Effect of Water Quality on Rejection of Selected Human and Veterinary Antibiotics by Nanofiltration and Reverse Osmosis Membranes

Amisha Shah, Graduate Research Assistant

Eddie McCallum, Graduate Student

Annie Park, Undergraduate Student

Ching-Hua Huang, Ph.D., Assistant Professor

Jae-Hong Kim, Ph.D., Assistant Professor

School of Civil and Environmental Engineering
Georgia Institute of Technology, Atlanta, GA 30332-0512

ABSTRACT

This study addresses the possibility for nanofiltration (NF) and reverse osmosis (RO) membrane processes to serve as an alternative to remove low levels of selected antibiotics from wastewaters and surface waters given changing water conditions such as pH and presence of natural organic matter (NOM). Preliminary experimental results obtained with a dead-end filtration apparatus suggest that adsorption of antibiotics onto the membrane surface is negligible. However, removal is greatly affected by changes in pH due to changes in electrostatic repulsion between charged antibiotics and membrane surfaces. Both the pore diffusion model for NF and the solution diffusion model for RO will be applied to further understand the mechanisms and trends behind the experimental data.

INTRODUCTION

In recent years, antibiotics have emerged as a group of contaminants with particular concerns because of their frequent detection at low levels (μg/L) in wastewaters and surface waters in the US, Canada, and parts of Europe[1, 2, 3]. Widely used for both human therapy and veterinary practices, antibiotics are only partially consumed by the users and can enter the environment through wastewaters and animal manure. Researchers indicate that such recalcitrance of these compounds at low levels may promote bacterial resistance and cause adverse human health effects[4, 5, 6].

Previous studies have shown that certain water treatment processes can affect the fate and transformation of antibiotics. Disinfectants such as free chlorine, chloramine, chlorine dioxide and ozone are proven to effectively oxidize various antibiotics[7, 8, 9], but the transformation of parent compounds into a more environmentally potent species is of growing concern. Pressure-driven nanofiltration (NF) and reverse osmosis (RO) membrane processes are emerging technologies which may be an important alternative towards removing antibiotics from

wastewaters and surface waters. Both NF and RO have been suggested as an effective barrier against organic compounds such as pesticides and steroid hormones under various water quality conditions[10, 11]. However, few studies have been reported on antibiotics removal by membrane processes to date. Considering their low molecular weight and charged speciation, removal of antibiotics might pose a unique challenge to water and wastewater treatment utilities.

The purpose of this study is to evaluate the potential removal of selected human and veterinary antibiotics, such as sulfamethoxazole (SMX), trimethoprim (TMP), erythromycin (ERY), ciprofloxacin (CF), and carbadox (CDX), by NF and RO membranes under varying water quality parameters and antibiotic concentrations, with the goal of understanding the underlying rejection mechanism(s). These antibiotics are selected considering their persistent use in the environment as well as variable pH-dependent charge speciation. As water quality parameters such as pH and the presence of NOM change, the effects of membrane surface charge, antibiotic concentration and antibiotic speciation on overall rejection and/or adsorption will be assessed. Both pore diffusion and solution diffusion models will be applied to experimental data to further elucidate antibiotic-membrane interactions.

EXPERIMENTAL METHODS

Materials. Five commercial NF and RO membranes were selected from two manufacturers, Saehan Industries Inc. and Filmtec (Dow Chemical Company). Saehan provided two NF membranes, NE 4040-90, NE 1812-70, and one RO membranes, RE 4040-BLN. Filmtec provided two NF membranes NF 270-400 and NF 90-400. All membranes are composed of a polysulfone support with a polyamide active layer but vary in divalent and monovalent ion rejection levels.

Chemicals and Solution Preparation. Ciprofloxacin hydrochloride and sulfamethoxazole were purchased from ICN Biomedicals, Inc. (Irvine, CA, USA). Trimethoprim, carbadox, and erythromycin were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Structure and relevant properties of the antibiotics used in this study are shown in Table 1. All the chemicals used were reagent or analytical grade, and all the synthetic solutions were prepared using Nanopure water produced from a Millipore water purification system. Antibiotic stock solutions were prepared at 25 or 100 mg/L in 10 to 50% methanol. Stock buffer solutions consisted of acetate (pH 4 -5), phosphate (pH 6-8), and borate (pH 9). Stock solutions were then spiked into test solutions to achieve a final antibiotic concentration of 100 μg/L or lower to represent both elevated and environmentally relevant concentrations. The final buffer concentration of test solutions is 1.0 mM. Solution pH is adjusted by addition of H_2SO_4 or NaOH.

Membrane Preparation and Performance Testing. Flat sheet membrane coupons were cut and soaked in Nanopure water for 24 hours prior to testing. To ensure comparable membrane quality, salt rejection was tested and compared to the manufacturer's specifications. Only membranes that passed the initial quality tests were used. For NF membranes, rejection was measured using a feed solution of 2,000 mg/L $MgSO_4$ at 70 or 75 psi. All the NF membranes passing the quality testing fell within $\pm$ 3 % of specified $MgSO_4$ rejection of 97% or greater. For RO membranes, rejection was measured using a feed solution of 1,500 mg/L NaCl at 150 psi.

All the RO membranes passing quality testing fell within $\pm$3% of specified NaCl rejection of 99%.

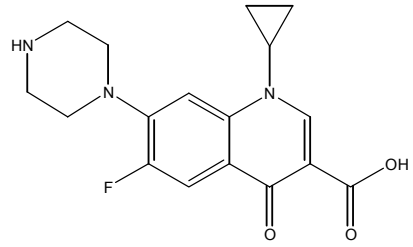
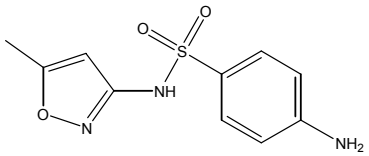
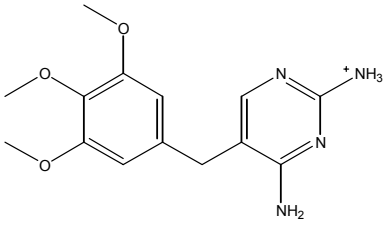
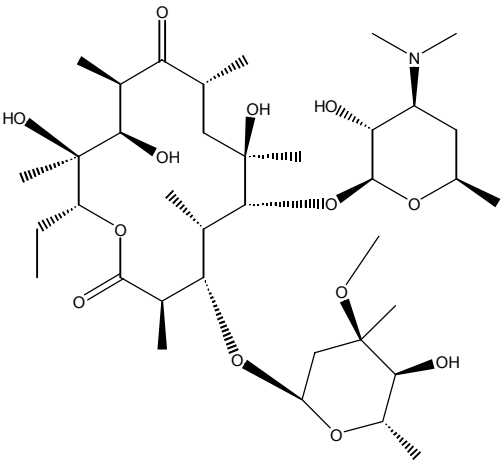
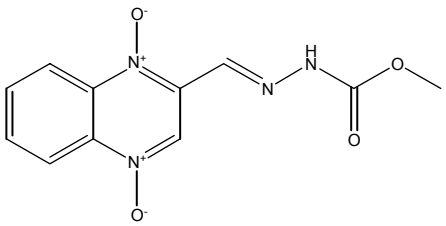
Dead-End Cell Experiments. All experiments were performed using a stainless steel dead-end stirred cell (300 ml volume, 14.6 cm^2 active membrane area). Water permeation flux was measured both when using Nanopure water for 30 min prior to the experiments as well as between sampling events during test runs. Comparable fluxes for all membranes were achieved by altering operating pressure. Membrane coupons were used repeatedly for multiple experiments. Antibiotics were desorbed from the membrane between experiments by running 150 mL of Nanopure water at 140 psi. Complete desorption was confirmed by comparing adsorption trends of replicate experiments. All experiments were performed at room temperature.

Efforts were made to maintain antibiotic concentration in the retentate as constant as possible by returning collected permeate to the feed every 10 min by depressurizing and opening the stirred cell for an overall run time of 60 to 100 min. Permeate samples (sample volume of ~ 0.5 ml) were collected repeatedly at times less than 10 minutes in order to evaluate adsorption effects. However, samples collected after 10 min were taken immediately following each permeate recirculation event in order to neglect effects of increasing retentate concentration. All the permeate samples were collected in 1.5 ml amber HPLC vials except for ciprofloxacin samples which were collected in 20 ml borosilicate amber vials and further acidified with 1.0 M H_3PO_4 before analysis.

The experimental design of all dead-end experiments followed a matrix where parameters such as pH, NOM concentration, and antibiotic concentration were varied. pH effects on antibiotic rejection were studied in NF by varying pH from 0.5 to 2.0 units away from environmentally relevant pKa values. In on-going study, NOM are added until when competition with significant levels of antibiotic adsorption is possible.

Analytical Methods. Concentrations of all antibiotics were measured using an Agilent 1100 Series HPLC system equipped with an RX-C18 reverse phase column. Concentrations of SMX, TMP, and CDX were measured using a UV/VIS diode-array detector while CF was measured using a fluorescence detector. Method development for CF, SMX, TMP, and CDX was taken from prior studies by using gradients of buffer solutions, 0.04 mM H_3PO_4 (CF)[7], 1.0 mM TFA (SMX and TMP)[8], or 20 mM acetic acid (CDX)[12], and acetonitrile. A method for ERY has not yet been developed. To assess antibiotic concentrations of a few µg/L, samples will be preconcentrated using solid phase extraction (SPE) prior to HPLC analysis.

Table 1. Structures and Properties of Antibiotics Used in This Study

	Structure	MW	pK_a	Charge at pH 7
Ciprofloxacin		331.4	$pK_{a,1}$=6.2 $pK_{a,2}$ = 8.8[13]	Zwitterion and Neutral
Sulfamethoxazole		253.28	$pK_{a,1}$= 1.7 $pK_{a,2}$ = 5.6[14]	Anion
Trimethoprim		290.3	$pK_{a,1}$=1.32[15] $pK_{a,2}$ = 7.45[16]	Cation and Neutral
Erythromycin		733.9	$pK_{a,1}$=8.90[17]	Cation
Carbadox		262.2	No pK_a found between pH 3-11[17]	N/A

RESULTS AND DISCUSSION

Experimental results obtained for the rejection of SMX and TMP at initial concentrations of 100 µg/L with the Saehan 4040 membrane at different solution pHs are shown in Figure 1. Water fluxes obtained with clean waters and test solutions for these experiments averaged 40.5 L/m^2/hr (± 5.1) and 34.9 L/m^2/hr (± 4.4), respectively. Experimental temperature ranged from 21 to 24 ºC. The permeate concentrations plotted versus filtration time (results not shown) suggested that rejection was greater during the initial stage of filtration due to the adsorption of SMX and TMP onto the membrane surface. After this initial period, the overall rejection was greatly affected by the charge speciation of both SMX and TMP as shown in the Figure 1. Both antibiotics showed higher level of rejection when pH increased above the pK_a values. SMX showed higher level of overall rejection compared to TMP when the solution pH was same. While the molecular weight of SMX is slightly lower than TMP, SMX is negatively charged and TMP are neutral beyond respective $pK_{a,2}$ values. Below the $pK_{a,2}$, SMX is neutral and TMP is positively charged. It is interesting to notice that rejection of SMX at below corresponding $pK_{a,2}$ (i.e., SMX is neutral) was comparable to that of TMP at above corresponding $pK_{a,2}$ (i.e., TMP is also neutral). These results suggest that the overall rejection of antibiotics is determined by electrostatic interactions between charged antibiotic species and membrane surfaces.

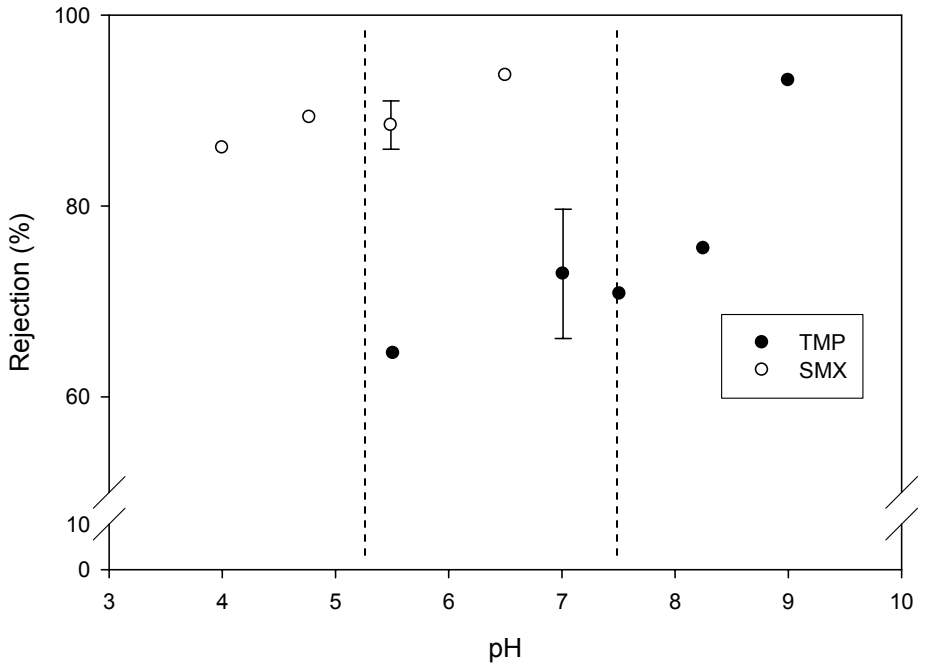


Figure 1. TMP and SMX rejection as a function of pH with Saehan 4040 membrane. Dotted lines represent pKa's of TMP ($pK_{a,2}$ =7.45)and SMX ($pK_{a,2}$ =5.6), respectively.

Experiments are currently on-going with other antibiotics and membranes at different water quality conditions. Completion of this study will help elucidate the mechanisms by which different water conditions drive efficient removal of low levels of antibiotics and subsequently provide valuable guidelines for utilities to use membrane technologies as a future alternative.

ACKNOWLEDGEMENT

The authors would like to acknowledge Saehan Industries Inc. for their financial support. Saehan Industries Inc. and Dow Chemical Company are thanked for providing NF and RO membrane samples for testing.

REFERENCES

1. Koplin, D.W., Furlong, E.T., Meyer, M., Thurman, E.M.,Zaugg, S.D., Barber, L.B., Buxton, H.T. (2002) Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance. *Environ. Sci. Technol.* 36, 1202.

2. Miao, X., Bishay, F., Chen, M., Metcalfe, C.D. (2004) Occurrence of Antimicrobials in the Final Effluents of Wastewater Treatment Plants in Canada. *Environ. Sci. Technol.* 38 (13), 3533.

3. Hirsh, R., Ternes, T., Haberer, K., Kratz, K. (1999) Occurrence of Antibiotics in the Aquatic Environment. *Sci. of Total. Environ.* 225, 109.

4. Guardabassi, L., Peterson, A., Olsen, J.E., Dalsgaard, A. (1998) Antibiotic Resistance in *Acinetobacter* spp. Isolated from Sewers Receiving Waste Effluent from a Hospital and a Pharmaceutical Plant. *Appl. and Env. Microbiology*. 64, 3499.

5. Goñi-Urriza, M. et al. (2000) Impact of an Urban Effluent on Antibiotic Resistance of Riverine *Enterobacteriaceaei* and *Aeromonas* spp. *Applied and Env. Microbiology*, 66, 125.

6. Daughton, C.G., Ternes, T.A. (1999) Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change?. *Environ. Heath Perspectives. 107, 907.*

7. Dodd, M.C., Shah, A.D., von Gunten, U., Huang, C.-H. (2004) Interactions of Fluoroquinolone Antibacterial Agents with Aqueous Chlorine: Kinetics, Reaction Mechanisms, and Transformation Pathways, *Environmental Science and Technology,* submitted.

8. Dodd, M.C., Huang, C.-H. (2004) Transformation of the Antibacterial Agent Sulfamethoxazole in Reaction with Chlorine. Kinetics, Mechanisms, and Pathways, *Environmental Science and Technology*, 38, 5607-5615.

9. Adams, C., Wang, Y., Loftin, K. Meyer, M. (2002) Removal of Antibiotics from Surface And Distilled Water in Conventional Water Treatment Processes. *J. of Env. Eng.* 128, 253

10. Van der Bruggen, B., Schaep, J, Maes, W. Wilms, D., Vandecasteele, C. (1998) Nanofiltration as a Treatment Method for the Removal of Pesticides from Ground Waters. *Desalination*. 117, 139.

11. Schafer, A.I., Nghiem, I.D., Waite, T.D. (2003) Removal of the Natural Hormone Estrone from Aqueous Solutions Using Nanofiltration and Reverse Osmosis. *Environ. Sci. Technol.* 37, 182.

12. Zhang, H., Huang, C.-H. (2004) Reactivity and Transformation of Antibacterial N-Oxides in the Presence of Manganese Oxide, *Environmental Science and Technology*, in press.

13. Vázquez, J. L., Berlanga, M., Merino, S.; Domènech, Ò., Viñas, M., Montero, M. T., Hernández-Borrell, J. (2001) Determination by Fluorimetric Titration of the Ionization Constants of Ciprofloxacin in Solution and in the Presence of Liposomes. *Photochem. Photobiol. 73*, 14.

14. Lucida H., Parkin J.E., Sunderland V.B. (2000) Kinetic Study of the Reaction of Sulfamethoxazole and Glucose under Acidic Conditions - I. Effect of pH and Temperature, *Int. J. Pharm.* 202 (1-2), 47.

15. Cao, J. Cross, R.F. (1995) Separation of Dihydrofolate Reductase Inhibitors and the Determination of pKa_1 Values by Capillary Zone Electrophoresis. *Journal of Chromatography A*. 695, 297.

16. Cocco, L., Roth, B., Temple, C., Montgomery, J.A., London, R.E, Blakley, R.L. Protonated State of Methotrexate, Trimethoprim, and Pyrimethamine Bound to Dihydrofolate-Reductase. (1983) *Archives of Biochem. And BioPhys.* 226 (2), 567.

17. Qiang, Z. Adams, C. (2004) Potentiometric Determination of Acid Dissociation Constants (pKa) for Human and Veterinary Antibiotics. *Wat.Res.* 38, 2874.