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Stability and removal of spironolactone from wastewater

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Stability and removal of spironolactone (SP) from wastewater produced at Al-Quds University Campus were investigated. Kinetic studies on both pure water and wastewater coming from secondary treatment (activated sludge) demonstrated that the potassium-sparing diuretic (water pill), spironolactone, underwent degradation to its hydrolytic derivative, canrenone, in both media. The first-order hydrolysis rate of SP in activated sludge at 25°C (3.80×10^{-5} s^{-1}) was about 49-fold larger than in pure water (7.4×10^{-7} s^{-1}). The overall performance of the wastewater treatment plant (WWTP) installed in the University Campus was assessed showing that more than 90% of spiked SP was removed together with its newly identified metabolites. In order to look for a technology to supplement or replace ultra-filtration membranes, the effectiveness of adsorption and filtration by micelle-clay filters for removing SP was tested in comparison with activated charcoal. Batch adsorption in aqueous suspensions was well described by Langmuir isotherms, showing a better removal by the micelle-clay material. Filtration of SP water solutions by columns filled with a mixture of sand and a micelle-clay complex showed complete removal of the drug at concentrations higher than in sand/activated-charcoal filled filters.

Keywords: Activated carbon, micelle–clay complex, Spironolactone, stability in sludge, wastewater treatment.

Introduction

Recently, a significant number of studies on the environmental occurrence and fate of pharmaceuticals used in developed countries has been published.[1] The escalating population growth and intensified agricultural and industrial activity have raised concerns not only in water- scarce regions but also in developed countries.[2] The reuse of treated water appears as an adequate solution for the future sustainable water cycle management.[3] One of the key issues in wastewater recycling is the emerging problem of micropollutants such as pharmaceuticals.[4] Pharmaceutically active compounds (PhACs) represent an overgrowing portion of trace organic contaminants in the urban aquatic environment that after human consumption reach wastewater treatment plants (WWTPs) in metabolized and/or un-metabolized form. WWTPs are frequently identified as main points of discharge of PhACs.[1–5]

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Most pharmaceutical compounds are, by nature, biologically active and hydrophilic. These two properties allow the human body to take them up easily. Furthermore, these compounds should be persistent so that early degradation before the curing effect could be avoided.When they enter a wastewater treatment plant, pharmaceuticals are not usually completely mineralized. They are either partially retained in the sludge, or metabolized to a more hydrophilic but still persistent form and, therefore, pass through the wastewater treatment plant (WWTP) and end up in the receiving waters.[4]

Among these pharmaceuticals Spironolactone (SP), a synthetic, yellowish, crystalline solid, is considered as one of the most-used drugs.[6] SP (**1**),(7α-acetylthio-3-oxo-17α-pregn-4-ene-21,17-carbolactone)[6] (Fig. 1), is a competitive aldosterone antagonist, which belongs to the steroid class of drugs. SP is practically insoluble in water, soluble in alcohol, and freely soluble in benzene and chloroform. SP is a potassium-sparing diuretic (water pill) that prevents the body from absorbing too much salt and keeps potassium levels from getting too low. It has been widely used to treat inflammation, allergy and diseases related to adrenal cortex insufficiency. Spironolactone is also known to

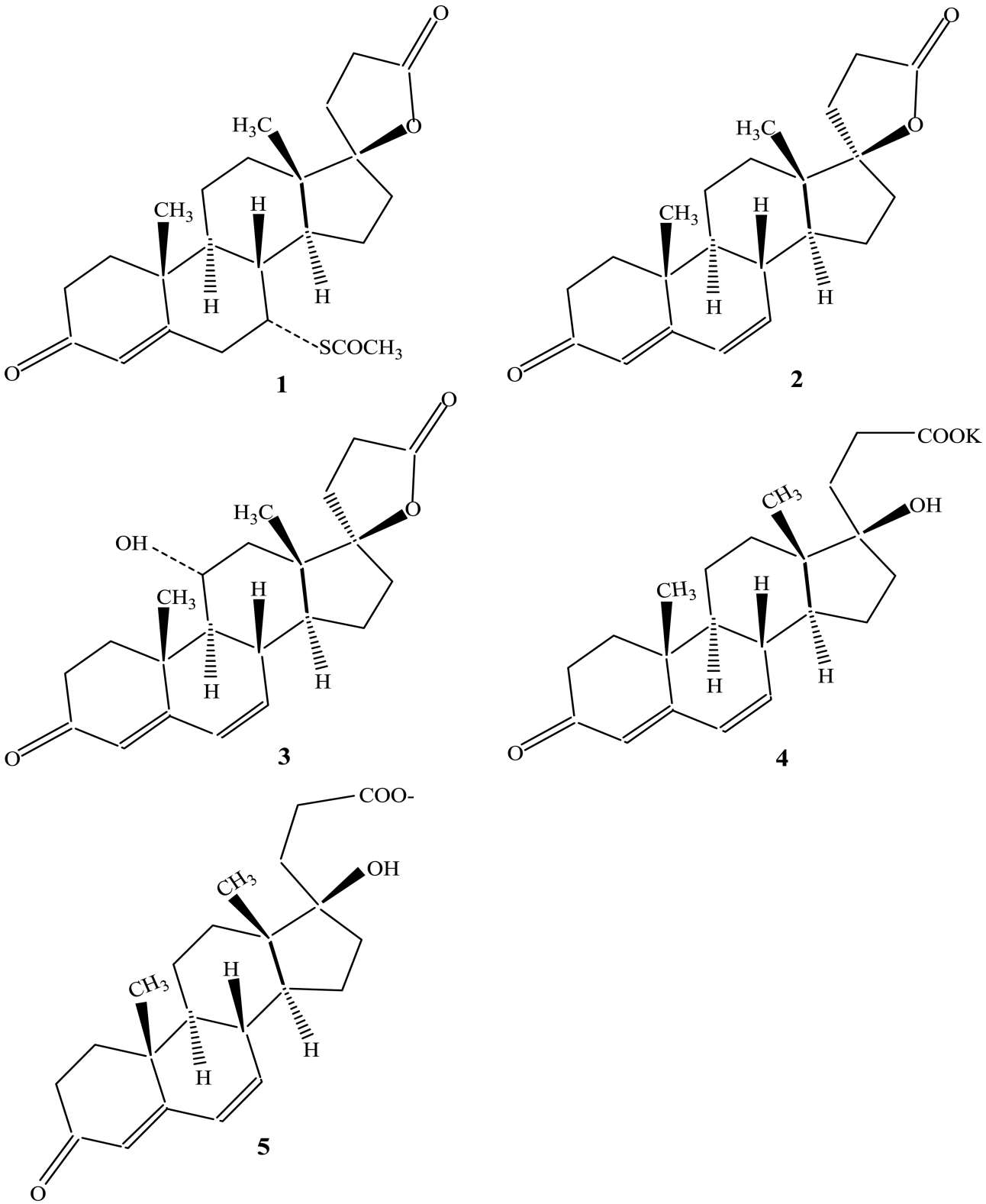

Fig. 1. Structures of Spironolactone (**1**), Canrenone (**2**), 11-α-hydroxy-canrenone (**3**), Potassium Canrenoate (**4**) and Canrenoate (**5**).

diagnose or treat a condition in which the body has too much aldosterone (hormone produced by adrenal glands to help regulate the salt and water balance in human body).[6]

SP is also used to reduce edema caused by heart, liver or kidney problems, hypertension, and hyper aldosteronism. Common side effects of SP include skin rash, headache, dizziness, and stomach pain.[6] Serious side effects of SP

include hyperkalemia, altered heart beats, confusion, tremors, decreased or no urine output, shallow breathing, muscle pain or weakness, and numbness.[6]

SP is rapidly and extensively metabolized in humans to 7α-thiomethylspirolactone and canrenone.[7] Sulfur-containing products are the predominant metabolites and are thought to be primarily responsible, together with spironol, for the therapeutic effects of the drug.[6] It is extensively used in medicine, though until recently it was considered only as potassium-sparing diuretic and antihypertensive drug. It may also reverse aldosterone-induced cardiac fibrosis and improve morbidity and survival of patients with congestive heart failure.[8,9] Furthermore, it is used in neonates, infants and children with congestive heart failure secondary to congenital heart disease.[10] As with many other frequently used drugs, SP is available only as tablets, rather than in liquid dosage form suitable for paediatrics use. Over the last 25 years, many extemporaneously prepared SP containing oral liquid formulations have been reported in the literature, as well as their physical and chemical stability.[11–17]

Pramar et al.[18] observed that the decomposition of SP consists of a series reaction (Spironolactone toCanrenoneand unidentified products) or a combination of series and side reactions, since some of the SP may also directly change into some unidentified products, which is probably the reason for the absence of the canrenone in the chromatograms. Moreover, it has also been suggested that in acidic medium the lactone is hydrolyzed reversibly.[18]

Canrenone (CR) (**2**), (10,13-dimethylspiro[2,8,9,11,12, 14,15,16-octahydro-1H-cyclopenta [α] phenan-threne-17,5′-oxolane]-2′,3-dione) (Fig. 1), is a cardiovascular drug, a sort of steroid; it is spironolactone's major metabolite a has been widely used clinically as a nonselective aldosterone receptor antagonist to treat heart failure, high blood pressure, edema, liver ascites, and other cardiovascular diseases.[19]

Canrenone (**2**) ($C_{22}H_{28}O_3$) is a pale yellow to pale green solid used as aldosterone antagonist. The production of (11-α-hydroxy-canrenone) (**3**) from canrenone (**2**) by the 11-α-hydroxylation reaction can be conducted by chemical synthesis or microbial transformation (Fig. 1).[6]

The 7α-acetylthio substituent is removed completely from 80% of the administered dose of SP yielding canrenone as the principal non-conjugated metabolite in plasma. Canrenone is active as a mineral corticoidant agonist in animals, and has been proposed as the principal pharmacologically active agent after administration of SP to humans. Potassium canrenoate (**4**) (Fig. 1), the potassium salt of asteroid acid, is also active as an aldosterone antagonist and has found clinical use in certain areas of the world. After administration of potassium canrenoate (**4**) to humans both canrenoate (**5**) (Fig. 1) and canrenone (**2**) are found in plasma. Evidence from in vitro studies indicates that canrenoate has a low affinity for aldosterone binding proteins, and is unlikely to contribute significantly to the pharmacological activity.[20] *In vitro* studies have also suggested that canrenone is the principal active metabolite of potassium canrenoate.[20]

The removal or elimination of pharmaceutical compounds can occur through various mechanisms during wastewater treatment process. Sorption onto sludge is one of the mechanisms and therefore the absorption and adsorption factors have to be taken into account. According to Carballa et al.,[21] absorption refers to the hydrophobic interactions of the aliphatic and aromatic groups of a compound with fats present in the sludge or with the lipophilic cell membranes of the microorganisms. Adsorption refers to the electrostatic interactions of positively charged groups of dissolved chemicals with the negatively charged surfaces of the microorganisms (characterized by the dissociation constant).

To evaluate the efficiency of different traditional and innovative tools for the elimination of pharmaceutical residues, we have performed a series of water purification experiments by using the WWTP installed at the Al-Quds, which includes sequential units, such as activated sludge (AS), ultra-filtration (UF), granular activated charcoal (GAC) and reverse osmosis (RO).[22] Problems arising from the management of such a plant can be due to the capability of the AS unit to favor the biodegradation of organic pollutants as well as the fouling phenomenon affecting membrane units, which must be often replaced with high costs.

In the present work we report a study on the efficiency of advanced wastewater treatment technologies adopted in the Al-Quds plant for the removal of "spironolactone" (SP), which was used as a model pharmaceutical compound due to its high solubility in water and 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 SP in pure water as well as in activated sludge collected from the plant was investigated and SP degradation products were identified.

Finally, the effectiveness of a micelle-clay (MC) filter for removing SP was tested and compared to a filter filled with granular activated charcoal. The SP adsorption equilibrium parameters and the adsorption Langmuir coefficients were determined for both micelle-clay and fine powderactivated charcoal (FAC) as adsorbent materials.

Micelle-clay composites have already been proven useful in the removal of about 20 neutral and anionic pollutants.[22–27] The micelle-clay composite which was used in this study is positively charged, has large surface area and includes large hydrophobic domains. It was shown by X-ray diffraction, electron microscopy and adsorption experiments that the material characteristics of the micelle-clay complex are different from those of an organo-clay complex, which is formed by adsorption of the same organic cation ODTMA (Octadecyltrimethylammonium) as monomers.[28]

Materials and methods

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 SP was obtained from Birzeit Pharmaceutical Company (Palestine) with 99% purity, and used as received. Fine powder activated charcoal (FAC) with particle size $\leq$ 60 μm, and granular activated charcoal (GAC) with particle size $\leq$ 700 μm were obtained from Sigma (Sigma Chemical Company, St. Louis, MO, 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 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 solid phase extraction (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 SP was determined by using a high pressure liquid chromatography system model 2695 HPLC from Waters (MA, USA), equipped with a Waters 2996 Photodiode array. Data acquisition and control were carried out using Empower software (Waters, MA, 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.

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HPLC conditions: mixture of water: acetonitrile (40:60; v/v) as mobile phase; flow rate of 1.4 mL min^{-1}; UV detection at a wavelength of 254 nm; Acrodisc syringe filters with GHP membrane (hydrophilic polypropylene 0.45-μm porosity) from Waters were always used for all analytical filtration requirements. The identification of SP degradation products was performed at University of Basilicata, Italy by 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). Full-scan experiments were performed in the ICR trapping cell in the range m/z 50–900. Mass-to-charge ratio signals (m/z) were acquired as profile data at a resolution of 100,000 (FWHM) at m/z 400. Negative and positive ion ESI-MS was used for the detection of by-products. The advanced wastewater treatment plant employed in this study is located at Al-Quds University-Palestine and was described in detail elsewhere.[22] Normally, the effluent from this plant is recycled for the irrigation of plants cropped in the field of university campus.

Methods

Characterization of wastewater used

The wastewater was characterized before the experiments according to the American Public Health Association procedures.[29,30]

Efficiency of WWTP for SP removal

The efficiency of different treatment units was ascertained by spiking separately the secondary effluent with 1.0 mg L^{-1} of SP in the activated sludge reservoir (1000 L). Samples were collected from different locations of the WWTP. SPE-C18 disposable cartridges were used to preconcentrate 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 SP. Recovery tests were performed using triplicate solutions of the three substances, and values ranging from 98% to 102% were obtained.

Stability of SP

Stability study of SP was performed using 100 mg L^{-1} solutions in pure water, or activated sludge taken from the WWTP installed at Al-Quds University and was described in detail elsewhere.[31–34] At specific time intervals (0 to 16 days) samples were collected from the above solutions (maintained under continuous orbital shaking), filtered, and analyzed by HPLC. The degradation by-products of SP were investigated using liquid chromatography/Fourier-transform ion cyclotron resonance/mass spectrometry (LC/FT-ICR-MS).

Micelle-clay complex preparation

The ODMTA micelle-clay complex was prepared by mixing the clay-mineral montmorillonite with the cationic surfactant octadecyltrimethylammonium (as bromide salt) with a critical micelle concentration (CMC) value of 0.3 mM as described previously.[26]

Batch adsorption experiments

Batch adsorption experiments of SP were carried out at different concentrations. Experiments were performed in a 250-mL Erlenmeyer flasks containing 200 mg of either micelle-clay complex or fine powder activated

Table 1. Physical, chemical and biological parameters of wastewater to be treated.

Parameters	*Results*	*Units*	*Parameters*	*Results*	*Units*
pH	7.32 ± 0.01	—	TSS	3710 ± 60	mg L^{-1}
Conductivity	1995 ± 20	$\mu Sm\ cm^{-1}$	BOD	940 ± 50	mg L^{-1}
Temperature	15.6 ± 0.3	°C	COD	1960 ± 60	mg L^{-1}
Turbidity	5050 ± 40	NTU	NH_4-N	55.5 ± 0.8	mg L^{-1}
DO	0.41 ± 0.03	mg L^{-1}	PO_4-P	13.1 ± 1.1	mg L^{-1}
TS	4240 ± 50	mg L^{-1}	FC (E. coli)	$2.9 \times 10^5 \pm 0.3 \times 10^5$	cfu/100 mL
TDS	615 ± 18	mg L^{-1}	TC	$6.5 \times 10^6 \pm 1.3 \times 10^6$	cfu/100 mL
Settable solids	260 ± 10	mg L^{-1}	TAC	$2.6 \times 10^7 \pm 1.3 \times 10^7$	cfu/100 mL

[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.

charcoal (FAC); 100 mL of each drug solution of known initial concentration were introduced into each flask. The flasks were shaken in an oscillating shaker for 3 h at room temperature, then 2.0-mL portions were filtered using 0.45-μm filters. The equilibrium concentrations of SP were obtained by HPLC, using the conditions reported above. The retention time of SP was 6.9 min.

Column filtration experiments

Column filtration experiments were performed using 50/1 (w/w) mixtures of quartz sand and either ODTMA-clay complex, or granular activated charcoal (GAC), which formed layers of 20 cm in borosilicate columns of 25 cm length and 5 cm 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 SP 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.

Results and discussion

Calibration curve

Linearity of the proposed analytical method was verified by analyzing standard SP solutions in the range of 0.1–100 mg L^{-1} in pure water. The calibration curve was obtained with a determination coefficient R^2 of 0.9999. The repeatability of triplicate successive injections ranged from 98.5% to 99.5%, depending on the sample concentration and type of analyte. The repeatability of morning/evening injections on the basis of 6-h elapsed time ranged from 97.5% and 98.0%, and was also affected by the concentration and type of analyte. Correction coefficients were used for experimental samples.

New calibration solutions were prepared using wastewater taken from the activated sludge reservoir of Al-Quds WWTP. The determination coefficient of calibration curves was 0.9999. The limit of detection, based on a signal/noise of 3, was 0.03 mg L^{-1} for SP. The limit of quantification, based on a signal/noise of 10, was 0.08 mg L^{-1}.

Table 1 summarizes the chemical, physical and biological characteristics of wastewater sampled from the activated sludge reservoir of Al-Quds WWTP. This table reveals that the wastewater contained high concentrations of suspended solids and large populations of bacteria, which are responsible of fouling phenomena affecting

Table 2. Removal of SP from wastewater by different treatment units in Al-Quds WWTP; average values of three replicates ± S.D.

Sample description		*Sampling site*	*Concentration of SP mg L^{-1}*	*Removal %*
The initial concentration of SP in storage tank (after addition of SP)		1	1.1 ± 0.06	
	Influent	2	0.83 ± 0.02	
UF-HF	Brine produced	3	0.49 ± 0.05	
	Effluent	4	0.25 ± 0.01	69.9
UF-SW	Brine	5	0.22 ± 0.02	
	Effluent	6	0.06 ± 0.04	92.8
GAC effluent		7	b.l.d.	≈ 100.0

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

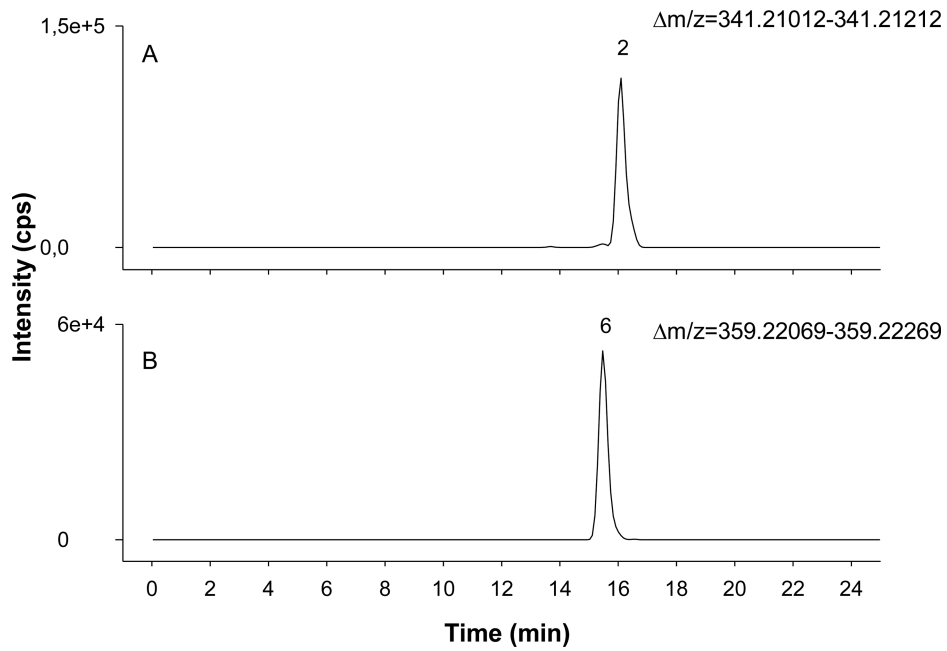
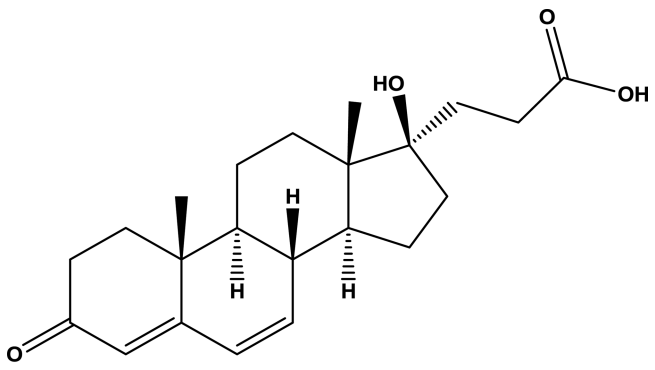


Fig. 2. Extracted ion chromatograms (XICs) by LC/ESI-FTICRMS acquired in positive ion mode of two main by-products obtained after one month of biodegradationof a SP solution. The ions monitored are displayed in each trace (A and B) and correspond to the most abundant protonated molecules, $[M+H]^+$, using a restricted window of ± 0.0010 m/z unit centered around each selected ion. Peak numbers correspond to Canrenone (**2**), Canrenoic acid (**6**).

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.

Efficiency of WWTP for SP removal

The efficiency of WWTP at Al-Quds University for the removal of SP was studied. The activated sludge reservoir was separately spiked with SP at concentration of 1.0 mg L^{-1}, which is close to literature reports.[3,35] Samples were taken from different collection sites of WWTP. Analytical results of water effluent from the hollow fiber ultra-filtration membrane (UF-HF) indicated that 69.9% of SP was removed at this stage, whereas about 92.8% of SP was removed after passing the spiral wound (UF-SW) membrane (Table 2). SP was completely removed by the GAC filter. However, it should be outlined that the concentrations of SP influent in the treatment units were diminishing along their sequence. This relationship reflected upon 100% removal by GAC filter, whose influent water contained only 0.06 mg L^{-1} of SP, on average, after the passage through the UF filters. This outcome enabled to skip using the 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 the contaminants are concentrated. For this reason additional methods of water filtration and purification should be tested experimentally.



6
Canrenoic acid

Fig. 3. Chemical structure for Canrenoic acid (**6**).

Stability of SP in pure water and in sludge

Because many pharmaceuticals might undergo degradation upon their standing in aqueous medium and sludge environment,[35,36] kinetic studies on SP stability in pure water and sludge conditions have been undertaken using SP concentration of 100 mg L^{-1}. The results showed that SP was unstable in both distilled water and Al-Quds

Table 3. Langmuir adsorption parameters (k and Q_{max}) and determination coefficients (R^2) obtained from the adsorption of SP on the micelle-clay complex and activated charcoal.

Adsorbent	k ($L\,mg^{-1}$)	Q_{max} ($mg\,g^{-1}$)	$k^* Q_{max}$ ($L\,g^{-1}$)	R^2
Micelle-clay complex	3.3 ± 0.3	17.8 ± 2.5	58.7 ± 1.5	0.935
Activated charcoal	2.7 ± 0.3	10.6 ± 2	28.6 ± 1.1	0.964

university activated sludge, being susceptible to water hydrolysis and bacterial degradation. In both cases the degradation followed a first order rate with values of the rate constants being $7.4 \times 10^{-7}\ s^{-1}$ and $3.6 \times 10^{-5}\ s^{-1}$, in pure water and in activated sludge, respectively. The degradation half-life was diminished from 10.7 days in pure water to 0.22 days in the activated sludge where the concentration of the parent molecule was found at a level of 4.6 mg L^{-1} after one day of incubation.

The accelerated degradation in sludge 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, Enterobactersakazakii, Citrobacterfreundii, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterobacter cloacae, Enterobacteramnigenus, Enterobacteraerogenes, Salmonella spp.*, and *Serratialiquefaciens*.[37] 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.

Monitoring the substances arising from the degradation of SP in the activated sludge indicated that SP underwent degradation to two by-products, as identified by mass spectrometry analysis. Extracted ion chromatogram (XIC) of the 16-day biodegraded sample is shown in Fig. 2. 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 molecule (i.e., $[M+H]^+ \pm 1.0$ mDa), greatly reduced the signal complexity of the total ion current trace (data not shown) allowing to completely characterize all degradation products.

Degradation products of SP in wastewater solution were identified through LC/MS analysis, which showed that SP can degrade to two metabolites, canrenone ($C_{22}H_{28}O_3$, experimental $[M+H]^+ m/z$ 341.21140, exact $[M+H]^+ m/z$ 341.21112, error 0.8 ppm) (**2**) (Fig. 1), with high percent and to other metabolites, e.g., canrenoic acid (**6**) (Fig. 3), which has the chemical formula $C_{22}H_{30}O_4$.(experimental $[M+H]^+ m/z$ 359.22183, exact $[M+H]^+ m/z$ 359.22169, error 0.4 ppm) with less amount; the analysis showed that SP was still present in solution but at very low concentration. All metabolites were identified with an error lower than 1 ppm.

There is no predominant metabolite containing sulfur, which is thought to be primarily responsible together with spironolactone for the therapeutic effects of the drug.[6] LC/MS analysis results agree with the conclusions of Pramar et al.,[18] who described spironolactone decomposition to canrenone by a series of reactions [spironolactone → canrenone→ unknown products]. Spironolactone is extensively metabolized in humans, and ≈79% of the spironolactone oral dose is converted to canrenone, its major biologically active metabolite.[18] Canrenone undergoes hydrolysis of its γ-lactone ring to canrenoic acid (CA), which is water soluble. Thus, after equilibrium is reached, similar plasma concentrations of CA and canrenone are reached.[20] LCMS analysis did not show the presence of metabolites containing potassium or sodium; this disagrees with other evidence from *in vitro* studies,[20] which suggested that canrenone is the principal active metabolite of potassium canrenoate.

Table 4. Removal of SP by filtration of 1 L of water solutions through laboratory filters, which included either MC or GAC mixed with excess sand at 1:50 (w/w) ratio; means of three replicates.[a]

Initial concentration ($mg\,L^{-1}$)	*Column type*[a]	*Average eluted concentration* ($mg\,L^{-1}$)	± *SD*
100	MC	13.5	3.5
100	GAC	24	4.2
10	MC	b.l.d.	—
10	GAC	b.l.d.	—
1.0	MC	b.l.d.	—
1.0	GAC	b.l.d.	—
0.01	MC	b.l.d.	—
0.01	GAC	b.l.d.	—

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

Adsorption isotherms

The adsorption of SP at several initial concentrations by the micelle-clay complex and activated charcoal was investigated. Equilibrium relationships between adsorbent and adsorbate can be described by Langmuir adsorption isotherm,[38] represented by Eq. (1):

$$\frac{C_e}{Q_e} = \frac{1}{kQ_{max}} + \frac{C_e}{Q_{max}} \quad (1)$$

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 complex or activated scharcoal, 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 complex.

The data fit well the Langmuir equation for SP giving $R^2 = 0.964$ for activated charcoal and 0.935 for the micelle-clay. The calculated Langmuir constants k and Q_{max} are presented in Table 3. The values of k and Q_{max} parameters for the adsorption isotherm obtained using the micelle-clay complex were 1.2- and 1.7-fold larger than the corresponding values deduced for activated charcoal. The analysis of the Langmuir equation yields that a deduction of an overestimate for the value of Q_{max} would yield an underestimate in the value of k and vice versa. The presentation of the Langmuir equation in another form as in ref.[25] emphasizes the fact that the quantity which controls the adsorption is the product $k^* Q_{max}$. Hence we added in Table 3 this quantity, whose values are 58.7 and 28.6 Lg^{-1}, for the adsorption of SP by the micelle-clay, or activated charcoal, respectively, which emphasizes that the former is the better adsorbent for SP removal.

Filtration

The results in Table 4 demonstrate removal of SP by filtration of 1 L of several solutions (100, 10, 1.0, 0.01 mg L^{-1}) through a filter that included mixtures of micelle-clay or activated charcoal with excess sand. Complete removal was observed for SP concentrations of 10 mg L^{-1} or less by both filters. In the case of the higher concentration of 100 mg L^{-1} the emerging concentration of SP through the activated charcoal filter was almost two-fold larger than through the micelle-clay filter. This result is in accord with the results of adsorption in suspension, indicating the higher efficiency of the micelle-clay complex to remove this pharmaceutical from water.

Previously reported experiments demonstrated the poor capability of activated carbon filters towards removing of anionic and certain neutral pollutants.[22–27] Karaman et al.[22] showed that micelle-clay filters are more efficient in the removal of diclofenac from drinking water and wastewater than activated carbon. Moreover, Khamis et al.[23] 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.[24] suggested that the integration of the micelle-clay complex filters in existing WWTPs may be helpful for improving removal efficiency of recalcitrant residues of nonsteroid anti-inflammatory drugs (NSAIDs). Polubesova et al.[25,26] showed efficient removal from water of several herbicides and antibiotics. Nir et al.[27] demonstrated that the removal from water by filtration was more efficient in the case of two herbicides, bromacil, which is neutral, and sulfentrazone, which is anionic. Elevation of the temperature to 35°C and 50°C did not affect the removal of these herbicides by the micelle-montmorillonite filter, whereas an equivalent filter which included activated carbon (GAC) yielded poor removal.

It can be argued that in addition to SP residues or other similar pharmaceuticals, wastewater usually includes other recalcitrant organic pollutants. In such cases GAC filters can be used as a 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, as well as pathogenic microorganisms.

Conclusions

The kinetic study conducted on SP stability revealed that SP was unstable in pure water and in sludge. The degradation products were identified by LC-MS and LC/MS/MS techniques, and those occurred in the sewage sludge were found to include not only the already known metabolite canrenone, but also another derivative, which was not known before. The WWTP of Al-Quds University showed that the sequence of advanced treatment technologies installed enabled a complete removal of SP from 1 mg L^{-1} spiked wastewater.

The batch and filtration experiments demonstrated an advantage of the micelle-clay complex over activated charcoal in removing SP from synthetic water. The large effectiveness and removal capacity of the micelle-clay complex are due to the high adsorption affinity towards the anionic SP by the relatively large number of positively charged and hydrophobic sites of the micelle-clay complex based on ODTMA.

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