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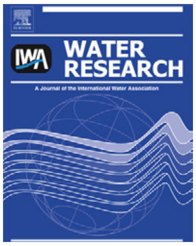

Enantiospecific fate of ibuprofen, ketoprofen and naproxen in a laboratory-scale membrane bioreactor

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ARTICLE INFO

Article history:
Received 10 June 2011
Received in revised form
8 September 2011
Accepted 10 September 2011
Available online 17 September 2011

Keywords:
Chiral analysis
Enantiomers
Non-steroidal anti-inflammatory drugs (NSAIDs)
Profens
Gas chromatography–tandem mass spectrometry

ABSTRACT

The enantiospecific fate of three common pharmaceuticals was monitored in a laboratory-scale membrane bioreactor (MBR). The MBR was operated with a hydraulic retention time of 24 h and a mixed liquor suspended solids concentration of 8.6–10 g/L. Standard solutions of ibuprofen, ketoprofen and naproxen were dosed into the synthetic feed of the MBR. Influent and permeate samples were then collected for enantiospecific analysis. The individual (*R*)- and (*S*)-enantiomers of the three pharmaceuticals were derivatised using a chiral derivatizing agent to form pairs of diastereomers, which could then be separated and analysed by gas chromatography–tandem mass spectrometry (GC–MS/MS). Accurate quantitation of individual enantiomers was undertaken by an isotope dilution process. By comparing the total concentration (as the sum of the two enantiomers) in the MBR influent and permeate, ibuprofen, ketoprofen and naproxen concentrations were observed to have been reduced as much as 99%, 43% and 68%, respectively. Furthermore, evidence of enantioselective biodegradation was observed for all three pharmaceuticals. (*S*)-Ibuprofen was shown to be preferentially degraded compared to (*R*)-ibuprofen with an average decrease in enantiomeric fraction (*EF*) from 0.52 to 0.39. In contrast, (*R*)-ketoprofen was preferentially degraded compared to (*S*)-ketoprofen with a relatively minor increase in *EF* from 0.52 to 0.63. The use of a relatively pure enantiomeric solution of (*S*)-naproxen resulted in a significant change in *EF* from 0.99 to 0.65. However, this experiment consistently revealed significantly increased concentrations of (*R*)-naproxen during MBR treatment. It is hypothesised that the source of this (*R*)-naproxen was the enantiomeric inversion of (*S*)-naproxen. Such enantiomeric inversion of chiral pharmaceuticals during wastewater treatment processes has not previously been reported.

1. Introduction

The presence of pharmaceutically active compounds in the environment has been an increasingly active area of investigation throughout the last decade (Halling-Sorensen et al., 1998; Heberer, 2002; Jones et al., 2005; Khetan and Collins, 2007; Ziylan and Ince, 2011). Many of the pharmaceuticals found in the aquatic environment are chiral chemicals; possessing at least one element of asymmetry and leading to the existence of two or more stereoisomers called enantiomers.

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doi:10.1016/j.watres.2011.09.020

Some pharmaceuticals are dispensed and consumed as mixtures of enantiomers, while others are dispensed as relatively pure single enantiomers (Murakami, 2007).

Enantiomers commonly differ in their biological and pharmacological activity as a result of their stereo-selective interaction with enzymes or other chiral molecules (Caldwell, 1995). Consequently, the relative enantiomeric composition of some chiral pharmaceuticals changes during human metabolism (de Oliveira et al., 2005). Similarly, changes in relative enantiomeric composition have been observed during biological treatment processes in wastewater treatment plants (WWTPs) (Fono and Sedlak, 2005; Kasprzyk-Hordern et al., 2010; MacLeod et al., 2007; MacLeod and Wong, 2010; Matamoros et al., 2009; Nikolai et al., 2006).

Enantiomers often have very different toxicity compared to each other (Stanley et al., 2006). In some cases, this may be an important consideration for environmental risk assessment since actual ecotoxicity implications will be highly dependent on the precise enantiomeric composition.

Many international studies have reported on the effectiveness of wastewater treatment processes for removing various chiral pharmaceuticals (Joss et al., 2005; Kim et al., 2007; Rosal et al., 2010). However, the vast majority of these studies have been undertaken using achiral analytical methods, which do not differentiate between enantiomeric pairs.

Although it is comparatively rarely reported, enantiospecific analysis of pharmaceuticals in wastewater and environmental samples has several important potential applications. For example, a number of authors have observed that the enantiomeric composition of specific chemicals has the potential to be a useful marker of biologically mediated degradation (Hashim et al., 2010; Kasprzyk-Hordern, 2010; Wong, 2006). In contrast to the alternative approach of measuring absolute concentrations before and after biological degradation, attention to the relative abundance of enantiomers has the advantages of not being affected by sample volume, short-term concentration fluctuations or variable extraction recoveries of different chemicals. Abiotic treatment processes such as physical settling, UV irradiation and membrane filtration are generally assumed to affect both enantiomers in equal proportion (Wong, 2006). Accordingly, microbial processes are presumed to be fully responsible for observed enantioselective degradation.

Quantitative description of relative compositions of (*R*)- and (*S*)-enantiomers is usually described as an enantiomeric ratio (ER) or an enantiomeric fraction (*EF*) (Harner et al., 2000). ER has most commonly been expressed as the ratio of (+)-enantiomer over the (−)-enantiomer. For some chromatographic separation studies, for which the enantiomeric conformation is unknown, ER has been defined as the ratio of the first eluting enantiomer over the second eluting enantiomer. *EF* is conventionally described as the concentration fraction of the (+)-enantiomer contributing to the total concentration of racemic mixture (Harner et al., 2000; Hashim et al., 2010).

Enantiospecific monitoring of the fate of pharmaceuticals during biological wastewater treatment and environmental residence has revealed that the pharmaceuticals ibuprofen, naproxen and propranolol do exhibit enantioselective degradation (Buser et al., 1999; Fono and Sedlak, 2005; MacLeod et al., 2007; Matamoros et al., 2009; Nikolai et al., 2006). On the other hand, non-enantioselective degradation has been observed for numerous other chiral pharmaceuticals including atenolol, fluoxetine, metoprolol, nadolol, sotalol, citalopram, salbutamol, 4-methylenedioxymethamphetamine and venlafaxine (Kasprzyk-Hordern et al., 2010; MacLeod et al., 2007; Nikolai et al., 2006).

In all reported enantiospecific studies of the fate of chiral pharmaceuticals during wastewater treatment, observed changes in ER or *EF* have been interpreted as having been caused solely by enantioselective biodegradation. The possibility of changes in ER or *EF* having been (partially) caused by interconversion of one enantiomer to the other has not previously been seriously considered. In order to more precisely investigate the enantiospecific fate of pharmaceuticals, the experiments described in this study were undertaken under stable controlled conditions in a laboratory-scale membrane bioreactor (MBR). The pharmaceuticals ibuprofen, naproxen and ketoprofen, were introduced to the feed of the MBR in precisely known enantiomeric composition. In particular, the use of enantiomerically pure (*S*)-naproxen enabled the observation of the appearance of (*R*)-naproxen during the biological treatment process.

2. Materials and method

2.1. *Chemicals and consumables*

Racemic ibuprofen, racemic ketoprofen, enantiomerically pure (*S*)-naproxen (99%), (*R*)-1-phenylethylamine (PEA) (99.5%), triethylamine (TEA) and ethyl chloroformate (ECF) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Racemic (α-methyl-D_3)-ibuprofen (D_3-ibuprofen), (α-methyl-D_3)-naproxen (D_3-naproxen) and (α-methyl-D_3)-ketoprofen (D_3-ketoprofen) were purchased from CDN Isotopes Inc., Canada. HPLC grade acetonitrile and methanol were purchased from Ajax Finechem (Tarron Point, NSW, Australia). Analytical grade ethyl acetate was purchased from Fisher Scientific, Australia. Kimble culture tubes (13 mm I.D. × 100 mm) were purchased from Biolab (Clayton, Vic. Australia). Two sizes of Oasis hydrophilic–lipophilic balance (HLB) solid phase extraction (SPE) cartridges (6 cc, 500 mg and 1 cc, 30 mg) were purchased from Waters (Rydalmere, NSW, Australia). Whatman filter papers (0.75 μm) were purchased from Millipore, Australia. The Oasis HLB sorbent is an achiral macroporous copolymer of two monomers, the lipophilic divinylbenzene and the hydrophilic N-vinylpyrrolidone.

2.2. *Laboratory-scale MBR system*

The laboratory-scale MBR system and the MBR experimental protocol used in this study have been previously described in detail (Alturki et al., 2010; Tadkaew et al., 2011, 2010). This MBR consisted of a glass reactor with an active volume of 9 L, a continuous mixer, two air pumps, a pressure sensor, and influent and permeate pumps. Two ZeeWeed-1 (ZW-1) submerged hollow fibre ultrafiltration membrane modules supplied by Zenon Environmental (Ontario, Canada) were