Improving the biodegradability of hospital urines polluted with chloramphenicol by the application of electrochemical oxidation

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Abstract

This work focuses on improving the biodegradability of hospital urines polluted with antibiotics by electrochemical advanced oxidation processes (EAOPs). To do this, chloramphenicol (CAP) has been used as a model compound and the influence of anodic material (Boron Doped Diamond (BDD) and Mixed Metal Oxide (MMO)) and current density ($1.25 - 5 \text{ mA cm}^{-2}$) on the toxicity and the biodegradability was evaluated. Results show that a complete CAP removal was attained using BDD anodes, being the process more efficient at the lowest current density tested (1.25 mA cm^{-2}). Conversely, after passing 4 Ah dm^{-3} , only 35 % of CAP removal is reached using MMO anodes, regardless of the current density applied. Furthermore, a kinetic study demonstrated that there is a clear competitive oxidation between the target antibiotic and the organic compounds naturally contained in urine, regardless the current density and the anode material used.

During the first stages of the electrolysis, acute toxicity is around 1 % EC₅₀ but it increases once CAP and its organic intermediates have been degraded. The formation and accumulation of inorganic oxidants may justify the remaining acute toxicity. This also helps to explain the trend observed in the rapid biodegradability assays. Finally, a 60 % of standard biodegradability (Zahn-Wellens test) was achieved which suggests that electrochemical oxidation with BDD anodes could be the most appropriate technology to reduce the hazard of hospital urines at the operating conditions tested.

Keywords: chloramphenicol; urine; hospital effluents; electrochemical oxidation; biodegradability

Highlights

- Electrochemical oxidation is suitable to reduce the hazard of hospital urines.
- CAP can be completely removed from hospital urines using BDD electrolysis.
- MMO-electrolysis leads to partial oxidation of CAP and nil mineralization.
- Urine toxicity decreases down to 74 % using low current densities.
- BDD-electrolysis at low current density increases biodegradability up to 60 %.

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1. Introduction

Nowadays, the presence of micropollutants in surface water has significantly increased since they are not properly degraded in conventional Waste Water Treatment Plants (WWTPs) (Fernández et al., 2014; Pérez et al., 2017). Hence, they are discharged into the aquatic ecosystems causing very serious environmental problems because of their hazardousness for both, aquatic ecosystems and human health (Badawy et al., 2009; Mohapatra et al., 2014). These micropollutants comprise industrial additives, surfactants, personal care products and pharmaceuticals. Within this last group, pollutants that kill target microorganisms such as antibiotics, antiparasitic and antifungal have been considered compounds of emerging concern by World Health Organization (WHO) due to the possibility of resistant strains developing (Korzeniewska et al., 2013).

One of the main routes through which drugs pass into the environment is hospital effluents (Michael et al., 2013). These effluents coming from the auxiliary kitchen/canteen, sanitary facilities of common use (not hospitalized) and gardening could be considered assimilable to urban/domestic effluents (Cotillas et al., 2018a; Kanama et al., 2018; Mohd and Malik, 2018). However, there is a wide variety of hospital effluents which could also involve serious chemical and biological risks such as those generated in radiology rooms (containing ICM, very recalcitrant compounds, in extremely high concentrations), laundries, laboratories of clinical analysis and operating rooms or areas of infected patients. Within them, urine is considered as one of the most dangerous pollution sources since high concentration of pharmaceuticals and their metabolites are excreted by this media (Solanki and Boyer, 2017).

In this context, the treatment of human urine from hospitalized patients is a great challenge that should be faced in the treatment of hospital effluents today. In literature, it

has been reported the efficiency of Advanced Oxidation Processes (AOPs) based on Fenton reaction to remove pharmaceuticals in urine matrixes (Giannakis et al., 2017; Papoutsakis et al., 2015). Likewise, the efficiency of electrochemical processes for the treatment of effluents containing organic pollutants not only pharmaceuticals but also alcohols, carboxylic acids or even more complex aromatic hydrocarbons has been widely reported (Al Aukidy et al., 2014; Azuma et al., 2019; Elmountassir et al., 2019; Lima et al., 2019; Vo et al., 2019).

Among the electrochemical processes, Conductive-Diamond Electrochemical Oxidation (CDEO) has been proven as a very efficient and robust technology for the removal of several pharmaceuticals from hospital effluents such as ibuprofen, cloxacillin, chloramphenicol, penicillin G, etc. (Cotillas et al., 2019; Cotillas et al., 2018a; Cotillas et al., 2018b). The majority of these studies were focused on the complete mineralization of the organic matter using high values of current density ($10\text{-}100\text{ mA cm}^{-2}$) that favor besides the direct transfer of electron at the anodic surface, the formation of reactive oxygen species from water oxidation such as hydroxyl radicals and/or other oxidants produced electrochemically from inorganic ions existing in the bulk such as chloride, sulfate, phosphate or carbonate (Cotillas et al., 2017; Garcia-Segura et al., 2019). These hard conditions justify the good performance of CDEO and the mineralization percentage attained but increase the energy requirement of the technology. Therefore, in the search of cost-effective treatments the use of these conditions does not seem to be a good practice and to make this technology competitive with conventional technologies, the application of softer conditions could be recommended in case the complete removal of organic load is not required (Mousset et al., 2018).

Within this background, this work proposes the use of electrochemical oxidation for the conversion of chloramphenicol (selected as model of antibiotic) in hospital urines into

other intermediate organics whose discharge into the sanitation network does not generate problems at wastewater treatment plants. To do this, the role of anodic material (Boron Doped Diamond (BDD) and Mixed Metal Oxide (MMO)) and current density (5, 2.5, 1.25 mA cm⁻²) will be evaluated. Likewise, a kinetic study will carry out to evaluate the competitive oxidation between the target antibiotic and other organic compounds naturally contained in urine at different current densities with both electrodes. Finally, to establish the optimum operation conditions and reaction time to reduce the chemical risk of hospital urines, it is proposed the use of both physicochemical characterization and biological indicators, such as the standardized method of toxicity (based on the use of a bioluminescent marine bacterium *Vibrio fischeri*) and rapid-biodegradability tests that allow to evaluate the enhancement of biodegradability in a short time using a real sludge from a wastewater treatment plant.

2. Material and methods

2.1. Chemicals

Chloramphenicol (C₁₁H₁₂Cl₂N₂O₅) was of analytical grade (> 98% purity) from Sigma-Aldrich. Urea, creatinine, uric acid, potassium chloride, magnesium sulfate, calcium phosphate, sodium carbonate and diammonium hydrogen phosphate employed for the formulation of synthetic urine were analytical grade and purchased from Sigma-Aldrich. Other chemicals employed for the determination of urea, oxidants (including free and combined chlorine species) and ions were also analytical grade from Sigma-Aldrich. Methanol used was HPLC grade and provided by VWR. All solutions were prepared with double deionized water obtained from a Millipore Milli-Q system (18.2 MΩ cm, 25°C).

2.2. Experimental procedure

Electrochemical oxidation experiments were developed in a single compartment electrochemical cell working under batch-operation mode (Cañizares et al., 2002). Boron Doped Diamond (BDD, purchased from WaterDiam) and Mixed Metal Oxide based on RuO_2 (MMO, purchased from Tianode) with a circular geometric area of 78 cm^2 were used as anodic materials, and stainless steel was used as cathodic electrode. The inter-electrode gap between both electrodes was 9 mm. A Delta Electronika ES030-10 power supply (0-30V, 0-10A) provided the electric current.

All experiments were carried out under galvanostatic conditions and the current densities applied were within the range $1.25 - 5 \text{ mA cm}^{-2}$. The simulated hospital effluent was prepared with 100 mg dm^{-3} of the target antibiotic and synthetic human urine as supporting electrolyte. The composition of urine has been reported elsewhere (Cotillas et al., 2018c) and it consists of organic compounds (urea, creatinine and uric acid) and inorganic salts (potassium chloride, magnesium sulfate, calcium phosphate, sodium carbonate and diammonium hydrogen phosphate).

2.3. Biodegradation assays

2.3.1. Rapid biodegradability assay

Rapid biodegradability assays were carried out in glass reactors (0.250 dm^3) with 0.095 dm^3 of endogenous activated sludge and 0.005 dm^3 of target sample. Before the assay, the activated sludge was maintained in starvation and continuous aeration overnight to eliminate any residual organic matter and to avoid oxygen limitation. The dissolved oxygen concentration was monitored by using an oximeter (HI9146, Hanna Instruments) as function of the experimental time. The rapid biodegradability of samples was calculated as a relation between rapid biochemical oxygen demand (BOD) and chemical oxygen demand (COD) by using Equation (1).

$$\text{Rapid biodegradability (\%)} = \frac{\text{BOD}_{\text{rapid}} \cdot V_{\text{biological reactor}}}{\text{COD}_{\text{sample}} \cdot V_{\text{sample}}} \cdot 100 \quad (1)$$

The rapid BOD is calculated as the difference, in terms of dissolved oxygen decay (mg O₂ dm⁻³), between the slope of endogenous activated sludge and the slope when sample is added into endogenous activated sludge.

2.3.2. Standard biodegradability assay

Standard biodegradability assays were carried out in Erlenmeyer glass reactors (250 mL) with 240 mL of sample following the Zahn-Wellens test (OECD 302 B 1992). Sludge in the ratio 1:3 for inoculum/TOC and mineral medium (10 mL A + 1 mL B + 1 mL C + 1 mL D + H₂O to 1 L) were added. The stocks solutions for the preparation of mineral medium were: A (0.85 g KH₂PO₄ + 2.175 g K₂HPO₄ + 3.34 g Na₂HPO₄·2H₂O + 0.05 g NH₄Cl + H₂O to 100 mL), B (2.75 g CaCl₂ + H₂O to 100 mL), C (2.25 g MgSO₄·7H₂O + H₂O to 100 mL) and D (0.025g FeCl₃·6H₂O + H₂O to 100 mL). The mixture was stirred softly at room temperature during 28 days. Samples were taken periodically to quantify the evolution of TOC and CAP concentrations. Glucose was used as reference compound to ensure the activity of microorganisms.

2.4. Acute toxicity assays

Acute toxicity towards *Vibrio fischeri* (marine luminescent bacterium) was measured using a Microtox® M500 Analyzer (Azur Environmental) according to standard Microtox test procedure (ISO 11348, 2007). Lyophilized bacteria are reconstituted by suspension in 1 mL of aqueous NaCl solution (0.7 % w/w). Samples were tested for 15 min of exposure at 15 °C. Toxic effects were monitored as a percent decrease of the light emission of *Vibrio fischeri* and compared to that obtained for the blank sample, which contains 2% NaCl in deionized water.

2.5. Analytical methods

Chloramphenicol concentration was measured by high performance liquid chromatography (HPLC) with an Agilent 1200 series coupled a DAD detector. A ZORBAX Eclipse Plus C18 analytical column was used and its temperature was maintained at 25 °C. The mobile phase consisted of 50 % methanol / 50 % milli-q water applying a flow rate of $0.6 \text{ cm}^3 \text{ min}^{-1}$, an injection volume of 20 μL and a DAD detection wavelength of 270 nm. The organic compounds from urine were determined by HPLC (uric acid), ion chromatography (creatinine) and colorimetric methods (urea) as described elsewhere (Cotillas et al., 2018a; Cotillas et al., 2018b). The Total Organic Carbon (TOC) was determined using a Shimadzu TOC-VCPH analyzer.

Inorganic ions were analyzed by ion chromatography (IC) with a Metrohm 930 Compact IC Flex coupled to a conductivity detector. A Metrosep A Supp 7 column was used to measure anions whereas cations were determined using a Metrosep C6 250 column. A mobile phase of 85:15 v/v 3.6 mM Na_2CO_3 /acetone with a flow rate of $0.8 \text{ cm}^3 \text{ min}^{-1}$ was used to measure anions and a mobile phase of 1.7 mM HNO_3 + 1.7 mM 2,6-pyridinedicarboxylic acid with a flow rate of $0.9 \text{ cm}^3 \text{ min}^{-1}$ was employed for the determination of cations. The volume injection of each sample was 20 μL .

Hypochlorite was analyzed by titration with 0.001 M As_2O_3 in 2 M NaOH (Freytag, 1959; Wilpert, 1957). Oxidants were determined iodometrically according to Kolthoff & Carr (Kolthoff and Carr, 1953). pH and conductivity were simultaneously measured using a Sension+ MM150 Portable Multi-Parameter Meter from HACH.

3. Results and discussion

Figure 1 shows the removal of CAP in urine matrix with the applied electric charge at different current densities (ranging from 1.25 to 5 mA cm⁻²) using BDD and MMO anodes. These low current densities were selected in order to favor a selective oxidation process and a competitive cost-effective technology. Likewise, BDD and MMO were tested as anodic materials due to their different electrocatalytic behavior as non-active and active anodes, respectively (Karaçali et al., 2019; Martínez-Huitle et al., 2015).

Figure 1. Evolution of chloramphenicol as function of the applied electric charge during the electrochemical oxidation of 100 mg dm⁻³ CAP in urine media. Current density: (■, □) 1.25 mA cm⁻²; (▲, Δ) 2.5 mA cm⁻²; (●, ○) 5 mA cm⁻². Anodic material: (black symbols) BDD; (white symbols) MMO.

As it can be seen, electrolysis with BDD and MMO exhibits different behavior in terms of CAP degradation efficiency. Specifically, a complete CAP degradation is attained with BDD anode after passing 6 Ah dm⁻³ of applied electric charge for the lowest current density (1.25 mA cm⁻²), being the most efficient condition. Likewise, the trend observed with this anodic material follows an exponential decay which is directly related to a mass-

transfer control of the degradation route (Martínez-Huitle et al., 2015; Panizza and Cerisola, 2009). The process efficiency is higher when working at very low current densities which suggests that other parasitic reactions take place at high current densities. Hence, electrons are used not only in the antibiotic removal but also are wasted in other side reactions such as oxygen evolution (Cañizares et al., 2007; Dominguez et al., 2018). Regarding CAP removal with MMO anodes, only degradation percentages of 15-35 % are attained at the operating conditions studied. These values are considerably lower than those obtained with BDD anodes. Specifically, BDD anode seems to be up to 5-fold more efficient on CAP degradation in urine media than MMO anode that requires higher applied electric charges to reach a similar CAP removal.

Urine is a complex water media containing different inorganic anions including sulphate, chloride, phosphate or carbonate (Dbira et al., 2015a; Dbira et al., 2015b). These compounds can be also oxidized during electrolysis, favoring the production of powerful oxidants such as hypochlorite, persulfate, peroxodiphosphate and percarbonates (Cotillas et al., 2019; Sánchez-Carretero et al., 2011b). In addition, hypochlorite is known to chemically react with ammonium ions during electrolysis, resulting in the in-situ production of inorganic chloramines (Lacasa et al., 2012). These generated inorganic oxidants may contribute to CAP degradation by mediated oxidation processes and, for this reason, their concentration was monitored during the process. Figure 2 shows the maximum concentration of total oxidants, hypochlorite and chloramines as function of the current density during the treatment of urines polluted with 100 mg dm⁻³ CAP.

Figure 2. Maximum molar concentration of total oxidants, hypochlorite and chloramines as function of the current density applied during the electrochemical oxidation of 100 mg dm⁻³ CAP in urine media. Anodic material: (a) BDD; (b) MMO. (■) total oxidants, (■) Cl-ClO⁻, (■) Cl-NH₂Cl, (■) Cl-NHCl₂, (□) Cl-NCl₃.

As can be observed, the higher the current density, the higher is the maximum concentration of total oxidants remained in solution. BDD anode leads to a lower concentration of total oxidants than MMO anode which is an unexpected behaviour according to literature (Cañizares et al., 2009; Cotillas et al., 2019; Sánchez-Carretero et al., 2011a). This fact is more noticeable at the highest current density (5 mA cm⁻²) where the maximum concentration of oxidants is 7.0 mmol dm⁻³ for BDD anode vs. 12.8 mmol dm⁻³ for MMO anode. This is mainly related to the stability of the different oxidants generated in each case. In this context, the formation of free and combined chlorine species (hypochlorite and chloramines) and peroxocompounds (persulfate, peroxodiphosphate, percarbonates) are expected to be generated using BDD anodes from the oxidation of the ions contained in urine (Cotillas et al., 2019) whereas only chlorine species are expected to be generated with MMO anodes due to their different electrocatalytic properties (Alfaro et al., 2006). Nonetheless, it is important to highlight that oxidants measured inform about the residual concentration of oxidants (after reaction

with organics) but not about the total amount of oxidants formed. This means that electrolysis with diamond anodes can be producing more unstable oxidants (peroxocompounds), helping to explain the higher oxidation of organics observed.

Regarding chlorine speciation, hypochlorite concentration attains a maximum value of 0.7 and 0.5 mmol dm⁻³ at a current density of 2.5 mA cm⁻² with BDD and MMO anodes, respectively. Likewise, the maximum concentration of chloramines formed is around two orders of magnitude lower in the case of using BDD and it seems to depend on the current density. The stronger oxidation conditions during BDD-electrolysis seems to favour chloramines decomposition (Cotillas et al., 2018a; Garcia-Segura et al., 2019), whereas the current density seems to favour the accumulation of dichloramine and trichloramine during MMO-electrolysis (McKay et al., 2013). Finally, it is noteworthy to mention that the generation of volatile active chlorine species such as Cl₂, Cl₂O or ClO₂ can be generated during the electrolysis with both anodes, enhancing the oxidation of organics in urine (Mostafa et al., 2018). However, the operating conditions tested (current density and pH) in this work promote the formation of hypochlorite as the main free chlorine species. On the other hand, chlorine compounds in high oxidation state such as chlorates or perchlorates are not detected anytime in solution at the operating conditions used. This is an excellent output that allow to consider electrochemical oxidation as an appropriate advanced oxidation process for the removal of pharmaceuticals in hospital urines. Combination of hypochlorite with ammonium to form chloramines prevent the further oxidation of hypochlorite to chlorates and perchlorates.

The oxidation of CAP by electrolysis can lead to the formation of other intermediate organic compounds or to progress up to the complete mineralization. The first case can be very worrying since other organics formed can be more hazardous than the initial pollutant, increasing the toxicity of the resulting effluent. On the other hand, the main

drawback associated to the complete mineralization is the increase in the energy requirements and, thus, in the treatment costs. At this point, it is important to highlight that the organic load of urine is not only related to the presence of CAP but also to other organics such as urea ($3333.33 \text{ mg dm}^{-3}$), creatinine ($166.67 \text{ mg dm}^{-3}$) and uric acid (50 mg dm^{-3}) present in its composition at much higher concentrations. For this reason, the evolution of urea, creatinine and uric acid, as well as, the mineralization were monitored during the treatment and results are presented in Figure 3.

Figure 3. Evolution of TOC (a) and urine organic compounds (urea (b), creatinine (c), uric acid (d)) as function of the applied electric charge during the electrochemical oxidation of $100 \text{ mg CAP dm}^{-3}$ in urine media. Current density: (■, □) 1.25 mA cm^{-2} ; (▲, Δ) 2.5 mA cm^{-2} ; (●, ○) 5 mA cm^{-2} . Anodic material: (black symbols) BDD; (white

symbols) MMO. TOC intermediates: (dark grey symbols) BDD; (light grey symbols) MMO.

The evolution of TOC is not significantly influenced by the current density applied, regardless the anodic material tested. A negligible mineralization is observed during electrolysis with MMO anode whereas 60 % of mineralization is reached for 8 Ah dm⁻³ using the BDD anode. In this last case, the residual organic matter is expected to be aliphatic compounds, mainly carboxylic acids such as formic, oxalic or oxamic acid (Chen et al., 2015; Cotillas et al., 2018a; Garcia-Segura et al., 2014). These results suggest that the operating conditions used (low current densities and electric charges) do not favour a complete removal of the organic matter present in the effluent, being more noticeable in the case of MMO anodes. Nonetheless, similar mineralization percentages can be attained with lower energy consumption and higher efficiency when working at very low current densities.

Regarding the evolution of organic compounds from urine composition, different behaviours can be seen in urea, creatinine and uric acid trends which are related to the different initial concentration of each compound and their different molecular structure (Cotillas et al., 2018c). The removal of urea is observed to follow a similar profile than mineralization shown in Figure 2a because initial concentration of urea corresponds to most of the initial TOC (83.7 %). Hence, there is a remarkable degradation of urea with BDD anodes whereas a negligible oxidation is observed using MMO anodes. This could be one of the main advantages of these electrodes (MMO) since it allows a selective oxidation of antibiotic, avoiding the potential degradation of urea. On the other hand, creatinine removal percentages range between 60-93 % in the case of using BDD anodes whereas only around 30 % of creatinine degradation is achieved using MMO anode despite the current density applied. Finally, uric acid is the most efficiently degraded

independently of the current densities tested and applied electric charges below 0.3 and 0.6 Ah dm⁻³ are required with BDD and MMO anodes, respectively.

In order to evaluate the competitive oxidation of organics contained in urine, a simple kinetic studied was carried out. Hence, experimental data of CAP, uric acid, creatinine and urea were fitted to a first order kinetics model according to Equation (2) (where C is the concentration of target compound at time t in mg dm⁻³, C₀ is the initial concentration of the target compound in mg dm⁻³, *k* is the kinetic constant in min⁻¹ and t is the time in min). Resulting kinetic constants are shown in Table 1.

$$\ln (C/C_0) = -k \cdot t \quad (2)$$

Table 1. *k* values for all the applied current densities and anode materials with the corresponding interval of adjustment, residual variance (*S*²_R) and coefficient of determination (*R*²).

CAP					
Anode / current density	Q (Ah dm ⁻³)	Time interval for complete removal (min)	<i>k</i> _{CAP} (10 ⁻³ min ⁻¹)	<i>S</i> ² _R (mg ² /L ²)	<i>R</i> ²
BDD / 1.25 mA cm ⁻²	8.00	0-3600	2.08±0.03	0.0176	0.9975
BDD / 2.5 mA cm ⁻²	8.00	0-1800	2.54±0.07	0.0149	0.9940
BDD / 5 mA cm ⁻²	6.46	0-720	3.04±0.07	0.0035	0.9941
MMO / 1.25 mA cm ⁻²	8.00	0-3600 (36.86 % CAP removal)	0.08±0.02	0.0033	0.7566
MMO / 2.5 mA cm ⁻²	8.00	0-1800 (25.88 % CAP removal)	0.11±0.02	0.0020	0.6842
MMO / 5 mA cm ⁻²	6.46	0-720 (16.26 % CAP removal)	0.18±0.04	0.0008	0.7232
URIC ACID					
Anode / current density	Q (Ah dm ⁻³)	Time interval for complete removal (min)	<i>k</i> _{UA} (10 ⁻³ min ⁻¹)	<i>S</i> ² _R (mg ² /L ²)	<i>R</i> ²
BDD / 1.25 mA cm ⁻²	0.57	0-160	29.46±0.06	0.0000	0.9999
BDD / 2.5 mA cm ⁻²	0.14	0-39	25.02±0.00	0.0000	1.0000
BDD / 5 mA cm ⁻²	0.14	0-20	49.96±0.07	0.0000	1.0000
MMO / 1.25 mA cm ⁻²	0.91	0-310	7.11±1.42	0.1066	0.8897
MMO / 2.5 mA cm ⁻²	0.57	0-80	14.46±2.67	0.0228	0.9341
MMO / 5 mA cm ⁻²	0.58	0-40	37.44±9.43	0.0712	0.8807
CREATININE					
Anode / current density	Q (Ah dm ⁻³)	Time interval for complete removal (min)	<i>k</i> _C (10 ⁻³ min ⁻¹)	<i>S</i> ² _R (mg ² /L ²)	<i>R</i> ²
BDD / 1.25 mA cm ⁻²	8.00	0-2880	1.42±0.10	0.0760	0.9638
BDD / 2.5 mA cm ⁻²	8.00	0-1440	2.60±0.07	0.0073	0.9949
BDD / 5 mA cm ⁻²	6.46	0-720	2.65±0.06	0.0023	0.9948
MMO / 1.25 mA cm ⁻²	8.00	0-3600	0.25±0.01	0.0019	0.9812
MMO / 2.5 mA cm ⁻²	8.00	0-1800	0.67±0.01	0.0005	0.9969
MMO / 5 mA cm ⁻²	6.46	0-720	1.10±0.05	0.0015	0.9808

UREA					
Anode / current density	Q (Ah dm ⁻³)	Time interval for complete removal (min)	k_U (10 ⁻³ min ⁻¹)	S^2_R (mg ² /L ²)	R^2
BDD / 1.25 mA cm ⁻²	8.00	0-3600	0.58±0.03	0.0165	0.9710
BDD / 2.5 mA cm ⁻²	8.00	0-1800	1.26±0.08	0.0203	0.9679
BDD / 5 mA cm ⁻²	6.46	0-720	1.68±0.07	0.0035	0.9806
MMO / 1.25 mA cm ⁻²	8.00	0-3600 (2.31 % urea removal)	3.68·10 ⁻³ ±0.00	0.0002	0.0533
MMO / 2.5 mA cm ⁻²	8.00	0-1800 (23.72 % urea removal)	0.10±0.03	0.0037	0.4645
MMO / 5 mA cm ⁻²	6.46	0-720 (7.63 % urea removal)	0.09±0.02	0.0001	0.7718

As can be observed, the kinetic constant of CAP (k_{CAP}) increases with the current density and the degradation rate is higher when working with BDD anodes, which is in line with lower removal percentage attained with MMO electrodes for a given electric charge passed. In the case of urine organic compounds, similar behaviours can be seen: the higher current density, the higher degradation rate for both electrodes, being the values higher with BDD anodes again. The kinetic constants values are higher for uric acid followed by CAP, creatinine and, finally, urea ($k_{UA} > k_{CAP} > k_C > k_U$). Hence, to evaluate the competitive oxidation of antibiotic with other organics naturally present in urine during electrolysis, the ratio k_{CAP}/k_i has been calculated where i is the target organic compound of urine (urea, creatinine or uric acid). Furthermore, electrochemical oxidation of 100 mg dm⁻³ CAP in 3000 mg dm⁻³ perchloric acid (inert media) as electrolyte was carried out and the ratio k_{CAP}/k_i has been also calculated. This study helps to explain the interferences of urine organic compounds on CAP removal. Figure 4 shows the ratio k_{CAP}/k_i as function of the applied current density during the electrolysis of 100 mg dm⁻³ CAP with BDD (Figure 4a) and MMO (Figure 4b) anodes. Values higher than 1 indicates that the degradation rate of CAP is favoured vs. the degradation of other organics whereas ratios lower than 1 reveals that the elimination rate of organics naturally contained in urine is favoured instead of antibiotic removal.

Figure 4. Ratio k_{CAP}/k_i as function of the current density for the different organic compounds from urine and the antibiotic in perchloric acid media. (a) BDD; (b) MMO; (■) urea; (▲) creatinine; (●) uric acid; (□) CAP in perchloric acid.

Overall, the ratio follows a similar trend regardless the anode material in urine: the higher current density, the lower ratio. Likewise, a similar behaviour is observed during the treatment of CAP in perchloric acid at different current densities. On the other hand, urea ratios (k_{CAP}/k_U) obtained are higher than 1 with both electrodes which reveals that degradation of urea is not favoured in comparison with CAP, despite its higher initial concentration (3333.34 mg dm⁻³ vs. 100 mg dm⁻³ of CAP). A similar behaviour can be seen in creatinine ratio (k_{CAP}/k_C) with BDD anodes (Figure 4a) where values are slightly higher. However, the ratios obtained for creatinine are very similar to CAP removal because the initial concentration of both organics are similar (166.67 mg dm⁻³ creatinine vs. 100 mg dm⁻³ CAP). In the case of MMO (Figure 4b), k_{CAP}/k_C decreases down to 1 when applying current densities higher than 2.5 mA cm⁻². This may indicate the important role of mediated oxidation in each case and of the molecule structure in the competitive oxidation of organics in urine media.

Regarding uric acid, the ratios for uric acid are lower than 1 for all the current densities and anode materials studied. This reveals that uric acid is more easily degraded than CAP during the electrolysis of urine. In this point, it is important to point out that uric acid is a more complex cyclic molecule and, it seems to be easier to break due to more available functional groups in its structure. Therefore, the rapid degradation of this compound suggests that it may be easily transformed to another intermediate organic compound. In this context, the removal of uric acid by electrolysis with MMO anodes has been reported in literature, being 2,3,6,7,8,9-hexahydro-1H-purine-2,6-diol and (5-amino-2,3-dihydro-1H-imidazol-2-yl)((hydroxymethyl)amino)methanol the first intermediates generated (Singla et al., 2019). The first one comes from the reduction of uric acid whereas the second is formed from the C-N bond cleavage of the first intermediate. Hence, 2,3,6,7,8,9-hexahydro-1H-purine-2,6-diol could be the main intermediate formed at the beginning of urine electrolysis at the operating conditions tested due to its easy formation.

Finally, the comparison of the degradation rate of CAP in urine and perchloric media may help to confirm the relevance of competitive oxidation in a complex media (hospital urine). Thus, ratios below 1 indicate the faster degradation of CAP in perchloric acid ($k_{\text{CAP-ClO}_4} > k_{\text{CAP-urine}}$) where other organics are not present. This confirms that there is a clear competitive oxidation between CAP and the other organics present in hospital urine. Additionally, in perchloric acid media, the degradation process can only take place by direct oxidation of the pollutant on the anode surface or mediated oxidation by hydroxyl radicals since perchloric acid is a non-reactive media at the operating conditions tested. Therefore, these results suggest that an increase in the current density leads to a potential production of oxidant species in the effluent and, consequently, an enhancement in organics removal.

378 The soft oxidative conditions studied allows to remove CAP from urine (up to 100 and
379 35 % CAP with BDD and MMO anodes, respectively, after passing 8 Ah dm⁻³) but
380 additional information is required to estimate the potential hazardousness of treated
381 effluents, due to intermediates formed. Figure 5 depicts the profiles of organic
382 intermediates formed during the electrochemical oxidation of 100 mg dm⁻³ CAP in urine
383 media. The chromatographic area is plotted as function of the applied electric charge to
384 evaluate the influence of the current density and the anodic material on the formation of
385 organic intermediates.

Figure 5. Profiles of intermediates chromatographic area as function of the applied electric charge during the electrochemical oxidation of 100 mg dm⁻³ CAP in urine media. (□) i1, (◇) i2, (Δ) i3, (x) i4, (*) i5, (○) i6, (■) i7, (◆) i8, (▲) i9, (●) i10. Anodic material / Current density: (a) BDD / 1.25 mA cm⁻²; (b) MMO / 1.25 mA cm⁻²; (c) BDD / 2.5 mA cm⁻²; (d) MMO / 2.5 mA cm⁻²; (e) BDD / 5 mA cm⁻²; (f) MMO / 5 mA cm⁻².

There are significant differences in the intermediate organic compounds formed when working with BDD or MMO anodes, regardless the current density tested. Specifically, large amounts of organics are monitored during MMO-electrolysis despite the low degradation of CAP observed in Figure 1. Nonetheless, the amount of these intermediates could decrease if higher electric charges will be applied, favoring the formation of aliphatic compounds (Chen et al., 2015; Cotillas et al., 2018a). Conversely, BDD anode lead to the formation of few organic intermediates and their maximum concentration in solution occurs at applied electric charges around 2 Ah dm^{-3} , and they disappear almost completely at the end of each test (8 Ah dm^{-3}). To shed light on the nature of the intermediates formed during the treatment of hospital urines polluted with CAP, electrochemical oxidation of 100 mg dm^{-3} antibiotic in 3000 mg dm^{-3} perchloric acid (inert media) as electrolyte was carried out (data not shown). This study allows to evaluate the intermediate compounds that come directly from the oxidation of CAP and not from other organics present in hospital urines. Results showed similar profiles in perchloric acid than that previously described during CAP degradation in urine media. Hence, most of organic intermediates monitored in this complex matrix can be attributed to CAP degradation instead of urea, creatinine or uric acid. Table 2 summarizes the ratio between the maximum area (MA) of intermediates in urine and perchloric acid during the electrolysis of CAP with BDD and MMO anodes at different current densities. Values higher than 1 indicates that the maximum area is higher during the electrolysis in urine matrix whereas ratios lower than 1 reveals that the maximum area of intermediates is higher when working in perchloric acid.

Table 2. Ratio $\text{MA}_{\text{urine}}/\text{MA}_{\text{perchloric acid}}$ of intermediates during the electrolysis of 100 mg dm^{-3} CAP with BDD and MMO anodes at different current densities.

Intermediate	$j_{\text{BDD}} / \text{mA cm}^{-2}$			$j_{\text{MMO}} / \text{mA cm}^{-2}$		
	<i>1.25</i>	<i>2.5</i>	<i>5</i>	<i>1.25</i>	<i>2.5</i>	<i>5</i>
i1	0.57	-	-	4.46	0.31	4.99
i2	-	8.90	33.40	-	-	-
i3	0.35	-	-	0.39	1.61	0.94
i4	0.49	-	-	0.69	0.57	0.53
i5	-	-	0.54	1.48	4.54	0.89
i6	-	-	-	11.72	4.69	3.03
i7	0.47	13.47	7.01	5.09	4.51	8.21
i8	0.38	0.13	0.49	2.64	8.12	2.54
i9	0.34	-	-	9.33	1.17	1.30
i10	-	-	-	3.84	1.58	0.52

416

417 Overall, the areas of intermediates formed are higher in urine matrix during the
418 electrolysis with MMO anodes since the ratio is higher than 1. However, an opposite
419 behavior can be seen when working with BDD anodes where the ratios are mainly lower
420 than 1. This can be due to mediated oxidation mechanisms. In this context, the removal
421 of CAP in perchloric acid can only take place by the direct oxidation of the pollutant on
422 the anode surface or by hydroxyl radical mediated oxidation. On the other hand, the
423 electrolysis in urine matrix leads to the formation of a cocktail of oxidants (hypochlorite,
424 peroxodisulfate, peroxodiphosphate...). Nonetheless, the nature of the anodic material
425 (electrocatalytic properties) favors the formation of different oxidants (Cotillas et al.,
426 2019). Specifically, MMO leads to the production of large amounts of hypochlorite as
427 main oxidant whereas BDD anodes promote the formation not only of hypochlorite but
428 also of other peroxocompounds. These last species present a higher oxidant capacity and,
429 hence, they can significantly contribute to a rapid degradation of the intermediates formed
430 during the electrolysis of CAP in urine. For this reason, the number and area of
431 intermediates are lower during the electrolysis in urine matrix with BDD anodes. In
432 literature, it has been reported the intermediates formed during the mineralization of
433 synthetic solutions polluted with CAP (Garcia-Segura et al., 2014). Table 3 shows the
434 main intermediate organic compounds reported in these works. However, the soft

operating conditions used in this work can lead to the formation of other intermediate compounds.

Table 3. Main intermediates formed during electrochemical treatment of CAP polluted effluents.

Chemical name	Molecular formula	Method	Reference
2,2-Dichloro-N-[1,3-dihydroxy-1-(4-hydroxyphenyl)-propan-2-yl]acetamide	$C_{11}H_{13}O_4NCl_2$	GC-MS / LC-MS	(Chen et al., 2015; Garcia-Segura et al., 2014; Nie et al., 2018)
4-(2-Nitro-1,3-dihydroxy-propanyl)-nitrobenzene	$C_9H_{10}O_6N_2$	GC-MS / LC-MS	(Chen et al., 2015; Garcia-Segura et al., 2014)
4-(2-Amino-1,3-dihydroxy-propanyl)-nitrobenzene	$C_8H_8O_4N_2$	GC-MS / LC-MS	(Chen et al., 2015; Garcia-Segura et al., 2014)
4-Nitrobenzoic acid	$C_7H_5O_4N$	GC-MS / LC-MS	(Chen et al., 2015; Garcia-Segura et al., 2014; Nie et al., 2018)
Allatoic acid	$C_4H_8N_4O_4$	GC-MS	(Cotillas et al., 2018a)
Ethyl hydrazine	$C_2H_8N_2$	GC-MS	(Cotillas et al., 2018a)
Dichloroacetamide	$C_2H_3Cl_2NO$	GC-MS / LC-MS	(Nie et al., 2018; Zhang et al., 2018)
Acetic acid	$C_2H_4O_2$	GC-MS / LC-MS	(Chen et al., 2015; Cotillas et al., 2018a)

In this point, it is essential to take in mind that these results only confirm the removal of CAP, its reaction intermediates and organics contained in urine but do not give information about the remaining riskiness of the treated urine. Thus, to assess the hazardousness of treated urine before their direct discharge to sewage network and the subsequent treatment in municipal WWTPs, toxicity analyses were carried out to the treated hospital urines. Specifically, acute toxicity towards *Vibrio fischeri* luminescence inhibition was evaluated by Microtox® standard method. Figure 6a shows the EC₅₀ percentages during the electrochemical oxidation of 100 mg dm⁻³ CAP in urine media with BDD anodes at different current densities. For comparison purposes, three different values of applied electric charge were selected in order to evaluate the hazardousness of the treated urine in three different stages: initially ([CAP]: 100 mg dm⁻³), when CAP has

been partial degraded and intermediates remain in the reaction media (2 Ah dm⁻³) and when CAP and total organic load has been almost degraded (6 Ah dm⁻³). Likewise, Figure 6b shows the EC₅₀ values obtained with MMO anodes but, in this case, CAP removal was only about 20 % at the maximum electric charge selected.

Figure 6. EC₅₀ values at different applied electric charges (0, 2 and 6 Ah dm⁻³) during the electrochemical oxidation of 100 mg dm⁻³ CAP in urine media. (□) 1.25 mA cm⁻²; (■) 2.5 mA cm⁻²; (■) 5 mA cm⁻². (a) BDD; (b) MMO.

As can be observed, EC₅₀ values are lower than 1 % at the beginning of the treatment with BDD anodes (Figure 6a) which can be directly associated to the presence of 100 mg dm⁻³ CAP in urine and, hence, it reveals the high toxicity of untreated hospital urine. This is an expected behaviour taking into account the pharmacological properties of CAP: a wide spectrum antibiotic (Balbi, 2004). At this point, it is important to highlight that urine matrix without CAP was analysed, resulting in EC₅₀ values around 90 %. This result confirms that urine matrix is not the responsible of hospital urine initial toxicity. Then, the EC₅₀ increases at 2 Ah dm⁻³ due to the CAP removal for all the current densities studied. However, there is not a significant increase in this parameter, which can be related to the presence of the primary intermediates from the oxidation of CAP (mainly

aromatics). At this point, it is important to highlight that oxidants were neutralized in samples with thiosulphate (1:1) when passing 2 Ah dm⁻³ and toxicity was measured again. After neutralization process, EC₅₀ values were quite similar (4-7 %) and, hence, this clearly indicates that the initial toxicity is due to the primary organic intermediates formed from CAP oxidation. Furthermore, a complete antibiotic removal was not attained at these conditions and, therefore, it also influences on the *V. fischeri* luminescence inhibition. Likewise, it can be seen that the current density has not a remarkable influence on toxicity at this charge value with BDD anodes. Finally, EC₅₀ values are higher at applied electric charges of 6 Ah dm⁻³ when working at current densities of 1.25 and 2.5 mA cm⁻². However, this parameter decreases at the end of the electrochemical treatment at 5 mA cm⁻². This different behaviour can be attributed to the residual CAP that has still not been removed at 5 mA cm⁻² (Figure 1). In addition, the different EC₅₀ values obtained at higher electric charges can be also influenced by the amount of oxidants accumulated in urine during the treatment. In this context, oxidants concentrations are lower at 1.25 mA cm⁻² than the results obtained at 5 mA cm⁻² (Figure 2). This is the expected outcome since the production of oxidants during electrolysis is strongly influenced by the current density (Cañizares et al., 2009). Hence, it suggests that the oxidants electrogenerated at low current densities are mainly wasted in the removal of organics whereas higher concentrations of these species remain in the bulk at 5 mA cm⁻² which can contribute to increase the toxicity towards *V. fischeri*. To check this hypothesis, toxicity analyses were carried out using a urine matrix without antibiotic and polluted with different concentrations of hypochlorite as model of oxidant that can be easily generated during electrolysis. Results showed that a low concentration of 1 mg dm⁻³ ClO⁻ leads to EC₅₀ values lower than 1 %. This reveals that the higher concentration of oxidants at 5 mA cm⁻² significantly influences the EC₅₀ values and, therefore, the toxicity of treated urines.

On the other hand, the EC₅₀ values obtained during the treatment of hospital urines with MMO anodes (Figure 6b) are lower than 1 % for all the applied electric charges studied. In this case, it can be attributed to the presence of antibiotic in urine (Figure 1) which has still not been removed (around 80 %). Furthermore, the toxicity can be also explained in terms of the generation of hypochlorite during the process, since MMO anodes mainly promotes the formation of large amounts of hypochlorite from the oxidation of chlorides due to their electrocatalytic properties (Polcaro et al., 2008).

Once the toxicity of hospital urines was evaluated, rapid biodegradability assays were performed during the treatment of urines polluted with 100 mg dm⁻³ CAP at different current densities using BDD and MMO anodes. This study is an approach that allows to evaluate if it will be possible to carry out an efficient conventional biological treatment after electrolysis (Ferreira et al., 2020). Therefore, rapid biodegradability together with toxicity analyses will initially allow to evaluate the final environmental and sanitary risk of treated hospital urine. Figure 7 shows the influence of the current density on the evolution of the rapid biodegradability during the treatment of hospital urines polluted with 100 mg dm⁻³ CAP using BDD and MMO anodes. This rapid test also informs about the biodegradability of treated effluents based on the oxygen consumed by microorganisms present in an activated sludge, relating the biological oxygen demand and the chemical oxygen demand.

Figure 7. Evolution of rapid biodegradability percentage as function of different applied electric charges during the electrochemical oxidation of 100 mg dm⁻³ CAP in urine media. Current density: (■) 1.25 mA cm⁻²; (▲) 2.5 mA cm⁻²; (●) 5 mA cm⁻². (a) BDD; (b) MMO.

The initial rapid biodegradability determined was around 25 % which can be attributed to an easy degradation of natural organic compounds present in hospital urine (urea, creatinine and uric acid). This initial value decreases during electrochemical oxidation at 2 Ah dm⁻³ despite the current density values and anodic materials. Lower percentages of rapid biodegradability are obtained with MMO anodes (4 – 15 %) in comparison with BDD anodes (11 – 15 %). This decrease may be a consequence of the maximum presence of poorer biodegradable organic intermediates than the parental ones. However, different performances are observed for the rapid biodegradability percentages during electrochemical oxidation at 6 Ah dm⁻³ depending on the current density and anodic materials. Specifically, the rapid biodegradability remains constant from 2 to 6 Ah dm⁻³ during electrolysis with MMO anodes, regardless the current density applied. This steady state in the rapid biodegradability may occurs due to different behaviors, simultaneously: the lower CAP removal efficiency, the amount of accumulated intermediates and/or the presence of oxidants (mainly hypochlorite). Therefore, higher electric charges should be applied to attain a complete CAP removal with MMO anode and a subsequent

biodegradability improvement. Conversely, rapid biodegradability increases during electrochemical oxidation with BDD anodes despite the current density. Here, similar trends are observed during electrolysis at 2.5 and 5 mA cm⁻² where rapid biodegradability increases up to 22 % (similar than initial rapid biodegradability value). Meanwhile, rapid biodegradability increases up to 36 % for the lowest value of current density. Overall, these performances lead to explain that rapid biodegradability is directly affected not only by the presence of organic intermediates (less biodegradable with MMO anodes, more biodegradable with BDD anodes) but mainly by the presence of antibiotic and oxidants electrogenerated in hospital urines.

Electrochemical oxidation of 100 mg dm⁻³ CAP in urine media with BDD anodes applying a current density value of 1.25 mA cm⁻² could be considered as the most appropriate technology to reduce the hazard of hospital urines under the experimental conditions studied since this technology completely removes the antibiotic, increases the EC₅₀ and the rapid biodegradability up to 23 and 36 %, respectively. For this reason, the Zahn-Wellens standard biodegradability test was performed to the resulting hospital urine after the electrolysis with BDD anodes at 1.25 mA cm⁻² and, the results obtained are plotted in Figure 8. The procedure control effluent of 1593 mg dm⁻³ glucose were also tested for comparison purposes.

Figure 8. Time-course of standard biodegradability percentage for the final effluent (8 Ah dm⁻³) obtained during the electrochemical oxidation of 100 mg dm⁻³ CAP in urine media with BDD anodes at 1.25 mA cm⁻². Effluents: (x) procedure control, (■) final effluent. Symbols: (black symbols) standard biodegradability, (white symbols) CAP removal.

The blank reference test (1593 mg dm⁻³ glucose) is observed to attain the 100 % of standard biodegradability after 28 days. This performance ensures the proper conditions of microorganisms from the activated sludge used to develop Zahn-Wellens procedure. After electrochemical oxidation where CAP is not presented in solution, the standard biodegradability rises almost linearly up to 60 % after 28 days. These results reveal that electrochemical oxidation can be considered as a promising technology to reduce toxicity and enhance biodegradability of hospital urines at the operating conditions tested (j: 1.25 mA cm⁻²; Q: 8 Ah dm⁻³) since, as reported in literature, effluents may be considered as easily biodegradable when its biodegradability percentage is above 40 % (Pulgarin et al., 1999; Zaghdoudi et al., 2017).

566

567 4. Conclusions

568 The following conclusions can be drawn from this work:

- 569 • Electrolysis with BDD anodes allows to decrease the hazardousness of hospital
570 urines, achieving a complete removal of the antibiotic CAP at very low current
571 densities and electric charges. Opposite to that, the use of MMO anodes only
572 attains a 20 % of CAP removal applying the same conditions. Free and combined
573 chlorine species and peroxocompounds are generated from the oxidation of the
574 ions contained in the effluent which are the main responsible of antibiotic removal
575 and also contribute to the degradation of natural organics present in hospital urine
576 (urea, creatinine and uric acid).
- 577 • A competitive oxidation takes place during the electrolysis of urine polluted with
578 CAP between the target antibiotic and the other organic compounds (urea,
579 creatinine and uric acid) for all the current densities and anode materials tested in
580 this work. Specifically, uric acid degradation rate is higher than CAP removal rate
581 whereas creatinine and urea show a lower rate.
- 582 • The acute toxicity evolution towards *Vibrio fischeri* luminescence inhibition
583 during the electrochemical oxidation at 6 Ah dm⁻³ showed EC₅₀ percentages up to
584 23 % with BDD-electrolysis at 1.25 mA cm⁻². However, EC₅₀ values lower than
585 1 % were obtained during electrolysis with MMO anodes despite the current
586 density applied. Likewise, the rapid biodegradability percentage increased in a
587 range from 22 to 36 % during the electrochemical oxidation with BDD anode at
588 6 Ah dm⁻³ whereas remained constant around 15 % with MMO anodes. Overall,
589 the evolution of both parameters seems to be directly related to the residual

antibiotic concentration, the amount of intermediates and the electrogenerated oxidants.

- Electrochemical oxidation with BDD anodes applying 1.25 mA cm^{-2} and electric charges of 8 Ah dm^{-3} can be considered as an appropriate technology for the pre-treatment of hospital urines before their disposal into municipal sewers since the final standard biodegradability (Zahn-Wellens method) was higher than 40 %.

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