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Extraction of 17β-estradiol in water using non-imprinted polymer submicron particles in membrane filters

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Abstract

17β-Estradiol (E2) is an endocrine disrupting chemical of harm to both animals and human beings at a low concentration level (ng/L). It cannot be completely removed by wastewater treatments, and is often detected in both environment and drinking waters. The purpose of this feasibility study, towards environmental engineering in the field of water analysis and treatment, was to remove E2 by extraction using non-imprinted polymer (NIP) submicron particles. Experimental results showed that 0.5 mg/L of E2 could be completely extracted by adding 10 mg of NIP particles directly into 10 mL of water. However, the extraction efficiency decreased to 64% for 100 mL of water. prefilling the NIP particles inside a membrane filter showed a potential for water treatment of a large volume, requiring no effort to distribute the particles uniformly in the water. High extraction efficiency (80 ± 10)% for E2 was achieved for 100 mL of water. A total mass of 0.29 mg E2 was extracted from 1000 mL of water containing 0.8 mg/L E2 (by using only 10 mg of NIP particles). Both efficiency and mass capacity can be increased, by scaling up the amount of NIP particles, towards environmental engineering applications.

Key words: 17β-estradiol; non-imprinted polymer; submicron particles; membrane filter; pollution abatement technology
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Introduction

Estrogenic compounds are endocrine disrupting chemicals (EDCs) which are causing concern in recent years due to their potential impacts on the environment (Chang et al., 2009). They are a group of steroids that function as primary female sex hormones. Their degradations in the environment take several days in optimal circumstances, and much more slowly in less ideal circumstances (Noppe et al., 2005). Estrogens are harmful to both animals and humans at a low concentration level (ng/L) (Heath et al., 2010). They can cause reproductive abnormalities and feminization of fish (Piferrer and Donaldson, 1992), and may decrease reproduction rate of birds (Cornil et al., 2009). They are also linked with the decline in sperm counts (Hess et al., 1995), the increasing incidence of breast cancer (Niemeier et al., 2010) and testicular cancer (Hirvonen-Santti et al., 2003), and earlier onset of puberty in humans (Chagin et al., 2007).

17β-Estradiol (E2) is one of the three main naturally occurring estrogenic compounds that are continuously released through different pathways, and cannot be completely removed by wastewater treatment plants (WWTP) (Barnabe et al., 2009). It was not only detected in sewage, treatment plant effluents, surface waters and ground waters, but was also found in tap waters at concentrations up to 2.1 ng/L (Cargouet et al., 2004). Therefore, it is important to develop a cost-effective method that can remove E2 from both wastewater and drinking water.

Among the technologies in use for wastewater cleanup and drinking water purification, nanofiltration (NF), reverse osmosis (RO), and granular activated carbon (GAC) are commonly applied for elimination of EDCs such as E2 (Robinson et al., 2006; Liu et al., 2009). However, these methods are facing different problems such as low removal efficiencies, release of estrogens from the NF and RO membranes during backwashing or at high pH variations (Kvanli et al., 2008), and the expensive regeneration of GAC (Kvech and Tull, 1997). So far, no technology can remove E2 from water in a cost-effective way.

Molecularly imprinted polymers (MIPs) are synthetic macroporous materials that have specific recognition cavities with a shape, size and functional groups complementary to the template molecule, thus establishing an interaction mechanism for molecular recognition (Yu et al., 2007). In recent years, solid-phase extraction (SPE) using MIPs is attracting more applications in the areas of environmental, food and pharmaceutical analyses (Jiang et al., 2009). It allows for the extraction (and hence clean-up) of different classes of compounds from various

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complex matrices due to the selectivity, stability, ease of preparation, and low cost of MIPs (Pichon et al., 2008). Non-imprinted polymers (NIPs) are synthetic macroporous materials that have functional groups to serve as binding sites for organic molecules. NIPs have the same chemical properties as MIPs except having the specific recognition cavities, and they exhibit strong non-specific binding with organic compounds due to hydrophobic interactions (Celiz et al., 2009). In previous research, NIPs were commonly used as a control polymer to test the selectivity of MIPs due to specific recognition cavities for the target analytes (Hu et al., 2010). Although MIPs generally showed higher selectivity and stronger binding to specific compounds, NIPs may have a higher potential for water treatment applications that involve the removal of several various contaminants. Moreover, the synthesis of NIPs is less expensive, and requires no time to remove the template, than the preparation of MIPs. NIP submicron particles can be synthesized by polymerization in a porogen mixture. They can distribute in water rapidly and uniformly due to their small size.

Membranes are commonly used for the removal of dissolved solids, color, and hardness in drinking water. They are also used for the reduction of suspended solids and sludge constituents in wastewater treatment (EPRI Community Environmental Center, 1997). Supor (hydrophilic polyethersulfone) membrane is hydrophilic and generally preferred for the filtration of water. It is a low protein binding membrane and does not bind biomolecules (Pall Life Science, 2008). Acrodisc syringe filters with 0.2 μm (pore size) Supor membrane were chosen to remove NIP particles (about 0.3 μm in diameter) from water, or for prefilling of NIP particles inside. The purpose of this work was to test the efficiency for low-cost extraction of E2 using NIP submicron particles, which were either added directly into the water or prefilled inside an Acrodisc filter.

1 Materials and methods

1.1 Materials

17β-Estradiol was purchased from Sigma-Aldrich (USA). HPLC-grade methanol and acetonitrile were obtained from Caledon (Canada). 18-MΩ·cm distilled deionized water (DDW) was obtained from a Millipore Milli-Q water system (USA). Non-imprinted polymer (NIP) submicron particles with an average diameter of 0.3 μm were prepared in our lab (Lai et al., 2010). The 1000 mg/L (mg/L) stock solution of E2 was prepared by dissolving E2 powder in methanol. Standard solutions of 0.3–1.0 mg/L E2 were prepared by dilutions of the stock solution with DDW. NIP submicron particles were suspended in DDW by sonication for 2 min in an ultrasonic bath. Acrodisc syringe filters with 0.2 μm Supor (hydrophilic polyethersulfone) membrane were purchased from Pall Life Sciences (Port Washington, USA).

1.2 Extraction of E2 using NIP submicron particles directly added into water

Various masses of NIP particles were added into standard E2 solutions of known volumes. Each sample was then sonicated for 2 min in the ultrasonic bath to distribute the particles uniformly. After a waiting period of 5 min, each sample was passed through a new syringe filter using a micropump at 500–9000 r/min (IDEX, USA). The filtrate was collected for analysis by high performance liquid chromatography with fluorescence detection (HPLC-FD).

1.3 Extraction of E2 using NIP submicron particles inside syringe filter

Each syringe filter was prefilled by passing 10 mg of particles in DDW suspension through the filter using a syringe pump. Then a standard E2 solution of known volume was pumped through the NIP prefilled syringe filter. The filtrate was collected for HPLC-FD analysis.

1.4 HPLC-FD analysis

Each standard E2 solution and all collected filtrates were analyzed by HPLC-FD to obtain their E2 peak areas. The HPLC-FD system consisted of a solvent pump (Shimadzu LC-6A, Japan), injector valve equipped with a sample loop, C18 column (3 μm, 150 × 2 mm) (Hypersil MOS-2, USA), fluorescence detector (PerkinElmer LC 240, USA), and data acquisition system (Baseline N2000 Chromatostation version 3.50, PRC). The mobile phase was prepared from DDW, HPLC-grade acetonitrile and HPLC-grade methanol (1:1:1, *V/V/V*). The flow rate was set at 0.4 mL/min. The excitation and emission wavelengths of FD were 220 nm and 310 nm, respectively. The extraction efficiency (f) for E2 in water was calculated by the following equation:

$$f = 100\% - A_f/A_{ss} \times 100\%$$

where, A_f and A_{ss} are HPLC area for E2 in filtrate and HPLC area for E2 in standard solution, respectively.

1.5 Spectrofluorometry of NIP submicron particles

A standard calibration curve of light scattering intensity versus concentration of NIP particles in water was constructed, using a fluorescence spectrophotometer (Varian Cary Eclipse, USA). The excitation wavelength was 700 nm, and the emission wavelength was scanned between 650 and 750 nm. Both excitation and emission slits were set at 5 nm. A detection limit for NIP particles was calculated as: Detection limit = 3 × Standard deviation of the blank (for seven replicate measurements)/Slope of standard calibration curve.

2 Results and discussion

2.1 Extraction of E2 by NIP particles

The performance of NIP particles directly added in water for E2 extraction was compared with NIP particles prefilled inside a syringe filter. As shown in Fig. 1, when various amounts of NIP particles were added in 25 mL

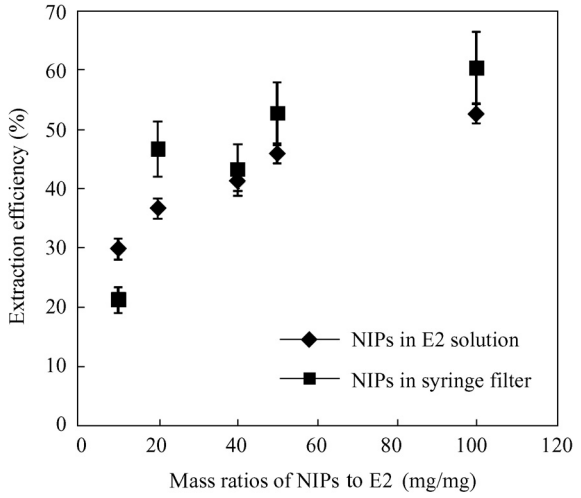

Fig. 1 Extraction of 1 mg/L E2 (25 mL of water containing 25 g of E2) by NIP particles added directly into water and prefilled inside a syringe filter. VIP: non-imprinted polymer.

of water containing 25 g of E2 in total, the extraction efficiency increased as the mass ratio of NIP particles to E2 was increased. So that the removal of E2 from water using NIP particles could be improved by increasing the amount of particles when other conditions were kept the same. This result agreed with a previous report (Kvanli et al., 2008), which indicated that larger amounts of SPE sorbent typically provided a better extraction of EDCs from water. However, the trend was not linear, but curved, representing equilibrium between unbound E2 molecules, unbound sites on NIP particles, and E2-bound sites on NIP particles. An increase in extraction efficiency for E2 was always provided with additional amount of NIP particles.

Compared to NIP particles added directly in water, NIP particles prefilled in a syringe filter generally showed a higher extraction efficiency for E2 especially when the mass ratio was higher than 10. This could be explained by the fact that in a syringe filter, the NIP particles were packed closely, and the E2 molecules in water would likely come to contact with the particle surfaces. On the other hand, the extraction efficiencies obtained using an NIP prefilled syringe filter had slightly more fluctuations. When NIP particles were directly added in water, a 5-min incubation period was allowed for the binding of E2 molecules onto the NIP particles. When NIP particles were prefilled in a syringe filter, the contact time of E2 molecules with NIP particle surfaces were much shorter. Also, the turbulent flow of water might randomly affect the binding of E2 molecules onto NIP particles. Nonetheless, as NIP prefilled in a syringe filter required less extraction time to obtain a higher extraction efficiency, it showed a higher potential for applications in water treatment to remove E2 (and other EDCs).

2.2 Extraction efficiency for NIP particles used inside a syringe filter

As the concentration of E2 in environmental waters is commonly found at a very low level, the extraction efficiencies for 0.8, 0.5 and 0.3 mg/L E2 in water were measured as a function of water volume. Using 10 mg of NIP particles inside a syringe filter, all the three concentrations followed a common trend of decreasing extraction efficiency as more and more water containing E2 was filtered (Fig. 2). When the volume of water was less than 200 mL, the extraction efficiencies for E2 exhibited more fluctuations, probably due to random errors associated with a turbulent water flow.

An important result obtained was that high extraction efficiency (80 ± 10)% was achieved for 50 mL of water or less. This efficiency could potentially be improved by prefilling more NIP particles inside the syringe filter. Figure 3 is a plot of mass of E2 extracted versus volume of water filtered. As expected, the mass of E2 extracted increased with the volume of water filtered. The set of data points for 0.5 mg/L E2 fell between those for 0.3 mg/L and 0.8 mg/L E2, and the trends for all three E2 concentrations were similarly curved. These curves indicated a binding equilibrium between E2 molecules and NIP particles before reaching the binding capacity for 10 mg of NIP particles, which should be higher than 292 μg.

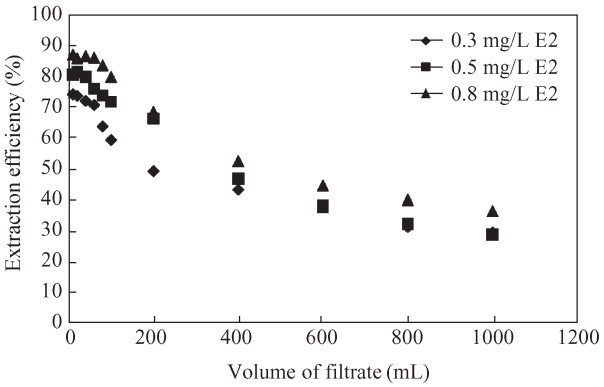

Fig. 2 Extraction efficiencies for 0.8, 0.5 and 0.3 mg/L E2 in water using 10 mg of NIP particles inside a syringe filter.

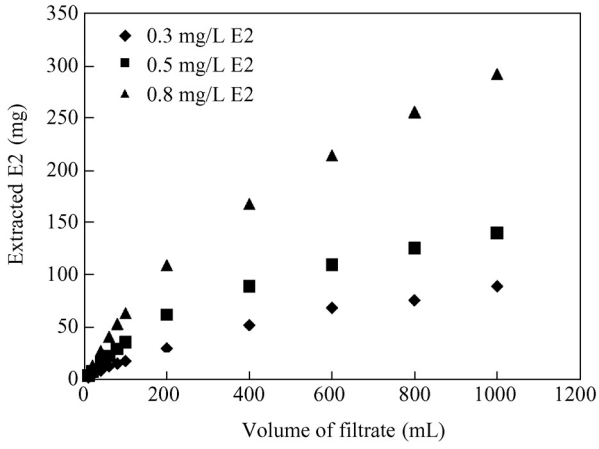

Fig. 3 Masses of E2 extracted from different volumes of water containing 0.8, 0.5 and 0.3 mg/L E2, using 10 mg of NIP particles inside a syringe filter.

2.3 Extraction efficiency for NIP particles added directly into water

A syringe filter, prefilled with 10 mg of NIP particles, achieved extraction efficiency as high as (80 ± 10)%. For comparison purposes, the extraction efficiencies for 0.8, 0.5 and 0.3 mg/L E2 by 10 mg of NIP particles directly added into water were measured as a function of water volume. The new results would determine which method showed a higher potential for water treatment to remove E2. The extraction efficiencies obtained by the two different methods (prefilling NIP particles inside a syringe filter versus adding NIP particles directly into water) generally showed similar trends for 0.3–0.8 mg/L E2 in water. Using the results obtained for 0.5 mg/L E2 (as shown in Fig. 4), the extraction efficiency for E2 decreased as the volume of water increased for both methods. When the volume of water was only 10 mL, NIP particles added directly into water showed a high extraction efficiency of nearly 100% for E2. However, the extraction efficiency soon began to decrease with increasing volume of water. In comparison, when NIP particles were prefilled inside a syringe filter, the extraction efficiency for E2 was essentially constant at 80% up to 40 mL of water. For larger water volumes, the efficiency decreased slowly. One plausible explanation is that when the particles were used inside a syringe filter, any increase in the volume of water for treatment did not affect the operating concentration of NIP particles. When NIP particles were directly added into water, an increase in water volume would decrease the concentration of NIP particles, thereby decreasing the chance for E2 molecules to collide with an NIP particle (and contact a binding site as a result). For these reasons, when the volume of water was large, 10 mg of NIP particles used inside a syringe filter could achieve a higher extraction efficiency (than addition of the particles directly into water). These results indicated that prefilling NIP particles inside a filter had a higher potential to be applied for the treatment of large water volumes.

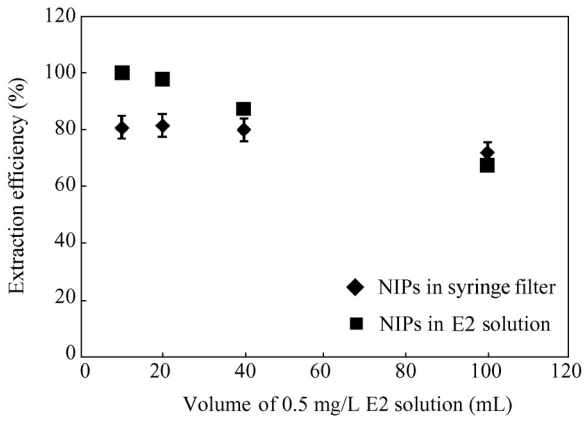

Fig. 4 Extraction efficiencies for 0.5 mg/L E2 in water using 10 mg of NIP particles prefilled inside a syringe filter or added directly into water.

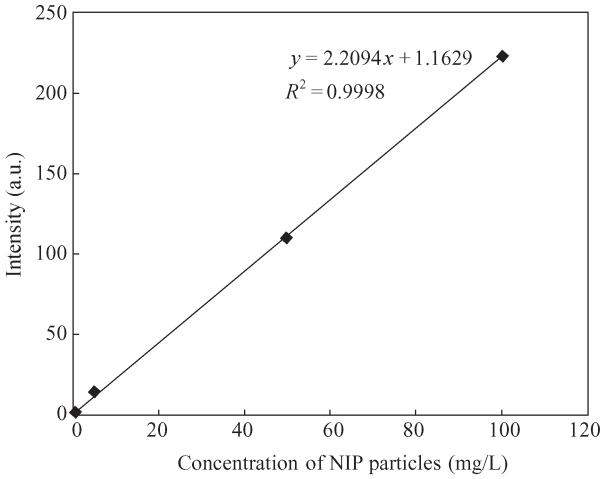

Fig. 5 Standard calibration curve of light scattering intensity versus concentration of NIP particles in water.

2.4 Detection of NIP particles by spectrofluorometry

The new method based on prefilling NIP particles inside a syringe filter was seen to have a higher potential for the treatment of large water volumes. It offered substantial savings on the amount of NIP particles needed to treat a large volume of water. No effort was required to distribute the particles uniformly in the water. All the particles were gathered inside the filter for easy periodic reactivation. Moreover, the water came out (through the filter) apparently clear of any observable amount of suspended NIP particles. When the filtrate water was analyzed on a spectrofluorometer, the concentration of suspended NIP particles was determined to be below the instrumental detection limit of 0.05 mg/L. Such a combination of simple treatment, low cost, and no post-cleanup effort makes the new method a very attractive technology for up-scaling adoption by water treatment plants.

3 Discussion and conclusions

With the objective to develop a cost-effective way for the removal of E2 in water, this work tested two different extraction methods using NIP submicron particles. Experimental results showed that the extraction efficiency for E2 increased as the amount of NIP particles was increased. This increase represented an equilibrium between unbound E2 molecules, unbound sites on NIP particles, and E2 bound on NIP particles. Nearly complete removal of E2 at low concentrations (0.5 mg/L) was achieved by adding 10 mg of NIP particles directly into a small volume (10 mL) of water. However, NIP particles prefilled inside a syringe filter required less time, and generally showed higher extraction efficiency for E2 (than NIP particles directly added into water), especially when the volume of water was large. Prefilling NIP particles inside a syringe filter showed a higher potential for the treatment of large volumes of water (than adding NIP particles directly into water). High extraction efficiency (80 ± 10)% was attained

for the low concentrations (0.8–0.3 mg/L) of E2 in less than 50 mL of water by 10 mg of NIP particles inside a syringe filter. This efficiency could potentially be improved by using more NIP particles in one filter (or several filters in series) to treat the water. Using 10 mg of NIP particles inside a syringe filter, 292 μg of E2 was removed without reaching the maximum binding capacity. This capacity is effectively good for the treatment of 10^3 L of water containing E2 at the 0.292 μg/L concentration level, or 10^6 L of water containing E2 at the 0.292 ng/L level.

Further research experiments are necessary to determine whether NIP particles are applicable in wastewater or environmental water treatment for extraction of estrogenic compounds in complex matrices. Both poisoning of binding sites (caused by dissolved organic matters) and competition for binding sites (by other organic compounds) can reduce the binding capacity significantly. Unlike GAC, the NIP particles are made of poly(methacrylic acid-co-ethylene glycol dimethacrylate) which is mechanically robust, chemically inert, and thermally stable. Periodic reactivation of these particles with strong acids/bases, organic solvents, or heat should be viable. NIP particles are also transparent to UV light, making photo-reactivation quite possible. Any residual E2 in these particles can be monitored readily on a spectrofluorometer, as reported elsewhere (Yang et al., 2010). To the best of available knowledge from the literature, poly(methacrylic acid-co-ethylene glycol dimethacrylate) particles pose no adverse health effect to either human or fish, even if they are ingested with a reasonable volume of water, particularly at concentration levels well below 0.05 mg/L. Magnetic NIP submicron particles are currently undergoing rapid development in our labs as the next abatement technology for E2 contamination control.

Acknowledgments

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