Efficiency of RO/NF membranes at the removal of veterinary antibiotics
D. Dolar, A. Vuković, D. Ašperger and K. Košutić

ABSTRACT

The production of pharmaceuticals has increased rapidly during the last several decades as they have been used for the health of both humans and animals. Routes of environmental exposure include the release of treated wastewater, the land disposal of livestock manures and municipal biosolids (i.e. sewage sludge), as well as the use of medicated aquaculture feed. This study deals with application of reverse osmosis (RO) and nanofiltration (NF) membranes for removing of antibiotic residues (sulfamethoxazole, trimethoprim, ciprofloxacin, dexamethasone and febantel) and their mixture. According to the results obtained in this work the use of RO (LFC–1 and XLE) and the tight NF (NF90) membranes are recommended to achieve a high level of retention (>95%) of all selected veterinary antibiotics (VAs). Nanofiltration NF270, NF and HL membranes showed a lower rejection of individual components, but much higher in a mixture solution, due to the synergistic effect.

Key words | removal efficiency, RO/NF membranes, veterinary antibiotics, water treatment

INTRODUCTION

It is now well established that pharmaceuticals are ubiquitous contaminants of wastewater effluents and amongst all the emerging pollutants, pharmaceuticals are those which have become of increasing concern. This is due to the possible appearance of resistant bacterial cultures as a consequence of their extensive use. Sources of pharmaceuticals in the environment are mainly due to the pharmaceutical industries, hospitals, households, and the disposal of expired or unused medicine, aquaculture and animal feeding (Shao et al. 2009). Veterinary antibiotics (VAs) belong to the biological class of pharmaceuticals and they are used to treat disease and to protect the health of animals. According to a report by the Union of Concerned Scientists (UCS), 16 million kg of antibiotics are used annually in the USA, and approximately 70% of them are used for non-therapeutic purposes. Antibiotic use in animal feeding has increased from nearly 91,000 kg in 1950 to 9.3 million kg in 1999. In the EU during the 1990s, a total usage of 5 million kg of antibiotics was reported (Zhao et al. 2010). Therefore, the presence of antibiotics in wastewater has increased in recent years and their abatement is a challenge.

Recently, a growing number of studies worldwide have shown the presence of a number of different VAs in animal wastes, surface water and ground water river sediments and in soils at concentrations that could have potential impacts on the ecosystem (Zhao et al. 2010). More often, these compounds occur at ng L⁻¹ or μg L⁻¹ concentrations (Batt et al. 2007; Christensen et al. 2009; Sim et al. 2010) and they pose potential risks, such as endocrine disruption and severe effects to the aquatic ecosystem. As they originally possess specific biological features, they are hardly biodegradable and thus give rise to pharmaceutical pollution which is an emerging environmental issue worldwide. They are commonly derived from municipal, agricultural and industrial wastewater sources and pathways, due to the often inadequate treatment in conventional wastewater treatment plants (WWTPs) (Brown et al. 2006; Karthikeyan & Meyer 2006; Batt et al. 2007; Gebhardt & Schröder 2007; Avisar et al. 2010). As a result, some of those micropollutants will be discharged into the receiving body of water or be used for irrigation, and may result in ground water pollution (Avisar et al. 2009). Membrane filtration processes using reverse osmosis (RO) and nanofiltration (NF) showed the excellent removal (>90%) of different pharmaceuticals (Kim et al. 2007; Watkinson et al. 2007; Radjenović et al. 2008). RO and NF are
recognized as the leading water treatment technologies for the 21st century, due to their high separation effectiveness and environmental acceptability. The intensive research and development of membrane preparation in the last decade has resulted in their reduced market prices and application in various fluid treatments. In this way RO and NF membranes have become competitive in clean technology of water treatment.

In this paper two RO and four NF membranes were used for the laboratory investigation of their efficiency in the removal of five different veterinary antibiotics (VAs).

### METHODS

#### Materials and standards

Chemical standards for trimethoprim (TMP), dexamethasone (DEXA) and febantel (FEBA) were purchased from Veterina (Kalinovica, Croatia), ciprofloxacin (CIPRO) from Pliva (Zagreb, Croatia) and sulfamethoxazole (SMETOX) from Sigma (Steinheim, Germany). All antibiotic standards (Table 1) used were of high purity grade (>93.6%).

Single solute (one compound dissolved in Milli-Q water) of individual VA standards (10 mg L\(^{-1}\)) were prepared in

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Structure</th>
<th>(M_w) (g mol(^{-1}))</th>
<th>p(K_a)^a</th>
<th>log (K_{ow})^b</th>
<th>(d_c) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfamethoxazole</td>
<td><img src="structure1.png" alt="Structure" /></td>
<td>253.28</td>
<td>p(K_1) 1.83</td>
<td>0.89</td>
<td>0.734</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p(K_2) 5.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim</td>
<td><img src="structure2.png" alt="Structure" /></td>
<td>290.32</td>
<td>p(K_1) 3.23</td>
<td>0.91</td>
<td>0.779</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p(K_2) 6.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin (\times) HCl (\times) H(_2)O</td>
<td><img src="structure3.png" alt="Structure" /></td>
<td>331.35</td>
<td>p(K_1) 6.68</td>
<td>0.28</td>
<td>0.826</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p(K_2) 8.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dexamethasone</td>
<td><img src="structure4.png" alt="Structure" /></td>
<td>392.46</td>
<td>n.a.</td>
<td>1.94</td>
<td>0.889</td>
</tr>
<tr>
<td>Febantel</td>
<td><img src="structure5.png" alt="Structure" /></td>
<td>446.48</td>
<td>n.a.</td>
<td>1.53</td>
<td>0.941</td>
</tr>
</tbody>
</table>

^\(pK_a\) values were retrieved from Babić et al. (2007).

^log \(K_{ow}\) values are for neutral molecule form; based upon REPHAD Database: http://sunasce.com/fkit/rephad.php (accessed 25 July 2010).
Milli-Q water, with the exception of FEBA, which was first dissolved in a very small amount of ethanol due to its poor solubility in water. Two mixtures were also prepared, one was SMETOX and FEBA, and the second one consisted of all five antibiotics.

**Reverse osmosis and nanofiltration waterworks**

The removal of VAs was investigated in the laboratory set-up as described previously ([Dolar et al. 2014](https://iwaponline.com/wst/article-pdf/65/2/317/442220/317.pdf)). Six home-made membrane cells of the same type and dimensions were connected parallel. The surface area of the membranes was 10.75 cm². Six commercially available RO and NF membranes were tested at a working pressure of 15 bar and cross-flow velocity of 300 mL min⁻¹. Reverse osmosis membranes were XLE and LFC–1 and NF membranes NF90, NF270, NF and HL. Preserved membranes were washed first with demineralized water and then pressurized at 20 bar for 5 h. After the pressure pretreatment, the pure water flux of membranes was measured.

Conditions for determining pore size and pore size distributions (PSDs) in the selective membranes’ layer, have been described previously ([Košutić et al. 2006](https://iwaponline.com/wst/article-pdf/65/2/317/442220/317.pdf)).

**Chemical analysis**

The concentrations of inorganic salts (sodium and calcium chloride, $c_i = 300$ mg L⁻¹) were determined by a conductometer (SCHOTT Instruments Lab 960, Germany), and those for markers (total carbon, TC) were determined by a carbon analyzer (Shimadzu TOC-VWS, Japan). Markers are listed in the previous paper by Košutić et al. ([2006](https://iwaponline.com/wst/article-pdf/65/2/317/442220/317.pdf)). Concentrations of sulfates were determined with an ion chromatograph (Dionex ICS-3000, USA).

The analyses of VAs were performed using a Varian ProStar 500 (Walnut Creek, California, USA) HPLC system consisting of a ProStar autosampler, ProStar 230 tertiary pump system, ProStar 330 diode array detector, and thermostatted column compartment. The column temperature was set to 30 °C and the injection volume was 30 μL. C18 Synergy Fusion 150 mm × 4.6 mm, particle size 4 μm column (Phenomenex) was used to separate all investigated compounds in the mixture. The mobile phase used in the chromatographic separation consisted of a binary mixture of solvents A (0.01% formic acid in water) and B (0.01% formic acid in acetonitrile). A simultaneous mobile phase gradient program was used: the elution started with a 2.5 min linear gradient from 100% A, to 8% B, followed by a 3.5 min linear gradient to 10% B, a 5 min linear gradient to 50% B, a 4 min linear gradient to 60% B, and finally a 3 min linear gradient to 95% B which was maintained for 10 min and then a 0.1 min linear gradient back to 100% of A. The flow rate was 0.5 mL min⁻¹. The separation was monitored at an absorbance wavelength of 254 nm.

**RESULTS AND DISCUSSION**

**Membrane characterization**

Rejection factors, $R$ of inorganic salts, NaCl, CaCl₂ and MgSO₄, are displayed in Table 2. $R_{\text{NaCl}}$ and $R_{\text{CaCl}_2}$ are different for different NF membranes; low for loose (NF, HL and NF270) and relatively high for dense (NF90) membranes, and rejections of magnesium sulfate are in the agreement with manufacturers data. The appreciably higher rejection factor for sodium and calcium chloride by the RO rather than the NF (except NF90) membranes are evident. In the terms of RO membranes, there is little difference between the rejections of sodium and that of calcium chloride while the differences of their rejection factors regarding NF membranes is significant. Both facts point to differences in the porosity of active layer of the examined membranes, which have been shown in PSD curves (Figure 1).

**Table 2**

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>$R_{\text{NaCl}}$ (%)</th>
<th>$R_{\text{CaCl}_2}$ (%)</th>
<th>$R_{\text{MgSO}_4\times7\text{H}_2\text{O}}$ (%)</th>
<th>$J_w$ (L m⁻² h⁻¹)</th>
<th>$N_{\text{eff}}$ (10¹⁶ m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFC–1</td>
<td>96.5</td>
<td>98.1</td>
<td>99.7</td>
<td>38.38 ± 2.18</td>
<td>0.874</td>
</tr>
<tr>
<td>NF90</td>
<td>89.9</td>
<td>98.0</td>
<td>99.4</td>
<td>77.92 ± 6.05</td>
<td>1.413</td>
</tr>
<tr>
<td>NF270</td>
<td>13.6</td>
<td>28.1</td>
<td>90.7</td>
<td>109.16 ± 18.82</td>
<td>1.188</td>
</tr>
<tr>
<td>NF</td>
<td>24.6</td>
<td>37.3</td>
<td>98.2</td>
<td>98.53 ± 14.57</td>
<td>1.004</td>
</tr>
<tr>
<td>XLE</td>
<td>89.5</td>
<td>96.4</td>
<td>99.2</td>
<td>69.38 ± 11.00</td>
<td>1.199</td>
</tr>
<tr>
<td>HL</td>
<td>27.1</td>
<td>82.9</td>
<td>94.1</td>
<td>88.48 ± 7.05</td>
<td>0.758</td>
</tr>
</tbody>
</table>
PSDs of all the NF membranes are located at clearly wider pores, which are responsible for the lower sodium chloride retention. Thus, the size exclusion mechanism is prevailing. The higher rejection of calcium chloride than NaCl observed with NF membranes is the consequence of the additional charge exclusion which resulted from the repelling action of the NF membranes’ electric charge on the divalent ions. Membrane XLE had slightly less rejection of sodium chloride than expected but it can be explained by the higher pore size as shown by the PSD curve.

There are few transport models for RO/NF: the solution-diffusion, irreversible thermodynamic model and the fine-pore model. According to Matsuura (1995) the fine-pore model is the most practical and typical and was used in this work. The newest model taking into account influence of solute-membrane affinity (Verliefde et al. 2009) could not be used because authors did not have data for solute and membrane surface tensions. The pore size and PSDs were determined for all membranes via the modified examination method, based on the specific solute’s (markers) transport according to Košutić et al. (2000). The results are presented in Figure 1 and there are noticeable differences in the PSD curves between RO and NF membranes. The PSDs for RO membranes are unimodal, with most pores of the size 0.78 nm (LFC–1) and 0.88 nm (XLE). The pores are smaller than 1 nm, and it is confirmation that these are RO membranes. The PSDs of two tight NF membranes have their main peak at a similar pore size, 0.79 nm for the NF90, and 0.70 nm for the NF270 type membrane. In the case of the NF270 type membrane, there is also an additional peak at 1.56 nm, making distribution similar to those of the loose NF membranes. Hence, loose NF membranes show bimodal PSD curves with two clearly separated peaks. The pores with the highest incidence in this case are those of 0.72 nm (NF) and 0.73 nm (HL) followed by large pores, sized between 1.32 and 2.03 nm.

**Removal of veterinary antibiotics**

Rejection \( R \) of each investigated compound was calculated as:

\[
R(\%) = \frac{c_f - c_p}{c_f} \times 100 \%
\]

where \( c_f \) and \( c_p \) are concentrations (mg L\(^{-1}\)) of a compound in feed and permeate stream, respectively. The rejections of the selected veterinary antibiotics in laboratory scale RO and NF treatment are depicted in Figure 2.

As systematized by Bellona et al. (2004), rejection of solutes on RO/NF membranes will be affected by solute and membrane properties, feed composition and operating conditions. Solute can be rejected on RO and NF membranes by one mechanism, or a combination of three basic mechanisms: size exclusion (siee steric effect), charge exclusion (electrical, Donnan) and physico–chemical interactions between solute, solvent and membrane.

In the last column of Table 1, the values for the effective diameter of an organic component in water \( (d_c)\) are presented. The relationship between the molecular weight, \( M_w \) of an organic component and its effective diameter in water (Geens et al. 2006) is:

\[
d_c = 0.065(M_w)^{0.438}
\]

Although molecular weight is not a direct measure of the molecule dimension, it still reflects the molecular size, and it is a readily accessible parameter. This correlation is valid for the molecular weight range where nanofiltration typically operates (up to ±600 Da) (Van der Bruggen & Vandecasteele 2002). It was an expected rejection increase, with the increase of effective diameter for all investigated membranes.

The smallest component (SMETOX) has \( d_c \) of 0.734 nm and it is smaller than the pores of all investigated membranes (from 0.73 up to 2.03 nm). Therefore, it was not completely removed from the water (15–97%) and rejection increased with the decreasing of the pore size of the membranes. The smallest difference between pore sizes of LFC–1 and NF90 membranes and diameter of SMETOX
resulted in their highest rejection rates. TMP has a similar diameter to SMETOX and the rejections were almost the same. A slightly lower rejection of SMETOX and TMP with the XLE membrane can be explained by its having bigger pores than LFC–1 and NF90 (Figure 1). Diameter of CIPRO, DEXA and FEBA is 0.826, 0.889 and 0.941 nm,
respectively and are higher than pore sizes of RO and NF90 membranes, which resulted in their complete removal.

Due to the effect of compound size and pore size (steric hindrance mechanism), rejection increases more rapidly for bimodal membranes (NF270, NF and HL). The PSD curves showed second pick or pores higher than 1.4 nm. The number of these pores was small but still they have an influence on the rejection of VAs and membrane flux. This can be seen for SMETOX, TMP and CIPRO. The NF270 membrane had the smallest incidence of pores located at 0.70 nm and therefore the smallest rejection for SMETOX (15%). The rejection for SMETOX was similar for NF and HL membranes, 29 and 24%, respectively. The PSD curves were also very similar but this small difference can be attributed to a narrower first pick for the HL membrane.

The increase of rejection with the increase of molecular weight, i.e. effective diameter in water, shows the effect of the steric hindrance mechanism, i.e. rejection of larger molecule is higher.

The mixture of SMETOX and FEBA and the mixture of all VAs (Figure 2) show a higher level of rejection than that of the single solute. This proves the positive synergy (an effect arising between two or more compounds that produces an effect greater than that of their individual effects) concerning the rejection effectiveness. This might primarily be attributed to a hydrogen bonding ability between solutes in the feed solution caused by the amino-hydroxyl- and keto-groups presented in the solute molecules. This resulted in a partial solute molecules aggregation and consequently higher separation results.

The results from this work showed the retention efficiency of selected VAs by different RO and NF membranes examined in a laboratory set-up. The next step of this work will be the investigation of the removal of VAs in different water matrices and also the feasibility of this work will be the investigation of the removal of VAs in real wastewater treatment. The membrane cleaning procedure, cleaning chemicals and the frequency of cleaning will be determined and optimized in a pilot plant.

2. A great removal of all VAs was achieved with RO (LFC, XLE) and tight nanofiltration (NF90) membranes and a good removal of bigger VA molecules was achieved with other NF membranes. The obtained results confirm the usability of the examined RO and NF membranes for the removal of veterinary antibiotics from water, especially the XLE and NF90 membranes, due to their high productivity.

5. According to rejection factors of veterinary antibiotics (in single and mixture solutions) as well as determined membrane characteristics, it can be concluded that the size exclusion prevail as the separation mechanism in the case of RO and tight NF membranes while the synergistic effect and physico–chemical interactions had an effect on loose NF membranes.

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