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Removal of amoxicillin and cefuroxime axetil by advanced membranes technology, activated carbon and micelle–clay complex

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Two antibacterials, amoxicillin trihydrate and cefuroxime axetil spiked into wastewater were completely removed by sequential wastewater treatment plant's membranes, which included activated sludge, ultrafiltration (hollow fibre and spiral wound membranes with 100 and 20 kDa cut-offs), activated carbon column and reverse osmosis. Adsorption isotherms in synthetic water which employed activated carbon and micelle–clay complex (octadecyltrimethylammonium–montmorillonite) as adsorbents fitted the Langmuir equation. Q_{max} of 100 and 90.9 mg g^{-1}, and K values of 0.158 and 0.229 L mg^{-1} were obtained for amoxicillin trihydrate using activated carbon and micelle–clay complex, respectively. Filtration of antibacterials in the ppm range, which yielded variable degrees of removal depending on the volumes passed and flow rates, was simulated and capacities for the ppb range were estimated. Stability study in pure water and wastewater revealed that amoxicillin was totally stable for one month when kept at 37°C, whereas cefuroxime axetil underwent slow hydrolysis to cefuroxime.

Keywords: antibacterials; amoxicillin; cefuroxime axetil; wastewater treatment; ultrafiltration; reverse osmosis; clay; clay-micelle complex; activated carbon; adsorption

1. Introduction

According to the UN and WHO, about one-fifth of the world population lives in regions with water scarcity. The challenge of water stress is not only caused by the increasing demand of water as a result of the rapid population growth. In many cases the water scarcity is related to the lack of clean water due to contamination. Besides the 'classic' contaminants, there has been growing attention during the last years on new groups of organic micropollutants, such as pharmaceutical residues. The appearance of these emerging pollutants is believed to pose a serious risk to human health and the environment.[1–3]

Various methods for wastewater treatment have been utilised, including conventional, such as activated sludge and biofilters and others slightly less conventional, such as oxidation ditches, aerated lagoons and natural treatment system, such as waste stabilization ponds.[4]

Despite progress in water treatment methods, varying amounts of chemicals exist in what is referred to as 'clean' water. This is an ever growing problem especially with the amount of pharmaceuticals found in water. Certain pharmaceuticals may be retained in the treated water and in certain cases be degraded to more harmful metabolites whose removal is needed. Furthermore, several pollutants are not efficiently removed by conventional treatments. For the past three decades, data have been accumulated on the concentrations of pharmaceuticals in drinking water.[5]

The occurrence of pharmaceutically active substances (PhACs) in the environment has become an important issue in the last few years. These agents along with their metabolites, which can be even more harmful than their parent compounds, are continuously released into the environment, mainly through disposal of unused or expired drugs or directly from pharmaceutical discharges.[6] The efficiency of their removal is influenced by the chemical properties of the specific pharmaceuticals or metabolites, by microbial activity and environmental conditions.[7–9] Recent studies have clearly shown that the elimination of PhACs from municipal wastewater treatment plants (WWTPs) is often incomplete.[10,11]

Among the pharmaceutical residues that have been detected in different environmental compartments are analgesics and anti-inflammatories (ketoprofen, naproxen, ibuprofen, indomethacin, diclofenac, mefenamic acid, acetaminophen, propyphenazone), lipid regulators and cholesterol-lowering statin drugs (clofibric acid,

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gemfibrozil, bezafibrate, pravastatin, mevastatin), psychiatric drugs (carbamazepine, fluoxetine, paroxetine), antiulcer agent (lansoprazole) and histamine

H1 and H2 receptor antagonists (loratadine, famotidine, ranitidine), antibiotics (erythromycin, azithromycin, sulfamethoxazole, trimethoprim, ofloxacin) and β-blockers (atenolol, sotalol, metoprolol, propranolol). Although, these pharmaceuticals were found at trace levels (ng L^{-1} to low µg L^{-1}) in the environment, and can be quite effective to cause toxicity. The detection of antibiotics and steroids is of particular concern since the former may cause resistance among natural bacterial populations and the latter might affect the induction of oestrogenic responses.[12]

The term antibiotic is used to denote any natural or synthetic drug that selectively kills bacteria or other single-celled microorganisms. Antibacterial agents are classified according to the type of organism against which they are active. Most antibacterials are utilized to treat bacterial infections and include agents from the penicillin, tetracycline, macrolide, quinolone and sulphonamide classes. Penicillins (e.g. amoxicillin), macrolides (e.g. erythromycin) and sulphonamides (e.g. sulfamethoxazole) are the most frequently used antibiotics in human and veterinary medicines to treat and prevent diseases.[12–16] Studies have shown that several classes of antibiotics are present in domestic effluents and aquatic environments,[17] since often they are not fully assimilated by humans and animals during treatment.[18]

The penicillins' antibacterial agents containing a β-lactam ring exert their antibacterial activity through the inhibition of the synthesis of the bacterial peptidoglycan cell wall. Penicillins consist of a thiazolidine ring bonded to a β-lactam ring, to which a side chain is attached. Penicillins G and V are among the more important of penicillins and are effective against gram-positive cocci. Within the penicillin class, amoxicillin is the most used antibiotic, followed by penicillin V. Other examples of the class are cefuroxime, cloxacillin and dicloxacillin.

Amoxicillin trihydrate is a semi-synthetic β-lactam antibiotic (Figure 1), the only phenolic penicillin which is used as an antibacterial drug.[19,20]

Cefuroxime axetil, (RS)-1 hydroxyethyl (6R,7R)-7-[2-(2-furyl) glyoxyl-amido]-3-(hydroxylmethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylate,72-(Z)-(O-methyl-oxime),1-acetate3-carbamate) (Figure 2) is a second generation oral cephalosporin antibiotic used to treat or prevent infections that are proven or strongly suspected to be caused by bacteria. It is an acetoxyethylester prodrug of cefuroxime which is effective when used orally. The activity of cefuroxime depends on the *in vivo* hydrolysis of the axetil prodrug and on the release of the cefuroxime moiety.[21]

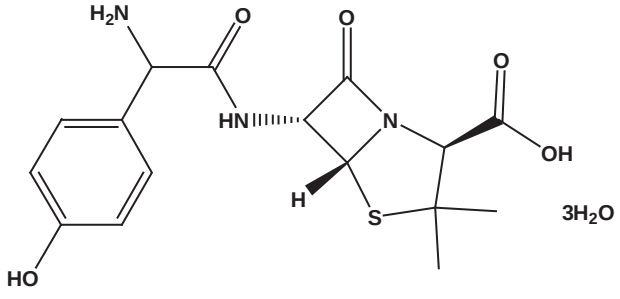

Figure 1. Chemical structure of amoxicillin trihydrate.

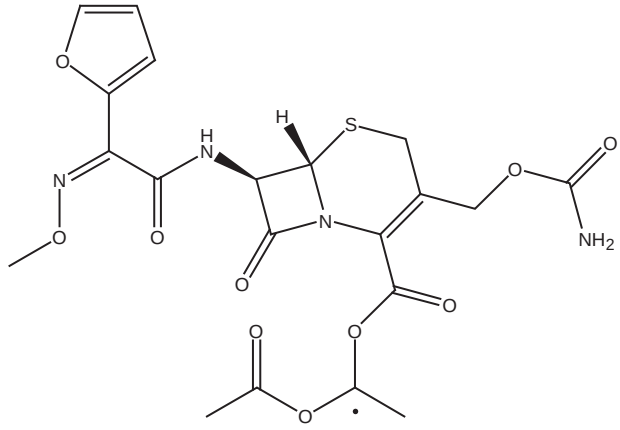

Figure 2. Chemical structure of cefuroxime axetil

Among the wide variety of drug residues reported, antibiotics such as trimethoprim, sulfamethoxazole, erythromycin, roxytomycin, oxytetracycline, ofloxacin, chlortetracycline and amoxicillin can be assumed to be commonly detected in Sewage Treatment Plants effluents. The concentration levels commonly found for these antibiotics are at ng L^{-1} or low µg L^{-1} (ppt–ppb).[11,12]

The goal of this study is to explore innovative sorbents using different filter materials for improved water treatment, and to develop an advanced method for wastewater reuse. In order to promote the introduction of new highly effective and cost-efficient treatment steps we propose treating selected organic contaminants (amoxicillin and cefuroxime axetil) by applying clay-composite filters. An appropriate combination of different clay composites, such as micelle or polymer-clay, can be tailored for optimized water treatment. These specifically designed clay composites can adsorb particular pollutants with higher efficiency than general sorbents, such as activated carbon.[22]

2. Experimental

2.1. Materials and equipment

2.1.1. Materials

Pure standards of amoxicillin trihydrate ($>99\%$) and cefuroxime axetil ($>99\%$) were obtained as a gift from Beit Jalah pharmaceutical company (Palestine). Acetonitrile, methanol and water high performance liquid chromatography (HPLC) grade, charcoal activated fine powder with particle size ≤ 60 micron, charcoal activated

granules with particle size ≤ 700 micron and octadecyltrimethylammonium (ODTMA) [22] were purchased from Sigma Chemical Company (Germany). The clay used was Wyoming Na-montmorillonite SWY-2 obtained from the Source Clays Registry (Clay Mineral Society, Columbia, MO, USA).

C18 (5 g) cartridges 6cc single use for general laboratory use were purchased from Waters Company (Milford, MA, USA).

2.1.2. Equipment

HPLC-photo diode array (PDA) system consists of an alliance 2695 HPLC (Waters: Milford, MA, USA), and a waters Micromass® Masslynx™ detector with PDA (Waters 2996: Milford, MA, USA). Data acquisition and control were carried out using Empower™ software (Waters: Milford, MA, USA). Analytes were separated on a 4.6 × 150 mm C18 XBridge® column (5 μm particle size) used in conjunction with a 4.6 mm × 20 μm XBridge™ C18 guard column. A microfilter (0.45 μm) was used (Acrodisc® GHP, Waters).

The concentrations of the drugs in the samples were determined spectrophotometrically (UV-spectrophoto meter, Model: UV-1601, Shimadzu, Japan) by monitoring the absorbance at λ_{max} for each drug.

Liquid chromatography-mass spectrometry (LC–MS): HPLC analysis was performed on an Accela High Speed LC system (Thermo Fisher Scientific Inc.) which consists of an Accela Pump, Accela Autosampler and Accela PDA detector. HPLC separations were carried out using a Kinetex Hexyl-Phenyl column (2.1 × 150 mm, particle size 2.6 µm, Phenomenex). The Accela LC system was coupled with the LTQ Orbitrap Discovery hybrid Fourier transform (FT) mass spectrometer (Thermo Fisher Scientific Inc.) equipped with an electrospray ionization source. The mass spectrometer was operated in positive ionization mode; ion source parameters were as follows: spray voltage 3.5 kV, capillary temperature 300°C, sheath gas rate (arb) 30 and auxiliary gas rate (arb) 10. Mass spectra were acquired in the *m*/*z* 130–1500 Da range. The LC–MS system was controlled and data were analysed using Xcalibur software (Thermo Fisher Scientific Inc.).

pH values were recorded on a pH meter (model HM-30G: TOA electronics™).

The Labofuge® 200 Centrifuge was used (230 V 50/60 Hz CAT. No. 284811, made in Germany). Some of the solutions of pharmaceuticals were shaken with an electronic shaker (Bigbill shaker, Model No.: M49120-26, 220–240 V 50/60 Hz) at 250 rpm.

The WWTP at Al-Quds University collects a mixture of black, grey and storm water. The treatment plant consists of a primary treatment (two-stage primary settling basins) and a secondary treatment (activated sludge with a hydraulic retention time of 16–20 h, coagulation and chlorination). Then, the secondary effluent is introduced to a sand filter before entering the ultrafiltration membrane (hollow fibre and spiral wound (SW)). After the ultrafiltration process, the effluent is subjected to an activated carbon column followed by reverse osmosis (RO) (advanced treatment). Then, a blend of all effluents is used for irrigation. The ultrafiltration process is made up of two small-scale membrane treatment plants with a capacity of 12 m^3 day^{-1}. The first ultrafiltration unit is equipped with 2 × 4 inch pressure vessels with pressure resistance up to 150 psi. Each vessel holds two separation membranes (SW with 20 kDa cut-offs which is equivalent to 0.01 micron separation rate). The designed permeate capacity of the system is 0.5–0.8 m^3 h^{-1}. This membrane can remove bacteria, suspended solids, turbidity agents, oil and emulsions. The second unit is equipped with two pressure vessels (AST technologies, model number 8000 WW 1000–2M) that house the hollow fibre membranes with 100 kDa cut-off (AST technologies, Model no. 8000- WWOUT-IN-8080). The two units are designed to deliver 1.5 m^3 h^{-1}. The RO membranes are made from thin film polyamide which consists of 1 × 4 inch pressure vessels made from composite material with pressure resistance up to 400 psi. The vessel holds two 4-inch special separation membranes (manufactured in thin film polyamide with pH range 1–11 models BW30-4040 by DOW Film Tec.). A membrane anti-scalent (Product NCS-106-FG), made up of phosphate in water with an active ingredient of phosphoric acid disodium salt, is continuously dosed to the RO feed at a concentration of 4 ppm in order to prevent deposition of divalent ions. The system is designed to remove major ions and heavy metals. The designed RO permeate capacity of the system is 0.45–0.5 m^3 h^{-1}.[23]

2.2. Methods

2.2.1. Calibration curves

(a) *Stock solution*: Stock solutions were prepared by dissolving amoxicillin trihydrate or cefuroxime axetil standards in water to a concentration of 1000 ppm for the use in (b).

(b) *Calibration curves using the solid phase cartridge*: The C18 cartridges were preconditioned by passing the first 10 mL of water through the cartridge and then 10 mL of methanol. The cartridges were then air dried. Several solutions of amoxicillin trihydrate and cefuroxime axetil with different concentrations (1.0, 5.0, 10.0, 20.0, 50.0, 100.0, 200.0 and 500.0 ppm) were prepared. Ten millilitres of each of these solutions were passed through the cartridge. The adsorbed amoxicillin trihydrate and cefuroxime axetil were eluted from the adsorbent of the cartridge using 10 mL of methanol. Afterwards, 20 µL of the eluate was injected into the HPLC and analysed using the HPLC conditions for amoxicillin trihydrate and cefuroxime axetil.

Peak areas vs. concentration of amoxicillin trihydrate and cefuroxime axetil were then plotted, and correlation coefficients of the plots were recorded. Linear plots with correlation coefficient (R^2) of 0.999 were obtained.

2.2.2. *Kinetic studies on the stability of amoxicillin and cefuroxime axetil in pure water and wastewater*

Stability of amoxicillin and cefuroxime axetil was attained using 100 mg L^{-1} solutions in pure water, or activated sludge taken from the WWTP installed at Al-Quds University. Samples at specific time intervals (0 to 30 days) were collected from the stability solutions (maintained under continuous orbital shaking), filtered and analysed by HPLC. The degradation by-products of amoxicillin and cefuroxime axetil were investigated using LC/FT ion cyclotron resonance/MS.

2.2.3. *Efficiency of the WWTP of Al-Quds University for the removal of amoxicillin trihydrate and cefuroxime axetil*

The efficiency of different units (hollow fibre (HF-UF), spiral wound (SW-UF), activated carbon and RO membranes) in the removal of amoxicillin trihydrate and cefuroxime axetil from wastewater was studied by spiking amoxicillin trihydrate and cefuroxime axetil in the storage tank of the WWTP at a concentration of 20 ppm (by dissolving 10 g of amoxicillin trihydrate and cefuroxime axetil in the storage tank containing 500 litres of activated sludge wastewater). Samples were taken from the following points of the WWTP: (1) storage tank (before running WWTP) (2), (3), and (4) feed-, brine- and product-points of the HF-UF membrane, respectively, (5) and (6) concentrated and permeated ultrafiltration points of the HF–SW membranes, respectively, (7) activated carbon point and (8) RO point. These sampling points are shown in Figure 3. The samples were treated using SPE C18 cartridge as follows: 10 mL of sample was loaded into a C18 cartridge, and allowed to pass through the cartridge by effect of gravity. Amoxicillin trihydrate and cefuroxime axetil adsorbed on the C18 cartridge were then eluted using 10 mL of methanol; 20 µL of the eluted solution were injected into the HPLC and analysed using the HPLC conditions for amoxicillin trihydrate and cefuroxime axetil methods of analysis.

2.2.4. *Micelle–clay complex preparation*

The micelle-clay complex was prepared by stirring 12 mM of ODTMA (Figure 4) with 10 g L^{-1} clay for 72 h. Suspensions were centrifuged for 20 min at 15,000 g, supernatants were discarded and the complex was lyophilized. The obtained complex by virtue of its positive charge and hydrophobic region is capable of efficiently binding negatively charged organic molecules.[22–26]

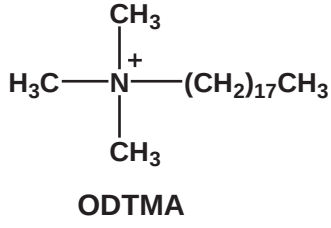

Figure 4. Chemical structure of ODTMA.

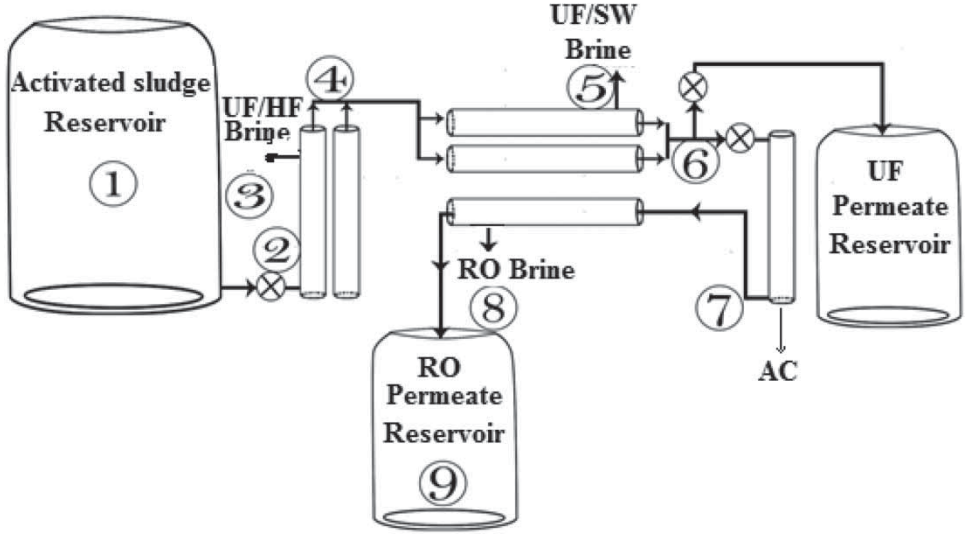

Figure 3. Flow diagram showing the process of WWTP which consists of HF-UF filters (hollow fibre) and SW-UF (spiral wound), AC (activated carbon) and RO filters. Sampling locations are indicated by numbers.

2.2.5. Adsorption studies on micelle–clay complex and charcoal

2.2.5.1. Batch adsorption isotherms Equilibrium relationships between adsorbents (micelle–clay complex and activated charcoal) and adsorbate (amoxicillin trihydrate and cefuroxime axetil) are described by adsorption isotherms which were obtained at adsorbate concentrations of 100, 200, 300, 400 and 500 ppm, prepared in distilled water at pH 8.2 (adjusted by 1M NaOH).

The following procedure was applied: 100 mL from each solution was transferred to a 200 mL Erlenmeyer flask; 0.500 g of the micelle–clay complex or activated charcoal was added to the flask. Then the flask was placed on the shaker for 180 min. Afterwards, each sample was centrifuged for 5 min, and filtered using a 0.45 μm filter.

A study on the kinetics of adsorption was conducted by introducing 100 mL of 100 ppm amoxicillin trihydrate and cefuroxime axetil solutions into 250 mL flasks containing 0.500 g of either micelle–clay or charcoal and determining the concentration of amoxicillin trihydrate and cefuroxime axetil. The concentration of amoxicillin trihydrate and cefuroxime axetil as a function of time was determined spectophotmetrically by recording the absorbance at λ_{max} of 273 and 278 nm, respectively.

2.2.6. Analysis of adsorption isotherms

Equilibrium relationships between adsorbents (micelle–clay complex and charcoal) and adsorbate (i.e. amoxicillin trihydrate or cefuroxime axetil) were described by the Langmuir adsorption isotherm which is considered the most widely used modelling for equilibrium data and determination of adsorption capacity.[27]

The linear form represented by Equation (1) was employed:

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

in which C_e is the equilibrium concentration of amoxicillin trihydrate or cefuroxime axetil (mgL^{-1}), Q_e the equilibrium mass of the adsorbed amoxicillin trihydrate or cefuroxime axetil per gram of complex or activated carbon ($mg\ g^{-1}$), K the Langmuir binding constant ($L\ mg^{-1}$) and Q_{max} the maximum mass of amoxicillin trihydrate or cefuroxime axetil removed per gram of complex ($mg\ g^{-1}$).

2.2.7. Column experiments

In the first experiment, a 25/1 (w/w) mixture of quartz sand and ODTMA-clay complex or granular activated carbon (GAC) (20 cm layer) was included in a column (25 × 5 cm). The bottom of the column was covered with a 3 cm layer of quartz sand. The quartz sand was thoroughly washed by distilled water and dried at 105°C for 24 h prior to its use. A wool layer of 2 cm was placed at the bottom of the column. One thousand millilitres of 100 ppm amoxicillin trihydrate solution were passed through the column at a fixed flow rate of 2 mL min^{-1}. For cefuroxime axetil, 1000 mL of 50 ppm cefuroxime axetil solution were passed through the column at a fixed flow rate of 2 mL min^{-1}. Eluted fractions of 100 mL (each) were collected, and the concentrations of amoxicillin trihydrate and cefuroxime axetil were determined spectrophotmetrically at λ_{max} of 273 278 nm, respectively. All experiments described were conducted in triplicates.

Additional filtration experiments employed the same columns, but with a 50/1 (w/w) mixture of quartz sand and ODTMA-clay complex or GAC (13 g) and the volume passed was several litres, at flow rates of 50 or 60 mL min^{-1}.

2.2.8. Adsorption and convection in a column filter

The adsorption and convection are described by Equation (2) whose numerical solutions were executed by a FORTRAN program.[28] Briefly, a column of length L is filled with material, whose initial molar concentration of adsorbing sites is R_0, whose concentration changes later to $R(X, t)$. The beginning and end of the filter are at the coordinates $X = 0$ and $X = L$, respectively. We consider that the pollutant concentration at the inlet, C_0 is constant, that is, $C(X, t) = C_0, X \leq 0$, where t denotes time:

$$dC(X,t)/dt = -v\partial C/\partial X - C_1 * C(X,t) * R(X,t) + D_1(R_0 - R(X,t)) \ldots, \qquad (2)$$

where C_1 ($M^{-1}\ min^{-1}$) represents rate constant of forward adsorption, D_1 (min^{-1}) the rate constant of desorption and v ($cm\ min^{-1}$) the flow velocity.

The statistical criteria for the goodness of the fits were the closeness of R^2 to unity, and RMSE, the root mean square error, which is given by

$$\mathrm{RMSE} = \left[\sum (y_{i,exp} - y_{i,calc})^2/(n-m)\right]^{0.5}, \qquad (3)$$

in which $y_{i,exp}$ and $y_{i,calc}$ are experimental and calculated values of per cent removal from water of the pollutant by the filter, n the number of data points and m the number of parameters. In our case, the parameters were R_0, C_i and D_i.

3. Results and discussion

3.1. Kinetic studies on the stability of amoxicillin and cefuroxime axetil in pure water and wastewater

Stability of amoxicillin and cefuroxime axetil was studied using 100 mg L^{-1} solutions in pure water and activated sludge taken from the WWTP installed at Al-Quds University. The HPLC and LC-MS results indicate that amoxicillin was completely stable for 30 days in both media, whereas in pure water cefuroxime axetil (an ester) was quite stable, but it underwent very slow hydrolysis to cefuroxime base (a carboxylic acid) as deduced from

the LC-MS analysis. Cefuroxime axetil is an ester which undergoes hydrolysis. In contrast, amoxicillin is a free acid which makes it stable in a neutral medium. The slow hydrolysis ($t_{1/2}$ = 14 days) of cefuroxime axetil might be catalysed by esterases present in the activated sludge.

3.2. *Characteristics of wastewater in the Al-Quds plant before and after purification*

The characteristics showed variations in the range of 10–60%. Typical values of total dissolved solids (TDS), chemical oxygen demand (COD) and biological oxygen demand (BOD) were 970, 380 and 240 ppm, respectively; Na 130 ppm, Cl^- 270 ppm, NH_4 90 ppm and NO_3 15 ppm. These values were reduced after RO to TDS, COD and BOD of 30, 20 and 10 ppm, respectively, and less than 10 ppm for the other species, except to 13 ppm in the case of Cl^-.

3.3. *Efficiency of the WWTP at Al-Quds University for the removal of amoxicillin trihydrate and cefuroxime axetil*

The efficiency of the WWTP at Al-Quds University for amoxicillin trihydrate and cefuroxime axetil removal was studied. The results demonstrated that amoxicillin trihydrate was removed up to 69.9% at the hollow fibre stage (UF-HF), while about 90.3% of amoxicillin trihydrate was removed at the SW stage, (Tables 1 and 2). At the activated carbon adsorbent point of the WWTP, 96.5% of amoxicillin trihydrate was removed. The results also indicated that complete removal (100%) of amoxicillin trihydrate was achieved after passing through the RO membrane.

A similar pattern was observed for cefuroxime axetil, which was removed up to 70.9% at the hollow fibre stage (UF-HF), 91.3% was removed after the SW stage and 96% was removed after the activated carbon stage.

It should be noted that the above percentages of removal represent cumulative values. Table 3 shows the actual relative efficiencies of removal of the antibacterials by UF-HF, UF-SW and activated carbon. This table demonstrates that for both antibacterials the efficiency of removal at the Al-Quds WWTP was similar for both the ultrafiltration elements, about 70% removal, which was above the per cent removal by the activated carbon.

3.4. *Adsorption of amoxicillin trihydrate and cefuroxime axetil on a micelle–clay complex (ODTMA) and activated charcoal*

Amoxicillin trihydrate removal by a micelle–clay complex and activated charcoal was investigated. Samples

Table 1. Removal of amoxicillin trihydrate and cefuroxime axetil through the hollow fibre (UF-HF), spiral wound (UF-SW), activated carbon adsorbent and RO from the WWTP at Al-Quds University.

No.	Sample location name		Concentration of amoxicillin trihydrate (ppm)	Concentration of cefuroxime axetil (ppm)
1	Blank (before addition of amoxicillin trihydrate and cefuroxime axetil)		0	0
2	The initial concentration of amoxicillin trihydrate and cefuroxime axetil in the storage tank (after addition of amoxicillin trihydrate and cefuroxime axetil)		19.5	19.1
3	HF-UF	Feed point	18	18.60
		Brine point	12.33	13.20
		Product point	5.67	5.73
4	HF–SW	Concentrated ultrafiltration point	3.72	40.1
		Permeated ultrafiltration point	1.95	1.34
5	Activated carbon point		0.41	0.63
6	RO	Permeated RO point	0.0	0.0

Table 2. Cumulative % removal of amoxicillin trihydrate and cefuroxime axetil.

	Trial No.	Hollow fibre (HF) (%)	SW (%)	Activated carbon (%)	RO (%)
Amoxicillin trihydrate	1	–	89.0	93.8	100.00
	2	68.9	92.0	97.8	100.00
	3	70.9	90.0	97.9	100.00
	Average	69.90	90.3	96.5	100.00
	SD	2.0	2.0	2.0	–
Cefuroxime axetil	1	71.0	90.3	95.5	100.00
	2	71.9	93.0	96.7	100.00
	3	69.8	90.5	95.9	100.00
	Average	70.9	91.3	96.0	100.00
	SD	2.0	2.0	1.0	–

Table 3. Relative efficiency of purification elements by a comparison of average % removal of amoxicillin trihydrate and cefuroxime axetil.

	Hollow fibre (HF) (%)	SW (%)	Activated carbon (%)
Amoxicillin trihydrate	69.9	67.8	63.9
SD	2	2	2
Cefuroxime axetil	70.9	70.1	54
SD	1	2	1

were taken at different time intervals (0–180 min). The results demonstrated that both the micelle–clay complex and activated charcoal were effective for the removal of amoxicillin trihydrate from spiked samples (100 ppm) at pH = 8.2. The removal of both adsorbents was complete, but the adsorption of amoxicillin trihydrate by the micelle–clay complex was faster when compared to that by the activated charcoal; about 81.6% of amoxicillin trihydrate was removed in the first 5 min while only 50.2% was removed by the activated charcoal.

Similarly, the results revealed that the micelle–clay complex and charcoal were effective for the removal of cefuroxime axetil from spiked samples (20 ppm) at pH = 8.2. The removal was 95% after 3 h. The results showed that the adsorption of cefuroxime axetil by the micelle–clay complex was faster when compared to that by the activated charcoal. After first 5 min the percentages of removal by the micelle–clay complex and charcoal were 72.2% and 49.5%, respectively.

Table 4. Adsorption isotherm parameters of amoxicillin trihydrate and cefuroxime axetil onto the adsorbent activated charcoal (pH = 8.2 and T = 25°C).

	C_i (ppm)	C_e (ppm)	Q_e (mg g^{-1})	C_e/Q_e (g L^{-1})
Amoxicillin trihydrate	100	1.5	19.7	0.08
	200	3.46	39.308	0.09
	300	10.29	57.942	0.18
	400	32.52	73.496	0.44
	500	68.15	86.37	0.79
Cefuroxime axetil	20	3.5	3.3	1.06
	50	2.3	9.54	0.24
	100	9.8	18.04	0.54
	200	61.4	27.72	2.22
	300	179.1	24.18	7.41

3.5. *Analysis of adsorption isotherms*

The adsorption of amoxicillin trihydrate using concentrations of 100, 200, 300, 400 and 500 ppm and cefuroxime axetil using concentrations of 20, 50, 100, 200 and 300 ppm on micelle–clay complex and activated charcoal were studied. C_e and Q_e were determined for both pharmaceuticals as shown in Tables 4 and 5 and Figure 5. C_e/Q_e vs. C_e was plotted for amoxicillin trihydrate and cefuroxime axetil adsorbed onto both micelle–clay complex and activated charcoal (Figure 5).

The two parameters Q_{max} and K for the adsorption of amoxicillin trihydrate and cefuroxime axetil on micelle–clay complex and activated charcoal can be calculated from the slopes and y-intercepts of the equations obtained from the plots (Q_{max} = slope^{-1}, K = (y-intercept)$^{-1}$(Q_{max})$^{-1}$). Table 5 lists the values of Q_{max} and K for amoxicillin trihydrate and cefuroxime axetil adsorbed on both the micelle–clay complex and the activated charcoal.

As shown in Figure 5, the relationship between C_e/Q_e and C_e for the two pharmaceuticals is linear; R^2 was larger than 0.98, in accord with the linear form of the Langmuir isotherm. The results demonstrated those adsorbents, micelle–clay complex and activated charcoal, yielded comparable Q_{max} values, 90.9 and 100 mg, of amoxicillin trihydrate per gram of complex or activated charcoal. The K values which reflect the binding affinity of amoxicillin were larger by 45% for the micelle–clay than for activated carbon. The results for cefuroxime axetil revealed that the adsorption isotherm for the micelle–clay complex has larger Q_{max} and K values than those for activated carbon, thus rendering the former a better adsorbent for the removal of cefuroxime axetil than the latter (Table 5). In this case, the K value for micelle–clay is more than

Table 5. Langmuir adsorption parameters (K and Q_{max}) and the correlation coefficient (R^2) values obtained from the adsorption of amoxicillin trihydrate and cefuroxime axetil on a micelle–clay complex and activated charcoal.

		Langmuir		
Pharmaceutical	Adsorbents	K (L mg^{-1})	Q_{max} (mg g^{-1})	K•Q_{max}(L g^{-1})
Amoxicillin trihydrate	Micelle–clay complex	0.229 ± 0.005	90.91 ± 0.86	20.8
	Charcoal	0.158 ± 0.003	100 ± 0.35	15.8
Cefuroxime axetil	Micelle–clay complex	0.271 ± 0.01	31.25 ± 0.65	8.5
	Charcoal	0.122 ± 0.005	26.31 ± 0.70	3.2

Notes: Results of K and Q_{max} are reported as value ± SD; SD, standard deviation of three replicates. Values of R^2 were 0.985, 0.997, 0.999, and 0.981 for the four rows in the table, respectively.

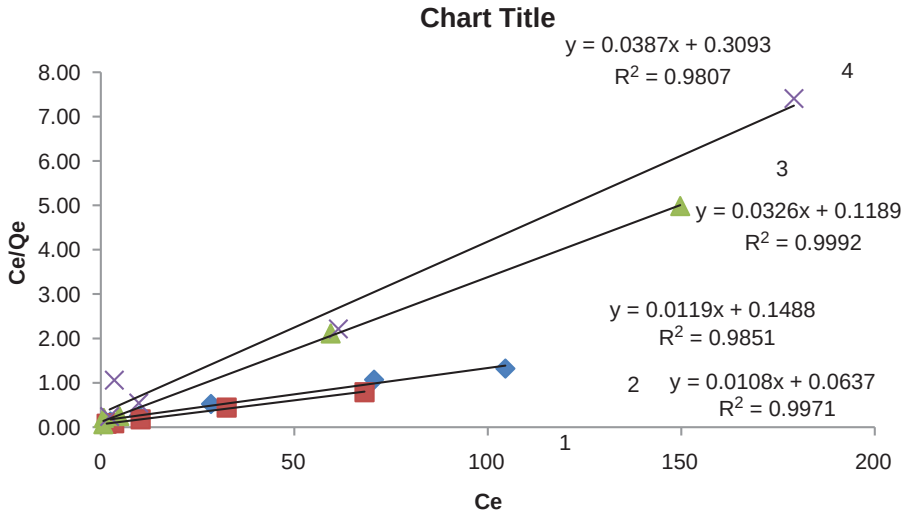

Figure 5. Langmuir isotherms for the removal of amoxicillin trihydrate by micelle–clay complex and by activated charcoal (plots 1 and 2, respectively), and for the removal of cefuroxime axetil by micelle–clay and by activated charcoal (plots 3 and 4, respectively) (pH 8.2, 25°C).

twofold larger than for charcoal. We added another column to Table 5, $K \bullet Q_{max}$ (L g^{-1}), which can give an easy first-order indication for the efficiency of adsorption. The rationale is that this is the quantity which is determined most reliably by analysis of the Langmuir equation, that is, in many cases the analysis can yield excessively large value of K at the expense of a small value for Q_{max}, or vice versa. This column indicates that the order of efficiency of adsorption is micelle–clay > charcoal and the adsorption of amoxicillin trihydrate is more efficient than that of cefuroxime axetil. The values of K for both antibacterials adsorbed by the micelle–clay complex are larger than that of another pharmaceutical, diclofenac (0.07 L mg^{-1}.[23]

Another form of presentation of K values is by the unit L mol^{-1}.[29] This presentation yields K values of about 96,000 and 138,000 (L mol^{-1}) for the adsorption of amoxicillin trihydrate and cefuroxime axetil by the micelle–clay complex, respectively, which are at the top of K values listed elsewhere.[25]

3.6. *Column experiments*

One thousand millilitres of amoxicillin trihydrate and cefuroxime axetil (100 ppm) were eluted in triplicate through column filters. The results (Table 6) indicate that a filter which includes the micelle–clay complex (ODTMA)-montmorillonite or charcoal may be efficient in purifying water from amoxicillin trihydrate and cefuroxime axetil, but the efficiency is somewhat larger in the former case. However, the flow rate employed and the volumes passed were rather small. Accordingly we extended the filtration results and applied modelling.

Table 7 presents the results of filtration of amoxicillin trihydrate solution at an average concentration of 7.7 ppm by two column filters in series, where each filter included 13 g of activated carbon mixed with 650 g of sand. The flow rate was 50 mL min^{-1}, that is, 25-fold larger than in Table 6, and the volume passed was 12-fold larger. On the other hand, the concentration of amoxicillin trihydrate was 14-fold smaller than in Table 6. The results demonstrate the effect of the flow rate (or rather flow velocity) on reducing the efficiency of removal of the pharmaceutical. In this context, we point out that the model [26] and experimental verification demonstrate that doubling the filter length yields at least the same capacity of the filter (in terms of volume passed per kg of the active material), but at a twofold larger flow velocity. The calculations employed the parameters $R_0 = 0.13$ M for the molar concentration

Table 6. Removal of amoxicillin trihydrate and cefuroxime axetil by filtration of its solution (100 ppm) through a laboratory filter, which included either a micelle–clay complex or activated carbon mixed with excess sand at 1:25 (w/w).

Pharmaceutical	Volume filtered (mL)	Concentration (ppm)	Column type	Emerging concentration (ppm)	Removal %
Amoxicillin trihydrate	1000	100	Micelle–clay	0.5 ± 0.1	99.5
	1000	100	Activated carbon	1 ± 0.2	99
Cefuroxime axetil	1000	50	Micelle–clay	2.10 ± 0.3	95.8
	1000	50	Activated carbon	3.5 ± 0.2	93

Table 7. Removal of amoxicillin trihydrate by two filters in series, which included each 13 g of activated carbon mixed with 650 g of sand.

Volume of solution passing through the filter (L)	Column no.	Per cent of antibacterial emerging from the first and second columns[a]	
		Exp.	Cal.
3	1	47 ± 6	47.5
	2		73.1
6	1	44 ± 6	44.9
	2	71 ± 4	70.5
9	1	41 ± 8	42.5
	2		68.0
12	1	36 ± 8	40.0
	2	67 ± 5	65.6

Notes: $D_1 = 10^{-3}$ min^{-1}. Average concentration of amoxicillin trihydrate was 7.7 ppm. The flow rate was 50 mL min^{-1}.
[a]The values of parameters used in Equation (2) were $R_0 = 0.13$ M; $C_1 = 25$ M^{-1} min^{-1}.

of adsorbing sites; $C_1 = 25$ M^{-1} min^{-1} for the forward rate constant of adsorption and $D_1 = 10^{-3}$ min^{-1} for the rate constant of desorption. The statistical analysis yielded RMSE = 1.8 and $R^2 = 0.979$.

For a passage of 5 ppm of amoxicillin trihydrate solution at a flow rate of 50 mL min^{-1}, a filter which included 13 g of micelle–clay complex yielded complete removal of the pharmaceutical after 9 L. In this case, the values of the parameters employed were: $R_0 = 0.026$ M; $C_1 = 80$ M^{-1} min^{-1} and $D_1 = 10^{-3}$ min^{-1}. In comparison, filtration of cefuroxime axetil (8 ppm) under the same conditions yielded only 27% removal after 9 L by the first column.

In reference [21] we demonstrated that the model simulations of filtration of diclofenac in the ppm range enabled prediction for the ppb range. Tentative calculations yielded that an exclusively filled micelle-clay filter (by granules) whose length is 80 cm can purify a solution which includes 100 ppb of amoxicillin trihydrate to an emerging level of 0.1 ppb at a capacity of 7.5 m^3 per one kg of the complex.

4. Conclusions

The results of sequential WWTP, which includes ultrafiltration, activated carbon and RO, showed that ultrafiltration and activated carbon were not sufficient for removing either amoxicillin trihydrate or cefuroxime axetil to a safe level, but addition of RO enabled their complete removal. On the other hand, the spiked concentrations were rather large. Adsorption studies on micelle–clay complex (ODTMA) and charcoal revealed that both adsorbents are efficient for the removal of amoxicillin trihydrate and cefuroxime axetil from synthetic water. However, the former was much more efficient, presumably due to a relatively high affinity for adsorption of the anionic antibacterials amoxicillin trihydrate and cefuroxime axetil by the relatively large number of positively charged and hydrophobic sites of the micelle–clay complex of ODTMA.

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Disclosure statement

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