Removal of endocrine disrupting compounds from wastewater using polymer particles
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ABSTRACT

This study evaluated the use of particles of molecularly imprinted and non-imprinted polymers (MIP and NIP) as a wastewater treatment method for endocrine disrupting compounds (EDCs). MIP and NIP remove EDCs through adsorption and therefore do not result in the formation of partially degraded products. The results show that both MIP and NIP particles are effective for removal of EDCs, and NIP have the advantage of not being as compound-specific as the MIP and hence can remove a diverse range of compounds including 17β-estradiol (E2), atrazine, bisphenol A, and diethylstilbestrol. Removal of E2 from wastewater was also tested to determine the effectiveness of NIP in the presence of interfering substances and natural organic matter. Removal of E2 from wastewater samples was high and increased with increasing NIP. NIP represent an effective way of removing a wide variety of EDCs from wastewater.

Key words | adsorption, endocrine disrupting compounds, MIP, molecularly imprinted, NIP, wastewater treatment

INTRODUCTION

Endocrine disrupting compounds (EDCs) have received increased attention in recent years. This is due in part to the discovery of the many negative ecological and health impacts of these chemicals, in part to improved detection abilities with new high-end analytical instruments, and in part to increased domestic, commercial and industrial use of these compounds leading to greater concentrations in water resources. The main sources of EDCs in natural environments are wastewater effluents and agricultural runoff (Chen et al. 2006; Caliman & Gavrilescu 2009).

The effects of EDCs on humans are difficult to quantify because humans are exposed to a wide variety of natural and synthetic chemicals over their lifetimes. Suspected health effects of EDCs on humans include increased risk of testicular and breast cancers (Maffini et al. 2006); decreases in sperm quality (Maffini et al. 2006); obesity (Newbold et al. 2008); earlier onset of puberty for girls (Euling et al. 2008); and development of autism (Braun et al. 2014).

Conventional water and wastewater treatment plants are neither designed nor equipped for removal of EDCs, and advanced treatments are required. EDCs are partially degraded during biological wastewater treatment, but these degraded compounds can be more toxic than the original contaminants (Clara et al. 2005). Ozonation or advanced oxidation can be used to remove EDCs, but partially degraded by-products can be a concern (Lee et al. 2004; Korschin et al. 2006). Activated carbon or membrane technologies can be used to completely remove EDCs from water while avoiding the formation of by-products. However, activated carbon can be affected by competition from natural organic matter (NOM), which reduces the efficiency and increases the cost of treatment (Fukuhara et al. 2006; Snyder et al. 2007). Membrane technologies can also remove EDCs without the formation of by-products, but can be expensive, and produce concentrated waste streams requiring disposal (Braeken & Van der Bruggen 2009).

Molecularly imprinted polymers (MIP) and non-imprinted polymers (NIP) represent an innovative alternative to conventional treatment methods because they do not lead to the formation of daughter products and past research suggests that they are not influenced by the presence of NOM (Sellergren 1999; Masqué 2001). MIP are cross-linked polymers containing cavities specific to a template molecule. The specific cavities are created by copolymerization of cross-linking monomers and functional monomers with an imprinting molecule or template.
Following polymerization, the template is removed, leaving a cavity specific to the template. MIP selectively re-bind to the template molecule or molecules with similar shapes and structures. NIP are synthesized using the same procedure of polymerization as MIP, with the exception that no template is present, and removal occurs via non-specific surface adsorption (Sellergren 1999; Masqué 2001; Alexander 2006). The main difference between MIP and NIP is thus their specifcity. MIP can selectively target and remove targeted compounds whereas NIP can remove different organic compounds. NIP’s non-specific binding is attributed to hydrophobic interactions between organic contaminants and the polymers.

Development of new, effective and affordable technologies for the removal of EDCs from water without producing any toxic degradation products has become a priority. The goal of this research was to determine and compare the binding efficiencies of MIP and NIP for selected EDCs, and to evaluate their capacity to remove EDCs from wastewater. While MIP have been studied extensively for removal of EDCs during water and wastewater treatment (Meng et al. 2005; Le Noir et al. 2007a; b, 2009; Lin et al. 2008; Zhongbo & Hu 2008; Fernández-Alvarez et al. 2009; Li et al. 2009; Guo et al. 2011), NIP have not been considered for treatment applications (Murray & OrmeCi 2012). This research also investigates the use of NIP as a potential treatment for EDC-contaminated water.

MATERIALS AND METHODS

Preparation of MIP and NIP

The MIP and NIP were prepared as proposed by Wei et al. (2006). For the preparation of MIP, E2, methacrylic acid (MAA) and ethylene glycol dimethacrylate (Sigma-Aldrich; Oakville, Canada) were dissolved in porogen solvent with the ratio of 1 mmol:8 mmol:6.7 mmol. For the preparation of NIP, no E2 was added (Wei et al. 2006).

The resulting polymer particles were collected using a centrifuge (Revolutionary Science; Lindstrom, USA) at 10,000 rpm, dried at 104 °C overnight, and broken up manually. The particles were then rinsed four times with acetonitrile (Fisher; Ottawa, Canada) and once with water. MIP were then cleaned three times with 100% triethylamine (TEA) (Sigma-Aldrich; Oakville, Canada) to remove the E2 template, rinsed once with acetonitrile, and then once with deionized water. The NIP were not cleaned with TEA as they contained no template.

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was used to determine the particle size. All SEM images were obtained with a JSM-6400LV (JEOL; Japan) microscope. MIP and NIP particles were deposited onto individual carbon tape cartridges, and coated with less than 5 nm of gold using radio frequency sputter (Anatech Hummer; Union City, CA, USA).

Analytical measurements

Analytical measurements were conducted in duplicate using high-performance liquid chromatography (HPLC). The HPLC setup included a solvent pump (Shimadzu LC-6A; Kyoto, Japan), injector valve (Valco Cheminert VIGI C2XL; Houston, Texas, USA) with a 25 μL sample loop, column (Keystone Scientific Spherisorb 3 μm 50 x 2 mm; State College, Pennsylvania, USA), and data acquisition system (PeakSimple; Torrance, California, USA). UV detection (Gilson 115 UV detector; Middleton USA) was used for diethylstilbestrol and bisphenol A (BPA), and atrazine and fluorescence detection (Millipore Waters 470) was used for E2.

Removal efficiencies of MIP and NIP

Removal experiments were conducted by preparing standard solutions in deionized water for E2 up to 10 ppm and in a 1:1 mixture of methanol and deionized water for higher concentrations (20–100 ppm corresponding to 1–5 mg endocrine disruptor/g particles) of E2, diethylstilbestrol, BPA, and atrazine.

Particle samples were prepared by weighing 20 mg of MIP or NIP into 2 mL centrifuge tubes. One millilitre of each standard was then added. The tubes were shaken manually for mixing, followed by 5 minutes of sonication and waiting, respectively. The tubes were then centrifuged at 10,000 rpm for 2 minutes to separate the particles from the supernatant. The supernatant was removed and analyzed by HPLC to determine percent removal.

Treatment of spiked wastewater with NIP

Secondary clarifier effluent samples were taken from the Robert O. Pickard Environmental Centre (ROPEC) (Ottawa, Canada) for treatment with NIP. ROPEC employs conventional primary and secondary wastewater treatment, and effluent samples were taken after secondary sedimentation and prior to disinfection. Wastewater was spiked with E2 and treated with NIP. The wastewater was filtered
with a 0.2 μm micro-syringe filter. Tests were then conducted with a constant E2 concentration (10 ppm) and varying NIP. Binding determination by HPLC analysis was conducted as outlined above.

RESULTS AND DISCUSSION

Following the synthesis of the MIP and NIP, they were characterized using SEM. The images in Figure 1 show that both the MIP and NIP are spherical. The MIP are slightly larger and slightly more uniform in size. The MIP had an average estimated size of 314 ± 96 nm and the NIP had an average size of 292 ± 147 nm. Prior to the SEM imaging, the particles were physically ground; thus it is evident from the image that physical grinding did not separate the aggregates into single particles. The particles were also not uniformly dispersed in water, and thus would not have all available adsorption sites accessible for contaminant removal. Increasing the dispersion of the particles in the solution to be treated would increase the treatment efficiency.

Binding efficiencies of MIP and NIP were compared using E2 in concentration ranges of 0.01–0.05 (Figure 2) and 1–5 mg E2/g particles (Figure 3). Each data point is given as the average of duplicates and the error bars represent the maximum and minimum values. In Figure 2, NIP exhibited slightly higher binding efficiencies compared to MIP, and 100% binding was achieved at 0.05 mg E2/g particles for NIP. In comparison, the highest binding efficiency of the MIP was 94%. At 0.01 mg E2/g particles, the binding efficiencies were 75% for MIP and 74% for NIP. Increasing the E2 concentration increased the binding efficiencies.

Percent removal of E2 for 1–5 mg E2/g particles in 1:1 solution of methanol and deionized water is illustrated in Figure 3. MIP and NIP again showed comparable binding efficiencies in this concentration range. The highest E2 removal was 90% for MIP and 91% for NIP. Increasing the E2 concentration resulted in a gradual decrease in binding efficiencies of the particles. In comparison to activated carbon, Snyder et al. (2007) studied removal of a range of EDCs and pharmaceuticals and personal care products (PPCPs) with activated carbon with 100 ng/L concentrations for 65 EDCs and PPCPs and 5 mg/L of powdered activated carbon, or a ratio of 1.3 mg total EDC and PPCP/g activated carbon, and found high levels of removal for most of the EDCs studied. Concentrations were chosen based on typical full-scale treatment concentrations (Snyder et al. 2007). Results shown in Figure 3 for MIP and NIP are comparable to those obtained with activated carbon, although factors such as contact time and concentrations of EDCs and adsorbents used are also important.

It should be noted that for all experiments conducted at greater than 1 mg E2/g particles, a 1:1 solution of methanol and deionized water was used to prepare the samples. This was necessary to increase the solubility of E2 in water to study the efficiency of MIP and NIP under a wider range of concentrations. Binding efficiencies are expected to be different in methanol/water compared to water samples (Pap et al. 2002). The compounds tested were more soluble in methanol than in water; thus methanol would decrease the removal in all cases. The data can therefore be interpreted as a worst-case scenario with the understanding that removal from water samples would be higher.

The results shown in Figures 2 and 3 indicate that NIP are equally effective as MIP in removing E2. NIP are also less...
expensive and easier to prepare and are capable of removing a variety of EDCs due to their non-specific binding. This suggests that NIP may be more suitable for wastewater treatment; therefore NIP were selected for use in the rest of the experiments.

Additional binding tests were conducted with diethylstilbestrol, BPA, and atrazine to evaluate the NIP for removal of a range of EDCs. These EDCs were selected due to their prevalence in the natural environment, and they represented a wide range of chemical and structural properties (e.g., molecular weight, acid dissociation constant and octanol–water partition coefficient). Figure 4 shows the removal efficiencies for diethylstilbestrol, atrazine, and BPA from a 1:1 mixture of deionized water and methanol. Atrazine removal decreased linearly with increasing atrazine with a slope of 5.4%, and the highest atrazine adsorption was measured as 75% at 1 mg atrazine/g NIP. BPA had the highest binding efficiencies among all the compounds tested and were greater than 92% for the entire concentration range. Diethylstilbestrol adsorption was lower than the adsorption of atrazine and BPA. The binding efficiency for diethylstilbestrol was only 27% at 1 mg diethylstilbestrol/g NIP, increased to 45% at 3 mg diethylstilbestrol/g NIP, and then decreased to 32% at 5 mg diethylstilbestrol/g NIP.

Presence of organic and inorganic compounds in water samples may affect the adsorption characteristics and thus removal efficiencies. NIP were tested on wastewater samples spiked with E2 to investigate their removal capacity for environmental samples. E2 was spiked into wastewater to give an E2 concentration of 10 ppm before treatment. As seen in Figure 5(a), there was a substantial decrease in the peak height, from 258 mV down to 27 mV, following application of just 5 mg of NIP per mL of wastewater. Increasing particle concentrations (5–20 mg of NIP/mL) were then applied to investigate whether increased E2 removal would result from increased NIP application (Figure 5(b)). The results show that the peak height decreased further with each increment in NIP concentration, and indicate that NIP can be effectively used to remove E2 from wastewater.

**CONCLUSIONS**

EDCs are typically released into the environment via wastewater and contaminate the surface waters that are the source of drinking water. Traditional treatment processes employed at water and wastewater treatment plants are neither successful nor designed to remove EDCs, and new technologies are needed. This study evaluated the use of MIP and NIP as a treatment method for removal of EDCs. MIP and NIP achieve removal of EDCs through specific and non-specific adsorption and do not result in the formation of harmful by-products. The results of this study show that both MIP and NIP are effective in the removal of EDCs. However, NIP are simple to prepare/manufacture and can remove a range of compounds including E2, BPA, diethylstilbestrol, and atrazine. Removal of E2 from wastewater was high in the presence of interfering substances.
and increased with increasing NIP application. Further work is required to determine the feasibility of both MIP and NIP for full-scale treatment.

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