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Cyclophosphamide removal from water by nanofiltration and reverse osmosis membrane

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ABSTRACT

The rejection of cyclophosphamide (CP) by nanofiltration (NF) and reverse osmosis (RO) membranes from ultrapure (Milli-Q) water and membrane bioreactor (MBR) effluent was investigated. Lyophilization–extraction and detection methods were first developed for CP analysis in different water matrices. Experimental results showed that the RO membrane provided excellent rejection (>90%) under all operating conditions. Conversely, efficiency of CP rejection by NF membrane was poor: in the range of 20–40% from Milli-Q water and around 60% from MBR effluent. Trans-membrane pressure, initial CP concentration and ionic strength of the feed solution had almost no effect on CP retention by NF. On the other hand, the water matrix proved to have a great influence: CP rejection rate by NF was clearly enhanced when MBR effluent was used as the background solution. Membrane fouling and interactions between the CP and water matrix appeared to contribute to the higher rejection of CP.

Keywords:
Cyclophosphamide
Nanofiltration
Reverse osmosis
MBR effluent
Removal

1. Introduction

The occurrence of pharmaceuticals in wastewater effluent (Lishman et al., 2006; Vieno et al., 2007), drinking water sources (Kasprzyk-Hordern et al., 2008; Lindqvist et al., 2005) and even in some treated drinking waters (Kim et al., 2007; Loraine and Pettigrove, 2006), although often detected at trace levels (sub-ng/L), has raised substantial concern in public and regulatory agencies. Compounds with a very potent mechanism of action, such as cytostatic drugs, are of particular environmental concern because of the high potential risk and the possible chronic adverse effects. Cyclophosphamide (CP) is one of the very commonly used alkylating cytostatic drugs,

involved in the chemotherapy of various forms of cancer, in the treatment of autoimmune diseases and used as an immunosuppressant after organ transplantations (Grisolia, 2002). In the body, it is converted to active alkylating metabolites (phosphoramide mustards) through cytochrome P450 enzyme systems, which cause DNA adducts (cross-links) and prevent cell division. This mechanism of action also accounts for its adverse effects on living organisms, such as the mutagenic, carcinogenic, teratogenic, and embryotoxic effects described in the literature (Anderson et al., 1995).

CP exhibits poor biodegradability in the traditional activated sludge process of wastewater treatment plant (WWTP). For example, only 17% of CP was removed from the waste

stream in a laboratory scale activated sludge plant after 39 days of a continuous dosing experiment (Steger-Hartmann et al., 1997). Buerge et al. (2006) also reported no CP degradation in activated sludge incubation experiments within 24 h at a concentration of 100 ng/L. The low adsorption potential on activated sludge is also to be expected because of the low octanol–water partition coefficient of CP (log $K_{ow} = 0.63$). Ternes et al. (2004) found there was almost no CP adsorption on the primary activated sludge of WWTP.

The high resistance to biodegradation and low adsorption ability of CP indicate that this drug will be extremely persistent in aqueous environments. Meanwhile, taking into account the fact that the excretion of the unchanged parent molecule in patients is around 20–45% (Bagley et al., 1973), CP could be present in the surface water and ground water via hospital or WWTP effluent. For example, Zuccato et al. (2000) found 2–10 ng/L CP in the river Lombardy near Milan, Italy. Buerge et al. (2006) also detected 0.15–0.17 ng/L CP in the river Limmat, Switzerland. These exposure concentrations are several orders of magnitude lower than the concentrations at which acute ecotoxicological effects have been reported in the literature. However, limited knowledge is available on chronic health effects related to the consumption of drinking water containing trace amounts of pharmaceuticals or their metabolites (Johnson et al., 2008). In addition, the cytostatic drug removal efficiencies of conventional drinking water treatment processes, which were not designed to control these emerging micro-pollutants, are limited (Ternes et al., 2002). Therefore, it is unanimously accepted that preventing such molecules from entering the aquatic environment is essential. A precautionary approach is also desirable once these compounds emerge during wastewater reuse and drinking water production.

Nanofiltration (NF) and reverse osmosis (RO) are increasingly used for water treatment, because of their complete or nearly complete removal of a wide range of organic micro-pollutants (Van der Bruggen and Vandecasteele, 2003). Many researchers have evaluated the ability of several commercially available NF/RO membranes to trap various pharmaceuticals. Most of these studies try to explain the different mechanisms that play a role in compound removal by controlling the operating conditions or by comparing removal levels for various target molecules (Kiso et al., 2001; Nghiem et al., 2005). But the individual contributions of the influencing factors to membrane retention are not well identified. In addition, a broad range of pharmaceutical activated compounds (PhACs) were selected as target molecules for a study with NF/RO membrane (Verliefde et al., 2007). However, to the best of our knowledge, CP has never been included in the above research. Furthermore, very few studies have focused on the influence of natural water matrices on solute removal by membranes and the conclusions are conflicting in some cases. For example, Yoon et al. (2006) studied the removal of 52 endocrine disrupting compounds (EDCs) and PhACs by NF and UF membranes and found a decrease in compound rejection with an increase in natural organic matter (NOM) concentration. But Comerton et al. (2008) found that the rejection of 22 EDCs and PhACs by NF membrane was enhanced in the presence of organic matter when a natural water matrix was used. Nghiem and Hawkes (2007) reported that organic fouling

could both improve and lessen the retention of PhACs by NF membranes.

The objective of the present study was to investigate whether NF and RO membranes could be used to effectively remove CP from water at trace level, not only in the tertiary treatment of raw water but also as a post-treatment process of membrane bioreactor (MBR) in wastewater treatment, which exhibits insufficient elimination of CP and its metabolites as shown in a previous study (Delgado et al., 2008). The main influencing factors and removal mechanisms were also examined. Experiments were carried out in a dead-end batch filtration cell in order to limit the volume of solutions and control the operating parameters. The first series of experiments was performed with a single solution of CP molecule in Milli-Q water in order to evaluate the CP removal efficiency of NF and RO membranes. Then the main influencing factors, such as trans-membrane pressure, ionic strength and CP feed concentration, were also investigated. The second series of experiments was performed with mixed solutions of CP molecule in MBR effluent to study the influence of the water matrix on CP retention and to evaluate whether MBR–NF and MBR–RO combined systems could be used directly for CP removal from a target hospital discharge.

Before the filtration experiments, it was necessary to develop sensitive and specific analysis methods, which allowed quantification of CP at trace level in different water matrices.

2. Materials and methods

2.1. Experimental set-up and filtration protocol

All experiments were performed in a dead-end stirred filtration cell made of stainless steel (see Causserand et al., 2005). The effective membrane area was $3.52 \times 10^{-3}\,\mathrm{m}^2$ and the total effective volume was about 400 mL. Stirring speed was maintained constant (200 rpm) in all tests. Trans-membrane pressure was exactly regulated by pressurized air through an electronic pressure transducer (in the range $5 \times 10^{+5}$–$25 \times 10^{+5}\,\mathrm{Pa}$). The filtrate flux J (mL/min) was measured by an electronic balance via a computer with an accuracy of $\pm 0.1\,\mathrm{g}$. All experiments were performed at room temperature ($20 \pm 3\,^\circ\mathrm{C}$).

Before its first use, the membrane was soaked in ultrapure water (Milli-Q) for 24 h. Then Milli-Q water was filtered through it at $20 \times 10^{+5}\,\mathrm{Pa}$ for about 2 h until the flux had stabilized. The pure water permeability of the new membrane was then determined. After the pre-compaction, the cell was emptied and filled with 380 mL of feed solution. Then the cell was pressurized and the filtration was conducted until 200 mL of permeate had been collected. The first 100-mL permeate sample (named 100 mL permeate) and the second one (named 200 mL permeate) were collected separately for CP extraction and further analysis. Feed and retentate samples were also collected at the beginning and end of experiments respectively. After the filtration run, the cell was emptied and the membrane was washed thoroughly with Milli-Q water. Its pure water permeability was measured again in order to determine the difference before and after the filtration.

The observed retention coefficient: $R_{obs} = (1 - C_p/C_r) \times 100\%$ was calculated from concentrations determined in the permeate C_p and the retentate C_r. When C_p was directly quantified through permeate sample analysis, C_r was deduced from mass balance, according to the fact that will be explained in Section 3.1 that CP loss (by adsorption or other phenomena) is negligible during filtration run. After each run, the membrane and the O-ring gasket were replaced by new ones in order to avoid cross contamination between runs by adsorption–desorption of the molecules.

2.2. Membranes

The NF membrane: Desal 5 DK (Osmonics) used in this study was a three-layer thin-film polysulfone-based membrane with a polyamide top layer (provided by the manufacturer). Its pure water permeability: 5.8 L/m²/h/bar (our measurements) is especially high compared with other NF membranes (Mazzoni and Bandini, 2006). According to the information provided by the manufacturer, this membrane possesses a molecular weight cut-off of 150–300 g mol⁻¹, a surface energy of 33.10^{+5} J m⁻², a mean pore radius of 0.47 nm and a negatively charged surface in the pH range of 4.4–8.3.

The selected RO membrane was a YMAKSP3001 (Osmonics). It is also a polyamide membrane which is commonly used in pharmaceuticals removal. It exhibits 99% NaCl retention (provided by the manufacturer) and its pure water permeability is 4.0 L/m²/h/bar (our measurements).

2.3. Chemicals and characterization

Cyclophosphamide monohydrate (CAS Number: 6055-19-2) was purchased from Fluka, France. All reagents (humic acid, sodium chloride, phosphoric acid) were norm-pure grade and obtained from Sigma–Aldrich, France. All solvents (methanol, acetonitrile, dichloromethane, ammonium formate) were of HPLC grade from Fluka, France. Ultrapure water was used for the preparation of all aqueous solutions and also as the high performance liquid chromatography (HPLC) mobile phase and as the eluent in liquid chromatography tandem mass spectrometry (LC/MS/MS).

The main properties of CP are listed in Table 1. This substance is hydrophilic, judging from the low octanol–water partition coefficient ($\log K_{ow} < 2$). The pKa value is given as a range because no exact value can be found in the literature. CP mainly exists in the neutral form within the normal pH range of water.

2.4. Water matrix selection and characterization

Ultrapure water and effluent from an MBR were selected for this study. The ultrapure water was produced from distilled water through the Milli-Q filtration system (Millipore). The pH value was in the range of 5–6 and was kept constant during the experiments.

The MBR system incorporated ZeeWeed 500 ultrafiltration membranes (GE Zenon, France) and produced approximately 6 L of permeate per day while operating at a mixed liquor suspended solid concentrations between 10 and 15 g/L. Hydraulic retention time (HRT) and sludge retention time (SRT) of MBR are 48 h and 50 days, respectively. Two small-scale membrane bioreactors were run in parallel. Both reactors were fed with a real municipal wastewater in which CP concentration is below detection limit. One reactor was operated as a blank: CP was never added to it; while the other was doped with CP molecule to investigate CP removal efficiency by the MBR process. The CP concentration supplied to this MBR was 5–10 µg/L in accordance with the actual concentration often found in the discharge from the target hospital (in southern France). Generally, CP removal efficiency in the MBR varied from 0 to 75% depending on the operating conditions. Thus the CP concentration in MBR effluent was about 1.5–10 µg/L (Delgado et al., 2008).

In the present study, the effluent from the blank reactor was collected and spiked with CP for the filtration experiments to study the influence of water matrix on CP rejection by controlling the initial CP concentration. The main parameters of MBR effluent are reported in Table 2.

In order to study the CP retention mechanisms of NF and RO membranes, CP concentration in the filtration experiments with ultrapure water was relatively high (10–600 µg/L) with respect to the detection limits of the analytical method. Low CP concentration (1–10 µg/L) was adopted in the filtration experiments with MBR effluent in order to simulate real levels of occurrence in raw wastewater and MBR effluent as mentioned before.

2.5. Sample extraction and analytical methods

2.5.1. Extraction
All CP samples were concentrated by a lyophilization–extraction procedure. Briefly, 200 µL isofosfamide (0.1 mg/mL) was added to a 100 mL CP sample as an internal standard. The 100 mL sample was frozen in a 500 mL glass bottle (Quickfit, England) in a liquid nitrogen bath in a rotation evaporator

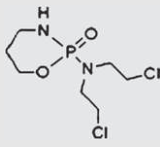| Table 1 – Main physicochemical properties of CP molecule.					
Molecular formula	Molecular weight (g/mol)	$\log K_{ow}$	pKa	Charge at pH = 6–7	Molecular structure
$C_7H_{15}Cl_2N_2O_2P$	261.09	0.63	4.5–6.5	Neutral	

Table 2 – Main parameters of MBR effluent.				
COD (mg/L)	Conductivity (mS/cm)	pH	N–NH$_3$ (mg/L)	Dissolved oxygen (mg/L)
150–300	20–50	7.5–8.0	2–5	6–8

Note: COD – Chemical Oxygen Demand.

(Phenomenex, France) for about 12 min. Then the frozen sample bottle was connected with the lyophilizer (CARLO ERBA, France) for one night under vacuum conditions. After lyophilization, the sample powder obtained was transferred into a 30 mL glass tube (Scientific, France). 10 mL dichloromethane was then put into the bottle and shaken manually for 10 min to completely dissolve the remaining powder. This operation was repeated twice with 5 mL dichloromethane and all the dichloromethane fractions were brought together in a 30 mL tube. The sample tube was shaken gently in a shaking bed (Stuart, France) for 30 min to further dissolve the CP in the dichloromethane. The sample was centrifuged for 10 min at 2000 rd/min. The dichloromethane phase was transferred into a 20 mL glass tube with a pipette and the tube was placed in the evaporator (PIERCE 18780, France) for complete drying under a gentle nitrogen stream. These operations were repeated twice with 5 mL dichloromethane. Finally:

- for HPLC–UV analysis: 1000 μL water–acetonitrile (80/20; v/v) was added to the tube after drying and completely mixed in a rotator.
- for LC–MS–MS analysis: 100 μL or 1000 μL (depending on CP concentration) methanol/ammonium formate buffer (50/50) was added, pH 5.7.

CP recoveries in the various water matrices were mostly greater than 75% and the overall variability of the method was below 8%. The extracted samples were stored at −80 °C for further analysis. CP analysis was conducted with HPLC or LC/MS/MS according to the ultimate concentration and detection limit.

2.5.2. HPLC–UV
The HPLC–UV method was used for CP analysis at high concentration and in ultrapure water. The HPLC equipment consisted of an Accela pump, coupled to an Accela PDA detector and an Accela auto sampler. The separation was completed on a C18 Gemini column (particle size 3 μm, 0.2 × 10 cm). A C18 Gemini guard column (Phenomenex) was also used. The UV detector was set at the wavelength of 195 nm for CP quantification. Column temperature was maintained at 20 °C. The injection volume was 5 μL. The mobile phase was acetonitrile/water (20/80, v/v) and phosphoric acid was added to the mobile phase to adjust the pH to 2.5. Elution was performed at a flow rate of 0.4 mL/min under isocratic conditions. The retention time of CP was about 5.0 min and the total run time was 12 min. The calibration curve was established by injecting five CP standard solutions, ranging from 4 to 48 mg/L. The correlation coefficient of CP calibration showed good linearity ($R^2 = 0.9990$). A typical

quantification limit for CP samples under these conditions is approximately 2 mg/L.

HPLC–UV is a time-saving, easily-controlled and relatively economical detection method. This method was initially adopted because of the large number of samples. However, it was not suitable for the CP samples in complex water matrices because of interference from other components (especially NOM). For this reason, it was necessary to develop another method for CP quantification in complex water matrices (MBR effluent).

2.5.3. LC–MS–MS
The LC–MS–MS method was applied for CP confirmation and quantification at lower CP concentration and in a complex water matrix. The injection volume was 20 μL. The mobile phase consisted of a gradient of methanol–ammonium formate buffer (CH$_5$NO$_2$ 2 mM, pH 5.7) circulated at an isocratic flow rate of 0.20 mL/min (see Table 3). The column used was a C18 Nucléosil (particle size 100 Å–5 μm, 0.2 × 12.5 cm). Column temperature was maintained at 30 °C. A guard column was also used: Frit SS Blk 0.5 μm, 0.094 × 0.065 × 0.250 (Cil Cluzeau Info Labo).

The MS was operated in positive electrospray ionization (ESI$^+$) mode using multiple reaction monitoring (MRM). The scan range was m/z [70–290] in the MS/MS mode, at a scan rate of 3 μ scans and 200 ms. Under ESI$^+$ conditions, an abundant protonated molecule [M + H]$^+$ at m/z 233 and the fragment ions at m/z 239, resulting from loss of chlorine, were observed. The cone voltage and collision energy for each transition were programmed through the Excalibur acquisition software.

The detection limit of the method was 10 ng/mL.

3. Results and discussion

3.1. CP rejection by NF membrane

3.1.1. Influence of trans-membrane pressure on CP retention
According to the detection limit of the HPLC–UV method and multiple CP concentration factors during the extraction procedure, 100 μg/L was the minimum CP concentration (100 mL sample volume) which was suitable for HPLC–UV analysis after extraction (100-fold concentration). At the same time, taking into account the expected removal efficiency of the NF membrane, CP initial concentration in the feed solution was fixed at 400 μg/L in this experiment. The solvent was ultrapure water at its natural pH of about 6.0. During the filtration run (less than 1 h) this pH remained stable. Fig. 1 shows the influence of trans-membrane pressure on CP retention by NF membrane.

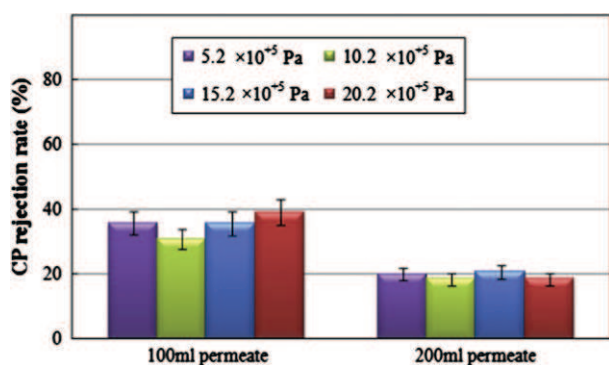| Table 3 – Methanol–ammonium formate buffer gradient.	
Time (min)	Mobile phase
0	80% buffer/20% methanol
9	80% buffer/20% methanol
9.5	55% buffer/45% methanol
25	55% buffer/45% methanol

Fig. 1 – Influence of trans-membrane pressure on CP rejection by NF.

It can be seen from this figure that the rate of CP rejection by NF membrane was relatively low, ranging from 20% to 40%. Considering the low $\log K_{ow}$ coefficient (hydrophobic compounds are characterized by $\log K_{ow} > 2$) and the neutral form of the CP molecule in the pH condition of the experiments, we could expect weak interactions between CP molecules and the membrane surface, which is hydrophobic and negatively charged (see Materials and Methods section). Taking into account the CP molecular weight (261.09 g/mol) and the molecular weight cut-off (MWCO) of NF membrane (180–300 g/mol, provided by the manufacturer), the low rejection rate of CP seems to be consistent with a solute transfer model mainly governed by a typical steric hindrance mechanism. A similar conclusion was drawn by Kiso et al. (2001) who showed that, for hydrophilic solutes, steric hindrance was the most important controlling factor for molecule rejection. Other evidences about this mechanism will be further analyzed later in this paper.

It was also found that CP concentration in the first 100 mL of permeate was always lower than that in the second 100 mL of permeate. Thus CP rejection rate decreased from almost 40% after the first 100 mL filtered to 20% after 200 mL of permeate filtered. This behavior can be attributed to weak adsorption of CP onto the membrane in spite of the hydrophilic character of the compound. In order to confirm this assumption, the total quantity of CP adsorbed onto the membrane was calculated from the mass balance after each filtration run. The calculated results under various trans-membrane pressures are reported in Table 4.

Given the low difference (between 5% and 7%) in the mass balance listed in Table 4, we cannot settle the question of the

contribution of experimental error and of adsorption in the values reported. However, this low quantity of CP loss during experiments clearly demonstrated that interactions between CP and the membrane surface were weak. We then assume that if weak adsorption is effective, it may increase the apparent CP retention rate at the beginning of the filtration experiment without affecting membrane permeability due to the low quantity adsorbed (see Section 3.1.4, Fig. 5). Further investigation involved in the precise adsorption evaluation is recommended. In consequence, 200 mL of permeate has to be filtered in order to obtain an accurate evaluation of CP retention by NF membrane.

However, for the same volume filtered, CP rejection rate was almost the same whatever the trans-membrane pressure investigated. Desal 5 DK membrane retention efficiency was not sensitive to pressure changes and exhibited almost constant rejection performance for CP in the pressure range $5.2 \times 10^{+5}$–$20.2 \times 10^{+5}$ Pa.

3.1.2. Influence of CP feed concentration on CP retention

The concentrations of CP found in sewage and drinking water sources are in the order of ng/L. Such concentrations are difficult to maintain in experiments due to analytical difficulties (detection limits). Consequently, a higher concentration was used for the experimental study. This raises the question of whether the conclusions drawn in the higher concentration range are consistent with those that would be deduced using lower concentrations. In this context, it was necessary to investigate the influence of CP feed concentration on CP retention. CP solutions were prepared in ultrapure water and filtered at $20 \times 10^{+5}$ Pa.

The CP concentrations selected here lay in two different ranges: one was from 200 to 600 µg/L, for which samples were analyzed by the HPLC–UV method, and the other was 10 µg/L, for which samples were analyzed by the HPLC–MS–MS method. There were two reasons for the selection here. Firstly, 10 µg/L of CP was to be used in the later filtration experiments with MBR effluent as explained before, so it was necessary to study whether CP retention remained constant throughout the concentration range used. Secondly, it was also indispensable to investigate the consistency of the two different analytical methods used in this study.

As shown in Fig. 2, no obvious effect of CP initial concentration on R_{obs} value was observed in the concentration range used in this experiment. A similar conclusion was drawn when pesticides were filtered through the NF membrane in the concentration range of several µg/L (Van der Bruggen et al., 1998). Thus, within the experimental error, it can be

Table 4 – Quantity of CP adsorbed on NF membrane.				
Trans-membrane pressure ($\times 10^{+5}$ Pa)	CP mass in the feed solution (µg)	CP mass in permeate and retentate (µg)	Total quantity adsorbed (%)	Total quantity adsorbed (mg/m^2)
5.2	98.3	92.0	6.4	1.79
10.2	96.5	91.0	5.7	1.56
15.2	105.8	100.1	5.4	1.62
20.2	92.85	86.6	6.7	1.78
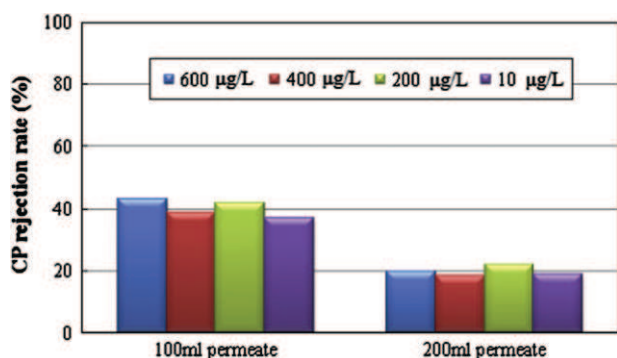
Fig. 2 – Influence of CP feed concentration on CP rejection by NF.

Fig. 4 – Influence of water matrix on CP rejection (CP feed concentration: 10 μg/L).

concluded that CP concentration in the feed solution has no influence on CP rejection by NF membrane. Furthermore, we can confirm that there was a good consistency between the HPLC–UV and HPLC–MS–MS analysis methods.

3.1.3. Influence of ionic strength on CP retention

Sodium chloride was used to adjust the ionic strength in the feed solution (25–50 mM). CP feed concentration in this experiment was fixed at 400 μg/L and trans-membrane pressure was $20 \times 10^{+5}$ Pa. Fig. 3 shows the CP rejection rate at different ionic strengths. We see in this figure that the R_{obs} value of CP was almost constant whatever the ionic strength of the feed solution, particularly after 200 mL of permeate filtered. From these results, it appears that the presence of salt does not affect CP retention by NF membrane.

In previous studies, some researchers have observed that the presence of salt can alter the solute rejection behavior (Nghiem et al., 2006; Bandini and Vezzani, 2003). For charged solute, the presence of salt decreases the solute rejection rate. This is due to the shield of electrostatic potential generated by membrane surface functional groups, leading to a decrease in electrostatic repulsive effects. On the other hand, the ''salting-out'' effect decreases the hydrated radius of the solute molecule and thus reduces the ''apparent size'' of the molecules. Both effects would be expected to influence solute rejection in similar ways and cannot be separated easily. However, in our experiments, CP mainly existed in neutral form in the feed solution as mentioned before. In these conditions, salting-out
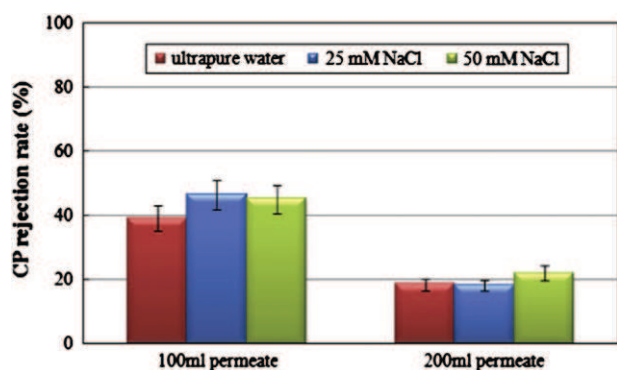
and electrostatic repulsive interactions had a negligible effect on the uncharged CP, the presence of salt had almost no influence on CP retention. This also demonstrated that the size exclusion mechanism governed CP retention by NF membrane, while the electrostatic repulsion mechanism played no role.

3.1.4. Influence of water matrix on CP retention

MBR effluent was used as a background solution to investigate the influence of the water matrix on CP retention and to evaluate the potential of nanofiltration as a post-treatment process of MBR. Experiments were performed at a trans-membrane pressure of $20 \times 10^{+5}$ Pa.

As the results in Fig. 4 show, the water matrix clearly influences CP rejection by NF membrane. When MBR effluent was used as the background solution, the CP rejection rate was much higher than that in ultrapure water. Furthermore, there was no obvious decrease in CP retention with the filtration volume (the two CP rejection rate values with MBR effluent are within experimental error). The CP retention enhancement was principally attributed to membrane fouling by the components (mainly natural organic matter) present in MBR effluent. A decrease of 21% in membrane water permeability was measured after filtration with MBR effluent as shown in Fig. 5. Both in-depth membrane fouling due to the
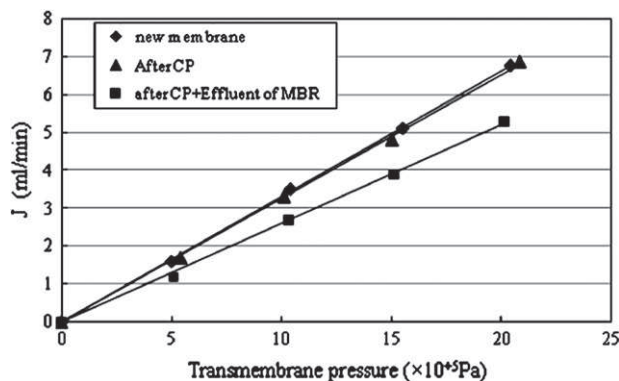

Fig. 5 – Membrane water flux determined on a new membrane, on the membrane after filtration of CP in ultrapure water (10 μg/L) and after filtration of the solution of CP in MBR effluent (10 μg/L).

Fig. 3 – Influence of ionic strength on CP rejection by NF.

Table 5 – CP rejection by RO membrane.

Trans-membrane pressure ($\times 10^{+5}$ Pa)	Water matrix	CP feed concentration (ng/L)	Concentration in 100 mL permeate (ng/L)	Concentration in 200 mL permeate (ng/L)	R_{obs1} (%)	R_{obs2} (%)
20.0	Ultrapure water	6338	218	364	96.57	94.26
20.0	Ultrapure water	4370	210	320	94.94	92.79
20.0	MBR effluent	1513	121	47	92.01	96.87
10.0	Ultrapure water	1252	61	42	93.70	96.62
10.0	MBR effluent	1382	103	43	92.53	96.67

Note: R_{obs1} is the CP rejection rate in the first 100 mL permeate; R_{obs2}, the CP rejection rate in the second 100 mL permeate.

NOM retention and NOM adsorption onto the membrane surface may have contributed to the membrane permeate flux decrease.

Considering the physicochemical properties of CP and the conclusions of Section 3.1.3, the modification of membrane surface properties (hydrophobicity, charge) caused by NOM may have little effect on CP retention. Hence, the pore restriction mechanism caused by NOM may play the greatest role in the increase of CP rejection. In addition, the binding of CP to NOM due to hydrogen bonding, forming NOM–CP complexes that are larger and have an increased negative charge, could also play a role in the removal of CP. Similar conclusions have been drawn in a previous work (Comerton et al., 2008). This study examined the rejection of 22 EDCs and PhACs from different water matrices by 'loose' and 'tight' NF membranes, and also an RO membrane. Among the investigated compounds, Sulfamethizole exhibits similar properties to CP's ones (Molecular weight is 270 g/mol, log K_{ow} is 0.54) In spite of the compound characteristics, molecule rejection from the natural waters by NF membrane was greater than that from the Milli-Q water. It was concluded that membrane fouling and compound interactions with the water matrix likely contributed to the higher rejection.

Summarizing the above conclusions, we can state that NF is not efficient enough in terms of CP removal to be considered as a tertiary treatment for raw water containing CP. However, it can be adopted as a post-treatment unit after an MBR system in wastewater treatment. For example, if CP concentration in the influent of the MBR was 10 µg/L (maximum concentration detected in wastewater from the target hospital), the removal efficiencies of the MBR and NF membrane in steady state conditions were evaluated at 75% and 60% respectively, so the CP concentration in the permeate of the MBR–NF system would be less than 1 µg/L. The total removal efficiency of the combined system would be greater than 90%. So an MBR–NF system is a promising process for the treatment of real wastewater containing CP in future applications. Furthermore, it can be expected that NF would remove CP in non-MBR permeate if the water contained similar organic material to the MBR permeate. Further investigation involved in the CP rejection by NF membrane alone from natural raw water and wastewater is recommended.

3.2. CP rejection by RO membrane

The CP rejection experiments using an RO membrane were conducted at relatively low concentrations (1–10 µg/L) in order to simulate the actual applications and the LC–MS–MS method was used for sample analysis. Table 5 shows the CP rejection by RO membrane under different operating conditions. As can be seen from this table, the RO membrane provided excellent CP rejection (>90%). Furthermore, the CP rejection rate was almost stable under all operating conditions. No obvious difference was observed when the trans-membrane pressure, CP feed concentration and water matrix were changed.

Considering the high efficiency in terms of CP retention whatever the operating conditions, an RO system could be considered as the low risk option for tertiary treatment of raw water containing CP as well as for a post-treatment unit after the MBR system in wastewater treatment. For the latter application, an MBR–RO system is an ideal choice for hospital wastewater treatment since it can be expected to totally remove many pharmaceutical compounds, such as CP, that are assumed to be present at quite high concentrations in this type of wastewater.

However, considering the filtrate flux obtained in the same operating conditions (CP concentration in MBR effluent: 10 µg/L, trans-membrane pressure: $20 \times 10^{+5}$ Pa): 3.5 mL/min with NF membrane and 1.2 mL/min with RO membrane, we can expect a higher energy cost in the case of MBR–RO. To obtain the same water productivity with an MBR–NF system, the applied trans-membrane pressure for the MBR–RO system would have to be increased.

4. Conclusions

In this paper, a lyophilization–extraction procedure was first established for CP enrichment of different water matrices. Sensitive and specific analysis methods based on HPLC–UV and LC–MS–MS were also developed, and allowed quantification of CP down to sub-ng/L concentrations. The rejection of CP by NF and RO membranes from Milli-Q water and MBR effluent was then investigated with CP feed concentrations ranging from 1 to 600 µg/L. The RO membrane provided excellent rejection (>90%) from the water matrices examined under all operating conditions. This suggests that a combined MBR–RO system could provide efficient removal of CP.

Concerning the NF process, this study has shown that the typical steric hindrance mechanism governed CP retention by NF membrane. Moreover, the results obtained demonstrated that the adsorption phenomenon between CP and membrane surface was weak but non-negligible as it led to an over estimation of the CP rejection rate at the beginning of the filtration experiment. In consequence, an accurate evaluation of CP removal by NF membrane was achieved after filtering 200 mL.

Rejection was poor from Milli-Q water: 20% in steady state conditions. Trans-membrane pressure, CP initial concentration and ionic strength of the feed solution had almost no influence on CP retention. On the other hand, the water matrix greatly influenced the CP rejection behavior by NF. The CP rejection rate was significantly enhanced when MBR effluent was used as the background solution. Both membrane fouling and interactions between CP and water matrix may have contributed to the higher CP retention.

From this point of view, both MBR–NF and MBR–RO combined systems can be considered as promising processes for the treatment of real wastewater containing CP in future applications.

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