UNIVERSITY OF SALERNO

DEPARTMENT OF CHEMISTRY AND BIOLOGY

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PhD in Chemistry XXXII Cycle

CHIM/01- Analytical Chemistry

Development and optimization of analytical methods for the analysis of drugs in wastewater samples and chronic aquatic toxicity assessment of the Crustacea Copepoda *Tigriopus fulvus*

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Abstract

Pharmaceutical compounds constitute one of the most important emerging classes of environmental pollutants. Recent studies have discovered their occurrence in environmental samples investigated worldwide, including different types of aqueous matrices. Drugs may be released into water sources and in the effluents from poorly controlled manufacturing or production facilities. In addition, the ubiquitous use of drugs has resulted in their relatively continuous discharge into wastewater, but conventional urban wastewater treatment plants (WWTPs) are not able to efficiently remove most of these pollutants. Thus, pharmaceuticals in surface waters, groundwater and treated water have relatively low concentrations ranging from ng/L to µg/L. This work has the purpose of exploring electrochemical and liquid chromatographic-mass spectrometric techniques for the analysis of several Pharmaceutical and Personal Care Products (PPCPs) and to assess the toxicological effects that some of these drugs have on aquatic organisms, such as *Tigriopus fulvus* (crustacea).

First phase of this study (Part A) covered the development together with electrochemical and spectroscopic characterization of new modified electrodes, i.e. glassy carbon/graphene oxide/polyvinyl alcohol (GC/GO/PVA) and glassy carbon/Betaine/Platinum (GC/BE/Pt) electrodes.

In the Part A of this work two modified electrodes, the first one based on polyvinyl alcohol (PVA) structured with particles of oxidized graphene (GO), and the second one on betaine-Pt on glassy carbon, were developed and characterized by voltammetric and spectroscopic techniques.

Two electrodes were morphologically characterized by Scanning Electron Microscopy (SEM) and superficially by X-Ray Photoelectron Spectroscopy (XPS). Furthermore, in order to evaluate the electroanalytical performance, the new electrode systems have been tested with the selected drugs.

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV), showed that both electrodes have a good response if compared to simple glassy carbon electrodes.

Second phase (Part B) is related to the optimization and validation of an HPLC-MS/MS method for the determination of amoxicillin, erythromycin, clarithromycin, omeprazole, metformin, carbamazepine, acetylsalicylic acid, ibuprofen, diclofenac, naproxen, estradiol and ethinylestradiol in wastewater samples. Firstly, mass spectrometric parameters i.e. ionization mode, capillary temperature, capillary potential and CID energy

for tandem mass analysis were optimized. Then, chromatographic parameters, i.e. selection of the chromatographic column, eluents, gradient and flow rate were optimized. Finally, the developed and optimized method has been validated for the analysis of the selected twelve drugs in wastewater samples.

Good linearity (coefficient of determination more than 0.993) and limits of detections in the range 0.0001 μg/L ÷ to 0.7211 μg/L were obtained. No significant matrix effect, evaluated by post extraction addition, was observed. Then, this methodology has been successfully applied to environmental study of pharmaceutical residues occurring in influent and effluent wastewater samples, from the main wastewater treatment plant in Potenza (Basilicata, Southern Italy), showing that for some drugs the purification process is not sufficient to degrade and remove them from the water matrix.

Finally, toxicological effects tests have been carried out to evaluate the effects that carbamazepine and amoxicillin have on aquatic organisms, such as *Tigriopus fulvus* (crustacea). Chronic test of 28 days was carried out on two subsequent generations of *T. fulvus* at a drug concentration actually found in the wastewater samples, i.e. 5 μg/L and 2 μg/L, for carbamazepine and amoxicillin, respectively. The results of the test show that no significative toxic effect of amoxicillin on the first generation of *T. fulvus* was observed, while carbamazepine effects on the number of broods were relevant. Concerning the second generation, amoxicillin once again appears to give no visible toxic effect, while carbamazepine shows toxic effects not only on the number of broods, but also on the number of total nauplii generated. Based on these results, carbamazepine could be considered a potential endocrine disruptor since a delayed reproduction and a reduced fecundity in *T. fulvus* was observed.