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Mitigating the impact of antineoplastic drugs on aquatic environment: nanofiltration as a promising tertiary wastewater treatment

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Abstract Pharmaceuticals known as antineoplastic agents are increasingly prescribed in the fight against cancer. This is causing some concern in the scientific community due their poor treatment in many conventional wastewater treatment plants and poor environmental biodegradability, posing a potential risk to eukaryotic organisms. Finding treatment solutions to prevent the entry and buildup of these compounds in the environment becomes urgent. In this study, ten antineoplastic drugs and one corticosteroid, five of which had never been examined before, were monitored in a real wastewater effluent and their removal using a nanofiltration pilot scale unit with a Desal 5DK membrane was evaluated. Average removals of 68±23% were attained for the eleven compounds, being the lowest one 30±10% for mycophenolate mofetil and the highest one 98.3±0.4% for megestrol. Except for cyclophosphamide, for which a high risk was estimated in the permeate, the remaining risks determined in the permeate for aquatic organisms from receiving bodies were low (capecitabine, mycophenolic acid and flutamide) or null. Phytotoxicity tests revealed that the permeate appears to have a lower negative impact on the growth of *Sinapis alba* plants' roots than the feed stream.

Keywords: wastewater effluents, anticancer drugs, cytostatic drugs, nanofiltration, toxicity screening

1. Introduction

The incidence of cancer has been increasing, and it is predicted that it will climb by around 21% through 2040. 2.68 million new cases of cancer were identified in European Union in 2020 [1]. Chemotherapy, which involves the administration of antineoplastic drugs, is the most widely used cancer treatment. Antineoplastic drugs can obstruct cell division in a variety of ways, by affecting a cell's metabolism or DNA [2, 3]. Although these drugs are used to treat cancer, they have long-term negative effects, such as secondary cancers [4]. Hence, the interaction with antineoplastic drugs should be limited to those who truly require them, thus preventing the exposure of healthy lives to these medications.

After administration, antineoplastic drugs are metabolized and excreted (either in their parent form or after being metabolized) and discharged into the sewage system as any other pharmaceutical. Their presence in environmental waters has been reported widely [5, 6] due to their poor removal by conventional treatments used in wastewater treatment plants (WWTPs). Some studies have already shown that there is a risk for aquatic organisms associated with the presence of antineoplastic drugs in surface waters [7]. Domestic effluents are the main source of contamination because these compounds are typically administered to outpatients [8, 9]; as a result, it is crucial to implement effective removal technologies for the elimination of antineoplastic drugs and other pollutants in

WWTP effluents to prevent their release to the environment.

Reverse osmosis and nanofiltration, with molecular weight cut offs (MWCO) ranging from 100 to 1000 Da, are the most suitable membranes to use for antineoplastics' removal because their MW typically ranges from 100 to 900 Da [10]. Their benefits include: (i) high removal rates of low molecular-weight (MW) organic pollutants; (ii) their integration with other treatment technologies in WWTPs; (iii) their ability to remove some compounds that are recalcitrant [11, 12]. The use of membrane-based processes for the removal of antineoplastic drugs from waters was reviewed by the authors in a previous study [12]; removals for cyclophosphamide, paclitaxel, capecitabine, fluorouracil, and cytarabine ranged from 35% to >95-100% [13-15]. While these treatment processes have been demonstrated to be viable technologies to use in WWTPs to remove antineoplastic drugs, information is lacking for some of the more dangerous and often used antineoplastic drugs (e.g., mycophenolic acid, mycophenolate mofetil or bicalutamide). To the best of the authors' knowledge, there is only one study that evaluated the performance of a pilot-scale nanofiltration unit in the removal of six antineoplastic drugs (capecitabine, cyclophosphamide, etoposide, ifosfamide, paclitaxel, and tamoxifen) present in wastewaters at realistic concentrations [14].

Triplicate nanofiltration experiments conducted over the course of three different days were performed in this work, aiming to investigate the effectiveness of a pilot-scale nanofiltration unit in the removal of ten antineoplastic drugs and one corticosteroid present in a secondary effluent at occurrence levels (without spike). Mycophenolic acid, bicalutamide, mycophenolate mofetil, megestrol, and tamoxifen are some of the selected antineoplastic drugs for which there are currently no studies available, despite the fact that their presence in water bodies has been linked to potential health risks for both humans and aquatic lives [7]. Additionally, in accordance with the guidelines for environmental risk assessment of pharmaceuticals, the effects of the unspiked nanofiltration matrices on plants and/or aquatic biota were estimated through: (i) phytotoxicity tests (thinking about the reuse of wastewaters for irrigation of land and crops), and (ii) determination of risk quotients (***RQ***) for aquatic organisms in receiving bodies (thinking about the discharge of the effluents) [16]. This study advances our understanding about treatment processes able to remove pharmaceuticals from wastewaters that may pose a threat to the environment.

2. Materials and methods

2.1. Chemicals and reagents

Bicalutamide, capecitabine, cyclophosphamide, flutamide, ifosfamide, megestrol, mycophenolate mofetil, mycophenolic acid, paclitaxel, prednisone and tamoxifen analytical standards of 98–99% purity, used in the calibration curve and validation experiments, were acquired from Sigma-Aldrich (St. Louis, USA) and Cayman Chemical Company (Ann Arbor, USA). Although prednisone is not considered an antineoplastic drug, it was added to this work since it is prescribed/administered in combination with several antineoplastic drugs during cancer treatment. Methanol, acetonitrile, isopropanol, Milli-Q water and ammonium acetate were supplied by Merck (Darmstadt, Germany). All solvents used were of LC–MS grade. Mycophenolic acid-d3 (MPA-d3) and cyclophosphamide-d4 (CYC-d4) were used as internal standards; both were acquired from Sigma-Aldrich (St. Louis, USA). Stock standard solutions were prepared at a concentration of 1000 mg/L in methanol, except paclitaxel that was prepared in acetonitrile. Working solutions were prepared at 10 mg/L in methanol, except paclitaxel that was prepared in acetonitrile. Formic acid and hydrochloric acid 1 M used for pH adjustment, were purchased from Sigma-Aldrich (St. Louis, USA). SPE cartridges, Oasis HLB (6 cc, 200 mg), were purchased from Waters (Milford, USA). Nylon membrane filters (Whatman 0.8 and 0.45 μm), used for sample filtration, were acquired from Sigma-Aldrich (St. Louis, USA).

2.2. Nanofiltration experiments and analysis of antineoplastic drugs by SPE-LC-MS/MS

Three nanofiltration studies were conducted between February and May 2022, using real effluents (without fortification or spiking of the target analytes) that were collected at an urban WWTP prior to river discharge. During the tests, a spiral wound Desal 5DK module was used as the nanofiltration membrane (model DK4040F30, Suez membranes, Lenntech, Delfgauw, Netherlands). This thin film composite membrane has a MWCO of 150-300 Da, a minimum $MgSO_4$ rejection of 98%, and an active surface area of 7.9 m^2, according to the manufacturer. Cristóvão et al., 2022 [14] defined the best operating conditions that minimized fouling for the pilot-scale nanofiltration unit using the same matrix. The two matrices from the nanofiltration process (the original feed and final permeate) were gathered in triplicate and extracted in quadruplicate. Two filtration processes were necessary for sample preparation, using 0.8 μm and 0.45 μm nylon membrane filters [6]. Then, 1 M of HCl was used to acidify the samples to a pH of 2. After being extracted by solid-phase extraction (SPE) and evaluated by liquid chromatography-tandem mass spectrometry (LC-MS/MS), the rejection of each antineoplastic drug in the nanofiltration unit was determined using Eq. 1:

$$\%\ Rejection\ = \left(1 - \frac{C_p}{C_f}\right) \times 100 \qquad \text{Eq. 1}$$

where $\boldsymbol{C_p}$ and $\boldsymbol{C_f}$ are the concentrations of the target antineoplastics or corticosteroids in the permeate and feed of the nanofiltration system, respectively.

The SPE and LC-MS/MS conditions are detailed in Gouveia et al., 2022 [6]. Validation of the methodology (method detection limits, recoveries and intra- and inter-day precisions) were also conducted as ascribed in previous works [6].

2.3. Toxicity screening and estimation of the risk

Using the MicroBioTests Inc. PHYTOTOXKIT for liquid samples, the toxicity of the two matrices was assessed. These investigations were carried out in accordance with

the steps suggested by MicroBioTests Inc., determining the percentage reduction of seed germination and the growth of plant roots and shoots in the examined matrices in comparison to the control (distilled water). *Lepidium sativum*, *Sinapis alba*, and *Sorghum saccharatum*, three distinct plant species, were utilized in this investigation.

The process began with the germination of the seeds, which were then grown for 72 hours at 25 ºC with 20 mL of each matrix added to quadruplicate tests with three seeds of each specie. Then, using ImageJ software, the quantity of seeds that had germinated as well as the size of the roots and shoots were determined. For calculating the percentage effect (%), Eq. 2 was used:

$$\%\ Percentage\ effect\ = \frac{(A-B)}{A} \times 100 \qquad \text{Eq. 2}$$

being A the number of germinated seeds or the length of the roots/shoots in the control sample and B the number of germinated seeds or the length of the roots/shoots in the studied matrices (feed and permeate).

Risk quotient (***RQ***) was assessed to determine whether the amounts of each antineoplastic observed in each matrix pose a harm to aquatic biota, particularly if employed for aquaculture purposes. ***RQ*** was determined using the ratio of the PNEC (Predicted No Effect Concentration), a number obtained from published toxicological data by applying an assessment factor, to the average concentration of each component measured in the matrix (MEC). The risk quotient was then clarified using a recognized criterion [17], where RQ≥1 denotes high risk, 0.01≤RQ<0.1 denotes low risk, and 0.1≤RQ<1 denotes moderate risk for aquatic biota.

3. Results and discussion

3.1. Presence and rejection of antineoplastic drugs in the nanofiltration pilot unit

In the feed of at least two of the three nanofiltration studies, all pharmaceuticals under investigation were found. The antineoplastic drugs that were found at higher concentrations in the feed of the nanofiltration unit were bicalutamide, megestrol, mycophenolic acid, and the corticosteroid prednisone, with concentrations equal to or above 40±6 ng/L (concentration of megestrol in the first experiment), with the highest one being 127±20 ng/L for mycophenolic acid in the third experiment. Even though the chemicals were present in the permeate at significantly lower proportions, all of them were still found there. Mycophenolic acid (16 ng/L to 24.9 ng/L), prednisone (20 ng/L to 38 ng/L), and bicalutamide (14 ng/L to 24 ng/L) were the substances detected in the permeate matrix at higher concentrations.

Regarding nanofiltration rejections, excluding flutamide, for which negligible rejections were achieved, the average rejection for all the other target antineoplastic drugs was relatively good: 68±23%, being the lowest one obtained for mycophenolate mofetil, 30±10%, and the highest one 98.3±0.4% for megestrol. Up to authors' best knowledge, there are no studies published that describe the removal of mycophenolate mofetil, megestrol, bicalutamide, mycophenolic acid or tamoxifen from liquid matrices by nanofiltration.

Variations in molecular weight, hydrophobicity and charge of the chemicals may affect the antineoplastic-membrane interactions, resulting in removals that differ from those anticipated. Table 1 represents the chemical characteristics of each drug, the expected rejections and the real rejections that were achieved in a schematic form.

Table 1 – Molecular weight, hydrophobicity, charge at neutral pH and rejections of the studied compounds.

Pharmaceuticals	Molecular weight (g/mol)	Hydrophobicity	Charge at neutral pH	Expected rejection	Real rejection	Average rejection±SD
Bicalutamide	430.4	Hydrophobic	0	●●	●	64±9
Capecitabine	359.4	Hydrophilic	0	●	●	89±7
Cyclophosphamide	261.1	Hydrophilic	0	●	●	53±36
Flutamide	276.2	Hydrophobic	0	●●	●	N/D
Ifosfamide	261.1	Hydrophilic	0	●	●	58±30
Megestrol	342.5	Hydrophobic	0	●●	●	98.3±0.4
Mycophenolate mofetil	433.5	Hydrophilic	0	●●	●	30±10
Mycophenolic acid	320.3	Hydrophilic	-	●	●	78±7
Paclitaxel	853.9	Hydrophobic	0	●●	●	65±6
Prednisone	358.4	Hydrophilic	0	●	●	60±10
Tamoxifen	371.5	Hydrophobic	+	●	●	82±10

Note: Rejections: ● → low; ● → moderate; ● → high; ● → very high; N/D- not defined.

Contrarily to what was expected, mycophenolate mofetil was poorly removed by nanofiltration (30±10%). These results may have also been influenced by elements like the chemistry of the feed and interactions between mycophenolate mofetil and other elements of the matrix. The elongated geometry of this compound, with a depth around 0.502 nm, may also favor its diffusion through the free volume of the membrane polymer top layer, interfering with final rejections.

3.2. Toxicity screening and estimation of the risk

Regarding phytotoxicity assays, statistics were used to compare the lengths of the species' roots and shoots to the control, and it was found that only the roots of *Sinapis alba* in feed matrix developed significantly less than controls. This indicates that, barring this one exception, the matrix utilized had no adverse effects on the growth of any other seeds.

Concerning risk assessment results, it can be concluded that since nanofiltration reduced the concentration of all the examined antineoplastic drugs, compounds' potential risk was also reduced. However, given the concentrations found in the permeate, four antineoplastics —capecitabine (low risk), flutamide (low risk), mycophenolic acid (low risk), and cyclophosphamide (high risk) —may still pose some damage to aquatic lives. These results are of utmost relevance as they highlight the need to develop efficient strategies for removing harmful chemicals from

wastewaters, considering the increased predisposition for wastewater reuse, particularly in agriculture and aquaculture purposes.

4. Conclusions

Bicalutamide, megestrol, mycophenolic acid, and prednisone were the antineoplastic drugs detected at higher concentrations in wastewater effluent samples (up to 127±20 ng/L for mycophenolic acid).

The nanofiltration system's average rejection for the eleven pharmaceuticals was 68±23%, being the lowest one for mycophenolate mofetil (30±10%) and the highest one for megestrol (98.3±0.4%).

According to phytotoxicity studies, the permeate appears to have less effect on the growth of *Sinapis alba* plants' roots than the feed streams. After nanofiltration treatment, the risk of adverse effects on aquatic life from receiving water bodies is reduced. However, there is still a potential risk associated with exposure to some of the studied compounds (e.g. cyclophosphamide). These results demonstrate the effectiveness of the nanofiltration method in reducing the pollution charge and toxicity of WWTP effluents, although a post-treatment is still recommended if a complete reduction in toxicity is desired.

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