that the wastewater contained high amounts of suspended solids and large populations of bacteria, which are responsible for fouling phenomena affecting ultra-filtration and reverse osmosis membranes. Moreover, high values of electrical conductivity and total dissolved solids are typical for municipal wastewaters, and should be reduced if WWTP effluents are re-used for crop irrigation purposes.

3.3. Efficiency of WWTP for ATO, RST and SIM removal

The efficiency of WWTP at Al-Quds University for the removal of ATO, RST and SIM was studied. The activated sludge reservoir (site 1 in Figure S1; Table S2) was separately spiked with ATO, RST or SIM at a concentration of 1.0 mg L^{-1}, which is an amount close to environmental values reported in the literature.[21,38] Samples were taken from different collecting sites of WWTP as described in Figure S1. Analytical results of water effluent from the hollow fibre ultra-filtration (UF-HF) membrane indicated that ATO, RST and SIM were about 84.6%, 69.2% and 73.6% removed at this stage, respectively, whereas about 100% of ATO, RST and 90.7% of SIM were removed after passing the spiral wound (UF-SW) membrane (Table 1). Besides, RST was completely removed in the effluent from the GAC filter. However, it should be outlined that the concentration of ATO, RST and SIM influent in the treatment units were diminishing along their sequence. This resulted in 100% removal by GAC filter, whose influent water contained only 0.067 mg L^{-1} of RST, on average,

after the passage through the UF filters. This finding made unnecessary the use of reverse osmosis for any further purification. Nevertheless, the advanced technology adopted in the WWTP of Al-Quds University did not overcome a problem common to all plants: the production of brine, in which a large portion of the contaminants ends up being concentrated there. For this reason additional methods of water filtration and purification were experimented.

3.4. Stability of ATO, RST and SIM in pure water and in sludge

Since many pharmaceuticals might undergo degradation upon their standing in aqueous medium and sludge environment,[38,39] kinetics studies on degradation of ATO, RST and SIM in pure water and in activated sludge conditions have been undertaken. Table 2 summarizes the hydrolysis results of the drugs in activated sludge and pure water at room temperature. For brevity we show the detailed results only for ATO (**A**) (Figure 2) and SIM (**B**) (Figure 2). The accelerated degradation of the statin drugs in sludge is expected to occur by the activity of bacteria attached to sites in the sludge. Hence, the process involves first sorption of the statin molecules by the sludge and then degradation by the bacteria. Consequently, even if the degradation by the bacteria is a first-order reaction, the

Table 2. Degradation rates and half-lives of ATO, RST and SIM in sludge and pure water and determination coefficients (R^2).

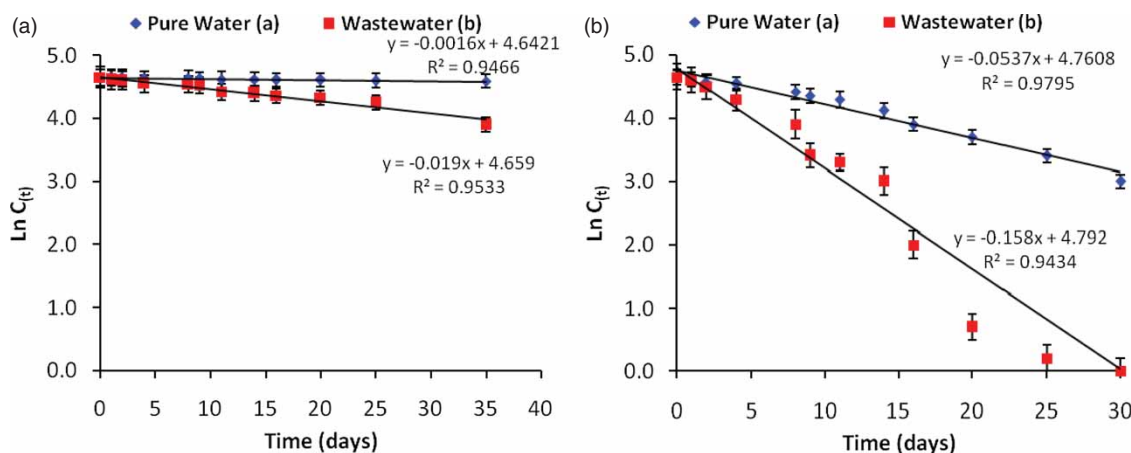| Drug	Medium	k (s^{-1})	Half-life (d)	R^2
ATO	Sludge	2.2×10^{-7}	36.3	0.9538
	Water	1.9×10^{-8}	433.1	0.9557
RST	Sludge	1.8×10^{-7}	45.9	0.9962
	Water	2.2×10^{-8}	364.7	0.9177
SIM	Sludge	1.8×10^{-6}	4.4	0.9483
	Water	6.2×10^{-7}	12.9	0.9892

Figure 2. Kinetics of ATO (**A**) and SIM (**B**) degradation in pure water (plot a) (♦) and activated sludge (plot b) (■). Data are reported as natural logarithm of concentrations ($C_{(t)}$) vs. time. Initial concentration ($C_{(0)}$) = 100 mg L^{-1}. Plotted values are the means of three replicates; bars represent the standard deviations calculated for each average value, $T = 25°C$.

overall reaction may deviate from a first order. However, in our case, where the rate of degradation is slow relative to the adsorption process, the overall rate of the degradation can appear as first order. Table 2 and Figure 2 demonstrate that the assumption of a first-order rate of degradation is largely justified.'

The results in Table 2 indicate that the rates of degradation for ATO, RST and SIM in sludge are about 12-, 8- and 3-fold faster than in pure water, respectively. The rate of degradation of SIM in sludge is about an order of magnitude faster when compared with ATO and RST, where the ratio is about 30-fold in water. The higher rate of SIM degradation compared with ATO and RST stems from the fact that SIM exists as a lactone moiety which is readily hydrolysed to the corresponding carboxylic acid form in the presence of acid or base catalysis, whereas ATO and RST exist in the free carboxylic acid forms.

The accelerated degradation in sludge compared with that in pure water can be attributed to bioactivity of the activated sludge. The morphological characterization of bacterial community in Al-Quds activated sludge allowed to identify many bacterial species: *Escherichia coli*, *Enterobacter sakazakii*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Enterobacter cloacae*, *Enterobacter amnigenus*, *Enterobacter aerogenes*, *Salmonella* spp. and *Serratia liquefaciens*. Further challenge will be the isolation of strains constituting the bacterial colonies, aiming at the identification of the more active strains capable of utilizing the pharmaceutical molecules as energy source.

Literature survey on the stability and degradation of SIM indicates that the drug can undergo metabolic degradation in humans.[40] Upon incubation of SIM with liver microsomal preparations from human donors, four major metabolic products were formed (3*-hydroxy SIM, 6*-exomethylene SIM, 3*,5*-dihydrodiol SIM, and the active hydroxy acid, SIMA), together with several

minor unidentified metabolites.[40] Based on four different in vitro approaches, namely (1) correlation analysis, (2) chemical inhibition, (3) immune inhibition and (4) metabolism by recombinant human P450, it is concluded that CYP3A is the major enzyme subfamily responsible for the metabolism of SIM by human liver microsomes.[40] Similar results were obtained by Robert et al., [41] who showed that CYP3A4 was the main enzyme involved in ATO metabolism resulting in the major metabolite ortho-hydroxy ATO (*o*-ATO) and in active metabolites ATO lactone and *o*-ATO lactone (Figure 3). These results are in agreement with a study by Liu et al., [42] who showed that ATO is administered in its calcium salt active form and is converted into active ATO acid, which is then metabolized into two other active metabolites, *para*-hydroxy ATO, and *o*-ATO. These three active compounds are subsequently equilibrated with their corresponding lactone forms at the ratio of approximately 1:1 [42]. Jani et al. [43] found that ATO in plasma can be metabolized by CYP3A4 to two hydroxylated metabolites, *o*-hydroxyl atorvastatin and *p*-hydroxy atorvastatin (Figure 3).

RST is a new generation of HMG-CoA reductase inhibitor, which exhibits some unique pharmacologic and pharmacokinetic properties. It has low extra hepatic tissue penetration, low potential for CYP3A4 interaction and substantial LDL-C lowering capacity and therefore has distinct advantages.[44] Metabolism of RST by cytochrome p450 (CYP) appears to be minimal and is principally mediated by the 2C9 enzyme, with little involvement of 3A4; this finding is consistent with the absence of clinically significant pharmacokinetic drug–drug interactions between RST and other drugs known to inhibit CYP enzymes.[45] The major metabolite of RST is N-desmethyl RST (active) via CYP450 2C9. Greater than 90% of activity is due to N-desmethyl RST. Clearance is not significantly dependent on CYP450 3A4.[44] Monitoring the derivative substances arising from the degradation of ATO, RST and
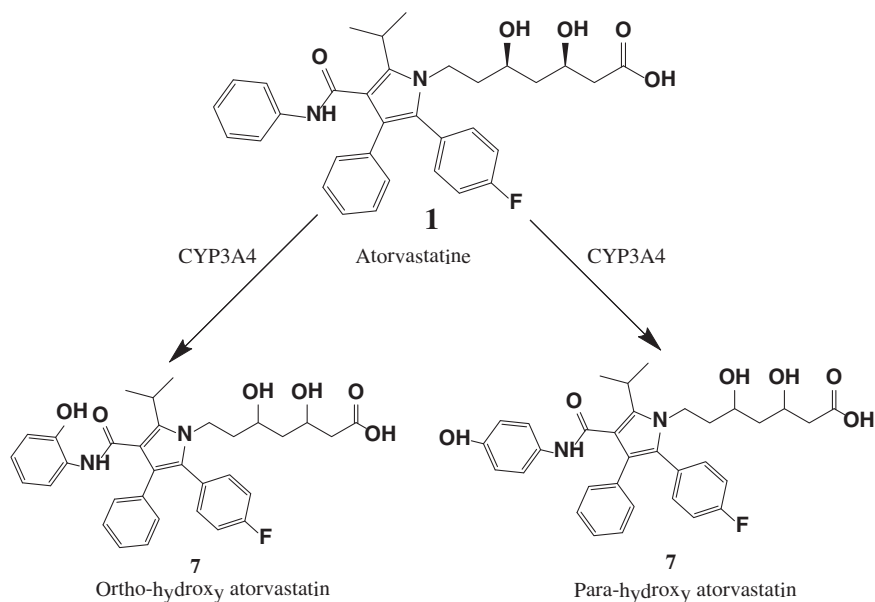
Figure 3.   ATO metabolism to *ortho*- or *para*-hydroxy atorvastatin by CYP3A4.

SIM in the activated sludge indicated that ATO underwent degradation to five by-products, whereas RST and SIM gave rise to only one derivative, respectively, as identified by mass spectrometric analysis. XICs of the 35 days biodegraded sample are shown in Figure 4. The benefit of using very selective XICs by FTICR MS, generated with a tight mass-to-charge ratio window of ± 0.0010 units around each selected protonated or deprotonated molecule (i.e. $[M + H]^+$ or $[M-H]^- \pm 1.0\,\text{mDa}$), greatly reduced the signal complexity of the total ion current trace (Figure 4(b)) allowing to completely characterize all degradation products.

Five major biodegradation products for ATO (Figure 4(a)B, exact $[M-H]^-$ *m/z* 557.24572) were found at retention times 7.5(**6**), 5.2(**7**), 4.3(**8**), 4.5(**9**) and 6.0(**10**) minutes, corresponding to compounds with exact $[M-H]^-$ *m/z* ratios 589.23555 (6, Figure 4(a)D), 573.24064 (7, Figure 4(a)C), 547.18860 (8, Figure 4(a)E), 545.17295 (9, Figure 4(a)F), 513.21951(10, Figure 4(a)H). Based on the accurate *m/z* ratios of deprotonated molecules, their retention times and relevant literature,[41] we propose for ATO biodegradation the structures reported in Figure 5. The major metabolite resulted from the biodegradation of ATO was compound (**7**), the para-hydroxy ATO, $C_{33}H_{35}FN_2O_6$, with exact $[M-H]^-$ *m/z* 573.24064 (error 0.6 ppm). *Ortho*-, *para*-dihydroxy atorvastatin was, also, recognized at exact deprotonated *m/z* ratio 589.23555 (error 1.2 ppm). The peaks belong to (**8**), (**9**) and (**10**) can be attributable to compounds obtained from losses of -CH₃COOH or isopropyl group from other biodegraded, oxidized products.

RST (Figure 4(b)B, exact $[M-H]^-$ *m/z* 480.16101) has one of the major biodegradation products at a retention time of 5.2(**5**) minutes (Figure 4(b)E). Based on the

accurate *m/z* ratios 462.15098 and relevant literature,[45] we suggest the structure of the metabolite as shown in Figure 1 (structure **4**). Other compounds derived from hydroxylation or polihydroxylation (Figure 4(b) C and D) showing an accurate $[M-H]^-$ ratio at *m/z* 496.15614 (exact *m/z* 496.15592, error 0.4 ppm) and 514.16660 (exact *m/z* 514.16649, error 0.2 ppm) and attributable at compounds with molecular formulas $C_{22}H_{28}FN_3O_7S$ and $C_{22}H_{30}FN_3O_8S$, respectively, which were also found in the biodegraded solution of RST. For SIM (Figure 4(c), exact $[M-H]^-$ *m/z* 573.24064) one major biodegradation product was formed at a retention time of 9.1 (**11**) minutes. Based on the accurate $[M-H]^-$ *m/z* ratio 435.27560 (error 0.9 ppm) and relevant literature,[40] we propose the structure of SIM metabolite as shown in Figure 5 (**11**). Interestingly, accurate mass data of biodegraded products, as protonated or deprotonated molecules, with a mass error lower than $+1.2$ ppm was found, indicating a very good mass accuracy.

It is worth noting, that no reports have been published on biodegradation of ATO, RST and SIM in wastewater.

3.5.   *Adsorption isotherms*

The adsorption of ATO, RST and SIM at several initial concentrations on micelle–clay complex and activated charcoal was investigated. Equilibrium relationships between adsorbent and adsorbate can be described by the Langmuir adsorption isotherm,[46] represented by the following equation:

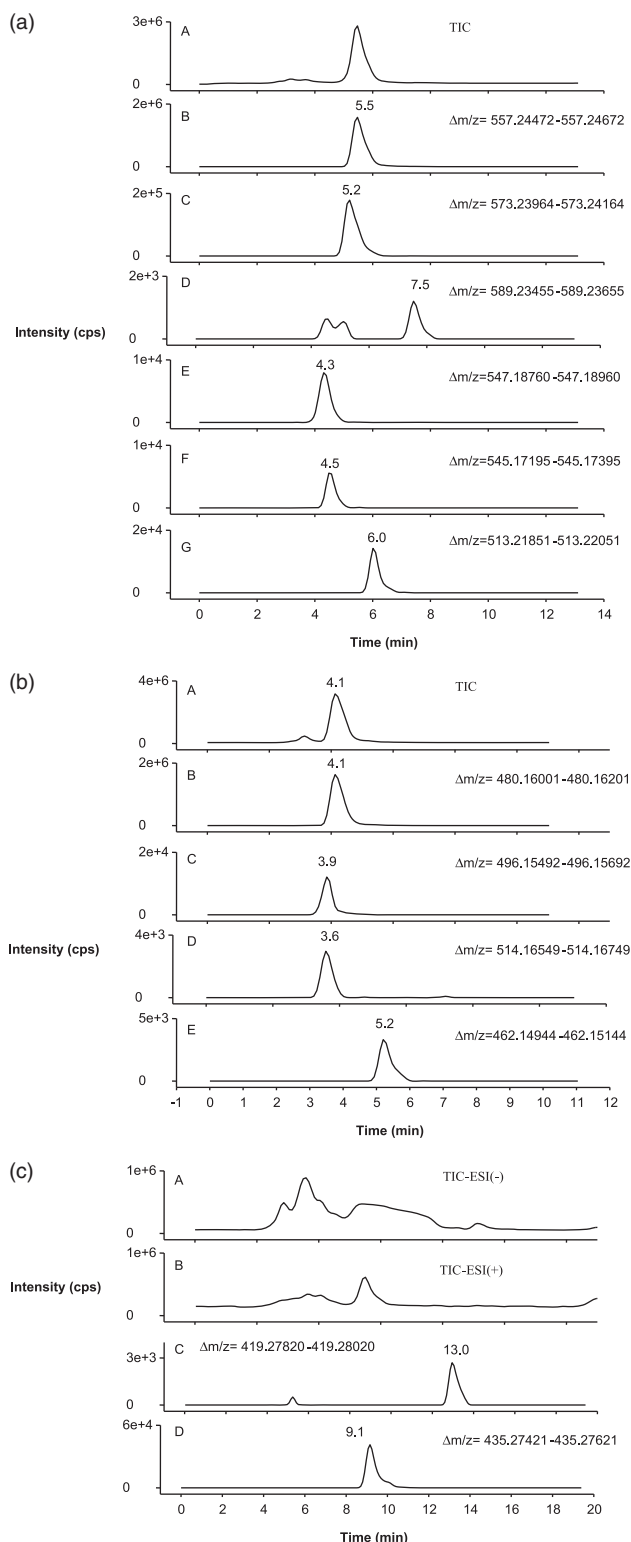$$\frac{C_e}{Q_e} = \frac{1}{KQ_{\max}} + \frac{C_e}{Q_{\max}}, \qquad (1)$$

Figure 4. XICs by LC/ESI-FTICR MS acquired in the negative ion mode of ATO solution (a), RST solution (b) and in negative and positive ion mode of SIM (c) after one month of biodegradation. The ions monitored are displayed in each trace and correspond to the most abundant protonated or deprotonated molecules, $[M + H]^+$ or $[M-H]^-$, using a restricted window of $\pm\,0.0010\,m/z$ unit centred on each selected ion.

where C_e (mg L^{-1}) is the equilibrium concentration of the drug in the solution, Q_e (mg g^{-1}) is the equilibrium mass of adsorbed drug per gram of the complex or activated charcoal, K (L mg^{-1}) is the Langmuir binding constant, and Q_{max} (mg g^{-1}) is the maximum mass of drug removed per gram of the complex.

Data fitted well the Langmuir equation for ATO, RST and SIM giving $R^2 = 0.9873$, 0.9748 and 0.9568 for activated charcoal and 0.9223, 0.9408 and 0.9154 for the micelle–clay, respectively (Table 3). The values of K and Q_{max} parameters for the adsorption isotherm obtained using micelle–clay complex were larger than those corresponding to activated charcoal, suggesting the former as the better adsorbent for ATO, RST and SIM removal.

3.6. Filtration results

ATO, RST and SIM solutions were passed separately through filtering columns, which included the micelle–clay complex or activated charcoal mixed with excess sand at 1:50 ratios (w/w). The results (Table 4) indicate a significant advantage of the micelle–clay filter in removing ATO, RST and SIM compared with the removal by activated charcoal. The removal efficiency of filters filled with activated charcoal and sand was acceptable only for the lowest ATO, RST and SIM concentrations, whereas the micelle–clay system removed the drugs completely at the higher concentrations. This finding is in line with the adsorption isotherms, which showed that the micelle–clay complex was more efficient than activated charcoal in adsorbing ATO, RST and SIM from water.

Previously reported experiments demonstrated the poor capability of activated carbon filters towards removing anionic and certain neutral pollutants.[30,30,34–36,47]

Khamis et al. [30] concluded that the incorporation of micelle–clay filters in sewage treatment systems with lose of tertiary capability can be a promising technology. In a recent paper, Karaman et al. [31] showed that micelle–clay filters were more efficient towards removal of diclofenac from wastewater than activated carbon.

Polubesova et al. [34] found very efficient removal of three anionic herbicides (imazaquin, sulfentrazone, sulfosulfuron) and four neutral pollutants (alachlor, acetochlor, chlorotoluron and bromacil) by micelle–clay complexes in aqueous dispersions. In another study,[35] column filters filled with a mixture of quartz sand and micelle–clay complex provided very efficient result for the removal of the antibiotics tetracycline and sulfonamide from water.

Moreover, Zadaka et al. [36] tested column filters with either a mixture of quartz sand and organic micelle – montmorillonite or zeolite; both filters were capable of removing well anionic pollutants such as sulfosulfuron, imazaquin and sulfentrazone, and neutral compounds such as bromacil and chlorotoluron from aqueous environments;
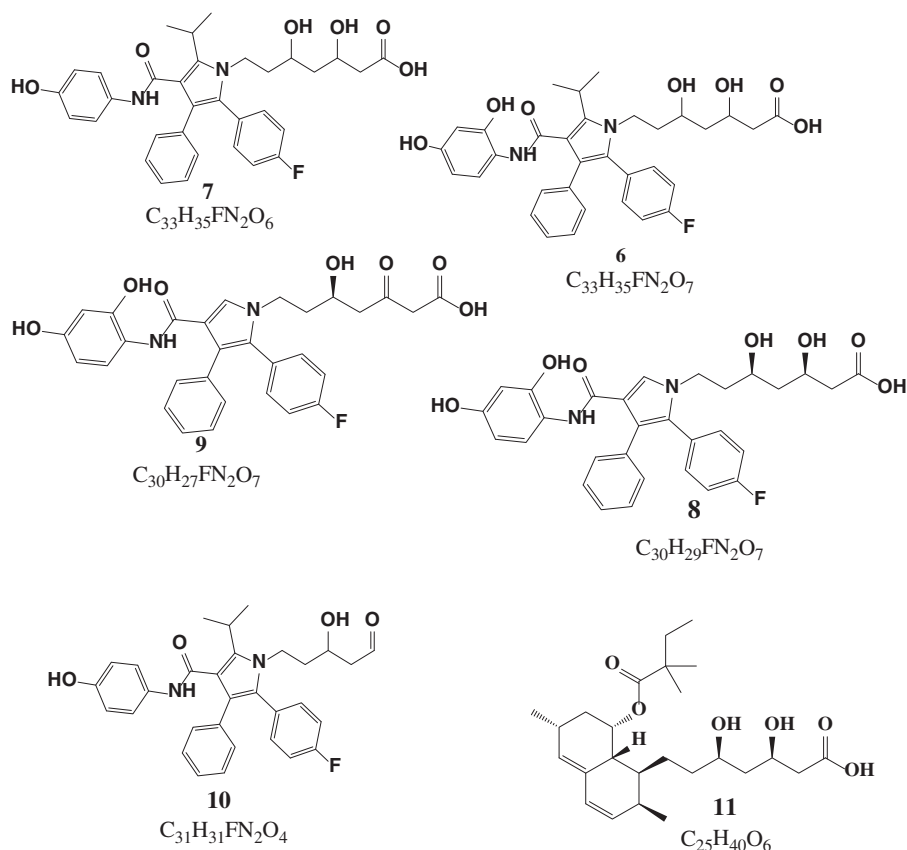
Figure 5. Chemical structures of ATO biodegradation products. *Ortho-*, *para*-dihydroxyatorvastatin, (7-[2-(4-Fluoro-phenyl)-4-(4-hydroxy-phenylcarbamoyl)-5-isopropyl-3-phenyl-pyrrol-1-yl]-3,5-dihydroxy-heptanoic acid), $C_{33}H_{35}FN_2O_7$ with exact *m/z* 589.23555 (**6**); para-hydroxy Atorvastatin (7-[2-(4-fluoro-phenyl)-4-(4-hydroxy-phenylcarbamoyl)-5-isopropyl-3-phenyl-pyrrol-1-yl]-3,5-dihydroxy-heptanoic acid),$C_{33}H_{35}FN_2O_6$ with exact *m/z* 573.24064 (**7**); 7-[4-(2,4-dihydroxy-phenylcarbamoyl)-2-(4-fluoro-phenyl)-3-phenyl-pyrrol-1-yl]-3,5-dihydroxy-heptanoic acid, $C_{30}H_{29}FN_2O_7$ with exact *m/z* 547.18860 (**8**); 7-[4-(2,4-dihydroxy-phenyl carbamoyl)-2-(4-fluoro-phenyl)-3-phenyl-pyrrol-1-yl]-5-hydroxy-3-oxo-heptanoic acid $C_{30}H_{27}FN_2O_7$ with exact *m/z* 545.17295 (**9**); 5-(4-fluoro-phenyl)-1-(3-hydroxy-5-oxo-pentyl)-2-isopropyl-4-phenyl-1H-pyrrole-3-carboxylic acid (4-hydroxy-phenyl)-amide, $C_{31}H_{31}FN_2O_4$ with exact *m/z* 513.21951 (**10**) and SIM acid $C_{25}H_{40}O_6$ with exact *m/z* 435.27560 (**11**).

Table 3. Langmuir adsorption parameters (K and Q_{max}) and determination coefficients (R^2) obtained from the adsorption of ATO, RST and SIM on the micelle–clay complex and activated charcoal.

Drug	Adsorbent	K (L mg^{-1})	Q_{max} (mg g^{-1})	R^2
ATO	Micelle–clay complex	10.7	23.2	0.9223
	Activated charcoal	5.0	9.1	0.9873
RST	Micelle–clay complex	8.2	29.4	0.9408
	Activated charcoal	7.4	27.3	0.9748
SIM	Micelle–clay complex	8.4	24.4	0.9154
	Activated charcoal	6.5	11.9	0.9568

in contrast a filter filled with the same weight of activated carbon and sand removed only partially these pollutants.

More recently, Khalaf et al. [47] suggested that the integration of clay–micelle complex filters in existing WWTPs may be helpful for improving removal efficiency of recalcitrant residues of non- steroid anti-inflammatory drugs.

It can be argued that in addition to ATO, RST and SIM residues wastewater usually includes other recalcitrant organic pollutants. In such cases, GAC filters can be used for the first-stage tertiary process to remove the majority of neutral pollutants, and additional micelle–clay filters can be adopted as second stage to eliminate anionic pollutants, and neutral compounds not retained by GAC filters.

4. Conclusion

The stability studies of ATO, RST and SIM revealed that the three statins were unstable in both water and sludge environments. In addition, it was found that the rate of degradation in sludge was higher than in water due to the presence of many bacterial species having a variety of enzymes which can catalyse the degradation processes for the mentioned three statins. It should be worth noting that

Table 4. Removal of ATO, RST and SIM by filtration of 1 L of pure water solutions (100, 10, 1.0, 0.01 mg L^{-1}) through laboratory filtering columns, which included either MC or GAC mixed with excess sand at 1:50 (w/w) ratio; means of three replicates.

Initial concentration (mg L^{-1})	Column type[a]	Average eluted concentration $\pm$ S.D: (mg L^{-1})		
		ATO	RST	SIM
100	MC	b.l.d.	b.l.d.	b.l.d.
100	GAC	62.2 ± 2.5	50.3 ± 4.2	52.7 ± 2.5
10	MC	b.l.d.	b.l.d.	b.l.d.
10	GAC	2.8 ± 0.48	b.l.d.	0.08 ± 0.03
1.0	MC	b.l.d.	b.l.d.	b.l.d.
1.0	GAC	0.25 ± 0.05	b.l.d.	b.l.d.
0.01	MC	b.l.d.	b.l.d.	b.l.d.
0.01	GAC	b.l.d.	b.l.d.	b.l.d.

Note:b.l.d., below the detection limit of the analytical method used.
[a]Flow rate, 2 mL min^{-1}; temperature, 25°C; pH, 7.2.

the higher degradation rate found for SIM compared with that of ATO and RST can be attributed to the relatively unstable lactone ring present in SIM.

The filtration study involving an advanced WWTP utilizing ultra-filtration, activated carbon and reverse osmosis demonstrated that activated carbon and RO are efficient in removing the commonly used anti-inflammatory ATO, RST and SIM from wastewater. But fouling problems due to the high bacterial load in the sludge and the production of brine cannot be avoided. For this reason a filter based on micelle–clay complex, (ODTMA)-montmorillonite, was tested and found to be very efficient in removing all three statins from solution in the mg L^{-1} or μg L^{-1} ranges. The large effectiveness and removal capacity are due to a relatively high affinity of adsorption of the anionic ATO, RST and SIM by the large number of positively charged and hydrophobic sites of the micelle–clay complex based on ODTMA. Furthermore, pilot experiments are needed to evaluate if filters based on micelle–clay could be included in WWTPs aiming at the reduction of membrane's use or at enhancing their efficiency and prolonging their life.

Disclosure statement

No potential conflict of interest was reported by the authors.

Supplemental data

Supplemental data for this article can be accessed at doi:10.1080/09593330.2015.1058422.

References

[1] Standley LJ, Rudel RA, Swartz CH, et al. Wastewater – contaminated ground water as a source of endogenous hormones and pharmaceuticals to surface water ecosystems. Environ Toxicol Chem. 2008;27(12):2457–2468.

[2] Purdom CE, Hardiman PA, Bye VJ, Eno NC, Tyler CR, Sumpter JP. Estrogenic effects of effluents from sewage treatment works. Chem Ecol. 1994;8:275–285.

[3] Jobling S, Nolan M, Tyler CR, Brighty G, Sumpter JP. Widespread sexual disruption in wild fish. Environ Sci Technol. 1998;32:2498–2506.

[4] Metcalfe CD, Metcalfe TL, Kiparissis Y, et al. Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (Oryziaslatipes). Environ Toxicol Chem. 2001;20(2):297–308.

[5] Tabata A, Kashiwada S, Ohnishi Y, et al. Estrogenic influences of estradiol-17, p -nonylphenol and bisphenol-A on Japanese medaka (Oryziaslatipes) at detected environmental concentrations. Water Sci Technol. 2001;43:109–116.

[6] Brody JG, Rudel RA. Environmental pollutants and breast cancer. Environ Health Perspect. 2003;111:1007–1019.

[7] Buser H-R, Muller MD, Theobald N. Occurrence of the pharmaceutical drug clofibric acid and the herbicide mecoprop in various Swiss lakes and in the North Sea. Environ Sci Technol. 1998;32:188–192.

[8] Kolpin DW, Furlong ET, Meyer MT, et al. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. Environ Sci Technol. 2002;36:1202–1211.

[9] Glassmeyer ST, Furlong ET, Kolpin DW, et al. Transport of chemical and microbial compounds from known wastewater discharges: potential for use as indicators of human fecal contamination. Environ Sci Technol. 2005;39:5157–5169.

[10] Buerge IJ, Poiger T, Muller MD, Buser H-R. Caffeine, an anthropogenic marker for wastewater contamination of surface waters. Environ Sci Technol. 2003;37:691–700.

[11] Heberer T. Tracking persistent pharmaceutical residues from municipal sewage to drinking water. J Hydrol. 2002;266:175–189.

[12] Tixier C, Singer HP, Oeller S, Muller SR. Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters. Environ Sci Technol. 2003;37:1061–1068.

[13] Webb S, Ternes T, Gibert M, Olejniczak K. Indirect human exposure to pharmaceuticals via drinking water. Toxicol Lett. 2003;142:157–167.

[14] Gros M, Petrović M, Barceló D. Multi-residue analytical methods using LC-tandem MS for the determination of pharmaceuticals in environmental and wastewater samples: a review. Anal Bioanal Chem. 2006;386:941–952.

[15] Tyler CR, Routledge EJ. Natural and anthropogenic environmental estrogens: the scientific basis for risk assessment. Estrogenic effects in fish in English rivers with evidence of their causation. Pure Appl Chem. 1998;70:1795–1804.

[16] Johnson AC, Belfroid A, Di Corcia A. Estimating steroid estrogens inputs into activated sludge treatment works and observations on their removal from the effluent. Sci Total Environ. 2000;256:163–173.

[17] Johnson AC, Sumpter JP. Removal of endocrine disrupting chemicals in activated sludge treatment works. Environ Sci Technol. 2002;35:4697–4703.

[18] Lee H-B, Peart TE, Lewina Svoboda M, Backus S. Occurrence and fate of rosuvastatin, rosuvastatin lactone, and atorvastatin in Canadian sewage and surface water samples. Chemosphere. 2009;77:1285–1291.

[19] Ma PTS, Gil G, Sudhof TC, Bilheimer DW, Goldstein JL, Brown MS. Mevinolin, an inhibitor of cholesterol synthesis, induces mRNA for low density lipoprotein receptor in livers of hamsters and rabbits. Proc Natl Acad Sci USA. 1986;83:8370–8374.

[20] Jones P, Kafonek S, Laurora I, Hunninghake D. Comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin, and fluvastatin in patients with hypercholesterolemia (the CURVES study). Am J Cardiol. 1998;81:582–587.

[21] Ternes TA. Occurrence of drugs in German sewage treatment plants and rivers. Water Res. 1998;32:3245–3260.

[22] Jemal M, Ouyang Z, Chen BC, Teitz D. Quantitation of the acid and lactone forms of atorvastatin and its biotransformation products in human serum by high-performance liquid chromatography with electrospray tandem mass spectrometry. Rapid Commun Mass Spectrom. 1999;13: 1003–1015.

[23] Bullen WW, Miller RA, Hayes RN. Development and validation of a high-performance liquid chromatography–tandem mass spectrometry assay for atorvastatin, ortho-hydroxy atorvastatin, and para-hydroxy atorvastatin in human, dog, and rat plasma. J Am Soc Mass Spectrom. 1999;10:55–66.

[24] Martin PD, Warwick MJ, Dane AL, et al. Metabolism, excretion, and pharmacokinetics of rosuvastatin in healthy adult male volunteers. Clin Ther. 2003;25:2822–2835.

[25] Cermola F, DellaGreca M, Rosaria Iesce M, Montanaro S, Previtera L, Temussi F. Photochemical behavior of the drug Atorvastatin in water. Tetrahedron. 2006;62: 7390–7395.

[26] Ridker PM, Danielson ED, Fonseca FAH, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med. 2008;359(21): 2195–2207.

[27] Hoffman WF, Alberts AW, Anderson PS, Smith RL, Willard AK. The organic chemistry of drug design and drug action. J Med Chem. 1996;29:849–852.

[28] Vickers S, Duncan CA, Chen IW, Rosegay A, Duggan DE. Metabolic disposition studies on simvastatin, a cholesterol-lowering prodrug. Drug Metab Dispos. 1990;18: 138–145.

[29] Ottmar KJ, Colosi LM, Smith JA. Development and application of a model to estimate wastewater treatment plant prescription pharmaceutical influent loadings and concentrations. B Environ Contam Tox. 2010;84:507–512.

[30] Khamis M, Karaman R, Qurie M, et al. Performance of micelle-clay filters for removing pollutants and bacteria from tertiary treated wastewater. J. Environ. Sci. Eng. 2012;1:160–168.

[31] Karaman R, Khamis M, Qurie M, et al. Removal of Diclofenac potassium from wastewater using clay-micelle complex. Environ. Technol. 2012;33:1279–1287.

[32] American Public Health Association (APHA). Standard methods for the examination of water and wastewater. 19th ed. Washington, DC: APHA; 1995.

[33] American Public Health Association (APHA). Standard methods for the examination of water and wastewater. 21th ed. Washington, DC: APHA; 2005.

[34] Polubesova T, Nir S, Zadaka D, et al. Water purification of organic pollutants by optimized micelle-clay systems. Environ. Sci. Technol. 2005;39:2343–2384.

[35] Polubesova T, Zadaka D, Groisman L, Nir S. Water remediation by micelle-clay system: case study for tetracycline and sulfonamide antibiotics. Water Res. 2006;40:2369–2374.

[36] Zadaka D, Mishael YG, Polubesova T, Serban C, Nir S. Modified silicates and porous glass as adsorbents for removal of organic pollutants from water and comparison activated carbons. Appl Clay Sci. 2007;36:174–181.

[37] Mishael YG, Undabeyita T, Rytwo G, Papahadjopoulos-Sternberg B, Rubin B, Nir S. Sulfometuron adsorption via alkylammonium cations adsorption as monomers and micelles on montmorillonite. J Agri Food Chem. 2002;50:2856–2863.

[38] Bendz D, Paxéus NA, Ginn TR, Loge FJ. Occurrence and fate of pharmaceutically active compounds in the environment, a case study. J Hazard Mater. 2005;122:195–204.

[39] Jones OAH, Voulvoulis N, Lester JN. Human pharmaceuticals in wastewater treatment processes. Crit Rev Environ Sci Technol. 2005;35:401–427.

[40] Prueksaritanont T, Gorham LMMA, Liu L, et al. In Vitro metabolism of simvastatin in humans [SBT] identification of metabolizing enzymes and effect of the drug on hepatic P450S. J. Pharmacol. Exp. Ther. 1997;25(10):1191–1199.

[41] Robert L, Katelijne E, Matthys G, et al. Pharmacokinetics of atorvastatin and its metabolites after single and multiple dosing in hyper cholesterol aemichaemo dialysis patients. Nephrol. Dial. Transplant. 2003;18(5):967–976.

[42] Liu DY, Jiang J, Zhou H, Hu P. Quantitative determination of atorvastatin and para-hydroxy atorvastatin in human plasma by LC–MS–MS. J Chromatogr Sci. 2008;46(10):862–866.

[43] Jani AJ, Faeth SH, Gardner D. A sexual endophytes and associated alkaloids alter an bhropod community structure and increase herbivore abundances on a native grass. Ecol Lett. 2010;13:106–117.

[44] Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. Science. 2001;292: 1160–1164.

[45] Olsson AG, McTaggart F, Raza A. Rosuvastatin: a highly effective new HMG-CoA reductase inhibitor. Cardiovasc Drug Rev. Winter; 2002;20(4):303–328.

[46] Dakiky M, Khamis M, Manasra A, Mereb M. Selective adsorption of chromium (VI) in industrial waste water using low cost abundantly available adsorbents. Adv Environ Res. 2002;6:533–540.

[47] Khalaf S, Al-Rimawi F, Khamis M, et al. Efficiency of advanced wastewater treatment plant system and laboratory-scale micelle-clay filtration for the removal of ibuprofen residues. J Environ Sci Health, Part B. 2013;48:814–821.