Chemosphere 127 (2015) 254–261

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Decomposition of drug mixture in Fenton and photo-Fenton processes: Comparison to singly treatment, evolution of inorganic ions and toxicity assay

Ardhendu Sekhar Giri, Animes Kumar Golder *

Department of Chemical Engineering, Indian Institute of Technology Guwahati, Assam 781 039, India

HIGHLIGHTS

- Slightly lower decomposition of CHPL, CIP and DIPY in their mixture than singly both in FP and PFP.
- Cl^- and F^- addition caused reduction in CHPL and CIP cleavage due to suppression of release of the same heteroatoms.
- Mixed drug system yielded more higher molecular weight intermediates.
- Equimolar (0.05 mM) mixture of drugs exhibited more than 50% death of *E. coli*.

ARTICLE INFO

Article history:
Received 9 August 2014
Received in revised form 24 January 2015
Accepted 2 February 2015
Available online 6 March 2015

Handling Editor: Klaus Kümmerer

Keywords:
Pharmaceutical effluent
Decomposition mechanism
Inorganic ions
Antimicrobial activity

ABSTRACT

The degradation of three pharmaceutical compounds i.e. chloramphenicol (CHPL), ciprofloxacin (CIP) and dipyrone (DIPY) singly and from equimolar (CCD) mixture has been investigated in Fenton and photo-Fenton processes. Drug mineralization was slightly less when present singly than their mixture. The degradation efficiency was likely hindered due to formation of common ions like Cl^-, F^-, NH_4^+ and NO_3^-. Addition of the same ions i.e. Cl^- and F^- in drug solution released upon cleavage of CHPL and CIP in CCD mixture suppressed the decomposition efficiency remarkably in both the oxidation processes. The major intermediates appeared in the mass spectra in combination of ion chromatograph were used to validate the routes of CCD decomposition and evolution inorganic ions. Furthermore, the bacterial toxicity assay was investigated using *Escherichia coli* (*E. coli*). The average reduction in cell death was about 38% in CCD system compared to 52%, 42% and 47% for CHPL, CIP and DIPY, respectively.

1. Introduction

In past few years, several attempts have been made to quantify the deleterious impact of pharmaceutical compounds rejected into the environment. The typical pharmaceutically active compounds (PhACs) include antibiotics, analgesics, anti-depressants, beta-blockers, and hormones & hormone mimics. The consumption of antibiotic drugs in India has increased between 6% and 7% annually with a gap of 5 years from 2007 to 2011. The Global Antibiotic Resistance Partnership (GARP) India research estimates nearly 1 900 000 neonatal deaths in each year due to sepsis, a bacterial infection and more than 30% are due to antibiotic resistances (THSA 2512475).

PhACs enter into the aquatic environment mostly through the discharge of improperly treated and untreated effluent. Advanced oxidation processes (AOPs) are promising for the decomposition of PhACs from industrial and municipal wastewater (Stumpf et al., 1999; Rivas et al., 2001). AOPs include both homogeneous and heterogeneous reacting systems in presence of light or in dark. They have common characteristics of formation of hydroxyl free radicals ($HO^{\cdot}$) (Arslan-Alaton and Olmez-Hanci, 2010). It causes consecutive unselective degradation of organic materials. Complete mineralization and/or oxidization occur even at very low concentration and the byproducts formed may be environmentally non-hazardous (Liu et al., 2013).

There are several reports that PhACs in a mixture could show added toxic effects than a single PhAC. Cleuvers (2003) investigated ecotoxicity of ten pharmaceutical compounds using three different aquatic organisms and most of the compounds showed moderate toxic effects. They showed that the mixture of different PhACs exhibited stronger toxicities than it is likely to be for a single drug. Pomati et al. (2006) studied the effects of mixture of thirteen

* Corresponding author. Tel.: +91 361 258 2269; fax: +91 361 258 2292.
E-mail address: animes@iitg.ernet.in (A.K. Golder).

http://dx.doi.org/10.1016/j.chemosphere.2015.02.010
0045-6535/

therapeutic drugs on human embryonic cells. The mixed drug system exhibited up to 30% reduction in cell proliferation.

In addition, PhACs treatment by AOPs could give different extent of decomposition in mixture and singly. The rate of composition of sulfamethoxazole, oxtetracycline, and ciprofloxacin is influenced by the solution pH when treated singly but not in their mixture using polychromatic UV treatment (Avisar et al., 2010). HCO_3^- and NO_3^- added separately reduced the rate of degradation of five PhACs out of six in UV/H_2O_2 process (Yuan et al., 2013). However, Cl^- did not show any specific impact on the decomposition even it could act as a $HO^{\cdot}$ radical scavenger (Anipsitakis et al., 2006).

Pharmaceutical effluents generally contain the mixture of a number of PhACs. Hence, it is prudent that research should focus on pharmaceutical mixture than a single compound. Three pharmaceutical compounds i.e., chloramphenicol (CHPL), ciprofloxacin (CIP) and dipyrone (DIPY) were selected for this study based on their consumption and occurrence (Kramer et al., 1984; Wiegel et al., 2004; Larsson et al., 2007; Lei et al., 2011). This work investigates how the mineralization efficiency and antimicrobial activity of CHPL + CIP + DIPY (CCD) could vary when present in a mixture and individually in Fenton and photo-Fenton processes (FP and PFP). The impact of three inorganic ions i.e. Cl^-, NO_3^- and F^- on drug decomposition was also tested and compared in CCD system. Moreover, the mechanism of CHPL, CIP and DIPY cleavage is explored through identification of intermediates in the mass spectra and inorganic ion formation in PFP.

2. Material and methods

2.1. Reagents

HPLC grade CHPL (purity > 99% w/w), CIP (purity > 98% w/w), DIPY (purity > 99% w/w), were procured from Sigma Aldrich (China). The chemical structure of the pharmaceuticals is illustrated in Fig. 1. Methanol (purity 98% v/v) of HPLC grade was obtained from Merck (India). Ammonium fluoride (purity > 95% w/w), ferrous ammonium sulfate heptahydrate (purity 99% w/w), sulfuric acid (purity 98% v/v), H_2O_2 (purity 50% v/v), NaOH (purity > 98% w/w), ethanol (purity > 99.9% v/v), sodium nitrate (purity > 99% w/w), sodium chloride (purity min. 99.5% w/w), sodium bicarbonate (purity 98% w/w) and sodium carbonate (purity 98% w/w) were purchased from Merck (India). *Escherichia coli* (*E. coli*) XL 10GOLD was collected from the Department of Biotechnology, Indian Institute of Technology Guwahati. Yeast (purity 99% w/w) and tryptone (purity 98% w/w) were obtained from Himedia (India). Mili-Q water (model: Elix 3, Millipore, USA) was used to prepare all reagents and solutions.

2.2. Analytical techniques

The concentration of drugs was determination by high performance liquid chromatography (HPLC) using an octadecyl carbon column (4.6 mm × 250 mm) (model: LC-20AD, Simadzu, Japan). Methanol and water (70:30 v/v) at a flow rate of 0.4 mL min^{-1} was used as the mobile phase for both individual drug and CCD mixture. Total organic carbon (TOC) analyzer of O.I. Analytical, USA (model: 1030C Aurora) was employed for the determination of TOC. pH of the solution was determined using a pH meter of Eutech Instruments, Malaysia (model: pH/ion 510). Liquid chromatography-time-of-flight mass spectrometry (LC-TOF-MS) (Waters Q-Tof Premier & Aquity UPLC) system was employed for the identification of drug fragments. An YMC (Wilmington, NC, USA) hydrosphere C_{18} reverse phase column (4.6 mm × 150 mm, 5 μm particle size) was used for the chromatographic separation. The mobile phase flow rate was 0.8 mL min^{-1} at 25 °C. It was consisting of H_2O and acetonitrile mixture with 0.1% (v/v) formic acid. A linear gradient varying from 95% to 50% H_2O was employed for 10 min. Electro-spray ionization (ESI) method in positive ion mode over the mass range of 260–560 amu was used to acquire the mass spectra.

The inorganic ions released during the mineralization reaction were measured by ion chromatography (model: 792 IC, Metrohm, India). Analyses were performed by injecting 25 μL aliquot of samples. Cl^-, F^- and NO_3^- concentration was determined with an ANION METROSEP A sup 5 column IC (250 mm in length with 4 mm in id). NH_4^+ was measured with a CATION METROSEP C2 250 column IC (250 mm in length with 4 in mm id). The circulating phase with a flow-rate of 0.7 mL min^{-1} was composed of 1.0 mM $NaHCO_3$ and 3.2 mM Na_2CO_3 (1:1) for anion determination. It was a mixture of pyridine dicarboxylic acid (0.75 mM) and tartaric acid (4.0 mM) at 1:1 (v/v) for cation. The flow rate was 1 mL min^{-1}. The growth inhibition test was conducted to determine the antimicrobial activity of CCD mixture and its degradation products using *E. coli* bacteria (Levard et al., 2013). The toxicity was evaluated by counting the difference between the number of colony forming units (CFU) with drugs and in absence of it. The Luria–Bertani (LB) media was used for a typical toxicity protocol (Liang et al., 2013). A calibration curve of number of CFU mL^{-1} versus intensity of absorption was made in control LB media. The cell number was counted from the calibration curve during the antimicrobial activity test.

2.3. Experimental procedure

All experiments were performed in a batch cylindrical reactor made of borosilicate glass (Ø 10.5 cm) of 1000 mL capacity. Drug solution of 400 mL was taken for the experimentation. The initial drug concentration (0.15 mM) was well within the earlier studies for DIPY (Assumpcao et al., 2013; Reis et al., 2013; Giri and Golder, 2014b). The experiments with mixed drugs were carried out with an equimolar concentration (0.05 mM) of CIP, DIPY and CHPL to have the same concentration of DIPY. The reaction temperature was around 23–25 °C maintained with proper cooling arrangement. The experiment with foreign anion was carried out at 10 mM concentration and the equivalent amount of salt was

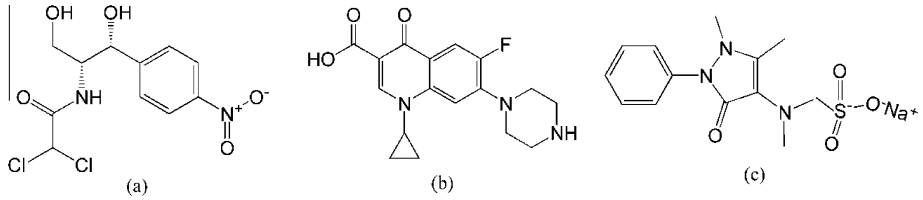

Fig. 1. Chemical structure of model pharmaceuticals. (a) Chloramphenicol [2,2-dichloro-N-((1R,2R)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl) acetamide], (b) ciprofloxacin [1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid] and, (c) dipyrone [(2,3-dihydro-1,5-dimethyl-3-oxo-2-phenyl-1-pyrazol-4-yl) methyl amino] methane sulfonate.