WATER RESEARCH 41 (2007) 2825–2831

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Selective removal of 17β-estradiol at trace concentration using a molecularly imprinted polymer

Mathieu Le Noir[a], *Anne-Sophie Lepeuple*[b], *Benoit Guieysse*[a,c,*], *Bo Mattiasson*[a]

[a]*Department of Biotechnology, Center of Chemistry & Chemical Engineering, Lund University, P.O. Box 124, 22210 Lund, Sweden*
[b]*Véolia Environnement, Centre de Recherche sur l'Eau, Chemin de la Digue, BP 76, 78603 Maisons-Laffite cedex, France*
[c]*School of Civil and Environmental Engineering, Nanyang Technological University, Block N1, Nanyang Avenue, Singapore 639798, Singapore*

ARTICLE INFO

Article history:
Received 7 December 2006
Received in revised form
8 March 2007
Accepted 9 March 2007
Available online 30 April 2007

Keywords:
Endocrine disrupter
Estrogen
Molecular imprinting
Trace contaminant
Yeast estrogen screen

ABSTRACT

A molecularly imprinted polymer (MIP) was synthesized with 17β-estradiol (E2) as template. It was then capable to recover this compound by 100±0.6% from a 2 μg/L aqueous solution. By comparison, E2 recoveries of 77±5.2%, 87.1±2.3% and 19.1±7.8%, were achieved using a non-imprinted polymer (NIP) synthesized under the same conditions (but without template), a commercial C18 extraction phase and granular-activated carbon (GAC), respectively. When fluoxetine hydrochloride and acenaphthene were added as interferences to the aqueous solution at 2 μg/L each, E2 was recovered by 95.5±4.0% from the MIP, compared to 54.5±9.4%, 76.0±2% and 14.3±0.1% from the NIP, C18 and GAC phases, respectively. Estrogenic activity equivalent to the effect caused by 22.4 ng E2/L was recorded in the MIP extract from a wastewater sample whereas no activity was detected in the NIP extract. This suggested the imprinted polymers removed estrogenic compounds. This study therefore demonstrates the potential of MIPs for the selective removal of endocrine-disrupting compounds. By using a synthetic analogue to natural hormone receptors, adsorption is based on the same property that makes the contaminants harmful. Biological treatment of enriched E2 was also demonstrated.

1. Introduction

Endocrine-disrupting contaminants (EDCs) can interfere with the regulatory network in humans and wildlife even when present at trace concentration of ng/L–μg/L (Daughton, 2002; Daughton and Ternes, 1999; Groshart and Okkerman, 2000; Jobling et al., 2003). They have been, for instance, linked to drastic problems such as the feminization of male fish in receiving waters (Diniz et al., 2005) and even the drop of human male fertility observed over the last decades (Golden et al., 1998). Many EDCs are natural hormones or consumer products such as pharmaceuticals and personal care products (Daughton, 2002; Daughton and Ternes, 1999). They are therefore continuously introduced in the environment from multiple diffuse sources and are ubiquitously found, at low concentration, in water resources (Cargouet et al., 2004; Fawell et al., 2001; Kolpin et al., 2002). It is precisely their potency at extremely low level combined with the large number at which they can be simultaneously found that make EDCs so worrisome, and requires the development of highly efficient water treatment methods.

Conventional wastewater and drinking water treatment processes are partially inefficient in removing EDCs (Jones et al., 2005), explaining why contaminants such as drug

*Corresponding author. School of Civil and Environmental Engineering, Nanyang Technological University, Block N1, Nanyang Avenue, Singapore 639798, Singapore. Tel.: +65 6790 6991; fax: +65 6791 0676.
E-mail address: bjguieysse@ntu.edu.sg (B. Guieysse).
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doi:10.1016/j.watres.2007.03.023

metabolites or human estrogens are frequently found in water resources (Stackelberg et al., 2004; Ternes et al., 2003). As typical example, adsorption to activated carbon is only efficient for hydrophobic contaminants (Ternes et al., 2002) and considerably impacted by the presence of interfering substances such as humic acids and surfactants (Fukuhara et al., 2006; Zhang and Zhou, 2005). Likewise, biological treatment of trace contaminants is often difficult because microorganisms preferentially metabolize other substances present at higher concentrations (Auriol et al., 2006; Petrovic et al., 2003). Although ozonation and advanced oxidation processes are efficient in removing many pollutants (Nakagawa et al., 2002; Ternes et al., 2002), they are also limited by the competitive removal of interfering organic matter as one study, for instance, reported that 5 mg/L of O_3 were necessary to remove 2 μg/L of nonylphenol in river water (Zwiener and Frimmel, 2000), which more than 800 times the amount theoretically required to fully mineralize this compound. Finally, the efficiency of microfiltration and ultrafiltration is limited to hydrophobic pollutants adsorbed to particles whereas nanofiltration and reverse osmosis are efficient for most compounds but are not specific and highly energy demanding (Duin et al., 2000). Hence, the lack of specificity of current methods considerably reduces their efficiency at trace concentration as most of the adsorption or oxidation capacity is wasted in the removal of other, often harmless, compounds.

With this perspective, this study presents a new method to remove EDCs by selective extraction using artificial molecular receptors synthesized by guided polymerization of functional monomers around a target molecule (the template), which leaves a specific recognition site after removal of the template (Makoto et al., 2003). By using an EDC as template, the adsorbing material synthesized becomes a synthetic analogue to the natural receptors targeted by the contaminant. Thus, pollutant removal ingeniously relies on the same property that makes the pollutants so harmful: their capacity to bind to natural receptors, which should permit to remove any molecules having the capacity to bind natural receptors (i.e. all endocrine disrupters). This would be extremely advantageous as it is impossible to identify, determine the environmental fate and understand the effects of all anthropogenic molecules (and their metabolites) entering the environment. 17β-Estradiol (E2) was chosen as model contaminant for being considered as the most active estrogen (Jobling et al., 1998) and for being frequently detected in wastewater (Ying et al., 2002).

2. Materials and methods

All chemicals were of reagent grade. E2, fluoxetine hydrochloride (FH), acenaphthene (Ac) and yeast nitrogen base (YNB) without amino acids were purchased from Sigma. *Saccharomyces cerevisae* BJ3505 was kindly provided by Professor Kevin W. Gaido (Center for Health Research, USA). Estradiol, [2,4,6,7-^{3}H(N)]- was supplied by Dr. L. Ye (Dept. of Applied Biochemistry, Lund University).

2.1. Molecularly imprinted polymer (MIP) synthesis

The MIP was prepared by dissolving E2 in acetonitrile in a dried 30 mL test tube, adding the functional monomer, the crosslinker and the initiator and gently mixing the solution for 5 min (Table 1). The mixture was then cooled on ice and purged with nitrogen during 5 min before the test tube was sealed and the mixture heated at 65 °C for 20 h. Then, the polymer monolith was withdrawn and smashed. The particles were ground in a mechanical mortar, passed through a 25 μm test sieve and washed with methanol. The flask containing fine particles of polymers in methanol was kept in a fume hood until the methanol was completely evaporated. The dried polymers were finally washed with methanol using a Soxhlet extractor for 24 h and dried in a dessicator for 24 h. The amount of MIP recovered was approximately 4 g. To serve as controls, a non-imprinted polymer (NIP) was prepared using the same protocol but without adding the template.

2.2. MIP characterization

The specific surface area (m^2/g), the specific pore volume (cm^3/g) and the pore size distribution of the polymers were characterized using the BET method (Brunauer et al., 1938) and nitrogen desorption (Groen et al., 2003) (multipoint Micromeritics ASAP 24000). Samples were de-gassed at room temperature for 16 h prior to measurements. Sorption of E2 to the polymer was assessed by suspending increasing amounts of polymer (5, 15, 25 and 40 mg) in 900 μL of toluene containing 428 fmol of radiolabeled 17β-estradiol (estradiol, [2,4,6,7-^{3}H(N)]- specific activity 2.59 TBq/mmol), NEN Life Science Products, Boston, USA in polypropylene microcentrifuge tubes. Controls were done by incubating radioactive E2 overnight in tubes supplied with toluene but no polymer. The tubes were then gently mixed on a rocking table and

Table 1 – MIP synthesis

Component	Compound	Quantity	Molecular ratio
Template	Estradiol	0.272 g	1
Monomer	4-Vinylpyridine	0.430 mL	4.05
Crosslinker	Ethylene glycol dimethacrylate	4.72 mL	25.06
Initiator	2,2′-Azobisisobutyronitrile	0.050 g	0.3
Solvent	Acetonitrile	8 mL	—