Characterization of Molecularly Imprinted and Nonimprinted Polymer Submicron Particles Specifically Tailored for Removal of Trace 17β-Estradiol in Water Treatment

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ABSTRACT: This study investigated the potential use of molecularly imprinted polymer (MIP) submicron particles for the selective removal of trace 17β-estradiol (E2) in water treatment. Methacrylate-based MIP submicron particles were synthesized, in a one-step suspension polymerization procedure, using ethylene glycol dimethacrylate (EGDMA) as the cross-linker. After template removal, the particles could be used as a smart material for specific binding of E2. The submicron size of MIP particles facilitated uniform dispersion in water for up to 17 days. These particles were meritorious in mass transfer behavior, allowing phase partitioning of E2 molecules in water during a short treatment time. After 1-mL water samples of different E2 concentrations were treated with 20 mg of MIP particles for 2 min, recovery percentages as high as 97% ± 3% were achieved. The specific binding capacity of these MIP particles was determined to be 15 mg E2/g. Nonimprinted polymer nanoparticles were also evaluated for nonspecific binding of E2, using 0.5 mg in 1 mL of water, to attain 64% ± 3% efficiency in 3 min towards general water treatment. A simple capillary electrophoresis method was successfully developed for the characterization of MIP and NIP particles. Apparently the less negative the electrophoretic mobility, the higher binding efficiency and faster binding kinetics the particles would exhibit with E2 due to less hindered Brownian diffusion. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 1499–1508, 2010

Key words: β-estradiol; molecularly imprinted polymer; submicron particles; binding kinetics; precipitation rate; water treatment

INTRODUCTION

In an era focused on environmental health, the water industry is faced with the challenge of ensuring a safe supply of drinking water from sustained sources of varying quality.[1] Water contaminants include toxic metals, carcinogenic organic compounds, synthetic chemicals, pharmaceuticals, illicit drugs, cosmetics, personal care products, and food supplements, together with their respective metabolites and transformation products.[2] These are of global concern because of their potential to adversely affect human health through consumption of drinking water. More emphasis needs to be placed on endocrine disrupting compounds (EDCs), including estrogens and androgens. The main sources of EDCs in the rivers and lakes worldwide are sewage effluent and agricultural runoff.

Estrogenic compounds are classified as EDCs which interfere with the synthesis, secretion, transport, binding, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and behavior.[3] These compounds have become a growing concern due to their potential of harming normal endocrine function and physiological health of humans and animals.[4,5] Compounds of this nature have been omnipresent in the environment since the existence of mammals, but it is the growing population of today's society that their effects are starting to be noticed. Natural estrogens are excreted daily by both humans and mammals. However, conjugated estrogens used in the treatment of cancer, hormonal imbalance, osteoporosis, and other ailments requiring hormonal therapy are also causal to the increase of estrogen pollution.[6] Descriptions of an evident increase in breast and testicular cancers, slow development in infants, and a corresponding decrease in human sperm quantity and quality have raised questions about the role of natural estrogens and

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synthetic estrogenic compounds.[7–9] 17β-estradiol (E2), 17α-ethynylestradiol (EE2), estrone (E1), and estriol (E3) are four estrogenic compounds contributing to pollution. E2 is the principal intracellular human estrogen and is substantially more active than its metabolites, E1 and E3;[10] their molecular structures and physiochemical properties have been detailed elsewhere.[11] The pharmaceutical EE2 is one of the active ingredients in birth control pills. Currently, pharmacological doses of estrogens (5–20 μg/day in oral contraceptives, 1000 μg/day in hormone replacement therapy) to achieve positive health outcomes are generally recognized as safe.

The main concern that arises from these compounds is their means of entering the aquatic environment from contraceptive residues, hormone replacement therapy residues, and human excretion. Synthetic hormones are generally more stable in water than natural hormones and have greater potency.[12,13] Studies in the United Kingdom have shown that the hormones E2, EE2 and E1, although excreted in inactive conjugates, can be degraded in sewage treatment plants to release the active steroid hormones.[14] More concerning is the fact that these estrogens resist degradation in the course of typical sewage treatment operation, exhibit resilience in activated sludge, and destine for release into river waters.[15] Sludge from wastewater treatment plants (WWTP) that is used in agricultural fields has high potential of leaching estrogenic compounds into surface and ground water.[16] When wastewater sludge is used as a raw material for biotransformation to value-added products, questions still remain on the persistence of EDCs and their toxic intermediates.[17] Even low concentrations (ng/L) can induce reproductive abnormalities, cause feminization of fish, and decrease the reproduction rate of birds.[18–20] UV radiation, ozonation, membrane filtration, reverse osmosis, and activated carbon adsorption are probable means of improving the removal of estrogens in WWTP.[21–23] Unfortunately, such methods are more costly than they are effective. Great endeavors have been reviewed on the removal of EDCs in wastewater, including physical means, biodegradation, and chemical advanced oxidation.[24] A photocatalytic reactor membrane pilot system has been evaluated for the removal of EDCs from water.[25] Although the total estrogenic activity were greater than 70% removed, the electrical energy consumption was as high as 4 kWh/m^3. An innovative biological removal technique by means of a nitrifier enrichment culture has also been applied in a membrane bioreactor.[26] Although >94% removal efficiency was achieved, the maximum removal rate was a mere 9 μgEE2/gbiomass/h. The foremost challenge is that WWTP have to face a complex and variable mixture of numerous organic and inorganic substances. This makes qualitative assignments doubtful and quantitative assays difficult owing to matrix-induced signal suppression effects or isobaric mass spectral interferences from the complex sample extracts.[27] The reporting limit for estrogens is 5 ng/L after tertiary treatment (sand filtration, ozonation, and UV disinfection). Relatively few methods have been developed for the processing of estrogenic pollutants in various environmental samples, especially water, due to their low concentrations present and hence the high sensitivity required. For worse, impoverishment of ecosystems arising from endocrine disturbance will have economic consequences on the efficiency of the food web, which ultimately controls the availability of foodstuffs for humans.[1]

The innovation of molecularly imprinted polymers (MIPs) can be utilized for highly selective isolation of specific analytes in sample preparation by SPE.[28,29] Synthesis of MIP is based on the copolymerization of a functional monomer-analyte (template) complex and a cross-linker. Design variables include the choice of monomer (which complexes with the target analyte at a high binding affinity),[30] selection of a cross-linker (where length may determine the cavity size), and method of polymerization (which dictates the nature of interactions between polymer matrix and the template to dictate the ultimate extraction efficiency). The versatility in MIP preparation allows optimization of template-polymer interactions to model after enzyme-substrate interactions in biochemistry that are highly selective but lack stability. Once the polymer is formed, the template is removed with a proper solvent, leaving a cavity that corresponds with the specific target analyte. In the subsequent process of rebinding for water purification, specific interactions would occur between the smart polymer and the target analyte molecules. In the present work, E2 is chosen as a model EDC contaminant for it is considered to be the most physiologically active estrogen[31,32] and has been frequently detected in wastewater.[33] Previous results by Zhu et al. indicated that submicron-scaled MIP particles had both good selectivity and high affinity to the template molecule E2 for chromatographic separation.[34] Molecularly imprinted solid phase extraction (MISPE) was also used for the isolation of estrogens from water and biological samples.[34–36]

METHODS

Preparation of MIP and nonimprinted polymer submicron particles

The preparation of MIP using 17β-estradiol as a template is well-known.[37] The template 17β-estradiol, the functional monomer methacrylic acid (MAA),

and the cross linker ethylene glycol dimethacrylate (EGDMA) or trimethylolpropane trimethacrylate (in the molar ratio of 1 : 8 : 4) were dissolved in the porogen acetone and acetonitrile (in the volume ratio of 1 : 3). After addition of the initiator 2,2′-azobisisobutyronitrile (in 2% by weight of the prepolymerization mixture), the solution was sonicated, deoxygenated with nitrogen for 5 min, and then thermally polymerized at 60°C for 24 h to produce MIP nanoparticles. As a control, nonimprinted polymer submicron particles were prepared by exactly the same synthetic scheme only in the absence of template. Scanning electron microscopy (SEM) was used to determine the size of MIP and NIP submicron particles. All SEM images were obtained with a JSM-6400LV (JEOL, Japan) microscope at an accelerating voltage of 5–10 kV. The wavelength for maximum light absorption by submicron particles was measured using a Cary 3 UV/VIS spectrophotometer (Varian, Mississauga, Ontario, Canada). Light scattering by submicron particles was measured using a Cary Eclipse spectrofluorometer (Varian, Mississauga, Ontario, Canada).

Binding efficiency, capacity and isotherm

Sorption binding of E2 to the MIP (after washing with 5% TEA in methanol) or NIP submicron particles was assessed by suspending a small amount of particles (0.5–20 mg) in each polypropylene microcentrifuge tube containing 1.00 mL of water with variable E2 concentrations (0.1–1.0 ppm). After incubation for 2–60 minutes at room temperature (20°C ± 1°C) and centrifugation at 1900 times gravity for 1–2 minutes, portions of the supernatant were withdrawn for analysis by high performance liquid chromatography (HPLC) with fluorescence detection (FD) to determine the amount of E2 remaining (i.e., not bound to the particles). All binding experiments and analytical measurements were done in duplicate. A binding isotherm was obtained by plotting the E2 peak area versus the mass of MIP or NIP particles.

HPLC determination of estrogens

A previously developed method of HPLC analysis[38] was modified for the rapid determination of estrogens in water before and after treatment with MIP nanoparticles. The HPLC setup consisted of a solvent pump (Shimadzu LC-6A, Kyoto, Japan), injector valve (Valco Cheminert VIGI C2XL, Houston, TX) equipped with a 25-μL sample loop, column (Keystone Scientific Spherisorb 3 μm 50 × 2 mm, State College, PA), fluorescence detector (Perkin–Elmer LC 240, Boston, MA), and data acquisition system (PeakSimple, Torrance, CA). The mobile phase (1 : 1 : 2 v/v acetonitrile-methanol-water) was pumped at a flow rate of 0.4 mL/min. FD of estrogenic compounds was performed at optimal excitation and emission wavelengths of 220 nm and 310 nm. These wavelengths were selected based upon fluorescence peaks observed during excitation–emission matrix (EEM) analysis of the compounds in the HPLC mobile phase. The EEM measurements were conducted using the Perkin–Elmer LC 240 detector in SCAN mode.

Capillary electrophoresis (CE) characterization of particles

Capillary electrophoresis (CE) analyzes were performed on a Beckman P/ACE 2100 instrument (Fullerton, CA). The background electrolyte was composed of 50 m*M* borate adjusted to pH 9.25. When the electrolyte was run under an applied voltage of 30 kV, the capillary was thermostatted at a temperature of 25°C. All samples were degassed by sonication, and hydrodynamic injections were made by applying N_2 gas pressure (∼0.5 psi) for 1 second. The new fused silica capillary (Polymicro Technologies, Phoenix, AZ, 100 μm i.d., 69 cm total length, 61.5 cm effective length to detector) was flushed with 1 *M* NaOH and rinsed with deionized distilled water. Before each analysis, the capillary was conditioned with the electrolyte under the N_2 gas pressure for 5 min followed by an applied voltage (20 kV) for 3 min. A UV absorbance detector in the P/ACE instrument monitored the elution of analytes at a wavelength of either 214 nm. The detector output signal was acquired by a personal computer running the system gold software.

RESULTS AND DISCUSSION

MIP submicron particles

MIP and NIP submicron particles were successfully prepared for E2, with an average particle size of 578 ± 23 nm and 366 ± 8 nm, respectively as characterized by SEM. The SEM images obtained in Figure 1 show that the dry nanoparticles possessed a homogeneous, porous, and rigid structure. Great suspensibility of these particles in water for more than 2–3 weeks, due to electrostatic repulsion forces among them, is a remarkable advantage which makes them suitable for preconcentration (and removal) of estrogenic compounds in water analysis (or wastewater treatment). The wavelength for maximum UV-visible light absorption (and scattering) was measured for both MIP and NIP submicron particles (prepared using EGDMA as cross-linker), which exhibited a broad absorption band at around 400 nm with no characteristic features. Solid-state NMR analysis

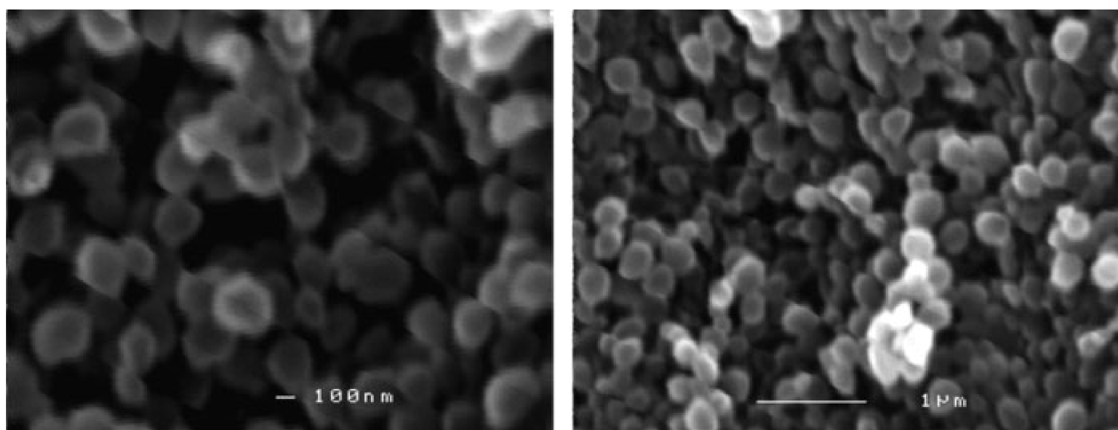

Figure 1 SEM images of MIP submicron particles on 100-nm scale (left) and 1-μm scale (right).

showed that the major difference between the MIP and NIP spectra was the appearance of two peaks in the MIP spectrum at chemical shifts of 126 ppm and 138 ppm (which were absent from the NIP spectrum). These extra peaks were attributed to the olefinic carbons (—C=C—) on the E2 template molecule, which was present in the MIP. Another interesting peak was observed at 18 ppm in the MIP spectrum. This peak could have been due to the methyl group (—CH_3) on the template, providing further proof of a template molecule in the MIP.

Using a Spherisorb 3 μm 50 × 2 mm column with acetonitrile/methanol/water (1 : 1 : 2 v/v) as the mobile phase running at 0.4 mL/min for HPLC analysis, E2 exhibited a retention time of 1.3 ± 0.1 min. The peak area scaled linearly (R^2 = 0.9963) with E2 concentration in the range from 0.01 to1.00 ppm. This mobile phase composition was versatile in that 1 : 1 : 3 v/v increased the retention time to 3.1 ± 0.1 min and 1 : 1 : 4.6 v/v extended the retention time further to 11.3 ± 0.1 min for confirmation of the E_2 peak. The standard calibration curves all exhibited a good correlation coefficient ($R^2 \geq 0.999$). After treating 1 mL of different initial E2 concentrations in water with 20 mg of MIP particles for 1 h, 15 min and 2 min, the remaining E2 concentrations were determined by HPLC-FD. The results in Figure 2 show that, after treatment with MIP particles, the remaining E2 concentrations in water were essentially zero (as indicated by a nearly zero HPLC-FD peak area). Quantitative binding (97% ± 3%) and hence complete removal of E2 was obtained for all three treatment times, across the entire range of E2 concentrations studied. Apparently, the binding was quick and complete even for a treatment time as short as 2 min. In 1 mL of water, a concentration of 1 ppm E2 translated to 1 μg of E2. It can now be established that 20 mg of the MIP particles can bind at least 1 μg of the estrogen, all completed within a short treatment time of 2 min at room temperature (under the control of Brownian motion and diffusion) with no mechanical agitation. Shorter treatment times (than 2 minutes) were not attempted because the subsequent centrifugation would take 1 minute to take down the MIP particles. Note that the combination of submicron particles in suspension with centrifugation, for water treatment, eliminates the risk of clogging in conventional methods based on a packed bed of microparticles (inside a column or cartridge).

Figure 3 presents the remaining E2 concentrations in water after treating 1 mL of different initial E2 concentrations with only 0.5 mg of MIP particles, for 2 min. Unfortunately, the remaining E2 concentrations in water were significantly higher than zero, going up nonlinearly with increasing initial E2 concentration. This trend suggested that 0.5 mg of MIP

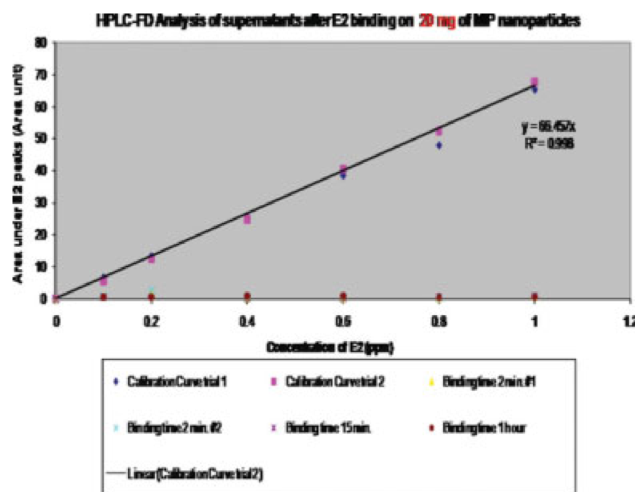

Figure 2 Remaining E2 concentrations in water after treating 1 mL of different initial concentrations of E2 with 20 mg of MIP submicron particles. No significant differences were observed among the data points for treatment times of 2 min, 15 min and 1 hr. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

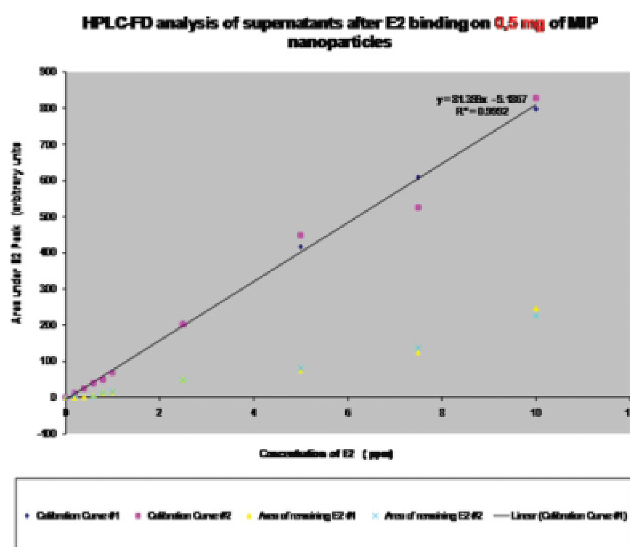

Figure 3 Remaining E2 concentrations in water after treating 1 mL of different initial concentrations of E2 with 0.5 mg of MIP particles for 2 min only. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

particles were inadequate for the quantitative binding of E2 in water, particularly at concentrations higher than 1 ppm. At the highest concentration of 10 ppm, only 7.5 μg of E2 in the 1 mL of solution was removed by the 0.5 mg of MIP particles. Hence, the specific binding capacity of these particles was estimated to be 15 mg E2/g. Despite such inadequacy of binding, these results demonstrated the importance of characterizing the working dynamics of E2 binding on MIP particles. They revealed that the binding capacity of particles became a limiting factor, as generally understood from the theory of solid phase extraction. With a specific binding capacity of 15 mg E2/g, these particles are a very suitable solid phase for the removal of E2 in water treatment.

Binding efficiency (or % recovery) was calculated as the ratio of (initial peak area − final peak area)/initial peak area, using the HPLC-FD data obtained in Figure 3 above for 0.5 mg of MIP particles with different initial E2 concentrations. The objective was that any improvement of binding efficiency with decreasing initial E2 concentration would confirm the working dynamics of E2 binding on a limited amount (0.5 mg) of particles. This can be seen clearly in Figure 4, where quantitative binding was achieved only at a low initial E2 concentration of 0.2 ppm. It was important, obviously, to use a sufficient amount of MIP particles for the complete removal of E2 in water treatment. This hypothesis was eventually proven by the calculation of binding efficiency using the data obtained in Figure 3 above for 20 mg of particles. As seen in Figure 5, quantitative binding was readily achieved for all the initial E2 concentra-

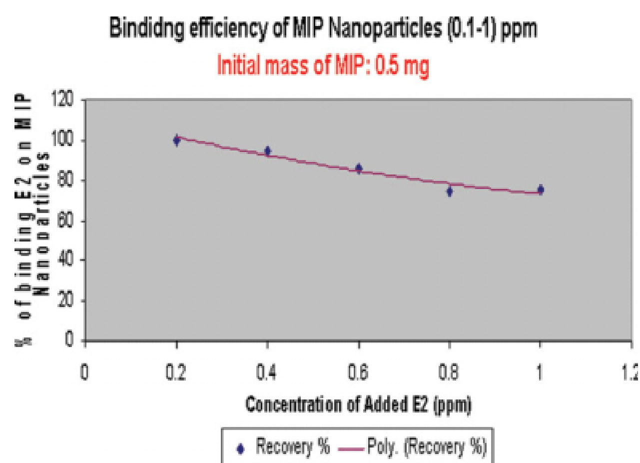

Figure 4 Binding efficiency (or % recovery) measured for 0.5 mg of MIP particles using different initial E2 concentrations. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

tions studied, up to 1.0 ppm, using MIP submicron particles. Our results are demonstrably better than the estradiol removal efficiencies of 80% achieved previously by Meng et al. with 1–2 μm MIP microspheres synthesized with α-estradiol as template,[39] 81–88% by Celiz et al.,[40] and 78–91% by Jiang et al.[41] Figure 6 shows a binding isotherm obtained from an initial E2 concentration of 1.0 ppm and different amounts of MIP nanoparticles. The remaining E2 concentrations, as measured by HPLC-FD, fully illustrated the need for a sufficient amount of MIP nanoparticles (5–10 mg) to completely remove (1 μg) estrogen under thermodynamic equilibrium.

Material cost analysis

It was deemed useful to examine economic factors in the preparation of MIP submicron particles. The

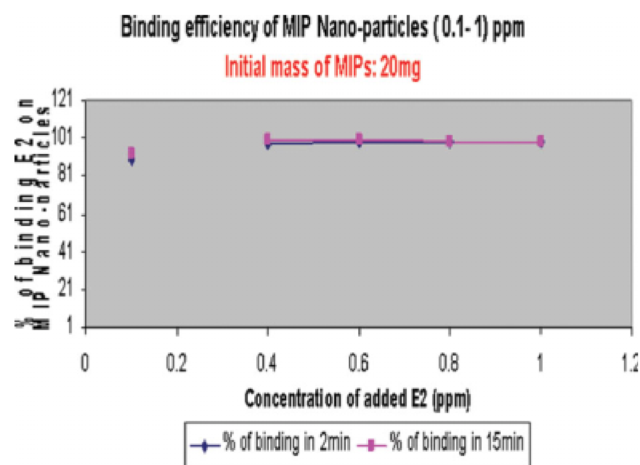

Figure 5 Binding efficiency measured for 20 mg of MIP particles using different initial E2 concentrations. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

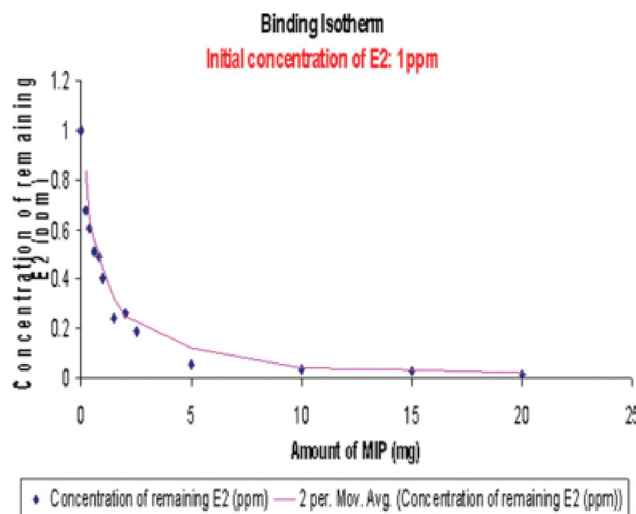

Figure 6 Binding isotherm for an initial E2 concentration of 1.0 ppm and different amounts of MIP particles. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

direct costs of monitoring and removing EDCs from industrial plant effluents, intensive agriculture, WWTPs, and drinking water treatment, would be considerable. This ultimately would affect the cost to the consumers of drinking water. These costs might be unavoidable but should be made known especially to municipal stakeholders. Table I shows a material cost analysis for the making of MIP particles in a small laboratory scale. Each batch (600 mg) of MIP particles cost $7 to make, which translated to 24 cents for treating 1 mL of water with 20 mg of particles (or $240 for treating 1 L of water). As the actual concentration of estrogeniccompounds in water is typically in the range of ppb down to ppt (which is 10^3–10^6 times lower than 1 ppm), it would not be necessary to keep the same ratio of 20 mg MIP nanoparticles to 1 mL of water as long as binding efficiency will support it. Note that MIP particles can be regenerated many times for the treatment of more water.

Among the six chemicals, E2 accounted for 90% of the total cost. If it is not required for the making of NIP submicron particles, the treatment of 1 L of water would cost only $24. In principle, functional carboxyl groups in the MAA monomer and EGDMA cross-linker would enable the NIP particles to extract species containing amino, hydroxyl, carbonyl, and carboxyl groups via hydrogen bonding. Although high nonspecific binding is generally not desired during traditional MIP applications such as chromatographic separation, it would not be a problem for water treatment as long as the NIP particles provide good binding efficiency and capacity for all organic contaminants including E2. All of these submicron particles are readily prepared in high yield in

TABLE I
Material Cost Analysis for Making a Batch (600 mg) of MIP Submicron Particles

Chemicals	Cost/batch
17β-Estradiol	$6.37
Methacrylic acid	$0.19
Ethylene glycol dimethacrylate	$0.16
Acetone	$0.05
Acetonitrile	$0.15
2,2-Azobisisobutyronitrile	$0.11
Total cost	$7.03

one step (within 24 h at 60°C). A recent study has found that the selectivity of MIP over NIP was relatively low, only 10% higher recovery.[40]

Kinetics of E2 binding with MIP and NIP submicron particles

In the earlier discussion as Figure 4 pointed out, 0.5 mg of MIP particles were inadequate for the quantitative binding of E2 in water, particularly at concentrations higher than 1 ppm. The binding efficiency as a function of treatment time over 2–60 min was fully investigated, using 0.005–0.5 mg of MIP or NIP particles for mixing with 1.0 mL of 1–5 ppm E2. A typical chart of % bindings on MIP and NIP versus time is presented in Figure 7, using 0.5 mg of particles for 1 ppm E2. The results obtained for MIP particles showed a small difference between 39% ± 3% binding for 3 min of incubation and 47% ± 3%

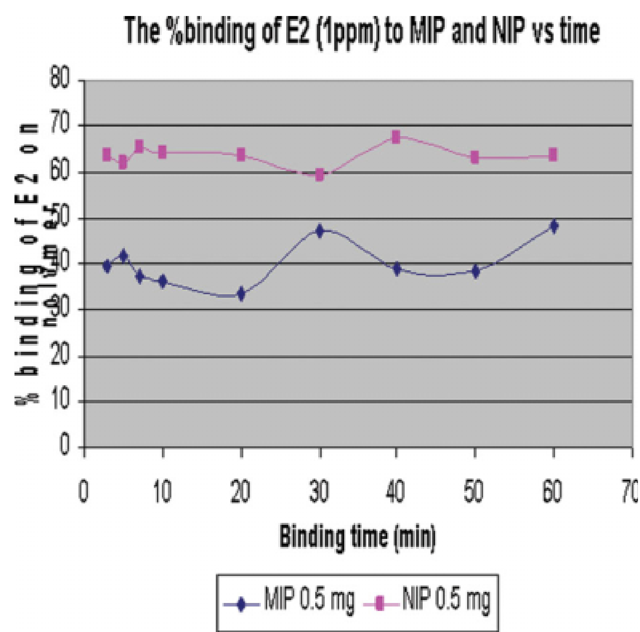

Figure 7 Percent binding of E2 with MIP and NIP submicron particles as a function of treatment time, using 0.5 mg of particles for 1 mL of 1 ppm E2. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

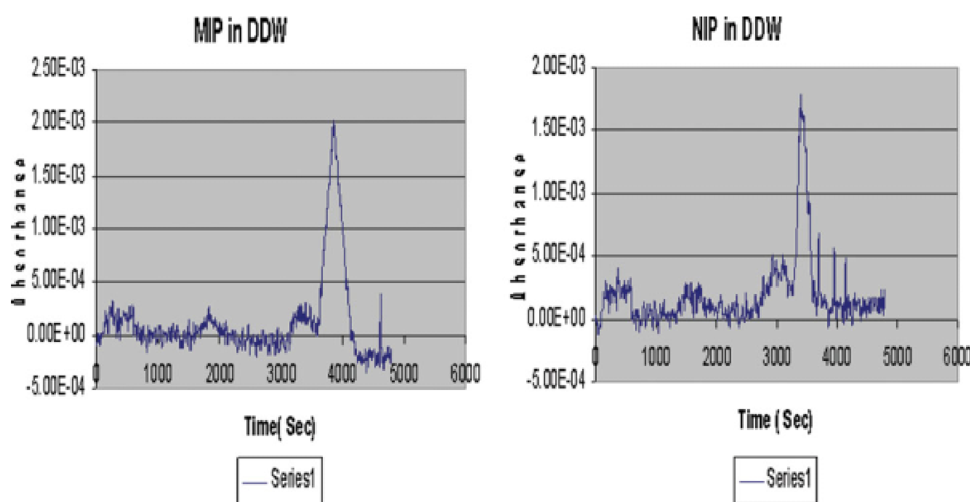

Figure 8 Characterization of MIP and NIP submicron particles by CE, using 50 m*M* borate (pH 9.25) as background electrolyte, at a UV detection wavelength of 210 nm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

for 60 min. One plausible explanation is that binding of E2 molecules with MIP particles required time for diffusion into the macroporous polymer matrix of imprinted cavities. The results obtained for NIP particles showed that the % binding was 64% ± 3% at 3 min. Additional incubation time up to 60 min did not increase the % binding. This indicates that 3 min of incubation is an optimal time to bind E2 with the NIP particles, for equilibrium had already been reached. This fast kinetics was ascribable to the good hydrophilic character of particles with a high content of MAA throughout the macroporous matrix. Further characterization would be a comparison of the ionic charge states, between NIP and MIP particles, by CE to better understand the discrepancy in their binding kinetics with E2 in water.

Capillary electrophoresis

The wavelength for optimal UV absorbance by MIP and NIP submicron particles was investigated by UV/VIS spectrophotometry. The results showed that there was a very broad absorption band at around 400 nm. Unfortunately, the UV detector in our Beckman CE instrument was not equipped with a visible light source. All CE characterization of submicron particles was hence detected at either 214 or 280 nm, instead of the optimal wavelength of 400 nm. In fact, 280 nm had been reported as a good wavelength for the detection of commercially available latex nanoparticles.[42] The electropherograms in Figure 8 show a nice CE peak for the MIP particles at a migration time of 6.4 ± 0.1 min, and NIP nanoparticles at 5.6 ± 0.1 min, using 50 m*M* borate (pH 9.25) as the background electrolyte. Both kinds of particles showed negative electrophoretic mobility in accordance with the anionic MAA functional groups on their surfaces. The electrophoretic mobility (μ_{ep}) was determined on the basis of the following equation: $\mu_{ep} = (Ll/V)(t_o - t_p)/t_o t_p$ where L, l, t_o, t_p, and V are the total capillary length, the effective capillary length (to detection window), the migration time of the EOF marker, the migration time of the particles, and the applied voltage, respectively. Electrophoretic mobility values of −21.3 ± 0.5 and −19.0 ± 0.5 cm^2/kV min were calculated from the observed migration times of MIP and NIP particles. This new finding is very exciting because, for the first time, MIP and NIP particles are demonstrated by CE analysis to be different in their μ_{ep} values. The difference can be attributed to their average particle sizes, ionic charge states,[43] and/or porosity, even though the MIP and NIP particles were prepared in essentially the same polymerization chemistry. Their difference in these physical properties would explain the discrepancy in binding efficiency between MIP (47% ± 3%) and NIP (64% ± 3%) discussed above (Fig. 7). It is note-worthy that the MIP particles exhibited a broad peak as a consequence of treatment with triethylamine (TEA) to extract the E2 template molecules. The separation efficiency is, therefore, limited by electrophoretic heterogeneity due to the variability in charge, size, and shape of the MIP particles. Before TEA treatment, the peak was sharp and it appeared at a significantly later migration time (than 6.4 min). These exciting capabilities of CE for the characterization of polymeric submicron particles will be discussed elsewhere. Previously, Okamoto et al. reported, how CE was used to separate cationic polymer microparticles with different sizes.[42] They investigated the electrostatic interaction between capillary wall and cationic polymer particles. By

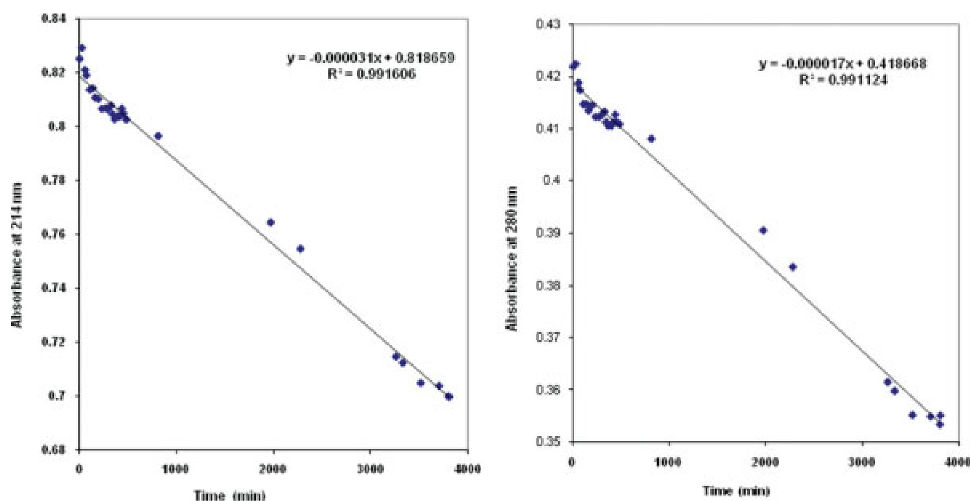

Figure 9 Precipitation of NIP submicron particles in water (35 mg/mL) under UV absorbance detection, at 214 nm and 280 nm, in a standard sample cuvette. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

employing a poly(vinyl alcohol)-coated capillary, a better size separation of amine-modified latex particles was obtained. In comparison, the present work has demonstrated that the composition, concentration, and pH of our background electrolyte were so good for the separation of MIP and NIP particles in a bare capillary. Surface adsorption of these anionic particles to the fused silica wall was not significant, and no aggregation of polymer particles was observed. Apparently the less negative the electrophoretic mobility, the higher binding efficiency the particles would exhibit with E2. One plausible speculation is that electrostatic repulsion among the more negatively charged MIP particles slowed down their Brownian diffusion towards E2 molecules in the water sample during the incubation time, resulting in hindrance to their binding kinetics and hence a decrease in binding efficiency as compared with the NIP particles (particularly at a short incubation time of 3 min).

Precipitation rate of submicron particles during water treatment

Good understanding of submicron particles can be gained, for water treatment applications, by determining a precipitation rate. This rate, in theory, is a function of particle size and zeta potential (which is simply the pH at which the ionic charge state of particles becomes zero. At this pH, the particles are neutral and not ion like). Our preliminary study of the pH effect showed that the precipitation of NIP submicron particles was faster at pH levels under 3. Another observation, at pH 6–7, was that NIP particles in water dispersion were not as stable as MIP particles. The precipitation of NIP submicron particles could be monitored by UV-visible spectrophotometry of their light absorbance and scattering properties. Based on the trend line obtained for 35 μg/mL by UV detection at 214 nm as shown in Figure 9, it would take 17.8 days (=25,585 min) for the absorbance to approach zero. At 280 nm, it would take 17.1 days (=24,630 min). Hence, both wavelengths were consistent in predicting a total precipitation time of 17.4 ± 0.4 days, which is long enough for most stationary water treatment processes. Similar precipitation times were obtained for 23 and 500 μg/mL of NIP particles. These results suggest that the precipitation rate was independent of the concentration of particles, which means insignificant aggregation due to collision (if any) of these anionic particles. Note that UV spectrophotometry could detect these NIP submicron particles at concentration levels as low as 10 μg/mL. It can hence be used to determine how little of the particles remain dispersed in water, after adequate time is allowed for their precipitation. This will assist in future promotion of these NIP particles for use in water treatment. New NIP submicron particles can be prepared using other cross-linkers that consist of a chromophore with strong UV light absorbance, instead of EGDMA.

Alternatively, a spectrofluorometer could be used to measure the intensity of light scattering by submicron particles in the visible region. Preliminary results (data not shown) indicated that the sensitivity of NIP submicron particle determination (based on the slope of standard calibration curve) was higher at 700 nm than at 650 nm for precipitation rates. A calculation of the detection limits, using light scattering data obtained for particle concentrations from 2.5 μg/mL down to 0.1 μg/mL, showed

that the 700 nm wavelength also afforded a slightly better detection limit below 0.1 μg/mL for residual particle levels.

CONCLUSIONS

Extensive monitoring of intake and discharge waters is becoming a routine in water treatment plants. Cost-effective practices for removal of EDCs in water treatment will require both careful development and knowledgeable implementation. MIP submicron particles have been successfully prepared for E2 in this work. With an average particle size of 578 nm for MIP (and 366 nm for NIP), and a specific binding capacity of 15 mg E2/g MIP, these particles are a very suitable solid phase for the removal of E2 in water treatment. They can be removed from the treated water by using a membrane filter (with a pore size of 0.2 μm). No estrogenically active transformation products are formed during treatment. This approach is technologically much simpler (and less costly) than the use of macroporous MIP/cryogel/PVA composite monolith systems for the removal of endocrine disrupting trace contaminants in water treatment.[44] In our future work, the binding capacity of MIP/NIP nanoparticles will be evaluated in wastewater containing >11 mg/L of total organic carbon that can compete for binding sites and/or block the submicron particle pores. It is likely to be a decade before human epidemiological studies have a major role in setting guidelines for safe levels of human exposure. In the meantime, the water industry needs to identify how best to maintain a sustainable supply of safe drinking water, which requires the detection and removal of potentially harmful contaminants.

Brownian motion is the random movement of nanoparticles suspended in a liquid. A mathematical model, often called the particle theory,[45] is under careful development in our laboratory to describe such random movements. This mathematical model would help to lead the technology of MIP and NIP submicron particles up to more real-world applications. They can potentially be used to bind E2 at very low concentrations at the ppt (or pg/mL) level. Cost-effective treatment technologies, based on the particle theory, will need to be assessed for their capability of removing EDCs to ensure a safe drinking water supply. Chronic amounts of estradiol may lead to hyperactivity, so they should be decreased to avoid its undesirable stimulatory effect on the thyroid structure and function.[46]

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