Influence of the doping level of boron-doped diamond anodes on the removal of Penicillin G from urine matrixes

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Abstract

The objective of this study is to understand the influence of the characteristics of boron-doped diamond on the degradation of Penicilin G in urine. To do this, five commercial BDD anodes with different boron doping levels (100 ppm - 8000 ppm) were studied. These electrodes were characterized by cyclic voltammetry, electrochemical impedance spectroscopy and electrolysis. Boron doping was found to correlate well with the electrochemical properties of the electrodes and the results indicate a distinct behavior in the drug degradation. The improvement in the toxicity and in the reduction of the antibiotic effect of urine were the most innovative inputs monitored. For this, the concentration of Penicilin G, toxicity in vibrio fisheri and antibiotic effect in E. Faecalis were monitored. The best results were found for the BDD with a boron content of 200 ppm, capable of removing 100% of the antibiotic, reducing toxicity by 90% and remove completely the antibiotic effect. These results indicate that lower doping levels are more efficient for urine anodic oxidation.

Highlights

- The lower the boron content, the lower the charge transfer
- All electrodes achieve complete penicillin G removal
- The lower the boron content the higher the penicillin G removal kinetics
- Penicillin G toxicity improves when low doping levels are employed
- The antibiotic effect of penicillin G is reduced on low boron doping electrodes

1. Introduction

In last decade, reducing environmental threat of pharmaceuticals (PhCs) has become a priority, and treatment of hospital wastewater has become the main target of several researches [ref], because they greatly affect the loads of pollutants arriving to the urban wastewater treatment plants (WWTPs). The inefficiency of treatment trains from WWTPs to treat sanitary effluents leads to the spread of chemicals and pathogens in the environment. This has led to numerous studies searching for technological solutions in order to decrease the impact of sanitary effluents into the environment, grouped in two main strategies: 1) boosting efficient treatments at WWTPs by the implementation of additional treatments, 2) pre-treatment before their discharge into WWTPs, that is in the pollution source [ref]. The second option is emerging as the key alternative, as it addresses the problem in more concentrated effluents and the volume of wastewater treated is further much lower than in the other case.

A great variety of technologies including biological and chemical oxidation treatments, membrane processes or Advanced Oxidation Processes (AOPs) have been tested to treat hospital effluents (ref). To overcome the drawbacks of this option (mainly related with the size of the treatment plants, as well as their construction and implementation costs), recent studies proposed the segregation of the most problematic hospital effluents and their at-source treatment (ref). Among these risky hospital effluents, urines are one of the most dangerous liquid wastes due to the variety and concentration of pharmaceuticals and pathogens contained. Thus, although urine accounts for only a small percentage of the total wastewater amount, the concentration of its contaminants may be up to 3-fold higher than that found in overall hospital wastewater [ref]. This makes urine treatment a key objective for reducing the environmental and health impact of hospital effluents and, also, energy costs for downstream WWTPs.

Until now, fewer studies have addressed the treatment of hospital urines on an individual basis. Among them, Prof. Pulgarin's group has conducted several studies on the application of

photo-assisted AOPs (UVC, UVC/H₂O₂, and UV/Fenton) or ultrasound radiation for the removal of PhCs and microorganisms in urine matrix [15, 19]. On the other hand, the use of electro-oxidation (ref) has also been evaluated as an alternative for urine treatment. Overall, it has been observed that this technology is capable of effectively degrading and mineralizing PhCs present in urine. Therefore, it seems to be a good alternative to reduce the risk of hospital urine but more scientific effort is needed for large-scale application, mainly in the aspect of reducing the formation of un-desirable by-products (such as perchlorate from electrolysis of chlorine contained in urine matrix) and the ecotoxicity risk, as well as improving energy efficiency by optimizing cell design to reduce operational cost. In fact, one of the challenges of research community is the design of appropriate electrochemical reactor to make this technology competitive with conventional technologies (ref). This design is a complex process in which many aspects must be taken into account and the overall performance of the electrochemical reactor will be determined by a complex interaction of different factors, including: the electrode material, the electrode configuration, flow dynamic conditions and, obviously, the operating conditions. Among them, the electrode material can be considered as the heart of the electrochemical cell, and the selection of the appropriate material is usually the cornerstone of electrochemical devices, in terms of its composition (metal oxide or diamond electrodes doped as anodes, for example) and geometry (planes, gratings, foams) [ref].

Within electrochemical AOPs, for many years, many researchers have been studying the performance of boron-doped diamond (BDD) anodes on the degradation of a great variety of compounds, such as dyes[1–3], carboxylic acids[4,5], pesticides[6–8] or drugs[9,10]. Anodic oxidation (AO) using BDD anode has demonstrated to be able to attain a high current efficiency due to the efficient generation of hydroxyl radicals and their contribution to the overall oxidation process (Eqs. 1-2) [13,14].

However, recent studies have demonstrated that diamond is not as robust as expected and its physicochemical properties such as sp^2/sp^3 , roughness, boron doping, thickness and resistivity may limit or promote different electrooxidation processes (ref).

With this background, the aim of this work is to analyze and determine the influence of boron doping level (100-8000 ppm of boron) of conductive diamond electrodes (supplied by Adamant Technologies) on the ability to decrease the antibiotic effect and the toxicity of synthetic hospital urine polluted with antibiotics. To do this, penicillin G was selected as model of antibiotic due to it is a widely used broad spectrum antibiotic (with excellent antibacterial properties associated to the disruption of the synthesis of bacterial cell walls) and it presents a low biodegradability. The results obtained will contribute to understand the behavior of diamond anodes in a complex matrix where inorganic and organic compounds compete to be oxidized, and they also will be useful to clarify the boron doping level recommended not for the complete mineralization of organic load but for the conversion of potentially hazardous pharmaceuticals into other less hazardous intermediate compounds.

2. Experimental

2.1 Chemicals

Penicillin G, urea, creatinine, uric acid and inorganic salts employed were of analytical grade (>98% purity) and purchased from Sigma-Aldrich. Table 1 shows details of synthetic urine media based on the composition given by Dbira et al., (2015). Methanol and formic acid used for the mobile phase of HPLC were purchased from Sigma-Aldrich. All aqueous solutions were prepared using high-purity water (Millipore Milli-Q system, resistivity > 18 MΩ).

Table 1. Synthetic urine composition.

Compound	Concentration (mg dm ⁻³)
CH ₄ N ₂ O	3333.34
C ₄ H ₇ N ₃ O	166.67
C ₅ H ₄ N ₄ O ₃	50.00
KCl	1000.00
MgSO ₄	170.00
(Ca) ₃ (PO ₄) ₂	28.34
Na ₂ CO ₃	166.67
(NH ₄) ₂ HPO ₄	83.34

2.2 Electrodes

The experiments were performed in an electrochemical cell using five different of BDD supplied by Adamant Technologies (Neuchatel, Switzerland). The most relevant properties of the anodes are summarized in Table 2.

Table 2. Properties of the five BDD anodes used in this work (from Adamant Technologies).

Code	Boron level/ppm	sp ³ /sp ²	Thickness / μm	Resistivity / Ohm	Roughness/ μm
BDD8000	8000	80	1.05	10	< 0.1
BDD2500	2500	43	1.13	10	< 0.1
BDD1300	1300	77	2.27	10	0.3 – 0.5
BDD200	200	75	1.14	100	0.3 – 0.5
BDD100	100	89	1.03	10	0.3 – 0.5

2.3 Analytical

A HPLC Agilent 1260 series, equipped with an Eclipse Plus C-18 column (4.6 mm × 100 mm; 3.5 μm) was used to measure the concentrations of organics. For the determination of Penicillin G a mixture of 50% methanol and 50% formic acid (0.1%) was used as mobile phase, with a flow rate of 0.6 mL min⁻¹. UV detector was set at 220 nm, the injection volume was 20.0 μL and the column temperature was set to 30°C. With these parameters the retention time of Penicillin G was 2.4 min. On the other hand, the mobile phase for the determination of uric acid consisted of 2% acetonitrile/98% aqueous solution with 0.1% of formic acid. A flow rate of 1 mL min⁻¹ and a DAD detection of 292 nm were used. The injection volume was 10 μL (retention time: 2.7 min).

Creatinine was determined by ion chromatography (IC) with a Metrohm 930 2 Compact IC Flex coupled to a conductivity detector. Anions were also analyzed by using the column Metrosep A Supp 7 with a mobile phase of 85:15 v/v 3.6 mmol dm⁻³ Na₂CO₃/acetone and flow rate of 0.8 mL min⁻¹. Moreover, cations were analyzed by using the column Metrosep C6 250 with a mobile phase of 1.7 mmol dm⁻³ 5 HNO₃ and 1.7 mmol dm⁻³ 2,6-pyridinedicarboxylic acid and flow rate of 0.9 cm³ min⁻¹. The volume injected was 20 μL. Urea concentration was determined by a spectrophotometric method using UV-1700 Spectrophotometer (Shimadzu). This method is based on the yellow-green color produced when p-dimethylaminobenzaldehyde is added to urea in dilute hydrochloric acid solution (Watt and Chrisp, 1954). The TOC concentration was monitored using a Multi N/C 3100 Analytik Jena analyzer. COD analysis were conducted by using a Spectroquant® test CSB from Merck.

2.4 Toxicity

The acute toxicity of initial and treated Penicillin G in synthetic urine solutions was measured by inhibition of bioluminescence of marine bacteria *Vibrio fischeri* by using Microtox® according to the protocol indicated by the manufacturer. The osmotic control of the samples was

adjusted prior to tests. The emitted bio-luminesce was recorded for 5 and 15 min of exposure and compared to that of the reference sample.

2.5 Antibiotic Activity Assay

The antibiotic effect of penicillin G was measured by the number of microorganisms using μ -Trac® 4200. The microorganisms were placed in contact for 3h with the samples and then taken to the equipment to quantify *E. Faecalis* colony forming units (CFU).

2.6 Experimental procedure

The electrochemical measurements were performed in a conventional three-electrode cell connected to a computer connect with an Autolab potentiostat/galvanostat model PGCTAT 302N. The cell used an Ag/AgCl (3 M KCl) as reference electrode and a platinum wire (Pt) as counter electrode. The working electrodes used were BDD anodes with a geometric surface area of 4 cm². The electrolysis were performed in galvanostatic mode at a fixed current density of 30 mA cm². The electrolysis of urine and polluted urine wastes were performed in the three-electrode cell using 150 mL of synthetic wastewater that was kept in a double-jacket cell at a constant temperature of 25 °C.

3. Results and discussion

In order to analyze the electrochemical behavior of the electrodes in urine media, cyclic voltammetries (**Figure 1a**) were performed with the redox couple $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at anodic and cathodic potentials. The role of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ has been widely studied in the literature[22,23], as it produces a redox system in the inner-sphere on the carbon-based electrodes. As can be observed, the electrodes containing higher boron doping showed high response currents, which can be attributed to better electrocatalytic activities. Within the low boron doping

electrodes, the role of other parameters can help to explain obtained results. Thus, BDD200 electrode has a Si resistivity 10 times higher than BDD100 and this factor may influence its electrochemical window, which seems to be wider in the BDD200. The importance of this parameter was previously studied in electrooxidation and electrosynthesis processes[12,24]. In order to better explore the electron transfer capability, electrochemical impedance spectroscopy (**Figure 1b**) was performed. In general, electrodes with high boron doped levels (8000, 2500 and 1300 ppm) exhibit semi-metallic conductivity, which is reflected on lower resistance to charge transfer. However other factors, such as sp^3/sp^2 ratio, also influence on their performance. According to Duo *et al*[11], a large amount of sp^2 carbon directly influences electron transfer, since sp^2 carbon acts as a charge mediator. On the other hand, the thickness of the diamond layer may help to explain the lower electron transfer resistance of the BDD1300 as compared to the BDD8000. In this case, the higher thickness (2.27 μm) of the BDD1300 seems to positively influence the reaction rate, as compared to the lower thickness (1.05) of the BDD8000. Results are similar to those found by Cañizares *et al*[12], where the electrode with the thickest layer had the most efficient electrochemical activity.

Figure 1: Electrochemical measurements performed in $K_3Fe(CN)_6$ and $K_4Fe(CN)_6 \cdot 3H_2O$ 10mmol L^{-1} in synthetic urine (a) cyclic voltammograms recorded at 100 mV and (b) Nyquist diagrams of electrodes

EIS again shows the outstanding characteristics of the BDD200, which taking into account the changes observed with the other electrodes seems to be more related to the Si support than to the boron doping.

Once evaluated its electrochemical behaviour, the use of these electrodes were tested for the degradation of penicillin G in urine media. **Figure 2** shows the changes in Penicillin G

concentration during the electrolysis of 50 mg L⁻¹ of the antibiotic in urine at a fixed current density of 30 mA cm⁻². As can be observed, all electrodes removed at least 85% of Penicillin G in 8 h of treatment (6.4 Ah dm⁻³ of electric charge passed). BDD100 was the unique electrode able to attain the complete depletion of Penicillin G while BDD2500 showed the worst efficiency. In all cases, experimental data fitted to a pseudo-first order kinetics (Ln(C/C₀) vs. time). For comparison purposes, the kinetic constants were estimated and plotted in the inset of the figure.

Figure 2: Penicillin G ($C_0 = 50 \text{ mg dm}^3$) removal on different doping level of boron (●) 8000 ppm, (■) 2500 ppm, (○) 1300 ppm, (◆) 200 ppm and (▼) 100 ppm in urine at a fixed current density 30 mA cm⁻².

Importantly, the medium urine is extremely complex and other organic compounds (urine, creatinine and uric acid) are contained even in higher concentrations than penicillin G. These compounds can also be oxidized during treatment, and thus they may consume electrons and decrease the efficiency of antibiotic removal[17,25]. **Figure 3** shows the changes in the

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concentration of these organics during electrolysis of urine media and urine polluted with penicillin G. As can be seen, uric acid is the sole compound completely removed, while urea and creatinine remain in the urine, at relevant concentrations, after passing 6.4 Ah dm^{-3} of electric charge. Additionally, oxidation rate is slightly affected by the presence of Penicillin G, mainly in the case of creatinine. This may be explained in terms of competitive oxidation of organics, and seems to confirm that the antibiotic is degraded first in spite of its lower concentration. Similar results were found by Cotillas *et al.*[10].

Figure 4 shows the removals of chemical oxygen demand (COD) and total organic carbon (TOC) attained in the electrolysis with different BDD anodes after passing 6.4 Ah dm^{-3} of electric charge.

Figure 3: Changes in the concentration of urea, uric acid and creatinine during the electrolysis with different BDD anodes at a fixed current density 30 mA cm^{-2} . (a) Urine media and (b) Urine polluted with Penicillin G. Conditions: pH:5.9, Penicillin G: 50 mg L^{-1} .

Figure 4. Final values of COD (a) and TOC (b) of the urine treated by electrolysis with different BDD anodes at a fixed current density of 30 mA cm^{-2} . Dotted lines: initial values

Results again depend on BDD used and removal percentages attained are lower in the presence of antibiotics. Additionally, BDD100 and BDD200 seem to be the most efficient electrodes for both mineralization and organic **oxidation**. To confirm this point, the kinetic constant obtained by fitting logarithmic decay of antibiotic, organic urine components and TOC and COD with a *pseudo-first-order kinetic* model are shown in Table 3.

Table 3. Pseudo-first-order kinetics constants calculated for Penicillin G, organic urine componentes and TOC and COD removals.

Anode	$k_{\text{penicillinG}}$ /min ⁻¹	R ²	K_{urea} /min ⁻¹	R ²	$K_{\text{uric acid}}$ /min ⁻¹	R ²	$K_{\text{creatinine}}$ /min ⁻¹	R ²	K_{TOC} /min ⁻¹	R ²	K_{COD} /min ⁻¹	R ²
BDD8000												
BDD2500												
BDD1300												
BDD200												
BDD100												

As can be observed.....

In this point, it is important to take in mind that these results only confirm the removal of Penicillin G and/or organic load but do not give information about the remaining hazardousness of the treated urine, associated with the presence of highly toxic and active organic and/or inorganic species. It is important to take in mind that the risk of hospital urines cannot be determined by a single technique due to complexity of this type of effluent. For this reason, it is necessary to combine different techniques based on biological indicators to evaluate riskiness of urine. In this point, it is important to highlight that hazardousness of urine is not only related to the toxicity of the pharmaceuticals/metabolites, but also to the resistance that certain antibiotics can produce when bacteria mutate in response to their use and their accumulation in the environment. Therefore, to clarify this point, besides physicochemical characterization, it is

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proposed the use of 1) standardized method of toxicity (based on the use of a bioluminescent marine bacterium *Vibrio Fishery*) and 2) of specific methods to monitor antibiotic resistance of treated urine using *Enterococcus faecalis* as target bacteria. This former method can be applied to different antibiotics, but the selection of this bacteria should be made on based of the type of antibiotic (wide or low spectrum) and its mechanism of action (bactericidal or bacteriostatic). The information obtained from these microbiological methods together with physicochemical characterization will allow to establish the optimum operation conditions and reaction time to reduce the chemical risk of hospital urines.

Figure 5 shows the remaining toxicity (expressed in terms of inhibition percentage, according to standard method) of the urine after passing with 2 Ah dm^{-3} . In this point, Penicillin G has been almost completely removed (see Figure 2) but not TOC and COD (see Figure 3). For comparison purposes, toxicity of the initial urine (with 50 mg L^{-1} of Penicillin G) is also shown.

Figure 5: Percentage of inhibition (after 15 min of exposure of samples to *Vibrio fischeri*) of the treated urine by electrolysis with different anodes (2 Ah dm^{-3} of electric charge passed). Dotted line: inhibition percentage of initial sample (urine with 50 mg L^{-1} of Penicillin G).

Initially, around 95 % of inhibition was obtained. After passing 2 Ah dm^{-3} of electric charge, it can be noted that, for all cases, bacterial inhibition is significantly reduced to less than 30% and, again, boron doping level shows a marked influence. As observed, low doped BDD electrodes seem to lead to the formation of less toxic byproducts and inhibition percentage below 10 % was measured. Surprisingly, BDD1300 shows worse results than BDD8000 and BDD2500. These results indicate that penicillin G initially present (together with the other chemicals as detailed in Table 1) in urine inhibits *Vibrio fischeri* (bacteria used as indicator in this method), but once Penicillin is degraded the toxicity of the urine decreases. However, final value depends on electrodes used and this could be indicative of the different degradation rate of electrodes tested and of the accumulation of intermediates (that can be toxic) in some cases. These reactions

intermediates can come from the partial degradation of Penicillin G or other organics, but also from the oxidation of inorganic species present in the reaction media. In fact, it should consider that synthetic urine used in this work presents large amounts of ions, being chloride the primary one. It can be electrooxidized and favor the formation of chlorine and hypochlorite (ref) that can be even further oxidized to chlorate and perchlorate (ref). These species are harmful to human health and environment, and hence, they should be avoided. Anyway, in this point it is important to consider that the presence of ammonium (also present in urine media in high concentration) can help to prevent the formation of these toxic chloro-byproduct due to ammonium rapidly reacts with hypochlorite to form chloramines by the well-known breakpoint chlorination reaction (ref). Furthermore, other ions can be found in synthetic urine (sulfate, phosphate, carbonate,...) and they can be also electrochemically oxidized to form powerful oxidants such as persulfate, peroxodiphosphate and percarbonate (ref). As stated in previous works (ref), the formation of highly oxidized chlorine species and of other types of oxidants also depend on BDD characteristics. Figure 6 shows the oxidants electrogenerated and the concentration of hypochlorite and perchlorate during the electrolysis with BDD anodes tested. Anyway, to exclude the contribution of oxidants, sodium sulfite was added to each urine sample (with a ratio of mol sodium sulfate/mol oxidants quantified by I^-/I_2 titration) before toxicity analysis. Thus, inhibition observed should be mainly related to the presence of toxic organic in the treated urine.

Comentado [CSJ5]: Tenemos estos datos?? Sería interesante comparar qué pasa y ver si hay diferencias

Comentado [MARR6]: ¿?

Figure 6. Final concentration of oxidants electrogenerated (a), concentration of hypochlorite (b) and concentration of perchlorate (c) formed during electrolysis of urine as function of BDD used.

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Additionally, to clarify the antibiotic effect of urine after treatment, a specific method was developed using *Enterococcus faecalis* as target microorganism. This method tries to evaluate the risk of treated urine using a target, due to Penicillin G is useful for the elimination of this type of bacteria. To do this, treated urine was merged with a pure culture of *E. faecalis* (10^7 CFU mL⁻¹) and after 3 h of contact time at 35 °C, the amount of CFU remained in the solution was quantified by µ-Trac® 4200. As previously mentioned, oxidants remaining in the solutions were removed by reaction with sodium sulfite added (1:1 molar ratio). Thus, the expected effect should be only related to the resistance of *E. Faecalis* to organics accumulation in urine. Each sample was measured by quadruplicate: without dilution, dilution 1:10, dilution 1:100 and dilution 1:1000. **Figure 7** shows results obtained in this test. As can be observed, antibiotic effect of treated urine is higher in the case of using high boron-doped electrodes (high mortality of *E. Faecalis*). This means that their potential ecotoxicity risk is higher due to organic contained in this treated urine still can induce the mutation of the bacteria in response to their accumulation in the environment. On contrary, urine treated by BDD100 does not show any effect on *E. Faecalis* population and thus its release to the environment should not have any negative effect beyond the typical urban wastewater. Therefore, results obtained from this test confirms again the main conclusion obtained with physicochemical and toxicity analysis: BDD anodes with low boron-doping level are more efficient to decrease the hazardousness of hospital urine.

Comentado [MARR7]: ¿?

Figure 6: Antibiotic activity removal of 50 mg/L of Pen G in synthetic urine using a charge of 2 A/h dm

Note that BDD1300 has a distinct behavior, microorganisms survive with treated effluents, showing that the antibiotic effect has been completely removed. This can be explained by the difference in boron layer thickness, which influences the catalytic response[12].

Comentado [CSJ8]: ¿este comportamiento no acaba de cuadrar con lo anterior?

4. Conclusions

From this work, the following conclusions can be drawn:

- All BDD anodes, regardless of doping, are reversible. The electrochemical response of anodes are influenced by physical characteristics. High boron content electrodes have a great electron transfer activity. BDDs with 8000, 2500 and 1300 ppm boron (higher doping levels) have semi-metallic conductivity due to the low energy impurity ranges that allow electron conduction. Because of this they have a lower load transfer

resistance. Levels with 100 and 200ppm boron (low doping) have semiconductor properties, thus low conductivity. Reflecting in greater resistance to charge transfer.

- All BDD anodes, regardless of their doping level, showed excellent efficiency in removing penicillin G in urine (> 90%). The kinetic constant was distinct for the anodes. Electrodes with low boron levels (100 and 200 ppm) presented a higher kinetic content when compared to electrodes with high boron doping (8000, 2500 and 1300 ppm).
- Regardless of the presence of penicillin G and the level of doping, all anodes can degrade the organic compounds present in synthetic urine (urea, creatinine and uric acid). However, uric acid is the only 100% degraded compound. Urea and creatinine achieve a reduction of 70 and 67% respectively.
- The reduction in TOC and COD is greater in low boron doping electrodes. This can be attributed to the favorable formation of oxidant species that the low boron content provides. In both cases (urine with and without penicillin G), more than 50% of these parameters were reduced.
- Toxicity tests indicated that with an applied load of 2A/h dm³ a huge decrease in toxicity occurred. However, for electrodes with low boron doping inhibition of *Vibrio fischeri* decreased by more than 80%. For the high doping electrodes, a maximum reduction of 70% was achieved.
- In general, for the antibiotic activity tests the 100 and 200 ppm electrodes presented a higher amount of UFC *E. Faecalis* showing that the effect of the antibiotic was completely eliminated by applying a load of 2A/h dm³

5. References

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