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Abstract

An electro-activated glassy carbon electrode (aGCE) was tested as sensor for the detection of acetaminophen (APAP) in surface water samples. The best measurement conditions for the determination of APAP by Differential Pulse Voltammetry (DPV), assisted by the aGCE, were optimised by means of a Design of Experiment approach. The analytical performance of the electrochemical procedure was than assessed in synthetic solutions and in real samples. The analytical response had a linear trend in a concentration range between 13.3 and 33 μg L^{-1}; the system could detect APAP concentrations higher than 4.4 μg L^{-1} in untreated river-water samples, and higher than 0.2 μg L^{-1} in river-water samples that were pre-treated by solid phase extraction. The electrochemical technique based on DPV with aGCE was then used for the quantification of APAP in river water samples collected in the Turin area (Piedmont region, NW Italy) and these

results agreed well to those obtained by liquid chromatography-mass spectrometry (HPLC with HRMS detection).

Keywords

Acetaminophen; Paracetamol; Glassy carbon electrode; Voltammetry; Electrochemical activation; Natural surface waters

1. Introduction

In the last two decades the occurrence of anthropogenic bioactive substances in surface waters has been widely reported, and this finding has urged scientists to investigate on the newly detected micro-pollutants [1]. The US EPA (United States Environmental Protection Agency) defines as Contaminants of Emerging Concern (CECs) the molecules that have not yet a legal regulation, but that have been recently detected in natural water bodies and could have an impact on the biological activity of natural waters. Pharmaceuticals belong to this category [2].

The increasing use of pharmaceuticals [3] and the unsuitability of the current Waste Water Treatment Plants (WWTPs) to decrease the concentration of these molecules [4,5] are the major reasons for their occurrence in surface waters. Several efforts are currently devoted to the assessment of the risks linked to the environmental dispersion of pharmaceuticals [6-9], to verify their effects and stability in the environment [10], to increase the abatement efficiency of WWTPs [11] and to develop new methods for the monitoring of water contamination. This work deals with contamination monitoring, and its goal is to test the applicability of a previously developed, electrochemically activated Glassy Carbon Electrode (aGCE) to quantify acetaminophen (APAP) in natural waters [12].

The conventional methods employed to quantify CECs in natural waters are based on High Performance Liquid Chromatography interfaced with Mass Spectrometry (HPLC-MS) [13]. These measurements are expensive and time consuming, they need highly qualified staff and cannot be executed in the field. Therefore, electrochemistry could be a good alternative thanks to its low cost, high sensitivity and potential portability. Several research papers report on the application of electrochemical methods for the quantification of drugs, and in the last ten years the use of carbon electrodes in this field has considerably increased [14]. Most of these applications use carbon electrodes modified by the application of materials that enhance electron transfer, such as MWCNT – Multi Walled Carbon Nano Tubes, C_{60} – Fullerene, AuNP – gold nanoparticles, SWCNT – Single

Walled Carbon Nanotubes, as well as BDD – Boron Doped Diamond [15-19]. More simply, the electro-activation method used in this work only requires the application of a potential for a defined time, on the GCE immersed in a buffer solution. As reported in previous work [12], the aGCE gives high signals with molecules like APAP that have a phenolic group and a substituent in para position. Thus, the electrochemical activation allows for an increase of the electrode selectivity and sensitivity towards APAP.

In this work, Differential Pulse Voltammetry – DPV – measurements were used for the quantification of APAP in both synthetic solutions and real samples. The DPV parameters were optimised by the application of an Experimental Design (DoE) method aimed at maximising sensitivity. To test the performance of the electrochemical technique, we determined the linearity range of the response, the calibration parameters and the limit parameters (limits of detection and quantification) in both ultra-pure water and real samples. The results obtained on real samples were then compared with those obtained by HPLC-HRMS (using Orbitrap High Performance Liquid Chromatography-High Resolution Mass Spectrometry) in order to confirm the reliability of the electrochemical results.

The real river water samples from the Turin area (Piedmont, NW Italy) were provided by ARPA Piemonte (the regional environmental protection agency), and they also allowed for the first-time assessment of the APAP concentration levels in this densely populated area of Italy. A total of 84 monthly samples were collected in 5 different sites, and they were analysed by DPV with aGCE.

2. Experimental

2.1 Reagents

Chemicals were purchased from Sigma Aldrich and were of analytical grade. Ultra-pure water was produced by a Milli-Q system (resistivity of 18 MΩ cm). The APAP stock solution was weekly prepared in Milli-Q water. The borate-phosphate buffer (hereafter, BPB solution) was prepared by

dissolving KH_2PO_4 and $Na_2B_4O_7$ salts in Milli-Q water to final respective concentrations of 45 and 5 mmol L^{-1}, or of 450 and 50 mmol L^{-1}, and by adjusting the pH to 9.0 with 1 mol L^{-1} NaOH.

2.2 Samples

The surface-water samples were collected by ARPA Piemonte from 5 sampling sites located in the Turin area, as shown in Figure 1. The sampling was carried out monthly for a year and half, starting from April 2016. The water samples were stored till analysis in 1 L dark glass bottles at 4°C. The rationale for the choice of the sampling sites is as follows: Dora Riparia and Banna are Po river tributaries; the Carignano sampling site is affected by typical urban pollution; the Brandizzo site is located directly downstream of the Turin WWTP, whereas the Lauriano site is located downstream of Brandizzo and receives additional water from an irrigation canal.

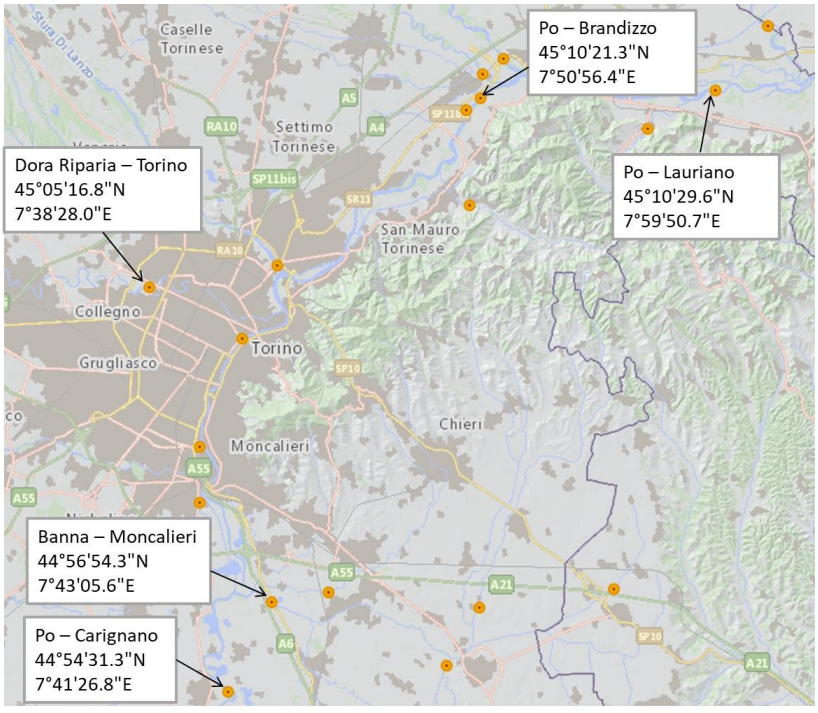

Figure 1 Sampling sites

Sampling sites and coordinates as latitude and longitude.

2.3 Measurement devices

The electrochemical measurements were carried out with a PalmSense3 potentiostat using a three-electrode system: a Glassy Carbon Electrode – GCE – (3 mm diameter, ALS Co., Ltd) as working electrode; a platinum wire (0.5 mm, surface area ~ 0.7 cm^2) as counter electrode, and Ag/AgCl, 3 mol L^{-1} KCl (ALS Co., Ltd, RE-1B) as reference electrode. The convective transport during the DPV measurements was ensured by magnetic stirring (stirring device by Velp Scientifica).

The solution pH was measured with a combined glass-membrane electrode (Metrohm) controlled by a 338 pH-meter (Amel Electrochemistry). The chromatographic measurements were performed by a HPLC interfaced with a High Resolution Mass Spectrometer (Thermo Scientific™ Orbitrap Fusion™ Tribrid™), equipped with a column Phenomenex Luna C18(2) 150 mm × 2.1 mm × 3 µm particle size and with a 20 µL sample loop.

2.4 Procedures

2.4.1 Electrochemical activation

Before activation the working electrode was mechanically polished with diamond powder (1 µm, ALS Co., Ltd) and alumina (0.3 µm, ALS Co., Ltd), and was carefully washed with acetonitrile and with ultra-pure water. As reported by Chiavazza et al. [12], activation consisted in the application of a 2.0 V anodic potential for 60 s under magnetic stirring (500 rpm), to the GCE immersed in a 50 mmol L^{-1} BPB solution. The activated electrode (aGCE) was then directly used for the measurements.

2.4.2 Solid Phase Extraction (SPE)

The purification and pre-concentration of the samples made use of Oasis® HLB 30µm 6cc (200mg) cartridges mounted on a SPE VacMaster-10 Sample Processing Station (Biotage, Sweden). Each cartridge was conditioned before use with 6 mL methanol followed by 6 mL ultra-pure water. Each

sample was filtered on a MF-Millipore membrane (HAWP, pore size 45 μm), after which a 200 mL aliquot was eluted through the SPE cartridge that was then washed with 1 mL of 95/5 water/methanol and let dry for 5 minutes. The elution of APAP was carried out with 6 mL pure methanol, and the eluate was dried under nitrogen flow. After that, for the measurements by DPV with aGCE the solid was recovered with 10 mL of 50 mmol L^{-1} BPB. For the HPLC-HRMS analysis, the recovery was carried out with 1 mL of methanol/0.1% formic acid 10/90.

2.4.3 HPLC-HRMS

The mobile phase used for the chromatographic separation was composed of 0.1% HCOOH in ultra-pure water (A) and methanol (B). The gradient elution was as follows: 0 – 20 min from 2% to 100% B, 20–21 min at 100% B, 21–23 min from 100% to 2% B, 23–33 min 2% B. The flow rate was 0.2 mL min^{-1}. In these conditions the retention time of APAP was 2.46 min.

As far as the MS conditions are concerned, the ESI(+) (Electrospray Ionisation) voltage was 3500 V, the capillary temperature was maintained at 325°C and the MS detection was conducted in full scan mode. The collision energy was 28% with mass range and resolution set at, respectively, 50-500 m/z, 60,000 (MS) and 30,000 (MS^2).

2.4.4 Electrochemical measurements

The electrochemical experiments were carried out at room temperature by DPV. With the exception of the samples that followed SPE processing, drying-up and buffer recovery as described in section 2.4.2, in the other cases 9 mL of solution were added added with 1 mL of the 500 mmol L^{-1} borate-phosphate buffer stock solution, in order to obtain a 50 mmol L^{-1} final buffer concentration (pH 9.0). Each measurement was preceded by a brief conditioning period (5 s) at 2.0 V, the potential scan was carried out between 0 - 0.400 V, and a slow stirring of the solution was maintained. The other parameters of the DPV, such as step height (E_{step}), pulse amplitude (E_{pulse}), pulse length (t_{pulse})

and scan rate (SC) were optimised by a DoE approach to maximise sensitivity. After the activation step and before processing each sample, ten DPV measurements of a blank solution were done to stabilise the signal and the last blank signal was subtracted to that of the sample. The measurement signal was the current value read at 250 mV, which corresponds to the potential of the APAP peak maximum. The peak height was used for quantification, and each analytical signal was recorded at least twice.

2.4.5 Design of Experiment (DoE)

In order to maximise the sensitivity of the DPV measurements, a DoE was applied to the DPV parameters. E_{step}, E_{pulse} and t_{pulse} were assessed on three levels each, respectively at 2, 4, 6 mV; 20, 60, 100 mV, and 0.005, 0.02, 0.05 s. Moreover, four levels were used for the SC: 5, 10, 15, and 20 mV s^{-1}. A D-optimal design (MODDE® Pro, MKS Umetrics) was selected and 23 experiments were done in triplicate, plus 3 for the central point, taking as experimental response the height of the APAP peak. All these experiments used the same APAP concentration, namely 30 µg L^{-1} APAP in 50 mmol L^{-1} BPB.

3. Results and Discussion

3.1 DoE outcome

The electrochemical signals of APAP in aqueous solution were increased by the application of the electro-activation procedure, as previously reported in the Ref. 12. However, several electrochemical measurement parameters, such as the step height (E_{step}), the pulse amplitude (E_{pulse}), the pulse length (t_{pulse}) and the scan rate (SC) affect significantly the intensity and the position of the electrochemical signal. Figure 2 shows the DPV peak obtained with different sets of electrochemical parameters, on a solution containing APAP 30 µg L^{-1}. In order to identify the

parameter values providing the highest measurement sensitivity, a DoE method was used. The details of DoE are reported in the experimental section (paragraph 2.4.5).

A first analysis of the confidence intervals of the model coefficients, calculated with a confidence level of 95%, suggested that the interaction terms of E_{step} with E_{pulse} and t_{pulse} and the squared terms of SC and E_{pulse} were not significant. Even by decreasing the confidence level down to 90%, the interaction terms of E_{step} and the squared terms of SC continued to be non-significant. Therefore, these terms were excluded from the model. The regression plot thus obtained is reported in Figure 3a (R^2 = 0.818). The Figure 3b shows the model coefficients and the coefficient signs, as the response surfaces, indicate that the highest current values were obtained for low values of SC and t_{pulse} and high values of E_{pulse}. An example of response surface is shown in Figure3c.

The maximum response was obtained for parameter values located at the border of the investigated ranges, thus experimental conditions that falling beyond the borders were tested in order to confirm that the maximum was certainly reached. The measurements with SC = 5 mV s^{-1} always gave the highest current values and, as reported before, the E_{step} value did not significantly affect the height of the peak. Therefore, a further refinement was carried out by recording the APAP signals with SC = 5 mV s^{-1} and E_{step} = 4 mV (central point), and by changing E_{pulse} and t_{pulse}. Figure 4b,c shows the signals obtained changing the E_{pulse} and the t_{pulse} only. The variability of the signals can be compared with the stability of the signal at the central point (Figure 4a).

The tests confirmed the appropriateness of the model and revealed that: *i*) the signals decreased with E_{pulse} > 100 mV; *ii*) the highest sensitivity was obtained with E_{pulse} = 100 mV, t_{pulse} = 8 ms, E_{step} = 4 mV and SC = 5 mV s^{-1}. Figure 4d shows the DPV signals obtained with these measurement parameters increasing the APAP concentration. All the following experiments were conducted in these conditions, unless otherwise specified.

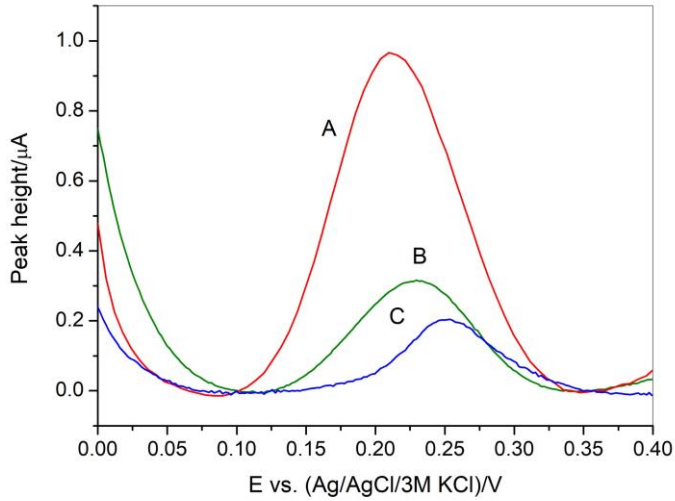

Figure 2 Effect of measurement parameters on DPV signals of APAP

DPV signals obtained on a solution of APAP 30 μg L^{-1} with different sets of measuring parameters: A - E_{step} = 6 mV, E_{pulse} = 60 mV, t_{pulse} = 5 ms, SC = 10 mV s^{-1}; B - E_{step} = 2 mV, E_{pulse} = 100 mV, t_{pulse} = 50 ms, SC = 20 mV s^{-1}; C - E_{step} = 2 mV, E_{pulse} = 20 mV, t_{pulse} = 20 ms, SC = 10 mV s^{-1}.

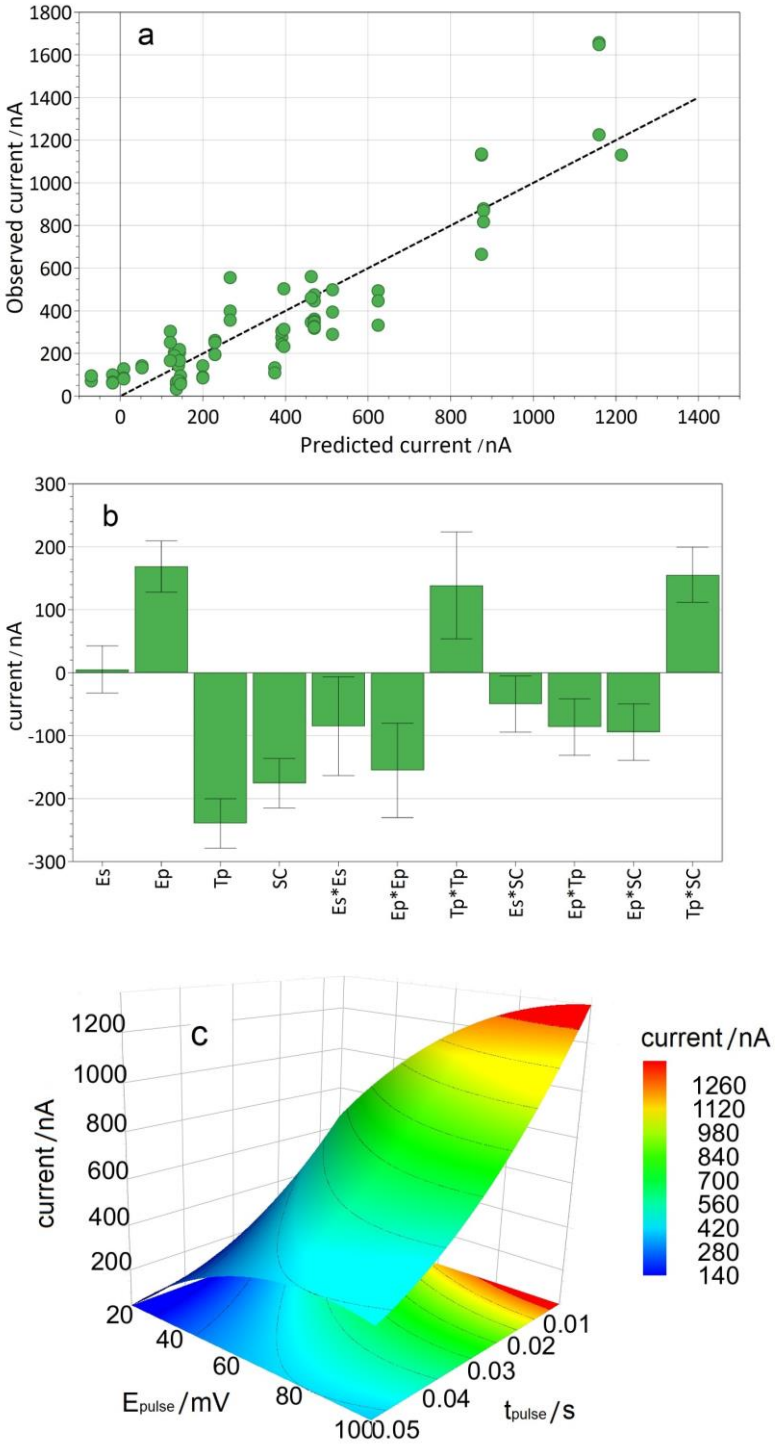

Figure 3 Design of experiment

a) Predicted values *vs* actual values; b) coefficients of the model; c) response surface obtained with SC = 5 mV s^{-1} and step height E_{step} = 4 mV. Confidence level 90%. Reference electrode Ag/AgCl/3M KCl.

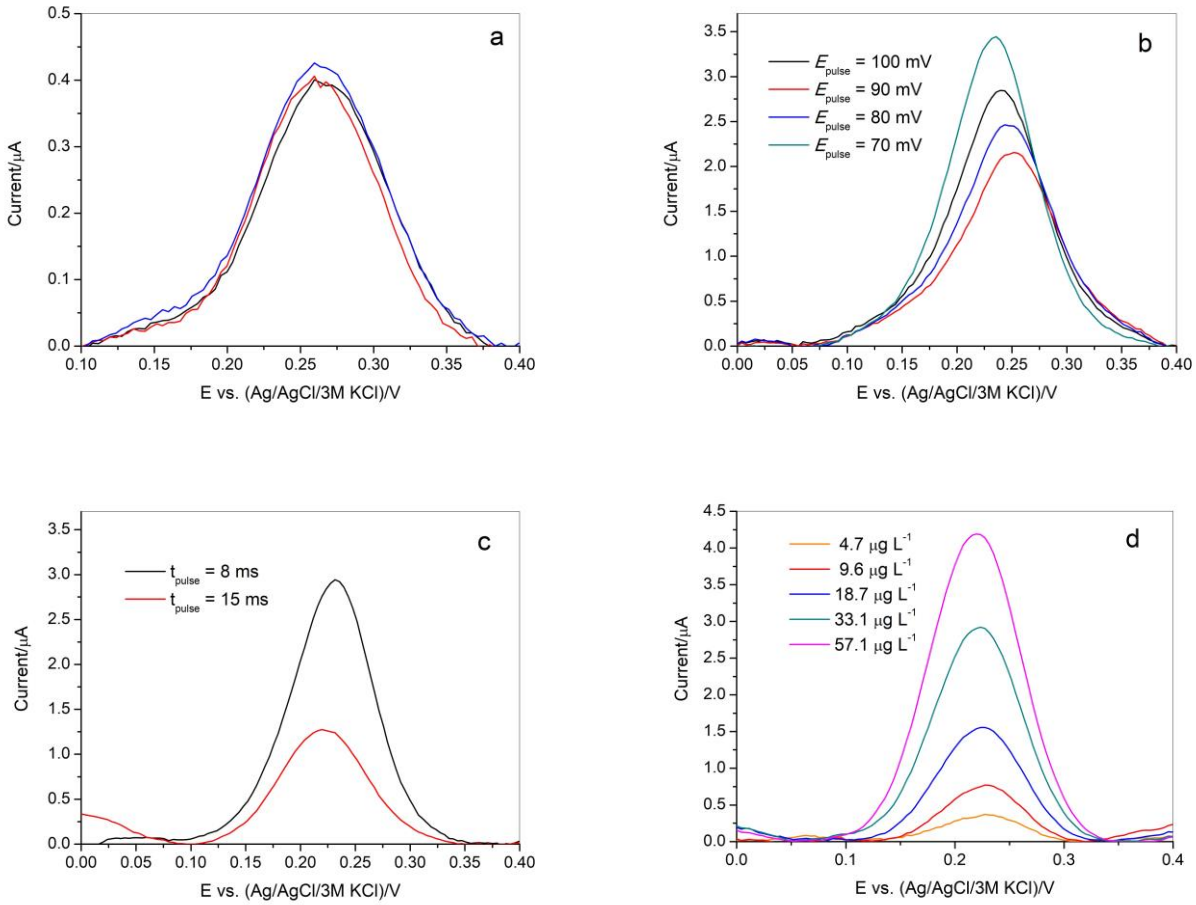

Figure 4 DPV signals of APAP obtained with different sets of measuring parameters

a - APAP 30 μg L^{-1}, E_{step} = 4 mV, E_{pulse} = 60 mV, t_{pulse} = 20 ms, SC = 15 mV s^{-1} (replicates of the central point of the DoE); **b** - APAP 30 μg L^{-1}, E_{step} = 4 mV, t_{pulse} = 8 ms, SC = 5 mV s^{-1}; **c** - APAP 30 μg L^{-1}, E_{step} = 4 mV, E_{pulse} = 100 mV, SC = 5 mV s^{-1}; **d** - APAP 4.7 - 57.1 μg L^{-1}, E_{step} = 4 mV, E_{pulse} = 100 mV, t_{pulse} = 8 ms, SC = 5 mV s^{-1}.

3.2 Calibration and limit parameters

The calibration and limit parameters of the DPV measurements were obtained by applying the DoE-optimised experimental conditions, varying the concentration of APAP between 0.68 and 476 μg L^{-1}. The linearity range and the calibration parameters obtained in ultra-pure water are reported in

Table 1. The calibration was performed by successive APAP additions to a 50 mmol L^{-1} BPB solution, and the DPV measurement was replicated at least twice on each calibration point.

The linearity range (5.5 - 33 μg_{APAP} L^{-1}) was not very wide, and two linear ranges could be detected (data not shown). Moreover, the optimal t_{pulse} = 8 ms is very short and unusual for DPV conditions. Unsurprisingly, these conditions became unsuitable for APAP concentration values above 100 μg L^{-1}. The calibration was thus repeated with t_{pulse} = 16 ms, and the plots obtained by using both t_{pulse} values are shown in Figure 5. Table 1 provides a comparison of the two calibration data sets. One can see that the use of t_{pulse} = 16 ms gave a more robust method, but it did not allow for the detection of very low APAP levels.

For the analysis of the real river-water samples, where APAP supposedly occurred at very low concentration, it was used t_{pulse} = 8 ms. The matrix effect on the measurements was tested by comparing the slopes of the regression lines obtained with and without the matrix. Therefore, increasing aliquots of APAP were added to: *i*) 50 mmol L^{-1} BPB solutions prepared with ultra-pure water, and *ii*) 50 mmol L^{-1} BPB solutions prepared with a real river-water sample that did not contain APAP. In all these cases we used t_{pulse} = 8 ms. The linear fits to the experimental points gave a mean slope of 0.088 ± 0.002 μA L μg^{-1} (mean ± standard deviation) and 0.069 ± 0.01 μA L μg^{-1} (mean ± standard deviation) for the systems without and with the matrix, respectively. The reported slopes are the mean values derived from three replicas of each calibration procedure and they are significantly different. Therefore, it is suggested that the matrix affects the measurements by decreasing sensitivity. The sensitivity decrease is probably linked to the presence of organic matter in river water, because measurements carried out on organic-matter poor tap water (data not reported) did not show a significant lowering of the regression line slopes compared to ultra-pure water. Because of the matrix effect we used the standard addition method to quantify APAP in real samples, which is a very common procedure in DPV measurements [20,21]. To avoid analyte overestimations, our APAP additions did not exceed the upper limit of the linearity range (33 μg

L^{-1}). In the presumably rare cases when the APAP concentration detected in a real sample is higher than (or very near) 33 μg L^{-1}, we recommend the use of t_{pulse} = 16 ms.

The above measurement conditions were used to determine the limit parameters as well. The Limit of Detection (LoD) was calculated by using the equation LoD = $k\ s_b\ S^{-1}$, where S is the slope of the calibration line, k = 3.3 is the coverage factor, and s_b is the standard deviation of 10 replicates of the blank signal (the blank solution contained only 50 mmol L^{-1} BPB).

The Limit of Quantification (LoQ) was calculated by using the same equation with k = 10. In Table 1 the limit parameters are reported for t_{pulse} values of both 8 and 16 ms. Limit values were also assessed in real river water in view of the forthcoming use of the DPV with aGCE technique on environmental samples. The applied procedure with river water was the same as previously reported, but the blank solution was prepared upon addition of 1 mL of 500 mmol L^{-1} BPB to 9 mL of river water. As river-water matrix for these experiments it was used the sample collected in June 2017 from the site Po-Brandizzo. In this case the noise was higher compared to the matrix-free BPB solution, which accounts for the increase in both LoD and LoQ values. The two limit parameters were also increased by applying t_{pulse} = 16 ms, because of the decrease in calibration sensitivity (S) and of the increase in the variability of the blank signals (s_b).

In order to show the reliability of the limit parameters, Figure 6 presents the DPV signals obtained on solutions containing APAP at concentration values corresponding to the LoD and LoQ, after a blank subtraction procedure.

Cernat et al. [22] discussed the advancements in the development of electrochemical sensors based on carbon nanomaterials for acetaminophen detection in an interesting review. They report the detection limit values obtained with electrodes modified with carbon nanotubes and graphene. The LoDs of the systems that use GCEs and DPV technique show values comprised between 0.3 μg L^{-1} and 118 μg L^{-1} and, for 5 cases out of 15, the LoDs is in the range 1.5 – 4.8 μg L^{-1} [23 – 27]. Only one case shows a LoD lower than the unit of μg L^{-1}. The sensors reported in the review were

variously modified with carbon based nanomaterials, whereas the sensor reported in this work only need of an electrochemical activation process and, despite this, it shows sensitivity comparable with the most performant sensors reported in the literature [23-28].

Table 1 APAP calibration parameters obtained by DPV & aGCE. Limit parameters were assessed in BPB + ultra-pure water (LoD_{BPB} and LoQ_{BPB}) or BPB + real sample (LoD_{matrix} and LoQ_{matrix}).

Calibration parameters	**t_{pulse} 8 ms**	**t_{pulse} 16 ms**
Linearity range	5.5 – 33 μg L^{-1}	8.9 – 290 μg L^{-1}
Slope[a]	0.088	0.0508
Slope standard error[a]	0.002	0.0007
Intercept[a]	-0.07	-0.04
Intercept standard error[a]	0.04	0.1
RSS[a b]	0.00506	0.24304
R[a]	0.9993	0.9993
Adjusted R^2 [a]	0.9979	0.9984
Limit parameters	**μg L^{-1}**	**μg L^{-1}**
LoD_{BPB}	1.8	9.0
LoQ_{BPB}	5.5	27.3
LoD_{matrix}	4.4	11.7
LoQ_{matrix}	13.3	35.5

[a] Regression line parameters derived from a single calibration procedure where the signal (each point signal was measured three times).

[b] RSS = Residual Sum of Square

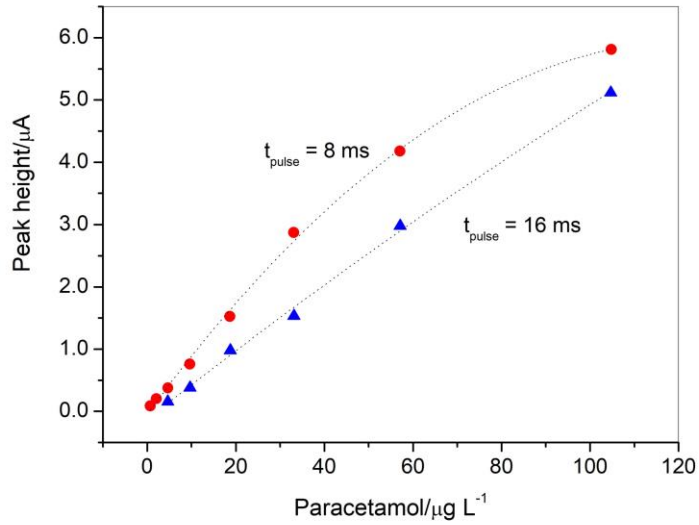

Figure 5 Calibration curves

Calibration curves obtained with SC = 5 mV s^{-1}, E_{step} = 4 mV, E_{pulse} = 100 mV and: a) t_{pulse} = 8 ms, or b) t_{pulse} = 16 ms.

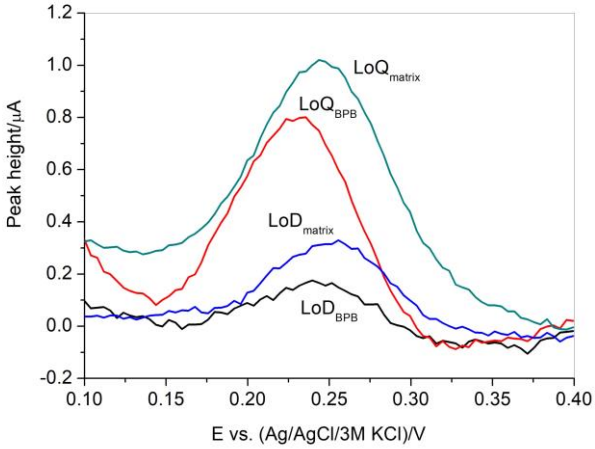

Figure 6 Signals at limit parameters

DPV signals obtained with t_{pulse} = 8 ms and after blank subtraction, on solutions with APAP concentrations equal to the limit parameters. LoD_{BPB} and LoQ_{BPB} were evaluated in a 50 mmol L^{-1} BPB solution prepared with ultra-pure water. LoD_{matrix} and LoQ_{matrix} were evaluated in a solution containing 50 mmol L^{-1} BPB, and prepared by spiking APAP to APAP-free river water.

3.3 Real samples

A total of 84 samples of Po river water were directly analysed by DPV with aGCE using the above optimised conditions without sample pre-treatment, upon application of the standard addition method because of the non-negligible matrix effect. Each DPV measure was repeated at least twice for each calibration point, and each sample was analysed in triplicate. Accordingly to the concentration levels of APAP detected in surface water and reported in the literature [29,30], all the analyzed samples showed signals not significantly different from the blank solution or comparable to that of LoD_{matrix}, therefore, in order to reach lower detectable concentration levels, some samples were subjected to pre-treatment by using the SPE technique, which allowed for a 20-fold sample concentration as reported in the experimental section. To assess the recovery of the extraction process, were carried out SPE elution of 15 and 1.5 μg L^{-1} APAP solutions prepared in both ultra-pure and river water. In the latter case, we spiked APAP to a river-water sample that did not show DPV signals of APAP after SPE pre-concentration. The extraction process was replicated three times for each system, with resulting mean recoveries ranging between (96 ± 6)% (with 15 μg L^{-1} APAP) and (99 ± 3)% (with 1.5 μg L^{-1} APAP) in ultra-pure water and, respectively, between (95 ± 3)% and (90 ± 5)% in river water.

The limit parameters were also estimated on real samples after processing with SPE, obtaining LoD and LoQ values that were comparable to those obtained by directly working on the real matrix, without SPE pre-treatment (Table 1). Therefore, the sensitivity enhancement carried out by SPE is expected to be totally accounted for by the concentration step.

Some samples showed detectable APAP concentration values after the SPE step, but they were still under the LoQ_{matrix}. The relevant samples were collected in the sites of Po-Brandizzo (January 2017 and September 2017) and Po-Lauriano (January 2017), and in these cases the APAP concentration can be approximately assessed as 0.2 - 0.7 μg L^{-1}.

The detection of APAP in January could be due to the extensive use of this drug by the population to treat cold-related diseases, whereas in the case of September 2017 the reason could be linked to a

prolonged period of drought that hit NW Italy from June to November 2017 (http://www.arpa.piemonte.gov.it/bollettini/elenco-bollettini-1/bollettino-idrologico, last access: 24th April 2018). The reduced flow of the Po River could have increased the pollutant concentration levels due to a lesser dilution of the WWTP effluent. The relation between the water scarcity and the water quality was studied by Petrovic et al. [31] and the vulnerability of the rivers located in the Mediterranean area, one of the regions most affected by the climate global change, was highlighted.

3.4 Selectivity: comparison between DPV with aGCE and HPLC-HRMS

To assess the selectivity/specificity of the DPV measurement and to ensure that the voltammetric signal can be confidently attributed to APAP, a spiked river-water sample was treated by SPE and APAP was then quantified by both DPV with aGCE and HPLC-HRMS. The spiked sample was a pool of river water samples collected at Po-Carignano. Six 200-mL aliquots of the pooled sample were spiked with 0.5 μg L^{-1} APAP, and three of them were also spiked with 0.5 μg L^{-1} APAP-D_4 that is the internal standard used in HPLC-HRMS. Each aliquot was treated by SPE as reported in the experimental section. After the drying step, three aliquots were recovered with 10 mL of 50 mmol L^{-1} BPB, thereby applying a pre-concentration factor of 20, and they were subjected to DPV measurement (carried out as reported above). The remaining three aliquots were recovered with 1 mL of methanol/0.1% formic acid 10/90 (pre-concentration factor of 200) and analysed by HPLC-HRMS. The chromatographic method used internal standard (APAP-D_4) and three-point calibration. The obtained APAP concentration values were 0.44 ± 0.12 μg L^{-1} (DPV) and 0.39 ± 0.10 μg L^{-1} (HPLC-HRMS) (confidence interval was obtained with 95% confidence, 2 degrees of freedom and applying the bilateral test). Interestingly, the mean SPE recovery at this concentration level was (82 ± 3)% and the two techniques gave results in quite good agreement and without significant difference (*t* test, 95% confidence, 4 degrees of freedom). Therefore, the DPV signal can be attributed to APAP with a high level of confidence.

4. Conclusions

In this work, we set-up the measurement parameters for the quantification of APAP by differential pulse voltammetry assisted by electrochemically activated glassy carbon electrode. We then tested the performance of the device on real samples. The optimisation of the measurement parameters by experimental design allowed for a good sensitivity to be achieved, so that the system could detect APAP concentrations higher than 4.4 μg L^{-1} in untreated samples. Because of non-negligible matrix effect, we suggest the use of the standard addition method for the quantification of APAP in real samples. Under conditions where the above sensitivity is sufficient, the DPV technique can be easily applied to directly detect APAP in real natural-water samples without previous treatment, therefore it could be employed for in-field measurements or for on-line detection of APAP in the effluents of Waste Water Treatment Plants.

The APAP concentration levels in surface waters were usually (and fortunately) lower than the LoD estimated above, but it is possible to overcome this limit by pre-processing the samples with SPE. The SPE sample treatment allowed for the LoD to be decreased down to 0.2 μg L^{-1}, still maintaining a good reliability of the overall analytical procedure as demonstrated by the comparison between the voltammetric results and those obtained by HPLC-HRMS.

The technique of DPV with aGCE developed here can be useful for screening analyses that require a fast and cheap measurement method, which easily recognises contaminated samples that can be submitted to successive analytical investigations. A complete measurement session, because of the need to apply the standard addition method, supposing to do four additions and three replicate each one, takes about 15 minutes. However, the electrochemical system presented here shows some interesting advantages: *i*) the apparatus is made up of commercial devices; *ii*) the electrochemical activation of the GCE is fast, easy and reproducible, and *iii*) the measurement of APAP by DPV shows considerable sensitivity and reliability.

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