

# Removal of 17 $\beta$ -estradiol (E2) and its chlorination by-products from water and wastewater using non-imprinted polymer (NIP) particles

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## ABSTRACT

Endocrine disrupting compounds and their chlorination by-products are two classes of emerging contaminants. Surface water and wastewater treatment technologies have limitations in removing these contaminants. This study evaluated the ability of non-imprinted polymer particles (NIP) to remove the endocrine disruptor 17 $\beta$ -estradiol (E2) and its chlorination by-products from water and wastewater. NIP effectively removed 98% of 10 mg/L E2 from wastewater. NIP were also effective in removing chlorination by-products of E2 by 84.9% after 10 mg/L E2 in water was chlorinated at 5 mg/L. In the presence of 5 mg/L humic acid, NIP were able to achieve removal of 10 mg/L E2 by greater than 99.9%. Furthermore, after chlorination of 10 mg/L E2 and 5 mg/L humic acid at 10 mg/L chlorine, NIP were also able to remove the chlorination by-products formed as well as the remaining E2 by greater than 99.9%. The presence of 5 mg/L humic acid did not adversely affect the adsorption efficiency. The results of this research indicate that NIPs have good potential as a final treatment step for surface water and wastewater treatment.

**Key words** | 17 $\beta$ -estradiol (E2), chlorination by-products, MIP, NIP, wastewater, water treatment

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## INTRODUCTION

Surface water as well as wastewater effluents have been shown to contain a range of synthetic and natural estrogens (Servos *et al.* 2005; Fernandez *et al.* 2007; Jeffries *et al.* 2010). Chlorination, widely used for disinfection of drinking water and wastewater, has been shown to achieve the removal of estrogens to an extent (Deborde *et al.* 2004); however, formation of chlorination by-products remains a concern (Korshin *et al.* 2006). Korshin *et al.* (2006) reported that total organic halogen concentrations during the chlorination of bisphenol-A increased for 4 h following chlorine addition.

For the removal of estrogens, adsorption has an advantage over chemical oxidation processes, as it does not result in the formation of by-products. Activated carbon is inexpensive and is commonly used for water treatment. However, competition for adsorption sites reduces its feasibility for wastewater treatment. Rowsell *et al.* (2006) found greater than 81% removal of estrogens from wastewater using activated carbon but also found that the removal was dependent on the bed volume and large volumes would be required to remove estrogens from wastewater.

Non-imprinted polymers (NIP) are polymeric particles prepared from cross-linked polymers. They have a macroporous structure and exhibit strong non-specific adsorption (Pichon & Chapuis-Hugon 2008). Molecularly-imprinted polymers (MIP), on the other hand, function through specific adsorption for the selected compound rather than non-specific adsorption, and have been studied by many researchers for the removal of endocrine disrupting compounds from water (Meng *et al.* 2005; Le Noir *et al.* 2007a, b, 2009; Randhawa *et al.* 2007; Lin *et al.* 2008; Zhongbo & Hu 2008; Fernández-Alvarez *et al.* 2009; Zhang & Hu 2009; Hajizadeh *et al.* 2010; Krupadam *et al.* 2010; Luo *et al.* 2011). In research studies, NIPs are used as blank polymer particles for MIPs to illustrate the specific adsorption of MIPs for the targeted endocrine disrupting compounds. NIPs have the advantage of not being as compound-specific as the MIPs and have the capability of removing a diverse range of endocrine disrupting compounds as well as other emerging contaminants simultaneously from water. In addition, NIPs are simple to prepare and can be regenerated and reused repeatedly

(Lin *et al.* 2008). In spite of their potential, previous studies have not considered using NIP for the removal of endocrine disrupting compounds from water and wastewater. The main goal of this study was to evaluate the use of NIPs for removal of 17 $\beta$ -estradiol (E2) and its chlorination by-products from surface water and wastewater, and to determine whether NIPs have significant future potential for water and wastewater treatment applications.

## MATERIALS AND METHODS

### Preparation of NIP particles

Functional monomer methacrylic acid (MAA) (Sigma-Aldrich; Oakville, Canada) and cross-linked ethylene glycoldimethacrylate (EGDMA) (Sigma-Aldrich; Oakville, Canada) were dissolved in a porogen with a ratio of 1 mmol:8 mmol:6.7 mmol (Wei *et al.* 2006). The porogen was composed of 40 mL of 1:3 (v:v) acetone (Caldon; Georgetown, Canada), and acetonitrile (Fisher; Ottawa, Canada). 2% (w:w) of 2-isobutyronitrile (AIBN) was added as the initiator (Sigma-Aldrich; Oakville, Canada). The mixture was sonicated (2510 Branson sonicator; Danbury, USA), deoxygenated with nitrogen for five minutes, and then placed in a 60 °C hot water bath for 24 h (Haake GH Fisons; Karlsruhe, Germany). The resulting polymer particles were dewatered using a centrifuge (Revolutionary Science; Lindstrom, USA) at 10,000 RPM, dried at 104 °C overnight, and ground manually. The particles were then rinsed four times with acetonitrile (Fisher; Ottawa, Canada) and once with water and dried before use.

### Sample preparation

E2 (17 $\beta$ -estradiol), which is the most potent natural estrogen (Shappell *et al.* 2010), was chosen as a model compound. A stock solution of E2 (Sigma-Aldrich, Oakville, Canada) at a concentration of 1,000 mg/L was prepared in methanol, and was spiked in water and wastewater samples to give an E2 concentration of 10 mg/L. This concentration is higher than concentrations present in the environment, but was chosen in order to evaluate the adsorption capacity of NIP for E2 and due to the detection limit of the instrument. Both water and wastewater samples were used in this study to better evaluate the potential of NIP for water and wastewater treatment.

Wastewater (secondary biological effluent) samples were collected from the Robert O. Pickard Environmental

Centre in Ottawa, Canada. The wastewater had a turbidity of 0.9 NTU and a chemical oxygen demand (COD) of 61 mg/L. The wastewater was filtered with a 200 nm micro-syringe filter. Adsorption tests were then conducted by spiking with 10 mg/L E2 and applying varying NIP concentrations in 1.5 mL centrifuge tubes (5, 10, 15, and 20 mg/mL). The tubes were shaken manually for mixing followed by 5 min of sonication and an additional 5 min contact time. The tubes were then centrifuged at 10,000 RPM, and the centrate was removed and analyzed with high-performance liquid chromatography (HPLC).

For chlorination experiments, two types of samples were prepared: deionized water spiked with 10 mg/L E2, chlorinated with 5 and 10 mg/L chlorine and treated with NIP; and deionized water spiked with 10 mg/L E2, 5 mg/L humic acid, chlorinated with 10 mg/L and treated with NIP. 1,000 mg/L sodium hypochlorite stock solution was prepared from Javex<sup>®</sup>. The stock solution was added to samples containing E2 or humic acid (Sigma-Aldrich; Oakville, Canada) to give an initial chlorine concentration of 5 and 10 mg/L. All samples were covered with aluminum foil to avoid light exposure and evaporation. Once chlorine was added, the samples were sonicated (2510 Branson sonicator; Danbury, USA) for 15 min during which the samples were well-mixed. Following the 15 min contact time, 40 and 80  $\mu$ L of sodium thiosulfate (Sigma-Aldrich; Oakville, Canada) were added to the 5 and 10 mg/L chlorine samples respectively to quench the residual chlorine. Sodium thiosulfate, chlorine solutions, humic acid solutions, and chlorinated humic acid solutions were all tested with the HPLC-FD prior to analysis to ensure that they did not generate any peaks that may have interfered with the analysis. Adsorption experiments were conducted as described above with 5 and 10 mg/mL of NIP particles.

### Analytical measurements

Analytical measurements were conducted in duplicate. The HPLC setup included a solvent pump (Shimadzu LC-6A, Kyoto, Japan), injector valve (Valco Cheminert VIGI C2XL, Houston, Texas, USA) with a 25  $\mu$ L sample loop, column (Keystone Scientific Spherisorb 3  $\mu$ m 50  $\times$  2 mm, State College, Pennsylvania, USA), and data acquisition system (Peaksimple, Torrance, California, USA). A fluorescence detector (Millipore, Waters 470; Billerica, USA) was used for analysis. Baseline correction was applied to all chromatograms. All chromatograms represent duplicate analytical samples. No pre-concentration or sample cleanup was carried out since the goal of the study was to conduct

a preliminary evaluation of the NIP for the removal of E2 and its chlorination by-products from water. Samples were simply directly injected into the HPLC. This resulted in a detection limit of 1 µg/L for E2. It was not possible to identify any of the compounds measured, but it was possible to observe changes in the HPLC peak areas and locations.

## RESULTS AND DISCUSSION

HPLC was used to measure the quantity of selected compounds before and after treatment with the NIP particles. The output was recorded as a series of peaks, and the differences in the peak areas were used to estimate the removal achieved with the polymer particles. Determination of the fate and concentration of E2 and its by-products was outside the scope of this study.

Table 1 shows the effect of adding increasing quantities of polymer particles (5–20 mg NIP/mL) to wastewater spiked with 10 mg/L E2. Percentage reduction values are averages of two analytical replicates and the percentage differences between two measurements are given. Treating the spiked sample with 5 mg NIP/mL alone resulted in 89.8% decrease in the peak area or E2

concentration. Increasing the NIP concentrations to 10, 15 and 20 mg NIP/mL continued to decrease the peak areas by 96.1%, 98.3%, and 97.7% consecutively (Figure 1). The results indicated that NIP were effective in removing E2 from wastewater, and 5 mg NIP/mL was adequate to remove the majority of the 10 mg/L E2 from the samples. Beyond 10 mg NIP/mL, increasing the NIP concentration did not result in a substantial improvement in E2 removal.

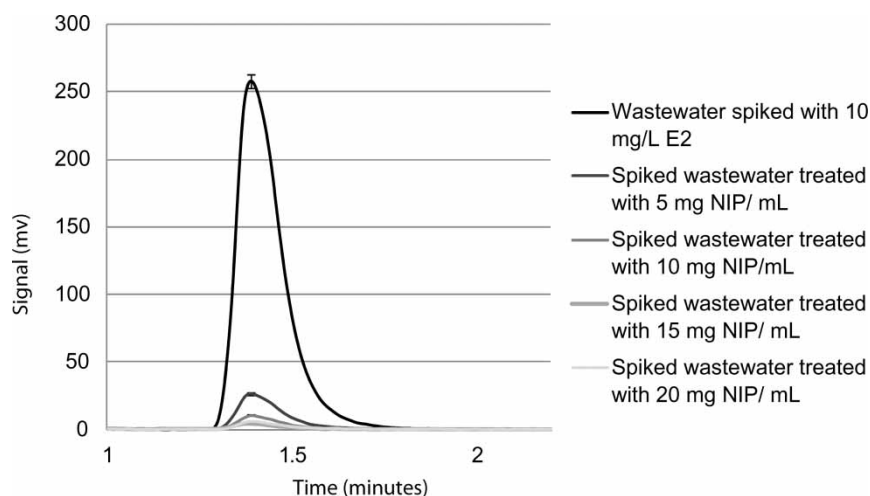
Chlorination experiments were carried out in deionized water, and not in wastewater, to avoid competing reactions from wastewater constituents with chlorine. Figure 2 shows the resulting chromatograms. Chromatograms are averages of two analytical replicates. The vertical bars, indicated only at the peaks for clarity, show the measured values for the two samples. Deionized water samples were first spiked with 10 mg/L E2 and were chlorinated at 5 mg/L, which resulted in a 61% decrease in E2 peak area (Figure 2(a)). The decrease was mainly caused by the oxidation of E2. A smaller peak appeared before the E2 peak indicating the formation of chlorination by-products of E2 after treatment with chlorine (Figure 2(b)). These chlorination by-products were not identified in this study. Hu *et al.* (2003) reported seven chlorination by-products

**Table 1** | Treatment of E2 under various conditions with NIP particles

NIP (mg/mL)	Sample	Chlorine (mg/L)	Humic acid (mg/L)	Peak	Percent reduction (%)	Percent difference (%)
5	Wastewater	0	0	Initial	89.8	0.15
10	Wastewater	0	0	Initial	96.1	0.38
15	Wastewater	0	0	Initial	98.3	0.03
20	Wastewater	0	0	Initial	97.7	0.08
5	DI water	5	0	Initial	98.4	0.32
5	DI water	5	0	By-product	61.9	6.98
10	DI water	5	0	Initial	>99.9	BD
10	DI water	5	0	By-product	84.9	1.69
5	DI water	10	0	Initial	>99.9	BD
5	DI water	10	0	By-product	>99.9	BD
10	DI water	10	0	Initial	>99.9	BD
10	DI water	10	0	By-product	>99.9	BD
5	DI water	10	5	Initial	>99.9	BD
5	DI water	10	5	By-product	>99.9	BD
10	DI water	10	5	Initial	>99.9	BD
10	DI water	10	5	By-product	>99.9	BD
5	DI water	0	5	Initial	>99.9	BD
10	DI water	0	5	Initial	>99.9	BD

DI water: Deionized water.

BD: Below detection.

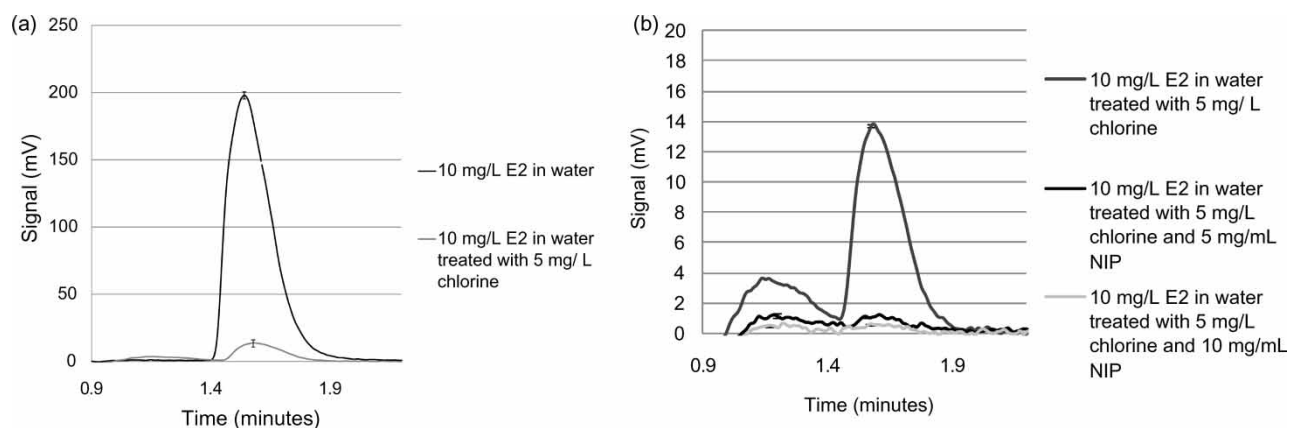


**Figure 1** | Wastewater spiked with 10 mg/L E2 and treated with NIP (5–20 mg NIP/mL).

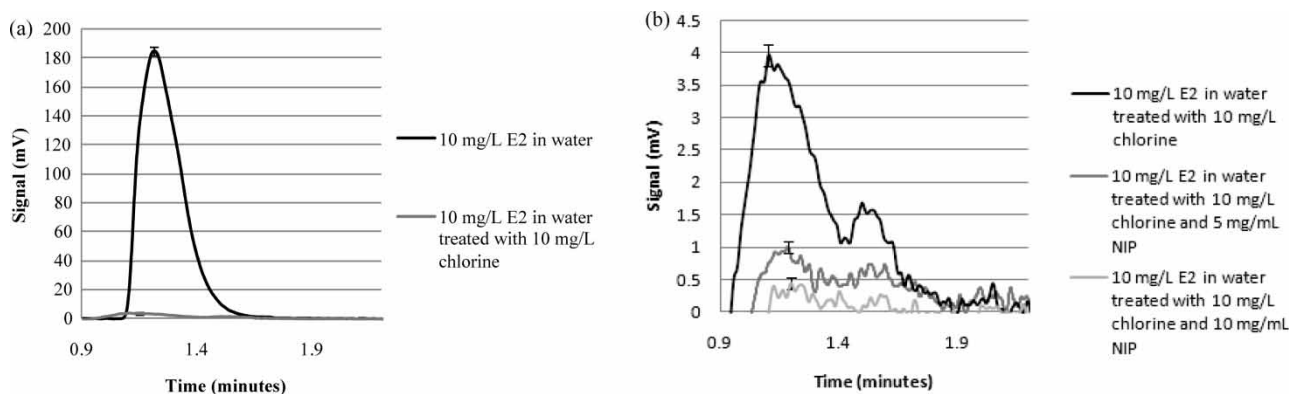
formed during the chlorination of E2 including 2-mono-chloroestrone, dichloroestrone, and dichloro-17 $\beta$ -estradiol.

When the same sample, which was spiked with 10 mg/L E2 and chlorinated at 5 mg/L, was treated with 5 mg/mL NIP particles, the E2 peak decreased by 98.4% and the by-product peak decreased by 61.9%, as shown in [Table 1](#) and [Figure 2\(b\)](#). Increasing the NIP to 10 mg/mL decreased the peak area by greater than 99% and 84.9% for E2 and by-product peaks consecutively. It should be noted that a by-product which has the same retention time as E2 may be detected under the E2 peak. Furthermore, a smaller peak does not necessarily mean the concentration of by-products were lower than E2, since by-products may have lower fluorescence than E2. In addition to the fluorescence detector, samples were also analyzed with a UV detector but no peaks were observed.

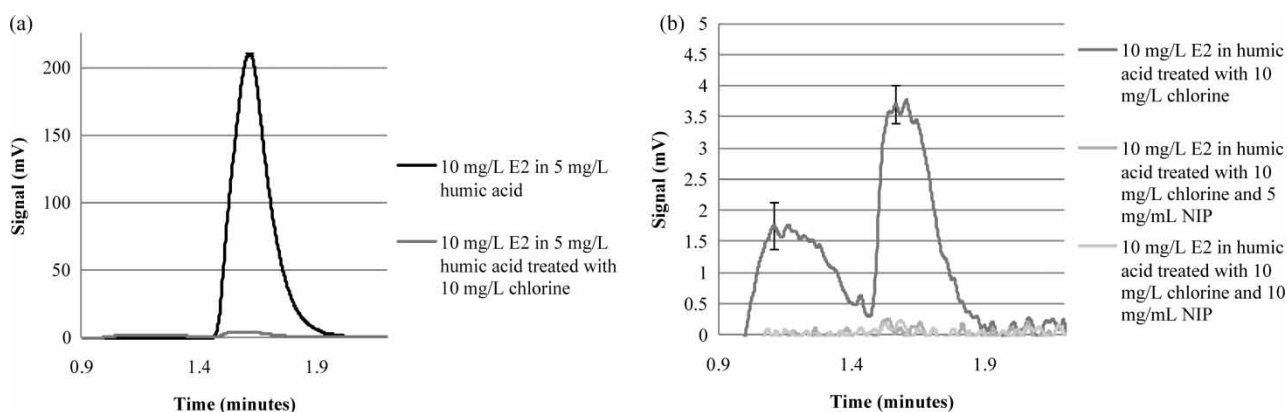
[Figure 3\(a\)](#) shows the chromatogram peaks when the E2 spiked sample was chlorinated with 10 mg/L. Vertical bars are shown at the peaks representing the signal values measured for the two samples averaged to obtain the chromatogram. Some of the vertical bars are not visible because they were small. A higher chlorine concentration resulted in higher oxidation of E2 and its by-products which resulted in smaller peaks compared to the samples that were chlorinated at 5 mg/L chlorine ([Figure 2\(a\)](#)). A 67.4% reduction in the E2 peak was observed at 10 mg/L chlorine compared to 61% observed at 5 mg/L chlorine. Chlorinated E2 samples were then treated with 5 and 10 mg/mL NIP. Addition of 5 or 10 mg/mL of NIP further reduced both peaks to below the detection limits of the instrument, achieving greater than 99.9% removal of E2 and its by-products ([Table 1](#) and [Figure 3\(b\)](#)).



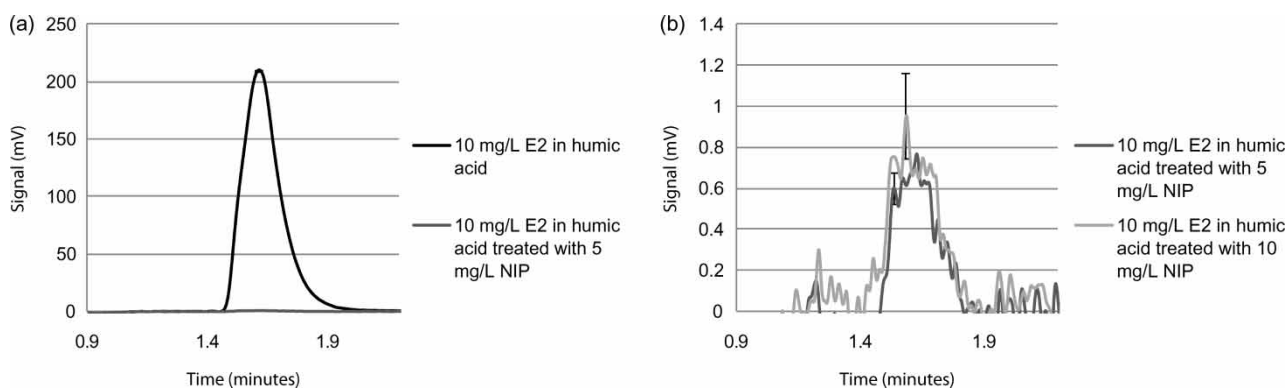
**Figure 2** | Deionized water spiked with 10 mg/L E2 and chlorinated at 5 mg/L (a); Deionized water spiked with 10 mg/L E2, chlorinated at 5 mg/L, and treated with 5 and 10 mg/mL NIP (b).



**Figure 3** | Deionized water spiked with 10 mg/L E2 and chlorinated at 10 mg/L (a); Deionized water spiked with 10 mg/L E2, chlorinated at 10 mg/L, and treated with 5 and 10 mg/mL NIP (b).



**Figure 4** | Deionized water spiked with 10 mg/L E2 and 5 mg/L humic acid, and chlorinated at 10 mg/L (a); Deionized water spiked with 10 mg/L E2 and 5 mg/L humic acid, chlorinated at 10 mg/L, and treated with 5 and 10 mg/mL NIP (b).



**Figure 5** | Deionized water spiked with 10 mg/L E2 and 5 mg/L humic acid, and treated with 5 mg/mL NIP (a); Deionized water spiked with 10 mg/L E2 and 5 mg/L humic acid, and treated with 5 and 10 mg/mL NIP (b).

In the above experiments, deionized water was used to isolate the chlorination by-products of E2. However, surface water and wastewater effluents contain a range of organic substances. Disinfection by-products that form in the

presence of organic matter have been proposed to have a wide range of health effects including birth defects and cancer (Kuo *et al.* 2009; Nieuwenhuijsen *et al.* 2009), and their removal is a priority.

In the next phase, NIP were tested for their effectiveness in removing chlorination by-products formed in the presence of E2 and humic acid. Figure 4(a) shows the chromatogram peaks obtained from deionized water samples first spiked with 10 mg/L E2 and 5 mg/L humic acid, followed by 10 mg/L chlorine. The chromatograms are averages of two replicates and the vertical bars show the measured values for the two samples. Not all of the vertical bars are visible due to their small size. Chlorination resulted in 97.7% reduction in the measured peak area (Figure 4(a)). The additional peak that appeared was likely caused by the chlorination by-products of E2 and humic acid. When treated with 5 or 10 mg/mL of NIP, both the initial peak and the chlorination by-product peak were reduced to below the detection limit (Table 1 and Figure 4(b)).

Chlorination is a widely used disinfection process, and provides the additional benefit of oxidizing organic and inorganic constituents remaining after treatment. If NIP are applied before chlorination, the adsorption sites on the particles would be exposed to higher concentrations of organic compounds which may block the adsorption sites and decrease the removal efficiency. In the last phase, the removal efficiency of the NIP was evaluated in water samples containing 10 mg/L E2 and 5 mg/L humic acid, but no chlorine. In a similar study, Lin *et al.* (2008) found that the efficiency of MIP for removal of estrogens increased in the presence of humic acid due to a decrease in pH and limited adsorption of humic acid. Treatment with 5 mg/mL NIP resulted in greater than 99.9% removal of E2 to below the detection limit, indicating that the NIPs were able to remove very high concentrations of E2 in the presence of humic acid (Table 1 and Figure 5(a)). When the NIP concentration was increased to 10 mg/mL, greater than 99.9% removal of E2 was again achieved (Table 1 and Figure 5(b)). The results indicated that NIP continued to have high removal of E2 in the presence of organic matter, and saturation of adsorption sites did not appear to be an issue.

These results demonstrate the potential for NIP as a possible treatment technology for water and wastewater. NIPs are cross-linked polymeric materials with adsorption sites for organic molecules, and they exhibit strong non-specific adsorption. The findings of this study indicate that NIPs can effectively and simultaneously remove both E2 and its chlorination by-products, and their adsorption capacity does not appear to be adversely affected in the presence of organic matter, which is a problem with activated carbon. NIP are easy and inexpensive to manufacture in

large quantities, and can potentially be used as a final polishing step at treatment plants. The application of the NIP after chlorination would also allow the removal of disinfection by-products and a lower concentration of NIP would be required due to the oxidation of organic compounds during chlorination. Future research is suggested to determine the removal efficiency of NIP for E2 and its by-products at much lower concentrations (less than 1 µg/L), which are more environmentally relevant.

## CONCLUSIONS

1. NIP effectively removed E2 from water and wastewater even at concentrations of 10 mg/L.
2. NIP effectively removed chlorination by-products of 10 mg/L E2 from water following chlorination at 5 and 10 mg/L.
3. NIP effectively removed chlorination by-products of 10 mg/L E2 and 5 mg/L humic acid following chlorination at 10 mg/L.
4. NIP effectively removed 10 mg/L E2 in the presence of 5 mg/L humic, and its adsorption efficiency was not significantly affected in the presence of humic acid.
5. NIP appears to have significant future potential for water and wastewater treatment applications.

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