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Stability and Removal of Several Statins from Wastewater Using Different Treatment Technologies.

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Abstract: *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 statins from wastewater produced at Al-Quds University Campus were investigated. Kinetic studies in pure water (abiotic degradation), in sludge (biodegradability) and in Advanced Oxidation Processes (AOPs) at room temperature were investigated. Heterogeneous photocatalysis using the semi- conductor titanium dioxide (TiO2) has proven to be a promising treatment technology for water purification. The degradation reactions of the three drugs in wastewater at room temperature follow first order kinetics with rate constants of $2.2 \times 10^{-7} s^{-1}$(ATO), $1.8 \times 10^{-7} s^{-1}$ (RST) and $1.8 \times 10^{-6} s^{-1}$ (SIM), which are larger than those obtained in pure water under the same conditions, $1.9 \times 10^{-8} s^{-1}$ (ATO), $2.2 \times 10^{-8} s^{-1}$ (RST) and $6.2 \times 10^{-7} s^{-1}$ (SIM).*

AOPs process was proposed and applied. TiO$_2$, used as catalyst, owns two important properties: high photo-catalytic activity and low cost. Photo-degradation of Atorvastatin was much faster under light irradiation in presence of TiO$_2$ (photocatalysis) ($t_{1/2} = 1.15$ hours, $0.704h^{-1}$) than under Suntest irradiation (photolysis) ($t_{1/2} = 5.0$ hours, $0.165h^{-1}$). Degradation products were identified by LC-MS and LC/MS/MS. The overall performance of the wastewater treatment plant (WWTP) installed in 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 and AOP's as a post treatment. The batch adsorption isotherms for the three statins were found to fit Langmuir equation, with larger number of adsorption sites and binding affinity for micelle-clay composite compared to activated carbon and filtration experiments of the three statins and their corresponding metabolites demonstrated a more efficient removal by micelle-clay filters.

Keywords: *Advanced oxidation processes; Photolysis and Photo-catalysis, Activated sludge, Activated charcoal, Micelle – clay complex.*

1.   Introduction

The substantial and extended use of pharmaceutical and personal care products (PPCPs) elucidate their frequent occurrence, which comprise a new class of pollutants that have been found in a wide range of aquatic environments including ambient surface water, groundwater, drinking water, and soil [1]-[2], and have high acute toxicity, tendencies to undergo bio-accumulation and bio-magnifications. They also have a high resistance against degradation and long half-life in the environment.

Nowadays another concern is arising due to pharmaceuticals or their metabolites found in environmental samples. The presence of pharmaceutical residues in environmental bodies has increased the probability of toxicity risks for animals and humans in the last years [3]-[5]. It has been reported that the aquatic environment can become polluted with pharmaceutically active compounds (drugs) at low concentrations because of the extensive consumption of pharmaceuticals in developed countries [6]-[7].

Persistence to biochemical degradation and polar structure, are indicated as mainly responsible for the incomplete removal of pharmaceuticals during conventional wastewater treatment plant [8]-[9] with efficiencies ranging between 60% and 90% for a variety of polar compounds [10]-[13].

To evaluate the efficiency of different traditional and innovative tools for the elimination of pharmaceutical residues, a series of water purification experiments were performed by using the WWTP installed at the Al-Quds University in Palestine as indicated in Figure 1 (appendix A), which includes sequential units as activated sludge (AS), ultra-filtration (UF), granular activated charcoal (GAC) and reverse osmosis (RO) [14].

Problems arising from the management of such a plant can be referred to the capability of the AS unit to favor the bio-degradation of organic pollutants as well as the fouling phenomenon affecting membrane units, which must be often replaced with high costs.

The present work reporting about the efficiency of advanced wastewater treatment technologies adopted in the Al-Quds WWTP for the removal of 'Atorvastatin' (ATO), Rosuvastatin (RST) and Simvastatin (SIM) which were used as model pharmaceutical compounds due to their large consumption in many Countries.

Aiming at the assessment of bacterial culture, which normally develops in the AS unit of Al-Quds WWTP, the stability of ATO, RST and SIM in pure water as well as in activated sludge collected from the plant were investigated and their degradation products were identified.

Finally, in order to check for different tools to be used instead of ultra-filtration membranes, the effectiveness of micelle-clay (MC) filter for removing ATO, RST and SIM was ascertained and compared to a filter filled with granular activated charcoal. Besides, the adsorption equilibrium parameters and the adsorption Langmuir coefficients were determined using both micelle-clay and fine powder activated charcoal (FAC) as adsorbent materials. In order to achieve a complete elimination of recalcitrant organic residue AOP's was used as a post treatment.

Advanced oxidation processes (AOPs) [15] constitute an alternative to filtration methods in removing organic matter from wastewater. The mechanism of AOPs is the generation of highly reactive free radicals such as hydroxyl radical (OH•) which is effective in destroying organic chemicals. Photo-catalytic oxidation is one of the most effective clean technologies able to reduce/remove contaminants from wastewater and drinking water. [16] A most used as photo-catalyst is TiO_2. This semiconductor can be used in a wide pH range, being able to produce electronic transitions by light absorption in the near ultraviolet range. TiO_2 is also characterized by its low cost, its high photo-catalytic activity, and its resistance to photo-corrosion. [16]

Atorvastatin (ATO, structure **1**, Figure 2), [(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.[17] Recently it has been observed that ATO, like other statins, can be efficient against Alzheimer's disease.[17] Less than 5% of a dose of ATO is recovered in urine following oral administration. The presence of ATO in sewage effluents and surface waters has been observed in concentration levels of $\mu g\ L^{-1}$. [18]

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.[19]-[20] However, the lactones were unstable as they hydrolyzed readily to their original acid forms. In contrast, RST was found not to be extensively metabolized, as 77% of it was excreted unchanged. [21] Approximately 90% of the non-metabolized RST was recovered in feces, 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. [21]

Atorvastatin was found to undergo a self-sensitized photo oxygenation by sunlight in water. [22] 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 singlet oxygen addition and an epoxide intermediate was suggested. [22]

Rosuvastatin, bis [(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl (methylsulfonyl) amino] pyrimidin-5-yl] (3R, 5S)-3,5-dihydroxyhept- 6- enoic acid] (RST, structure **2**, Figure 2, Appendix A), decreases the production of LDL cholesterol by blocking the action of the enzyme HMG-CoA reductase in the liver. This decreases the amount of cholesterol in the liver cells, which causes them to take up LDL cholesterol from the blood. The decreased cholesterol production and increased removal of LDL cholesterol from the blood ultimately results in lowered blood cholesterol levels.[23]-[24] In the pharmacokinetic study of RST, RSTL (structure **4**, Figure 1, Appendix A) was identified as one of the minor metabolites.[21]

Simvastatin, 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 2, Appendix A), is derived synthetically from a fermentation product of *Aspergillu sterreus*.[25] After oral ingestion, Simvastatin, which is an inactive lactone, is hydrolyzed to the corresponding β-hydroxyacid form. The latter is a potent inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, an essential enzyme involved in the in vivo synthesis of cholesterol. [26]- [27]

The goals of this study were (i) to explore the efficiency of the integrated advanced wastewater treatment technology installed at Al-Quds WWTP towards the removal of Atorvastatin, Rosuvastatin and Simvastatin and their metabolites from spiked wastewater samples; (ii) to show the behavior of Atorvastatin upon exposure to TiO_2 photodegradation (iii) to evaluate the degradation kinetics of the above mentioned compounds in water and sludge, and identify the biodegradation products; (iv) to determine the ability of micelle-clay composite and activated carbon towards removal of these drugs by adsorption from aqueous solutions. Furthermore, adsorption kinetics and adsorption isotherms will be evaluated and fitted to Langmuir equations. The micelle-clay composite which was used in this study is positively charged, has 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. [28]

2.Experimental

2.1 Materials and Equipments
2.1.1 Materials.

All 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, USA). Quartz sand (grain size 0.8—1.2 mm) was obtained from Negev industrial minerals (Israel). Octadecyltrimethylammonium (ODTMA) bromide was obtained from Sigma Aldrich. Pure ATO, RST and SIM were obtained from Birzeit Pharmaceutical Company (Palestine) with 99% purity, and all were used as received. Fine powder activated charcoal (FAC) with particle size ≤ 60.0 μm, and granular activated charcoal (GAC) with particle size ≤ 700.0 μm were obtained from Sigma (Sigma Chemical Company, USA). The powder was used for batch adsorption experiments while the granules were used in column experiments. Magnesium sulfate anhydrous, potassium dihydrogen phosphate as well as methanol, acetonitrile and deionized 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 1g C-18 6 mL disposable cartridges (Waters, Milford, MA, USA) were used.

TiO$_2$ P-25 (anatase/rutile = 3.6/1), surface area 10 m^2/g, non-porous) was performed from Degussa.

Activated sludge used in the stability test was obtained from the Advanced Wastewater Treatment Plant, located at Al-Quds University-Palestine and was described in detail elsewhere. [28]

2.1.2 Equipments.

Samples were shaken using Big Bill, (Banstaed/ Themolyne, USA). The disappearance of ATO, RST and SIM were determined by using a high pressure liquid chromatography system model 2695 HPLC from Waters (Israel), equipped with a Waters 2996 Photodiode array. Data acquisition and control were carried out using Empower ™ software (Waters, Israel). Analytes were separated on a 4.6 mm x150 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$_3$PO$_4$: acetonitrile (1:1; v/v) as 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 0.45μm porosity) from Waters were always used for all analytical filtration requirements. [29]

The identification of ATO, RST and SIM degradation products were 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 an 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, 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 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 (FWHM) 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 Sigma Plot 10.0 (Systat Software, Inc., London, UK).

The advanced wastewater treatment plant employed in this study is located at Al-Quds University-Palestine and was described in detail elsewhere. [28] Normally, the effluent from this plant is recycled for the irrigation of plants cropped in the field of university Campus.

2.2 Methods
2.2.1 Characterization of wastewater used.

The wastewater was characterized before the experiments according to the American Public Health Association procedures [30]-[31] by performing measurements listed in Table 1, appendix B.

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 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 1, Appendix A. 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 SPE cartridge was injected into the HPLC, and analyzed 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 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 analyzed by HPLC. The degradation by-products of ATO, RST and SIM were quantified using liquid chromatography/Fourier-transform ion cyclotron resonance/mass spectrometry (LC/FT-ICR MS).

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 octadecyltrimethylammonium (as bromide salt) at multiples of 10 g L^{-1} (clay) and 12 mM (ODTMA, **5** in Figure 2, Appendix A) as described elsewhere. [28] The obtained complex, which has net positive charge and includes hydrophobic regions, is capable of efficiently binding neutral and negatively charged organic molecules .[30]-[34]

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 fine powder activated charcoal (FAC). 100 mL of each drug solutions having known initial concentration were then introduced into each flask. The flasks were shaken in an oscillating shaker for three hours 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 minutes, 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 granular activated charcoal (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 hours before its use. Solutions in pure water (1 L each) containing different ATO, RST and SIM concentrations (0.01, 1, 10, and 100 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 mL min^{-1}. Eluted fractions were collected in all column experiments and analyzed.

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

2.2.7 Photochemical and Photocatalytic experiments.

Photochemical and photocatalytic experiments were conducted by using Suntest CPS Solar Simulator (Heraeus Instruments, Germany) equipped with a xenon lamp, with a temperature sensor and a water-cooling circuit. The xenon lamp was filtered by an optically stable borosilicate UV filter (Atlas Material Testing, France) delivering a light emission spectrum similar to that of the sun.

A stock solution (0.1mM) of Atorvastatin pure standard in water was prepared and kept in the darkness at + 4°C. Calibration and working solutions of Atorvastatin were prepared when used by dilution from stock solution.

Kinetic behavior of Atorvastatin was performed by using HPLC Waters 2695 equipped with a photodiode array. Data acquisition and control were carried out using Empower ™ software (Waters: Israel). Analytes were separated by using a C18XBridge® column (4.6 mm x150 mm, 5 μm particle sizes). pH meter HM-30G (TOA electronics™) was used to control the samples pH value.

3. Results and discussion
3.1 Calibration curves
Linearity of the proposed analytical method was verified by analyzing standard solutions in the range of 0.1 - 100 mg L^{-1} for ATO, RST and SIM in pure water. The calibration curves were obtained using HPLC method with 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 analytes. The reproducibility of morning/evening injections on the basis of 6-hours elapsed time ranged from 97.5% and 98.0%, and was also affected by the concentration and type of analytes. 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 of 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 mg L^{-1} for ATO and RST, and 0.02 mg L^{-1} for SIM. The limit of quantifications, based on a signal/noise of 10, was 0.08 mg L^{-1}, 0.08 mg L^{-1} and 0.06 mg L^{-1} for ATO, RST and SIM, respectively.

3.2 Wastewater characteristics
Table 2, Appendix B, summarizes the chemical, physical and biological characteristics of wastewater sampled from the activated sludge reservoir of Al-Quds WWTP. Table 2 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 1; Table 3) was separately spiked with ATO, RST or SIM at concentration of 1.0 mg L^{-1}, which is an amount close to environmental values reported in the literature.[35]-[36] Samples were taken from different collecting sites of WWTP as described in Figure 1. Analytical results of water effluent from the hollow fiber ultra-filtration membrane (UF-HF) 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 3, Appendix B). Besides, RST was completely removed in the effluent from 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 [36]-[37], kinetics studies on degradation of Atorvastatin, Rosuvastatin and Simvastatin in pure water and activated sludge conditions have been undertaken. Table 4, Appendix B, 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 Atorvastatin (**A**) (Figure 3, Appendix A) and Simvastatin (**B**) (Figure 3, Appendix A).
The results in Table 4, Appendix B, 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 to ATO and RST stems from the fact that SIM exists as a lactone moiety which is readily hydrolyzed 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 to 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.[38] 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.[38] 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.[38] Similar results were obtained by Robert et al. (2003)[39], who showed that CYP3A4 was the main enzyme involved in ATO metabolism resulting in the major metabolite ortho-hydroxy ATO and in active metabolites ATO lactone and ortho-hydroxy ATO lactone (Figure 4, Appendix A). These results are in agreement with a study by Liu et al. (2008)[40] who showed that ATO administered in its calcium salt active form was converted into active ATO acid, which is then metabolized into two other active metabolites, para-hydroxy ATO (p-ATO), and ortho-hydroxy ATO (o-ATO). These three active compounds are subsequently equilibrated with their corresponding lactone forms at the ratio of approximately 1:1[40]. Jani et al. (2010) [41] found that ATO in plasma can be metabolized by CYP3A4 to two hydroxylated metabolites, o- hydroxyl atorvastatin and p-hydroxyatorvastatin (Figure 4, Appendix A).

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.[42] 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.[43] 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.[42] Monitoring the derivative substances arising from the degradation of ATO, RST and 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. Extracted ion chromatograms (XICs) of the 35 days biodegraded sample are shown in Figure 5, Appendix A. The benefit of using very selective extracted ion chromatograms 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]- ±1.0 mDa), greatly reduced the signal complexity of the total ion current trace (Fig. 5(b)) allowing to completely characterize all degradation products.

Five major biodegradation products for Atorvastatin (Fig.5(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**, Fig.5(a)D), 573.24064 (**7**, Fig.5(a)C), 547.18860 (**8**, Fig.5(a)E), 545.17295 (**9**, Fig.5(a)F), 513.21951(**10**, Fig.5(a)H). Based on the accurate m/z ratios of deprotonated molecules, their retention times, and relevant literature [41], we propose for Atorvastatin biodegradation the structures reported in Figure 6, Appendix A. The major metabolites resulted the compound (**7**), the para-hydroxy Atorvastatin, $C_{33}H_{35}FN_2O_6$, with exact [M-H]- *m/z* 573.24064 (error 0.6 ppm). Ortho, para-dihydroxyatorvastatin 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 - CH_3COOH or isopropyl group from other biodegraded, oxidized products.

Rosuvastatin (Fig.5 (b) **B**, exact [M-H]- *m/z* 480.16101) has one of major biodegradation products at retention time of 5.2(**5**) minutes (Fig.5 (b) **E**). Based on the accurate *m/z* ratios 462.15098 and relevant literature [43] we suggest the structure of the metabolite as shown in Figure 2 (structure **4**). Other compounds derived from hydroxylation or polihydroxylation (Fig 5 (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 formula $C_{22}H_{28}FN_3O_7S$ and $C_{22}H_{30}FN_3O_8S$, respectively, which were also found in the biodegraded solution of Rosuvastatin. For Simvastatin (Fig.5 (c), exact [M-H]- *m/z* 573.24064) one major biodegradation product was formed at 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 [38], we propose the structure of SIM metabolite as shown in Figure 6 (**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 Langmuir adsorption isotherm [44], represented by equation (1):

$$C_e/Q_e = 1/(kQ_{max}) + C_e/Q_{max} \qquad (1)$$

where Ce (mg L^{-1}) is the equilibrium concentration of the drug in the solution, Qe (mg g^{-1}) is the equilibrium mass of adsorbed drug per gram of complex or activated charcoal, K (L mg^{-1}) is the Langmuir binding constant, and Qmax (mg g^{-1}) is the maximum mass of drug removed per gram of 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 5, Appendix B). The values of K and Q$_{max}$ parameters for the adsorption isotherm obtained using micelle-clay complex were larger than those correspond 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 6, Appendix B) indicate a significant advantage of the micelle-clay filter in removing ATO, RST and SIM compared to 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 of anionic and certain neutral pollutants [32]-[34].

Polubesova et al. [32] found very efficient removal of three anionic pollutants (imazaquin, sulfentrazone, sulfosulfuron) and 4 neutral pollutants (alachlor, acetochlor, chlorotoluron and bromacil) by micelle –clay complexes in aqueous dispersions. In another study [33] column filters filled with a mixture of quartz sand and micelle–clay complex provided very efficient result for the retention of tetracycline and sulfonamide pharmaceuticals from wastewater.

Zadaka et al. [34] tested column filters with either a mixture of quartz sand and organic micelle – montmorillonite or zeolite; both filters were capable to well remove ethylene dibromide, anionic pollutants as sulfosulfuron, imazaquin and sulfentrazone, and neutral compounds as bromacil and chlorotoluron from aqueous environments; in contrast a filter filled with the same weight of activated carbon and sand removed only partially these pollutants.

In a recent paper, Karaman et al. [28] showed that micelle-clay filters are more efficient towards removal of diclofenac

potassium from wastewater than activated carbon. Moreover, Khamis et al. [27] concluded that the incorporation of micelle-clay filters in sewage treatment systems with loose tertiary capability can be a promising technology. More recently, Khalaf et al. [45] 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 (NSAIDs).

It can be argued that in addition to ATO, RST and SIM residues wastewater usually includes other recalcitrant organic pollutants [46]-[50]. In such cases GAC filters can be used as 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.

3.7 Photolysis and Photocatalysis processes of Atorvastatin

Table 7, Appendix B, shows kinetic parameters of the photochemical reactions, considering three replicates for each experiment. In all cases, photo-reactions of the first order resulted, and the half-life of Atorvastatin under light irradiation was always lower than Atorvastatin under photocatalysis .The pure standard solutions used as a control in the darkness did not show any significant degradation during the experiment.

The results of photolysis and photocatalysis of ATO in aqueous solution are shown in Figure 7, Appendix A. A complete removal of this species was achieved, at the adopted experimental conditions, with half life of 5 hours under photolysis and 1.15 hours of irradiation under photo catalysis conditions, with the formation of four major intermediates.

It is known, that TiO$_2$ is a strong oxidizing agent. The band gap illumination of a semiconductor particle suspended in water causes electronic transitions from the valence band to the conduction band, leaving holes in the former. These electrons and holes then either migrate to the particle surface and become involved in redox reactions or they recombine and simply liberate heat. Conduction- band electrons are consumed in reactions that reduce oxidants while holes are filled via oxidation reactions. Hydroxyl radicals are generated by the oxidation of water at the valence band of TiO$_2$. [51]-[53]

The OH• radicals formed on the illuminated semiconductor surface are very strong oxidizing agents with an oxidation potential of 2.8 V. These can easily attack the adsorbed organic molecules or those located close to the surface of the catalyst, thus, at the final stage, leading to their complete mineralization (Figure 8, Appendix A). All photoproducts derived from both experiments were identified either by comparison of the mass spectra and retention times with literature spectra and by accurate masses.

Behnaz et al.[2011] [54] found that under the experimental conditions used, at high atorvastatin concentration (35.8 mM) the contribution of singlet oxygen (^{i}O$_2$) to the photodegradation of atorvastatin in natural waters was higher than that of hydroxyl radical, and accounted for up to 23% of the loss in aqueous solutions. Whereas, at a concentration of

35.8 nM,$\cdot$O$_2$ (and »OH) both played a minor role in the removal of this compound.

Flavio et al. [2006] [55] found that Atorvastatin undergoes a self-sensitized photo oxygenation by sunlight in water. The main photoproducts, isolated by chromatographic techniques, have been identified by spectroscopic means. They present a lactam ring arising from an oxidation of pyrrole ring and an alkyl/aryl shift.

Figure 9, Appendix A, shows the probable intermediates metabolites structures for photo chemical of atorvastatin, where more study on those compounds is in progress. Compound **12** showed a molecular peak at m / z 575.2046 [M+H]$^+$ suggesting a molecular formula C$_{33}$H$_{34}$FN$_2$O$_6$. Compound **13** showed a molecular peak at m / z 587.2355, [M+H] $_+$ in the ESI-MS spectrum suggesting a molecular formula C$_{34}$H$_{35}$FN$_2$O$_6$. All the previous results agree with [54]. Cermola et al., [2006] [56] suggest that all atorvastatin photoproducts characterized arise from an oxidation of pyrrole ring and an alkyl/aryl shift with formation of a lactam ring or via lactonization and cyclization, respectively. [56]

4. Conclusions

The stability studies of Atorvastatin, Rosuvastatin and Simvastatin revealed that the three statins were unstable both in 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 catalyze the degradation processes for the mentioned three statins. It should be worth noting that the higher degradation rate found for Simvastatin compared to

that of Atorvastatin and Rosuvastatin can be attributed to the relatively unstable lactone ring present in Simvastatin.

The filtration study involving an advanced wastewater treatment plant utilizing ultrafiltration, activated carbon and reverse osmosis demonstrated that activated carbon and RO are efficient in removing the commonly used statins: Atorvastatin, Rosuvastatin and Simvastatin from wastewater. However, 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 mgL^{-1} or µgL^{-1} ranges. The large effectiveness and removal capacity are due to a relatively high affinity of adsorption of the anionic Atorvastatin, Rosuvastatin and Simvastatin 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.

In conclusion, atorvastatin has been found to be sensitive to sunlight. So it was noticed that photolysis process has the ability to eliminate this drug from water, while photo catalysis shows much more significant degradation.

Both photo-degradation processes have followed a 1st order kinetic while the t$_{1/2}$ ranging from 1.15 under photo-catalysis to 5.0 h under photolysis.

References

[1] T. Heberer, (2002) "Tracking persistent pharmaceutical residues from municipal sewage to drinking water," Journal of Hydrology, 266(3-4): pp. 175-189, 2002. doi: 10.1016/s0022-1694(02)00165-8

[2] M.J Hilton, K.V. Thomas, "Determination of selected human pharmaceutical compounds in effluent and surface water samples by high-performance liquid chromatography–electro spray tandem mass spectrometry", Journal of Chromatography A, 1015, pp. 129-141, 2003.

[3] B. Hailing-Sorensen, S.N. Nielsen, P.F. Lanzky, F. Ingerslev, H.C.H. Lutzhoft, and S.E. Jorgensen, "Occurrence, fate and effects of pharmaceutical substances in the environment - A Review," Chemosphere, 36, pp. 357-394, 1998.

[4] C. Daughton, T. Jones-Lepp, eds., Pharmaceuticals and personal care products in the environment: scientific and regulatory issues, American Chemical Society, Washington DC, 2001.

[5] O.A. Jones, J.N. Lester, and N. Voulvoulis, "Pharmaceuticals: a threat to drinking water," Trends Biotechnology, 23, pp.163-167, 2005.

[6] T.A. Ternes, "Occurrence of drugs in German sewage treatment plants and rivers," Water Research, 32(1998) 3245-3260.

[7] C.G. Daughton, T.A. Ternes, "Pharmaceuticals and personal care products in the environment: agents of subtle change," Environmental Health Perspectives, 107, pp. 907-938, 1999.

[8] T. Heberer, "Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment; a review," Toxicology Letter, 131, pp.5-17, 2002.

[9] T.A. Ternes, "Occurrence of drugs in German sewage treatment plants and rivers," Water Research, 32, pp. 3245-3260, 1998.

[10] O.A.H. Jones, N. Voulvoulis, J.N. Lester, "Human pharmaceuticals in wastewater treatment processes," Critical Reviews in Environmental Science and Technology, 35, pp. 401-427, 2005.

[11] R. Hirsch, T.A. Ternes, K. Haberer, K.L. Kratz, "Determination of beta blockers and b-sympathomimetics in the aquatic environment," VomWasser, 87, pp. 263-74, 1996.

[12] D. Bendz, N.A. Paxéus, T.R. Ginn, F.J. Loge, "Occurrence and fate of pharmaceutically active compounds in the environment, a case study," Journal of Hazardous Material,122, pp. 195-204, 2005.

[13] G. Ohlenbusch, M.U. Kumke, F.H. Frimmel, Sorption of phenols to dissolved organic matter investigated by solid phase micro extraction, Science of the Total Environment, 253, pp. 63-74, 2000.

[14] M. Khamis, R. Karaman, F. Ayyash, A. Qtait, O. Deeb and A. Manssra, "Efficiency of Advanced Membrane Wastewater Treatment Plant towards Removal of Aspirin, Salicylic Acid, Paracetamol and p-Aminophenol," Journal of Environmental Science and Engineering, 5, pp. 121-137, 2011.

[15] R. Andreozzi, V. Caprio, A. Insola and R. Marotta, "Advanced oxidation processes (AOP) for water purification and recovery", Catalysis Today, 53, pp. 51-59, 1999.

[16] R. Munter, S. Preis, J. Kallas, M. Trapido, and Y. Veressinina, "Advanced oxidation processes (AOPs): Water

treatment technology for the twenty-first century" Kemi, 28, pp. 354-362, 2001.

[17] A. Goodman-Hilman, T. Rall, A. Nier, P. Taylor, "The Pharmacological Basis of Therapeutics," McGraw-Hill, New York, 1996.

[18] A. Schepers, M.M. Pires Nuno, D. Eefting, M.R. de Vries, J.H. van Bockel, P.H.A. Quax, "Short-term dexamethasone treatment inhibits vein graft thickening in hypercholesterolemic ApoE3Leiden transgenic mice," Journal of Vascular Surgery, 43, pp. 809-815, 2006.

[19] D.W. Muller, G. Golomb, D. Gordon, R.J. Levy, "Site-specific dexamethasone delivery for the prevention of neointimal thickening after vascular stent placement," Coronary Artery Disease, 5, pp. 435-442, 1994.

[20] E.P. Strecker, A. Gabelmann, I. Boos, C. Lucas, Z. Xu , J. Haberstroh, N. Freudenberg, H. Stricker, M. Langer, E. Betz, "Effect on intimal hyperplasia of dexamethasone released from coated metal stents compared with non-coated stents in canine femoral arteries," Cardiovascular Intervent Radiology, 21, pp. 487-496, 1998.

[21] R. Hoffmann, R. Langenberg, P. Radke, A. Franke, R. Blindt, J. Ortlepp, J.J. Popma, C. Weber, P. Hanrath, "Evaluation of a high-dose dexamethasone-eluting stent," American Journal of Cardiology, 94, pp. 193-195, 2004.

[22] *British Pharmacopeia*, Revised edition, British Pharmacopoeia Commission, London, 2007.

[23] *Martindale*: The Extra Pharmacopoeia, 30th ed., Pharmaceuticals Press, London, 1993, p 728.

[24] *Martindale*: The Extra Pharmacopoeia, J. E. F. Reynolds ed., Royal Pharmaceutical Society, London, 1996, pp. 700-706.

[25] J.C. Cloyd, R.L. Lalonde, T.E. Beniak, and G.D. Novack, "A single-blind crossover comparison of the pharmacokinetics effects of a new diazepam rectal gel with intravenous Diazepam," Epilepsia, 39, pp. 520-526, 1998.

[26] E. Rey, J.M. Treluyer, and G. Pons, "Pharmacokinetic optimization of benzodiazepine therapy for acute seizures. Focus on delivery routes," Clinical Pharmacokinetics, 36, pp. 409-424, 1999.

[27] M. Khamis, R. Karaman, M. Qurie, J. Abbadi, S. Nusseibeh, A. Manassra, S. Nir, "Performance of micelle-clay filters for removing pollutants and bacteria from tertiary treated wastewater," Journal of Environmental Science and Engineering, 1, pp. 160-168, 2012.

[28] R. Karaman, M. Khamis, M. Quried, R. Halabieh, I. Makharzeh, A. Nasser, A. Manassra, J. Abbadi, F. Qtait, S. Bufo, S. Nir, "Removal of Diclofenac Potassium from Wastewater Using Clay-Micelle Complex," Environmental Technology, 33, pp. 1279-1287, 2012.

[29] British Pharmacopoeia Commission, British Pharmacopeia, Revised edition, British Pharmacopoeia Commission, London, 2007.

[30] American Public Health Association (APHA). Standard Methods for the Examination of Water and Wastewater, 19th ed. APHA, Washington, DC, 1995.

[31] American Public Health Association (APHA). Standard Methods for the Examination of Water and Wastewater, 21th ed. APHA, Washington, DC, 2005.

[32] T. Polubesova, S. Nir, D. Zadaka, O. Rabinovitz, C. Serban, L. Groisman, "Water purification of organic pollutants by optimized micelle-clay systems," Environmental Science and Technology, 39, pp. 2369-2384, 2005.

[33] T. Polubesova, D. Zadaka, L. Groisman, S. Nir, "Water remediation by micelle-clay system: Case study for tetracycline and sulfonamide antibiotics," Water Research, 40, pp. 2369-2374, 2006.

[34] D. Zadaka, Y.G. Mishael, T. Polubesova, C. Serban. S. Nir, "Modified silicates and porous glass as adsorbents for removal of organic pollutants from water and comparison activated carbons," Applied Clay Science, 36, pp. 174-181, 2007.

[35] B. Hailing-Sorensen, S.N., Nielsen, P.F. Lanzky, F. Ingerslev, H.C.H. Lutzhoft, and S.E. Jorgensen, "Occurrence, fate and effects of Pharmaceutical substances in the Environment - A Review", Chemosphere, 36, pp. 357-394, 1998.

[36] D. Bendz, N.A. Paxéus, T.R. Ginn, F.J. Loge, "Occurrence and fate of pharmaceutically active compounds in the environment, a case study," Journal of Hazardous Material, 122, pp. 195-204, 2005.

[37] O.A.H. Jones, N. Voulvoulis, and J.N. Lester, "Human pharmaceuticals in wastewater treatment processes," Critical Review of Environmental Science and Technology, 35, 401-427, 2005.

[38] T. Prueksaritanont, L.M.M.A. Gorham, L. Liu, X. YU, J.J. Zhao, D.E. Slaughter, B.H. Arison, K.P. Vyas, "In Vitro Metabolism of Simvastatin in Humans [SBT] Identification of Metabolizing Enzymes and Effect of The Drug on Hepatic P450S". Journal of Pharmacology and Experimental Therapeutics, 25(10): pp. 1191-1199, 1997.

[39] L. Robert, E. Katelijne, G. Matthys, A. Verpooten, C. Patrick, P. Max Dratwa, S. Jean-Claude, H.L. Norbert, "Pharmacokinetics of atorvastatin and its metabolites after single and multiple dosing in hyper cholesterol aemichaemo dialysis patients", Nephrology Dialysis Transplantion, 18(5): pp. 967-976, 2003.

[40] D.Y. Liu, J. Jiang, H. Zhou, P. Hu, "Quantitative Determination of Atorvastatin and Para-hydroxy Atorvastatin in Human Plasma by LC–MS–MS". Journal of Chromatographic Science, 46(10): pp. 862-866, 2008.

[41] A.J. Jani, S.H. Faeth, D. Gardner, "A sexual endophytes and associated alkaloids alter a bhropod community structure and increase herbivore abundances on a native grass" Ecology Letters, 13: pp. 106-117, 2010.

[42] E.S. Istvan, J. Deisenhofer, "Structural mechanism for statin inhibition of HMG-CoA reductase". Science, 292: pp. 1160-1164, 2001.

[43] A.G. Olsson, F. McTaggart, A. Raza, "Rosuvastatin: a highly effective new HMG-CoA reductase inhibitor". Cardiovascular Drug Review, 20(4): pp. 303-28, Winter 2002.

[44] M. Dakiky, M. Khamis, A. Manasra, M. Mereb, "Selective adsorption of chromium (VI) in industrial waste water using low cost abundantly available adsorbents", Advances in Environmental Research, 6: pp. 533-540, 2002.

[45] S. Khalaf, F. Al-Rimawi, M. Khamis, D. Zimmerman, U. Shuali, S. Nir, L. Scrano, S.A. Bufo, R. Karaman, "Efficiency of advanced wastewater treatment plant system and laboratory-scale micelle-clay filtration for the removal of ibuprofen residues," Journal of Environmental Science and Health, Part B, 48, pp. 814-821, 2013.

[46] R. Karaman "The Effective Molarity (EM) – a Computational Approach," Bioorganic Chemistry, 38, pp. 165-172, 2010.

[47]. O. Almarsson, R. Karaman, T.C. Bruice, "The Kinetic Importance of Conformations of Nicotinamide Adenine Dinucleotide in the Reactions of Dehydrogenase Enzymes," J. Am. Chem. Soc., 114, pp. 8702-8704, 1992.

[48] R. Karaman, "Prodrugs Design Based On Inter- And Intramolecular Processes," in "Prodrugs Design – A New Era", R. Karaman (editor), Nova Publisher, USA, pp. 1-76, 2014.

[49] A. Abu-Jaish, G. Mecca, S. Jumaa, A. Thawabteh, R. Karaman, *"*Mefenamic acid Prodrugs and Codrugs- Two Decades of Development" World Journal of Pharmaceutical Research*,* 4(6), pp. 2408-2429, 2015.

[50] Y. Khawaja, R. Karaman, *"*A Novel Mathematical Equation For Calculating The Number of ATP Molecules Generated From Sugars In Cells,*"* World Journal of Pharmaceutical Research*,* 4(4), pp. 303-312, 2015.

[51] M.A. Fox, M, "Organic heterogeneous photo catalysis - Chemical Conversions sensitized by irradiated semiconductors", Account of Chemical Research, 16, pp. 314, 1983.

[52] R.W. Matthews, "Photo-oxidation of organic material in aqueous suspensions of titanium dioxide", Water Research, 20, 569, 1986.

[53] C. Minero, E. Pelizzetti, S. Malato, and J. Blanco, "Large solar plant photo catalytic water decontamination - degradation of pentachlorophenol", Chemosphere, 26, pp. 2103-2119, 1993.

[54] R. Behnaz, B.A. Sihem, S. Weihua, E.O. Kevin, and J.C. William, "Photochemical fate of atorvastatin (Lipitor) in simulated natural waters", Water research, 45, pp. 625-631, 2011.

[55] C. Flavio, D. Marina, R.I. Maria, M. Sara, P. Lucio and T. Fabio, "Photochemical behavior of the drug atorvastatin in water", Tetrahedron, 62, pp. 7390-7395, 2006.

[56] F. Cermola, M. DellaGreca, M. Rosaria Iesce, S. Montanaro, L. Previtera, and F. Temussi, "Photochemical behavior of the drug atorvastatin in water", Tetrahedron, 62, pp. 7390-7395, 2006.
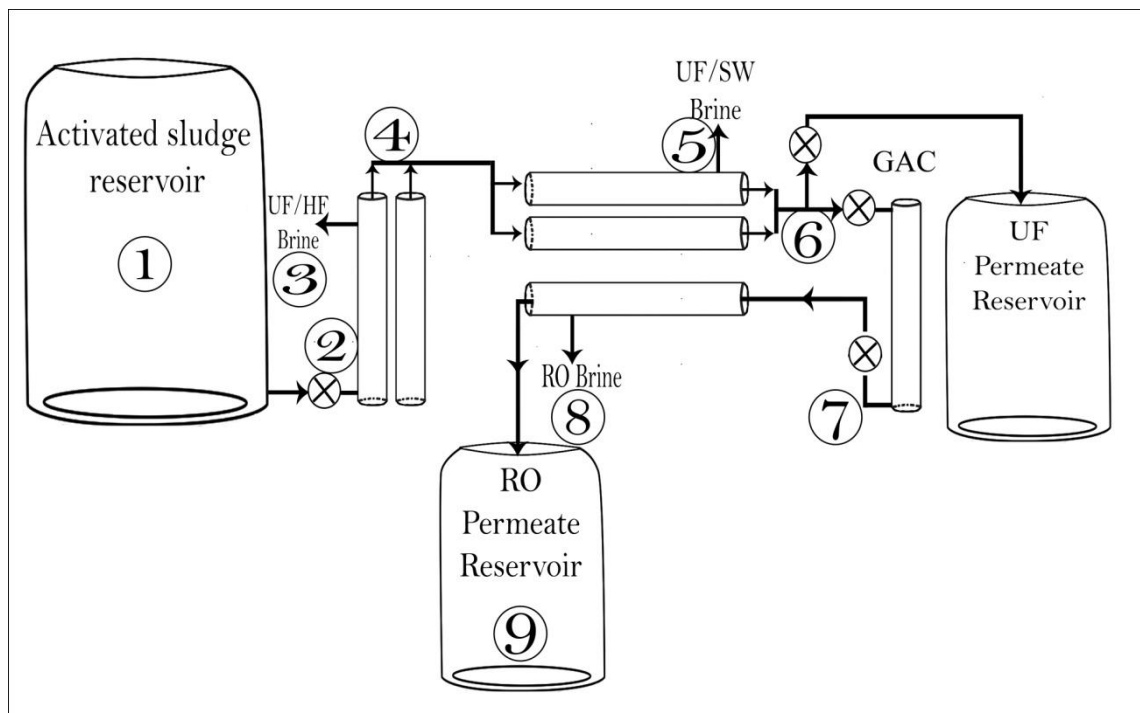
Appendix A

Figure 1: Flow diagram schematizing the WWTP at Al-Quds University. Sampling sites are indicated by numbers. UF/HF, hollow fiber ultrafiltration membrane; UF/SW, spiral wound ultrafiltration membrane; RO, reverse osmosis; GAC, granular activated charcoal filter.
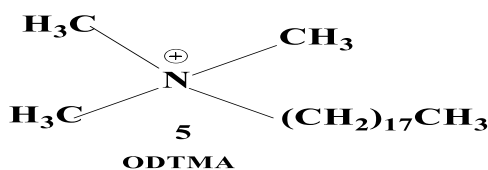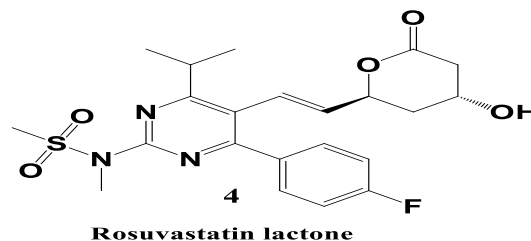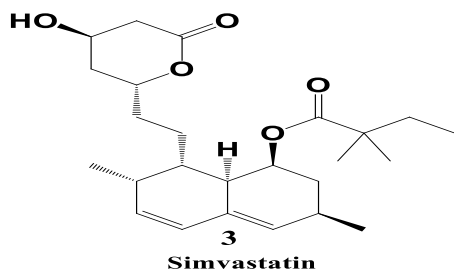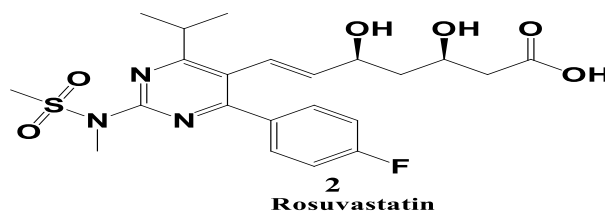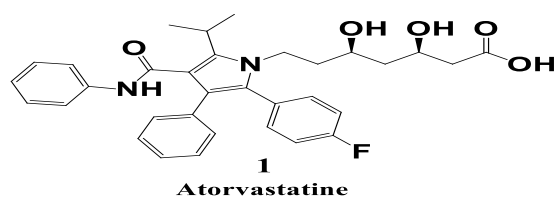
Figure 2: Chemical structures of Atorvastatin (**1**), Rosuvastatin (**2**), Simvastatin (**3**) and Rosuvastatin lactone (RSTL) (**4**), Octadecyltrimethylammonium ion (**5**).
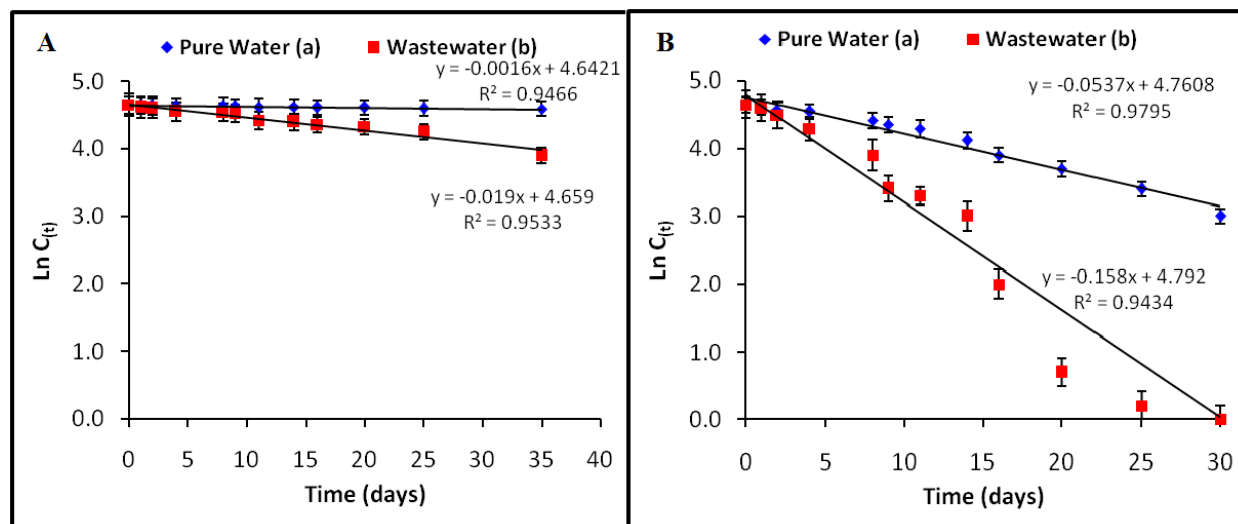

Figure 3: Kinetics of ATO (**A**) and SIM (**B**) degradation in pure water (plot a) (♦) and activated sludge (plot b) (■). Data are reported as natural logarithm of concentrations ($C_{(t)}$) *vs.* time. Initial concentration ($C_{(0)}$) = 100 mg L^{-1}. Plotted values are the means of three replicates; bars represent the standard deviations calculated for each average value, T = 25 °C.
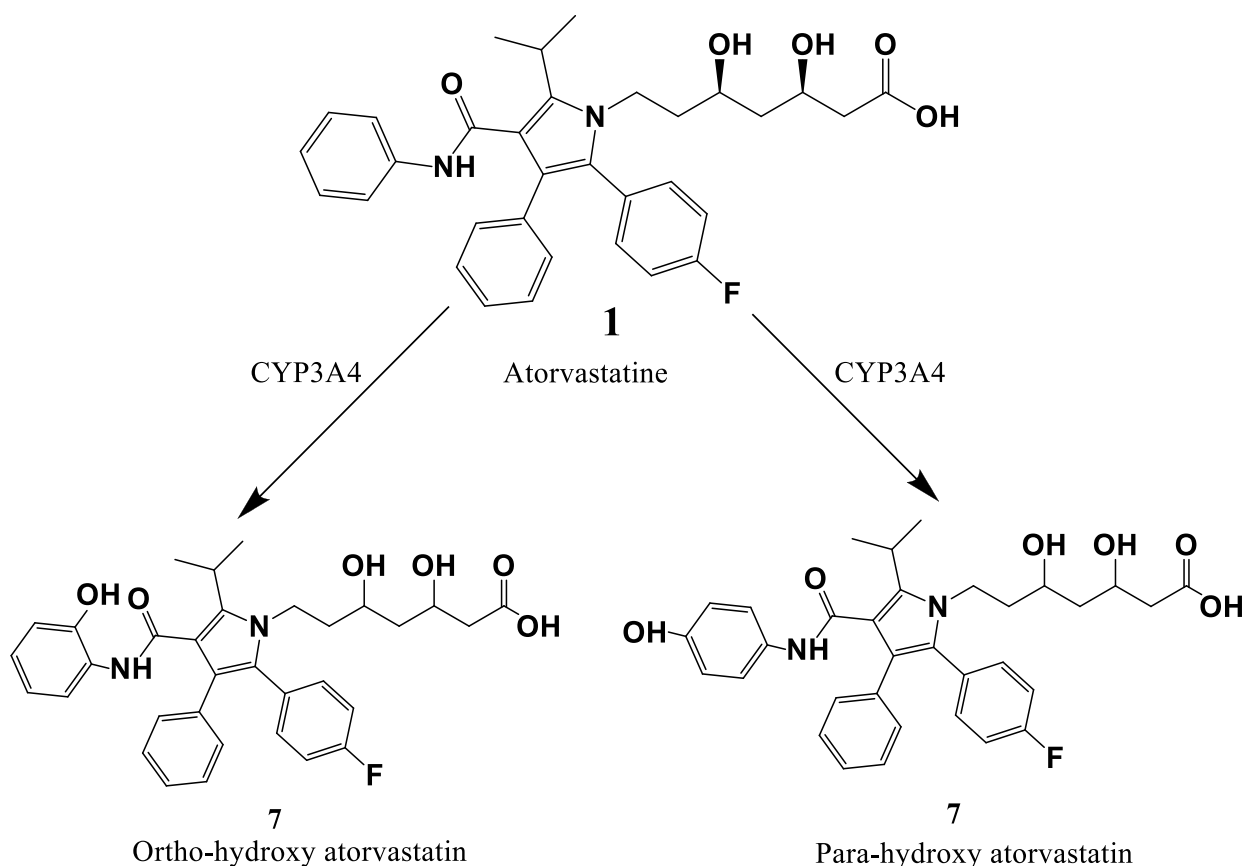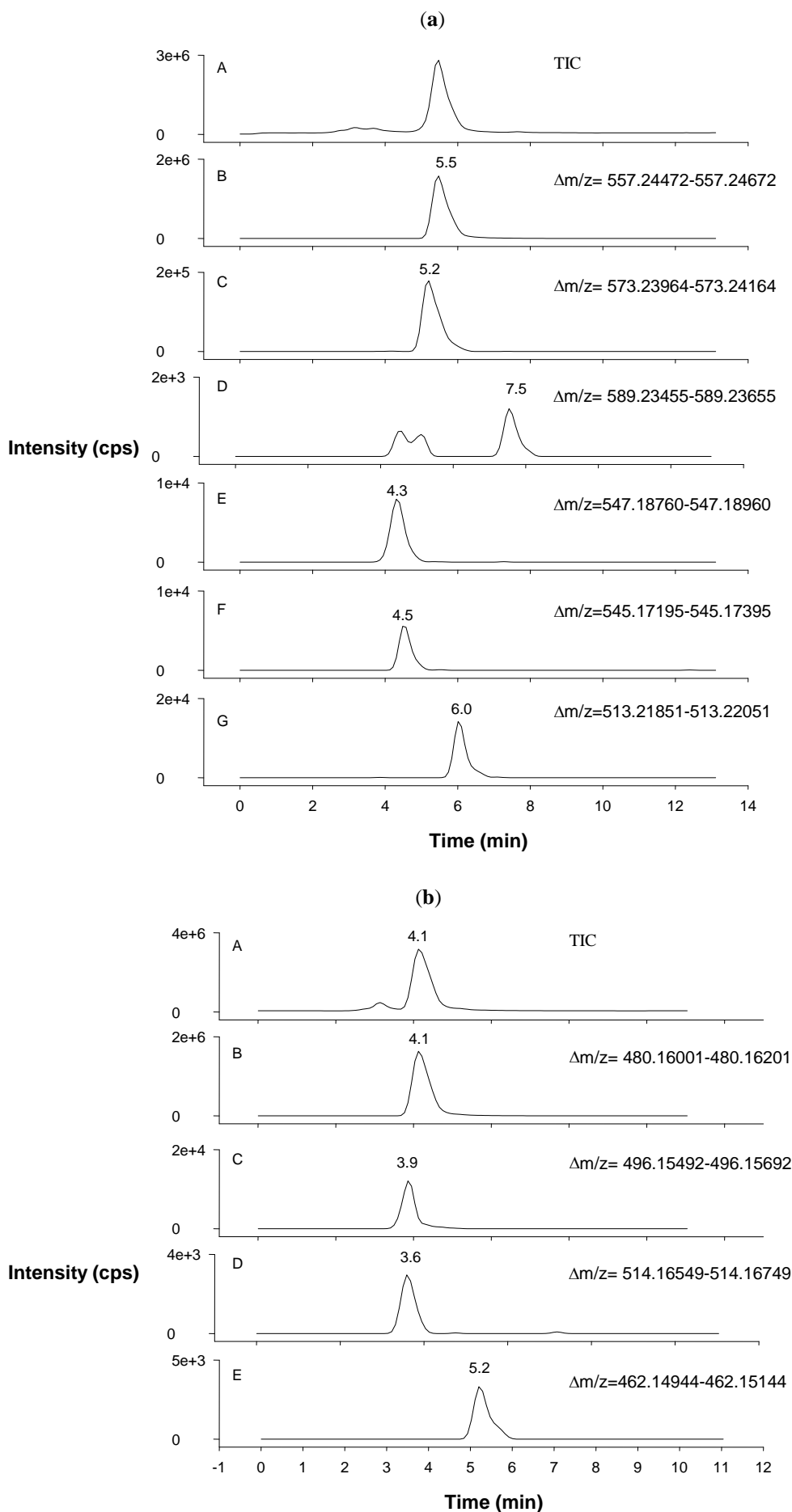
Figure 4: Atorvastatin metabolism to ortho or para hydroxy atorvastatin by CYP3A4.
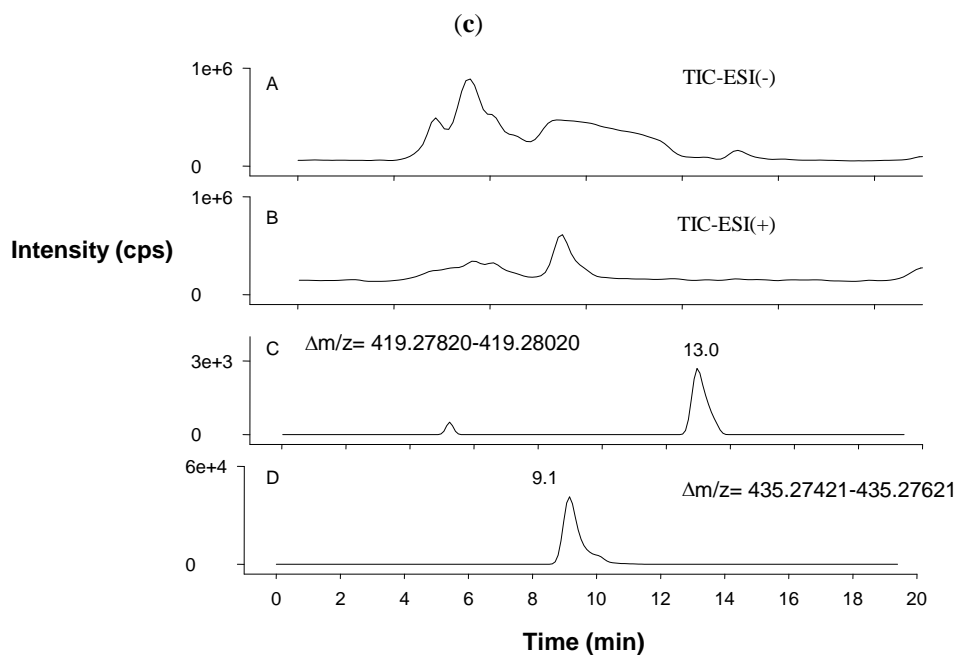
Figure 5: Extracted ion chromatograms (XICs) by LC/ESI-FTICR MS acquired in negative ion mode of ATO solution (**a**), RST solution (**b**) and in negative and positive ion mode of SIM (**c**) after one month of biodegradation. The ions monitored are displayed in each trace and correspond to the most abundant protonated or deprotonated molecules, [M+H]$^+$ or [M-H]$^-$, using a restricted window of ±0.0010 m/z unit centered on each selected ion.
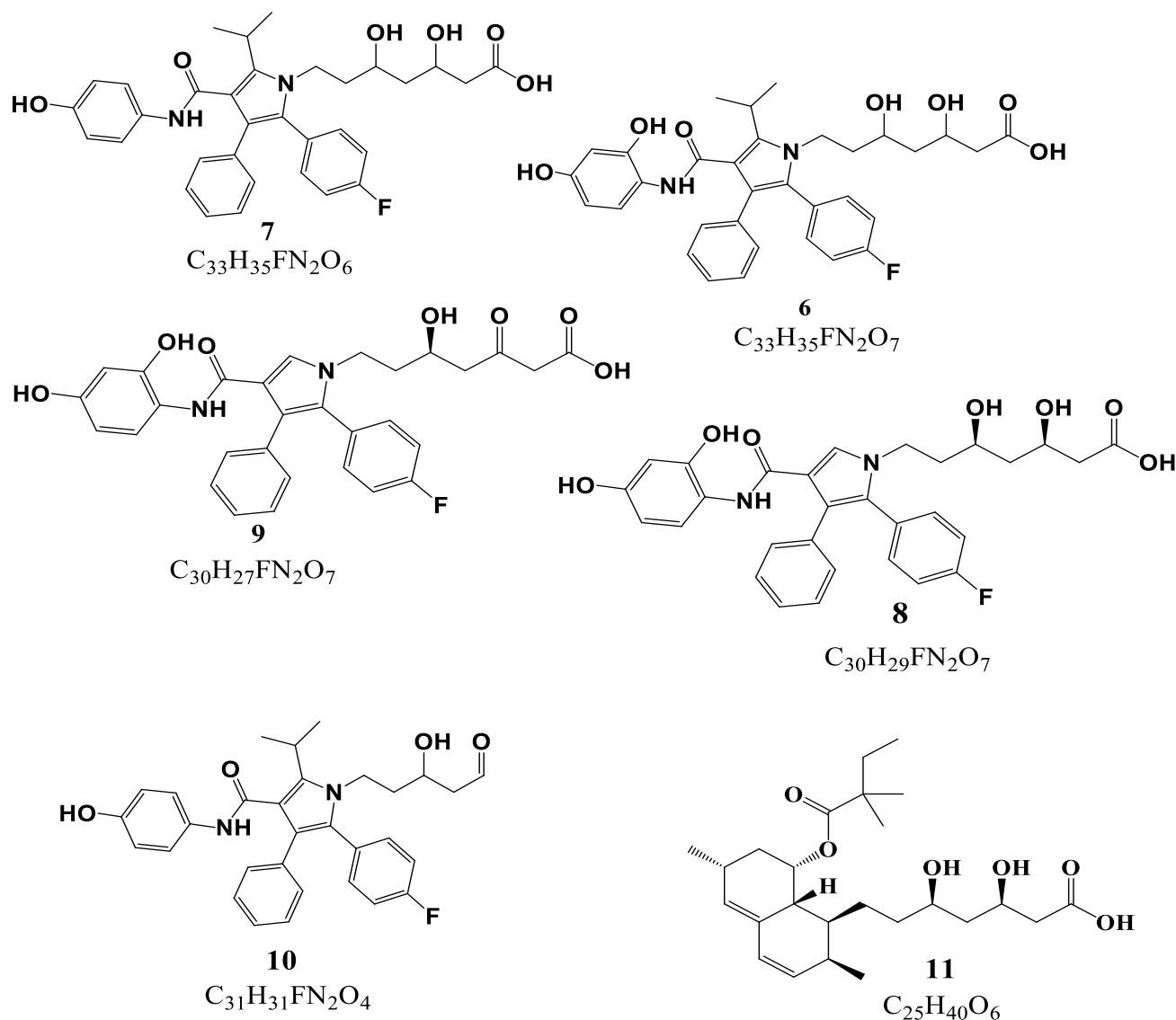
Figure 6: Chemical structures of Atorvastatin biodegradation products. Ortho, para-dihydroxyatorvastatin, (7-[2-(4-Fluoro-phenyl)-4-(4-hydroxy-phenylcarbamoyl)-5-isopropyl-3-phenyl-pyrrol-1-yl]-3,5-dihydroxy-heptanoic acid), $C_{33}H_{35}FN_2O_7$ with exact *m/z* 589.23555 (**6**); para-hydroxy Atorvastatin, (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-phenylcarbamoyl)-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 Simvastatin acid $C_{25}H_{40}O_6$ with exact *m/z* 435.27560 (**11**).

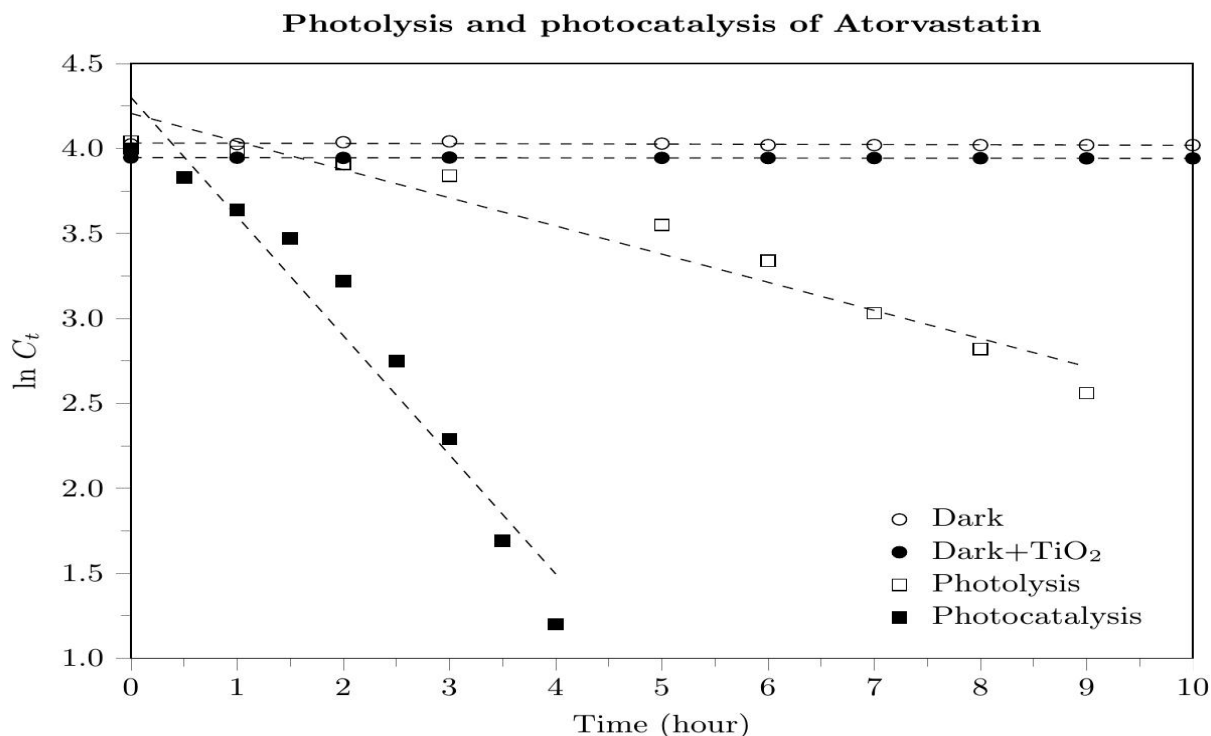**Figure 7**: Time course mineralization of Atorvastatin at 0.1mM initial concentrations in batch culture (Photolysis and Photocatalysis) solar (lamps Heraeus TNN 15/32). Experimental conditions: atorvastatin concentration = 0.1mM; [TiO_2] = 200mg /L; Solar intensity (sunny day) = 400 Wm^2.
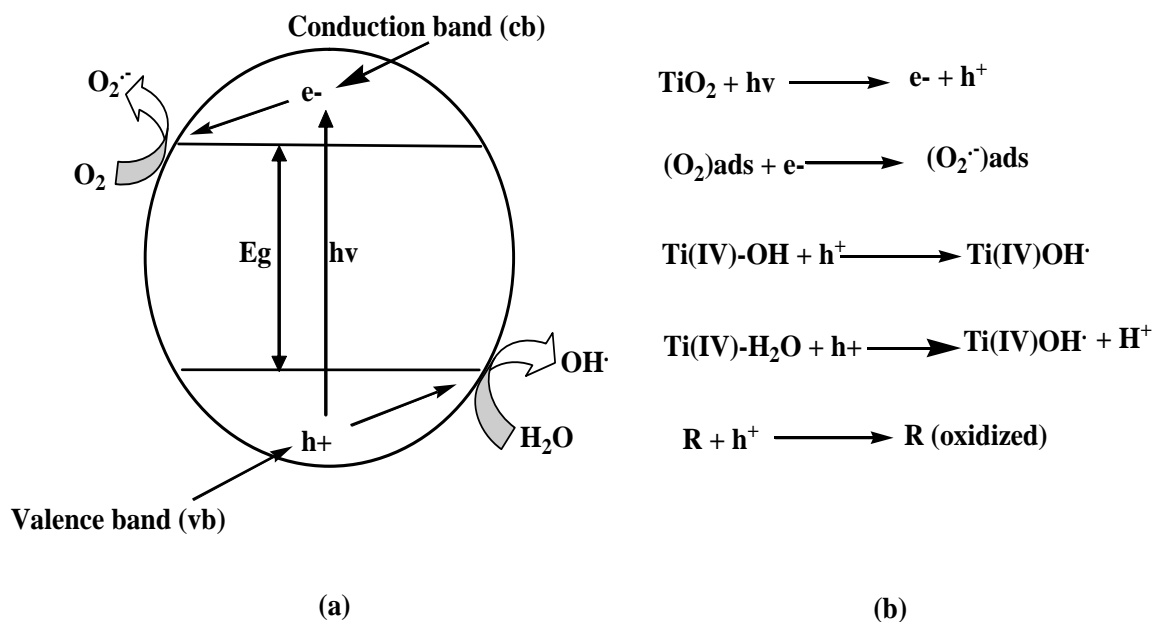

Figure 8: (a) The TiO_2 solution interface under UV- illumination. (b) Reactions on the TiO_2 surface under UV-illumination.
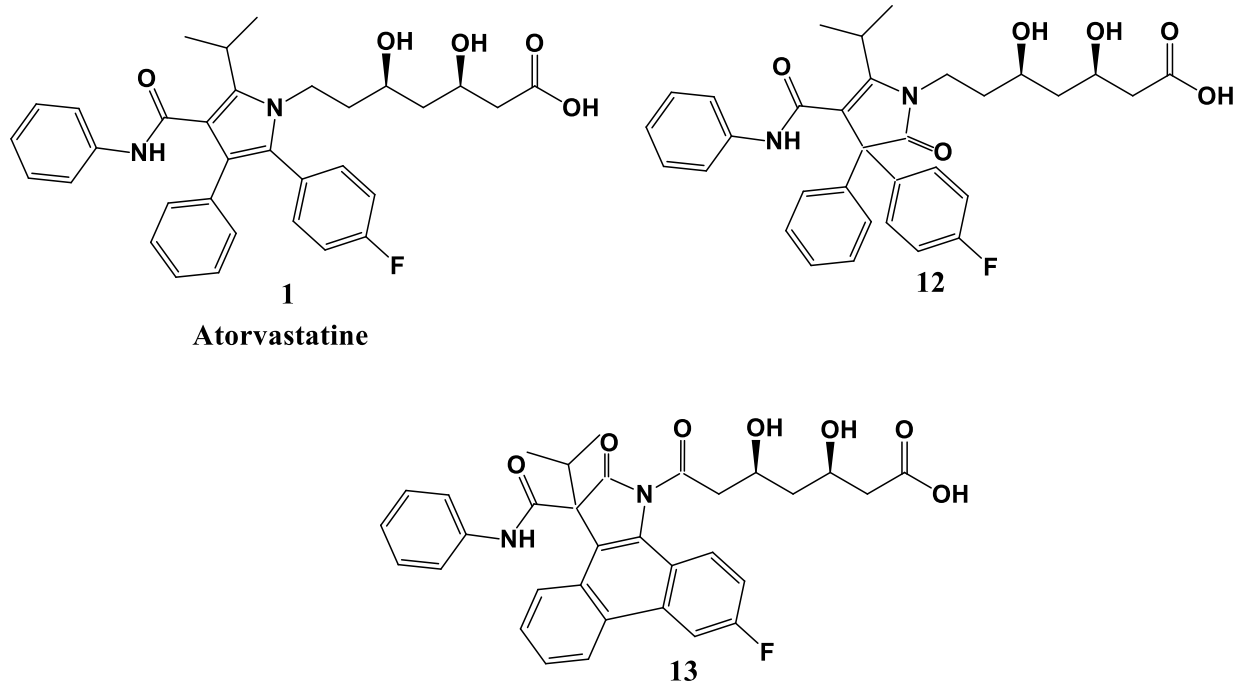
Figure 9: Structure of atorvastatin **(1)** and its photoproducts **(12-13)**

Appendix B

Table 1: Methods used and wastewater quality parameters measured in the Al-Quds WWTP.

Parameter measured	Instrument used for analysis	Method of analysis	Reference
pH	pH-meter 3320, Jenway	SM#4500-H+(B) (on site)	Direct measurement as manufacturer procedure
Conductivity	Conductivity meter 4320, Jenway	2520-B	APHA 1995, 19th ed. [30]
Total Coliforms and Fecal Coliforms	Membrane filter method	9222-B 9221-E	APHA 1995, 19th ed.
Orthophosphate	Automated ascorbic acid reduction	SM# 4500-PF	APHA 1995, 19th ed.
COD BOD$_5$	Hach COD reactor DO meter – Oxy 197	5210-B 5220-D	APHA 1995, 19th ed.
NH$_4^+$	Nesselarization method	4500A-NH$_3$	APHA 1995, 19th ed.
Total bacterial count	Pour plate method after serial dilutions	9215D	APHA 2005, 21th ed. [31]
Solids	Gravimetric methods	2540B 2540C 2540D	APHA 2005, 21th ed.

Table 2: Physical, chemical and biological parameters of wastewater to be treated.

Parameters	Results	Units	Parameters	Results	Units
pH	7.32 ±0.01	------	TSS	3668 ±6	mg L^{-1}
Conductivity	2000 ±8	µSm cm^{-1}	BOD	915 ±5	mg L^{-1}
Temperature	15.5 ±0.2	°C	COD	1936 ±6	mg L^{-1}
Turbidity	4960 ±7	NTU	NH$_4$-N	59.5 ±0.1	mg L^{-1}
DO	0.40 ±0.01	mg L^{-1}	PO$_4$-P	14.3 ±0.1	mg L^{-1}
TS	4218 ±8	mg L^{-1}	FC (*E. coli*)	$2.9 \times 10^5 \pm 0.3 \times 10^5$	cfu/100mL
TDS	618 ± 4	mg L^{-1}	TC	$6.5 \times 10^6 \pm 1.3 \times 10^6$	cfu/100mL
Settable solids	240 ± 3	mg L^{-1}	TAC	$2.6 \times 10^7 \pm 1.3 \times 10^7$	cfu/100mL

[a]DO, dissolved oxygen; TS, total solid; TDS, total dissolved solids; TSS, total suspended solids; BOD, biological oxygen demand; COD, chemical oxygen demand; FC, fecal coliforms; TC, total coliforms; TAC, total aerobic count.

Table 3: 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

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

Table 4: Degradation rates and half-lives of ATO, RST and SIM in sludge and pure water and determination coefficients (R²).

Drug	Medium	k (s^{-1})	Half-life (d)	R²
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

Table 5: Langmuir adsorption parameters (K and Q_{max}) and determination coefficients (R²) 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})	R²
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

Table 6: Removal of ATO, RST and SIM by filtration of 1L 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.

[a]Flow rate, 2 mL min^{-1}; temperature, 25 [0]C; pH, 7.2

b.l.d., below the detection limit of the analytical method used.

Table 7: Kinetic parameters of atorvastatin degradation: n, reaction order; $t_{1/2}$, half-life; k, kinetic constant; R^2, determination coefficient. Values were obtained on the basis of three replicate experiments.

Oxidation Process	n	$t_{1/2}$ (h)	$k(h^{-1})$	R^2
UV	1	1.15	0.165	0.94516
TiO$_2$/UV	1	5.02	0.704	0.94599