Removal of cytostatic drug 5-fluorouracil from water by electro-Fenton process using boron-doped diamond anode

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Abstract

Cytostatic drugs are one of the most troublesome classes of emerging pollutants in water owing to their toxicity and potential effects on DNA. This study addresses, for the first time, the optimization of the electro-Fenton process for the removal of cytotoxic drug 5-fluorouracil from water using a BDD anode and a carbon felt cathode. As demonstrated from chromatographic and TOC analyses, the optimal catalyst concentration was 0.2 mM Fe^{2+}, allowing the quickest drug degradation and almost complete mineralization at 300 and 1500 mA, respectively. The absolute rate constant for oxidation of 5-fluorouracil by hydroxyl radicals was found to be $(1.52 \pm 0.01) \times 10^9$ M^{-1} s^{-1}. During the electrolyses, linear carboxylic acids (oxalic and acetic) were formed and destroyed, whereas the final solution at 360 min mainly contained some inorganic ions (NH_4^+, NO_3^- and F^-) and < 10% TOC. Hence, electro-Fenton process constitutes an interesting alternative for the degradation of (bio)refractory drugs.

Keywords 5-Fluorouracil; Antineoplastic; BDD; Cytostatic; Electro-Fenton; Water decontamination

Introduction

Pharmaceuticals are non-regulated trace organic emerging contaminants that are ubiquitous in the aquatic environment due to discharge of effluents from pharmaceutical industry, hospitals and municipal wastewater treatment facilities (Feng et al. 2015, Petrie et al. 2015). In most cases, these pollutants are also present in the digested sludge, potentially entailing a high ecotoxicological risk (Martín et al. 2012). In order to avoid the propagation and subsequent accumulation of pharmaceutically active compounds in water bodies, specific prevention systems should be devised at major point sources of pollution. In the case of pharmaceuticals, such locations are hospitals and production sites. Unfortunately, removal efficacy data are scarce since the vast majority of the studies only focus on their impact on pharmaceutical loads.

The most hazardous representatives among these pollutants are those with a very powerful pharmacological action. Among them, the class of cytostatic (antineoplastic) drugs, used to prevent or inhibit the growth of malignant cells or tumors, can display severe negative effects on non-target organisms including humans, like carcinogenicity, cytotoxicity, genotoxicity, mutagenicity and teratogenicity (Kosjek et al. 2013). 5-Fluorouracil (Fig. 1A) belongs to the subclass of antimetabolites and its consumption reaches the range of tonnes in Europe. Several studies have reported its presence in natural water at concentrations ranging from ng L^{-1} to μg L^{-1} (Lutterbeck et al. 2016).

Conventional water treatment methods based on biological processes have been shown as insufficient to eliminate the majority of target pharmaceuticals because of their high recalcitrance and toxicity to bacteria (Kovalova et al. 2012). 5-Fluorouracil is not an exception and, furthermore, it has been demonstrated that photodegradation by sun is not suitable for its removal either, showing partial degradation and no mineralization (Lutterbeck et al. 2016). Therefore, it does not undergo natural attenuation in the environment and cannot be removed in traditional treatment plants. Chemical degradation with NaClO or H_2O_2 could be an option to face up to this troubling situation, as tested in the hospital environment (Castegnaro et al. 1997). Alternatively, several enhanced water treatment technologies have been developed in recent years. In particular, the advanced oxidation processes (AOPs), which utilize strong reactive oxidant species like hydroxyl radicals ($^{\bullet}OH$) generated on site, present appealing perspectives for treating biorefractory pollutants (Oturan and Aaron 2014). A relatively new group of AOPs is constituted by electrochemical methods (EAOPs) such

as electro-oxidation, Fenton-based EAOPs and photoelectrocatalysis, whose great performance during the treatment of pharmaceuticals has been thoroughly reviewed (Sirés and Brillas 2012, Feng et al. 2013, Brillas and Sirés 2015). The common feature of all the EAOPs is their ability to generate reactive oxygen species (ROS), as either superoxides (MO_x) or hydroxyl radicals ($M(^{\bullet}OH)$), adsorbed on the anode (M) surface from the oxidation of water. Active anodes like Pt promote the formation of the first kind of ROS, whereas non-active anodes like boron-doped diamond (BDD) foster the second type via reaction (1). However, only the Fenton-based EAOPs like electro-Fenton (EF) process are able to produce homogeneous $^{\bullet}OH$ from Fenton's reaction (2) thanks to the in situ generation of H_2O_2 from the two-electron cathodic reduction of O_2:

$$M + H_2O \rightarrow M(^{\bullet}OH) + H^+ + e^- \quad (1)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad (2)$$

The fundamentals and reactivity of EF process are well described elsewhere (Brillas et al. 2009). EF has been successfully employed for the treatment of acidic aqueous solutions containing antibiotics, antidepressants or β-blockers, among others, using undivided electrolytic cells with different anodes (Sirés et al. 2010; Dirany et al. 2012; Estrada et al. 2012; Panizza et al. 2014; Salazar et al. 2017). However, there have been no studies dealing with the application of EF to the degradation of cytostatic agents. Regarding 5-fluorouracil, only a few publications have reported its treatment by AOPs, including photocatalysis with TiO_2 (Lin and Lin 2014), UV/H_2O_2 (Lutterbeck et al. 2015), as well as Fenton and photo-Fenton processes (Governo et al. 2017; Koltsakidou et al. 2017). In this context, the aim of the present work is to optimize the key operation parameters for a fast drug decay and large mineralization of aqueous solutions of the emerging pollutant 5-fluorouracil by EF using a BDD/carbon felt cell, trying to confirm the great oxidation power of this method to treat highly (bio)recalcitrant molecules.

Experimental

The cytostatic drug 5-fluorouracil was of analytical grade (> 99% purity) from Sigma-Aldrich, and it was used as received. Solutions were prepared with ultra-pure water obtained from a Millipore Simplicity 185 water purification system with resistivity > 18 MΩ cm. Iron(II) sulfate heptahydrate used as source of Fe^{2+} ions (catalyst) and sodium sulfate added as supporting electrolyte were of analytical grade from Acros Organics

and Sigma-Aldrich, respectively. Sulfuric acid from Merck was added to adjust initial pH of all solutions. Salts used as standard in ion chromatography were of ACS grade (purity > 99.5%) from Sigma-Aldrich. Methanol used as organic solvent for high performance liquid chromatography (HPLC) was purchased from VWR.

A bench-scale, open, undivided glass cell was used in batch operation mode at room temperature to carry out galvanostatic electrolyses using an HM8040-3 Hameg power supply. The cell was filled with 200 mL of a 0.1 mM drug solution containing 0.05 M Na_2SO_4 and a given amount of $FeSO_4$, at pH 3.0 since it is the optimal value for performing EF treatments (Brillas et al. 2009). A carbon felt cathode (18.5 cm × 4.5 cm) from Carbone-Lorraine was placed covering the inner wall of the cell, and a PTFE mesh was used to keep it apart from the anode, a thin film BDD on niobium substrate (6.0 cm × 4.0 cm) centered in the cell. The solution was saturated with O_2 by sparging compressed air at 1 L min^{-1}, starting 15 min before electrolysis, and continuous stirring (450 rpm) was maintained during the trials. The experiments were run in triplicate.

Drug decays were monitored by reversed-phase HPLC using a Merck Lachrom chromatograph fitted with a Purospher Star RP-18, 5 µm, 25 cm x 4.6 mm (i.d.), column at 40 °C, and coupled with an Elite Lachrom L-2400 UV detector selected at λ = 277 nm. Analyses were carried out isocratically by using a 5:95 (v/v) methanol/water mixture, both with 0.1% acetic acid, at 0.4 mL min^{-1}, employing the EZChrom Elite 3.1 software. In competition kinetics experiments (see Panizza et al. 2014), the HPLC analysis was made with a 30:70 (v/v) methanol/water solution (0.1% acetic acid in each) as mobile phase at 0.3 mL min^{-1}. Short-chain carboxylic acids were determined by ion-exclusion chromatography using the same equipment, with a Supelcogel H, 9µm, (25 cm x 4.6 mm (i.d.)) column at room temperature, the detector set at λ = 210 nm and using 1% H_2SO_4 as mobile phase at 0.2 mL min^{-1}. Inorganic ions were analyzed on a Dionex ICS-1000 chromatograph that included self-regenerating suppressors. The system was equipped with a DS6 conductivity detector containing a cell heated at 35 °C. An anion-exchange column (IonPac AS4ASC, 25 cm × 4 mm) fitted with an IonPac AG4A-SC column guard was used to analyze the anions. For cations (NH_4^+), a cation-exchange column (IonPac CS12A, 25 cm × 4 mm) fitted with an IonPac CG12A column guard was used. A mixture of 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$ at 2.0 mL min^{-1} and a 9.0 mM H_2SO_4 solution at 1.0 mL min^{-1} were eluted as mobile phases. The mineralization of each solution was monitored from the total organic carbon (TOC) abatement, using the non-purgeable organic carbon method with ± 2% accuracy,

determined on a Shimadzu VCSH analyzer. The average values with an error < 2% are reported in all figures.

Results and discussion

Optimization of EF parameters for 5-fluorouracil removal

The catalyst content and applied current (*I*) are the most crucial operation parameters to be optimized in EF treatments. First, the effect of the initial Fe^{2+} concentration on 5-fluorouracil destruction was studied at 300 mA. The drug decay with oxidation time is represented in Fig. 1A as the ratio between the concentrations of pollutant at time *t* and before the electrolysis. As can be observed, the destruction of the drug was quick and complete in all cases. At 0.1 mM Fe^{2+}, total disappearance is attained in only 7 min, thanks to the synergistic action of $^{\bullet}OH$ formed in the bulk and BDD($^{\bullet}OH$) adsorbed on the anode surface (Brillas et al. 2009). The oxidation is accelerated when increasing the initial Fe^{2+} content to 0.2 mM, achievieng a significantly quicker decay at the early stages and an overall removal at 6 min. This enhancement can be explained by the larger production of $^{\bullet}OH$ from Fenton's reaction (2), since the carbon felt cathode electrogenerates enough H_2O_2 so as to react with the higher amount of catalyst and, furthermore, it is able to continuously regenerate Fe^{2+} from Fe^{3+} reduction. Conversely, the degradation rate decreases upon further increase of the Fe^{2+} content to 0.5 mM, needing again 7 min for complete disappearance. This can be better seen in Fig. 1B, which depicts the corresponding apparent rate constant (k_{app}) values for the analysis of decay trends assuming a pseudo-first-order reaction kinetics (R^2 > 0.985 in all cases). The change of k_{app} with Fe^{2+} concentration makes it evident the need for optimization this parameter. The existence of a maximum arises from the fact that an excessive amount of catalyst triggers a number of parasitic reactions that cause quenching of $^{\bullet}OH$ and BDD($^{\bullet}OH$), especially their consumption by Fe^{2+} to form a less reactive catalyst (Fe^{3+}).

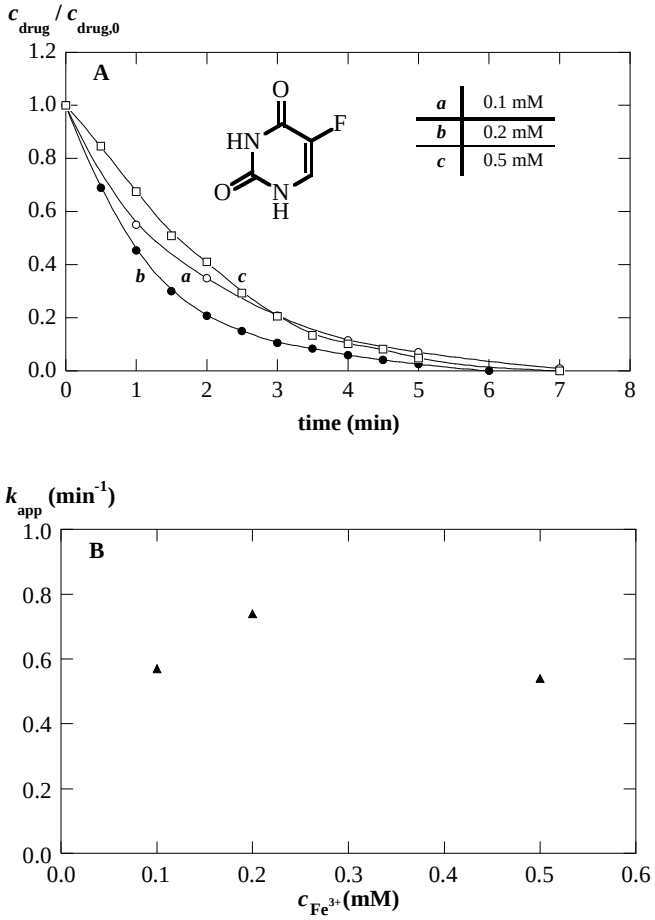

Fig. 1

Effect of Fe^{2+} concentration on the destruction of 0.1 mM 5-fluorouracil during electro-Fenton (EF) treatment at 300 mA. Plot (A): Drug decay with electrolysis time. Plot (B): Change of the apparent rate constant values with Fe^{2+} concentration, assuming a pseudo-first-order decay kinetics. Results show that 0.2 mM Fe^{2+} is the optimal concentration for degrading 5-fluorouracil by EF process

Afterwards, the effect of the applied current for treating acidic 0.1 mM 5-fluorouracil solutions was studied using 0.2 mM Fe^{2+}. The time-course of normalized drug concentration is shown in Fig. 2A. Again, a fast and total destruction is achieved, being required a shorter time as current increases. For example, the application of currents from 50 to 300 mA allows shortening the required time from 9 to 6 min. This is due to the gradually greater production of BDD($^{\bullet}$OH) from reaction (1), as well as of $^{\bullet}$OH via reaction (2) due to the upgrading of H_2O_2 cathodic electrogeneration. In contrast, the application of a higher current like 400 mA is detrimental, since the process becomes slower and 7 min are needed to completely remove the drug. The values of k_{app} ranged between 0.38 and 0.60 min^{-1}, with a maximum of 0.74 min^{-1} appearing at 300 mA. As in Fig. 1B, this deceleration can be justified by the larger extent of parasitic reactions, such

as the attack of hydroxyl radicals onto the extra H_2O_2 as well as onto the reaction intermediates (whose formation rate increases as current rises), along with the promotion of the four-electron reduction of O_2 to H_2O. Analogous dependence of k_{app} on current was found for EF treatments with 0.1 and 0.5 mM Fe^{2+}, although in those cases the k_{app}-range was remarkably lower, from 0.26 to 0.42 and 0.16 to 0.40 min^{-1}, respectively, confirming the superiority of 0.2 mM Fe^{2+}. From competition kinetics trials using *p*-hydroxybenzoic acid as reference (Oturan and Aaron 2014), the absolute rate constant (k_{abs}) for the reaction between 5-fluorouracil and hydroxyl radical was determined for the first time, obtaining $(1.52 \pm 0.01) \times 10^9$ M^{-1} s^{-1}.

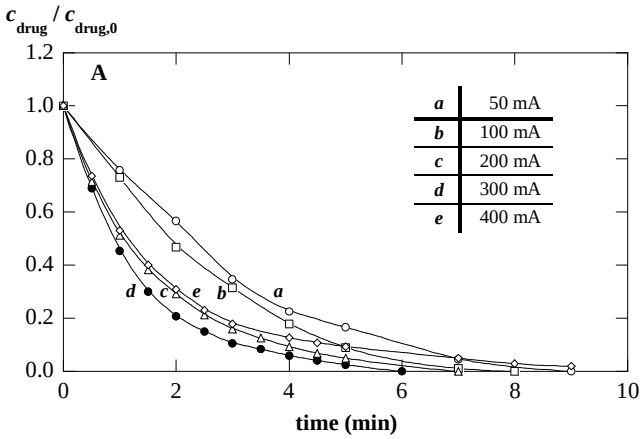

Fig. 2

Effect of applied current on the destruction of 0.1 mM 5-fluorouracil during electro-Fenton treatment with 0.2 mM Fe^{2+}. Drug decay kinetics is enhanced with increasing current, being the treatment optimal at 300 mA

Mineralization of 5-fluorouracil solutions and by-products generated

The effect of Fe^{2+} content and current on the mineralization ability of the EF process is shown in Fig. 3 as the percentage of TOC removal. Fig. 3A confirms that 0.2 mM Fe^{2+} is also the optimal concentration for the degradation of reaction intermediates, since the removal percentage increased from 76% to 86% at 360 min. Further increase of the catalyst content to 0.5 mM was detrimental, with a slower and lower final mineralization of 82%. In order to assess the possibility of attaining larger TOC removals, current was tested from 200 to 1500 mA (Fig. 3B). As can be seen, almost overall mineralization (> 94%) was achieved at the highest current value due to the simultaneous action of hydroxyl radicals onto the parent drug and its intermediates, whereas gradually lower percentages

were achieved as the applied current decreased. Depending on the energy cost, currents < 1500 mA can be chosen, still attaining > 90% mineralization at long time.

The attack of $^{\bullet}$OH and BDD($^{\bullet}$OH) on the cyclic structure causes its hydroxylation upon attack onto nitrogen atoms and/or –CH group (Lutterbeck et al. 2016), followed by ring cleavage with formation of carboxylic acids. This is demonstrated for 5-fluorouracil in Fig. 4A, where acetic and oxalic acids (as free neutral molecules or complexed with Fe^{2+} ions) are accumulated since the beginning of the electrolyses and then progressively destroyed, in agreement with the low TOC remaining in the final solutions. The oxidation of the drug is accompanied by the release of heteroatoms. Fig. 4B shows that F is almost completely released as F^-, whereas nitrogenated ions (NH_4^+ and NO_3^-) only account for < 60% of the initial N, which can be explained by the formation of *N*-containing soluble organic matter along with the production of volatile products such as N_2 and N_xO_y.

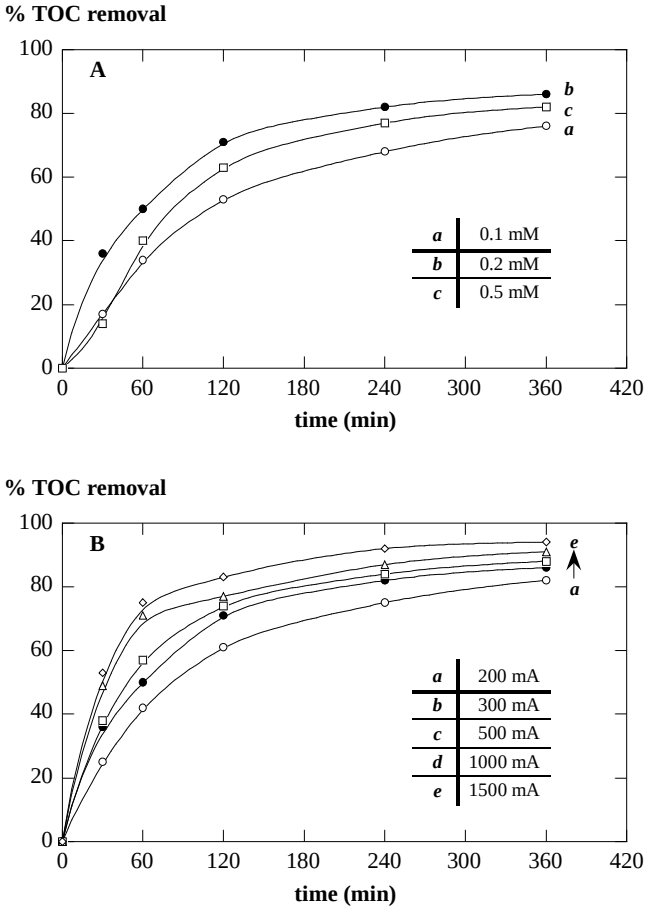

Fig. 3

Total organic carbon (TOC) abatement versus electrolysis time during electro-Fenton treatment of 0.1 mM 5-fluorouracil solutions. Plot (A): Effect of Fe^{2+} content as in Fig. 1. Plot (B): Effect of applied current as in Fig. 2, but within a higher range. Mineralization is optimal at 0.2 mM Fe^{2+} and 1500 mA

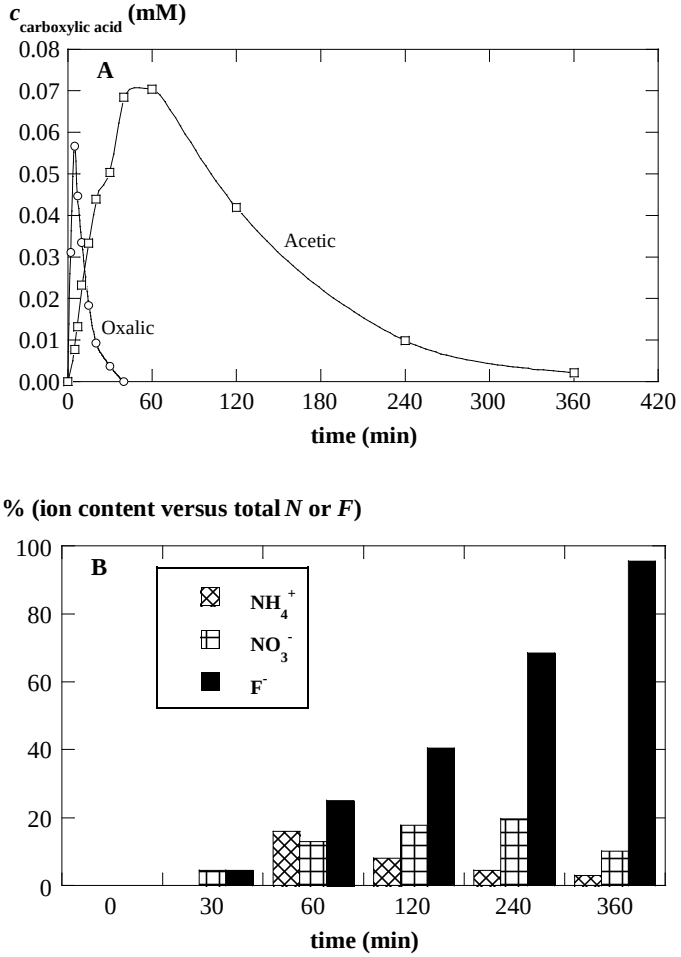

Fig. 4

By-products generated during the electro-Fenton treatment of 0.1 mM 5-fluorouracil with 0.2 mM Fe^{2+} at 300 mA. Plot (A): Evolution of oxalic and acetic acids. Plot (B): Accumulation of inorganic ions. The final solution contains no acids, and initial heteroatoms are accumulated as F^- along with low amounts of *N*-containing ions

Conclusion

The fastest destruction of cytotoxic drug 5-fluorouracil by electro-Fenton process (6 min at 300 mA) was reached at an optimum Fe^{2+} concentration of 0.2 mM. Regarding the mineralization of 5-fluorouracil solutions, the application of 1500 mA ensured the abatement of > 94% TOC. The second-order rate constant (k_{abs}) for oxidizing this drug with hydroxyl radical was determined for the first time. Electro-Fenton process is shown as highly effective for destroying cytostatic pharmaceutical residues in water.

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