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Electrochemical determination of pharmaceuticals in spiked water samples

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Abstract

The electrochemical behaviour of acidic and neutral pharmaceutical active compounds (PhACs) was studied by cyclic voltammetry and pulse voltammetric techniques on mercury, carbon nanotube paste, carbon paste and gold electrodes. The best results, in terms of sensitivity, linearity range and detection limits, were obtained by differential pulse voltammetry (DPV) for ofloxacin (LOD 5.2 μM), differential pulse polarography (DPP) for clofibric acid (LOD 4.7 μM) and normal pulse voltammetry (NPV) for diclofenac (LOD 0.8 μM) and propranolol (LOD 0.5 μM).

An enrichment step of approximately two orders of magnitude was performed by a solid-phase extraction procedure (SPE) in order to concentrate the samples. The developed method was optimized and tested on spiked river water samples.

Keywords: Pharmaceutical active compounds; Voltammetry; Mercury electrode; Carbon nanotube paste electrode; Gold electrode; Water analysis

1. Introduction

The growing use of pharmaceuticals is becoming a new environmental problem, as both via human and animal urinary or fecal excretion and pharmaceutical manufacturing discharges, increasing concentrations of pharmaceuticals reach sewage treatment plants (STPs). To date, many tons of drugs have been produced per year. In Germany, for example, up to 100 t of individual drugs are prescribed every year [1], but this amount underestimates the total usage of pharmaceuticals, as many of them are also purchased without prescription. Due to this extensive use, high concentrations of drugs are found in sewage, depending on their half-lives and metabolism. STP are therefore often ineffective in removing these substances, so that varying concentrations of them can be found in surface and ground waters.

In recent years, increasing attention has been paid to the determination of pharmaceuticals in water samples. Until now, many analytical methods reported in the literature have been carried out by gas and high-performance liquid chromatography, usually in combination with mass spectrometry (GC–MS, LC–MS) [2–14], capillary electrophoresis-mass spectrometry [15] and high-performance liquid chromatography-photochemically induced fluorimetry (LC-PIF) [16]. Unfortunately, all these reliable methods are very expensive, and it would be better to use different analytical methods, which do not require expensive instrumentation and which therefore could be used even in less highly developed areas.

To our knowledge, information on the electrochemical behaviour of pharmaceuticals is available in the literature [17–20], but there is nothing on determination in surface waters as a result of sewage treatment.

Our aim was to apply not the above methods but voltammetric techniques to determine pharmaceutical active compounds in spiked surface water samples. The drugs used in

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this study are neutral and acidic compounds: clofibric acid, diclofenac, ofloxacin and propranolol. They belong to several medicinal groups such as lipid regulators, non-steroid anti-inflammatory drugs, antibiotics and beta-blockers. In particular, the determination of clofibric acid was extremely important, as this drug has a half-life ($t_{1/2}$) of more than 21 years [10].

In summary, the concrete goals of the present work are: accurate electrochemical characterization of four pharmaceutical active compounds (PhACs) by means of various voltammetric techniques and electrodes; choice of the best electrochemical technique for the determination of each drug and application of these voltammetric techniques to the analysis of spiked surface water samples after a solid-phase extraction (SPE) enriching step.

2. Experimental

2.1. Materials

Diclofenac, ofloxacin and propranolol were obtained from Sigma (St. Louis, MO, USA). Clofibric acid, single-wall carbon nanotubes Carbolex (diameter 12–15 Å) and graphite particles (powder 1–2 μm) were obtained from Aldrich (Steinheim, Germany). Mineral oil was obtained from Fluka (Buchs, Switzerland).

Resprep C_{18} cartridges (1 mL) from Restek (Bellefonte, PA, USA) were used for solid-phase extraction.

All other chemicals were from Carlo Erba (Milan, Italy). All solutions were prepared with high purity water produced by a Milli-Q system.

2.2. Apparatus

Cathodic field measurements were performed with a computer-controlled potentiostat (Amel 433, Milan, Italy) with a dropping mercury electrode (DME) or a static mercury drop electrode (SMDE) as working electrodes, and a platinum disk and a Ag/AgCl/KCl(sat.) as counter and reference electrodes, respectively. Anodic field measurements were performed on an Autolab electrochemical system (Eco Chemie, Utrecht, The Netherlands) equipped with PGSTAT-12 and GPES software (Eco Chemie). The electrochemical cell was assembled with a conventional three-electrode system: a carbon nanotube paste (CNTP) working electrode (3 mm diameter), an Ag/AgCl/KCl(sat.) reference electrode, and a platinum disk as counter electrode. Carbon paste (CP) (3 mm diameter), glassy carbon (GC) (2 mm diameter) and gold disk (Au) (2 mm diameter) electrodes were also used as working electrodes. The cell was a one-compartment cell with an internal volume of 10 mL. All potentials are referred to the Ag/AgCl/KCl(sat.) electrode. The Au surface was polished with 0.003 μm alumina powder, sonicated in water for 10 min, and then washed with water. All measurements were carried out at $22.0 \pm 0.2\,^{\circ}C$ in a thermostatic bath and the supporting electrolyte was 0.2 M sodium perchlorate. A 0.1 M sodium hydroxide solution was used to analyse propranolol. All solutions were carefully de-aerated before use and maintained under a nitrogen atmosphere during voltammetric experiments.

Differential pulse polarography (DPP) and differential pulse voltammetry (DPV) measurements were carried out at a pulse amplitude of 0.010 V, pulse width of 10 ms, interval time of 1 s and at a potential scan rate of $0.002\,V\,s^{-1}$. Normal pulse voltammograms (NPVs) were recorded at a pulse width of 50 ms, interval time of 5 s, and potential scan rate of $0.002\,V\,s^{-1}$.

2.3. Electrode preparation

The carbon nanotube paste electrode (CNTP) was prepared by carefully hand-mixing (60 + 40)% (w/w) carbon nanotubes and mineral oil in a mortar and then packed into a cavity (3 mm diameter, 0.5 mm depth) at the end of a Teflon tube. Electrical contact was established via a copper wire connected to the paste. The carbon paste electrode (CP) was similarly prepared using graphite powder with a (60 + 40)% (w/w) graphite + oil ratio [21,22].

2.4. Pre-treatment and enrichment of surface water samples

A solid-phase extraction (SPE) was used for preconcentration of samples. Surface water samples from the river Tevere (Tiber, Rome) were spiked with 5×10^{-7} mol/L of the four drugs.

Two 1000 mL samples were brought to pH 3 and 7 with concentrated hydrochloric acid and sodium hydroxide, respectively. They were then passed through an SPE cartridge (conditioned with acetone, methanol and water) using a vacuum system. After the cartridges had been left to dry for 30 min, the drugs were eluted with 5 mL of methanol. The choice of methanol as extracting agent was suggested by its strong eluent ability and its inactivity on the electrodes used. Extracts were diluted with 5 mL of 0.2 M $NaClO_4$ before electrochemical analysis. For propranolol, 1 mL of the extract obtained at pH 7 was then diluted with 9 mL of 0.1 M NaOH before analysis.

3. Results and discussion

3.1. Electrochemical characterization of pharmaceutical active compounds

For the first aim of this work, the electrochemical behaviour of each compound was evaluated using various electrochemical techniques and electrodes. Preliminary experiments by cyclic voltammetry (CV) showed two trends in electrochemical response: clofibric acid and ofloxacin showed reduction peaks in the forward scan at SMDE

electrode, indicating reducible groups; diclofenac and propranolol showed anodic peaks in the forward scan at GC and Au electrodes, respectively, due to the presence of oxidizable groups. The electrochemical behaviour of the four drugs studied was therefore studied separately in the cathodic and anodic fields, respectively.

3.1.1. Cathodic field experiments

Fig. 1 shows the electrochemical behaviour of a clofibric acid solution with CV and DPP. Cyclic voltammetric measurements showed a reduction peak at about −1500 mV (versus Ag/AgCl) with no peak on the reverse scan, indicating the irreversibility of the electrode process (Fig. 1A). Fig. 1B shows the voltammograms obtained with DPP. Calibration curves are shown in Fig. 2. In this case, DPP showed the highest sensitivity, together with the largest linearity range.

The effect of the scan rate ν on peak current I_p in CV experiments was first studied in the range 10–1000 mV s^{-1}. A linear relationship was found between I_p and the square root of ν, indicating that the reduction process is diffusion controlled.

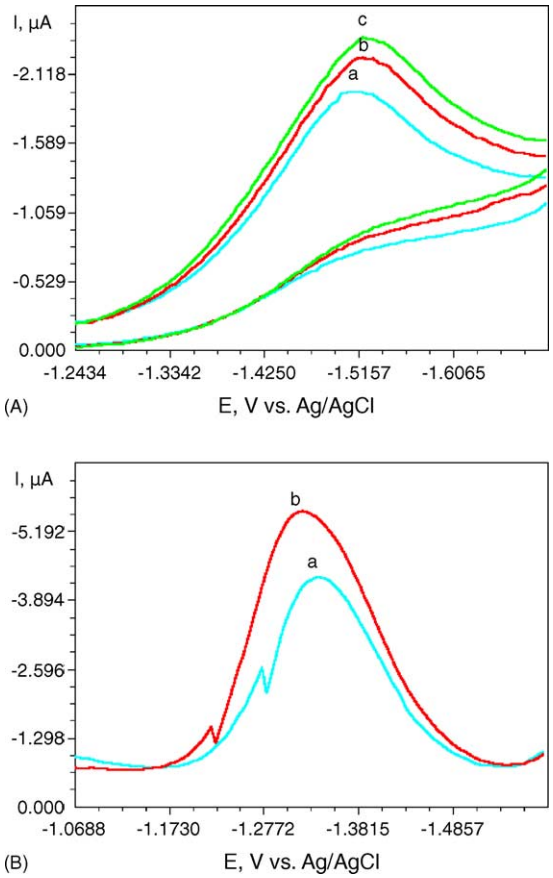

Fig. 1. (A) Cyclic voltammograms of 1.8×10^{-4} mol/L (a), 2×10^{-4} mol/L (b) and 2.2×10^{-4} mol/L (c) clofibric acid solutions at SMDE. (B) Differential pulse polarograms of 5×10^{-5} mol/L (a) and 6×10^{-5} mol/L (b) clofibric acid solution at DME. Experimental conditions: 0.2 M $NaClO_4$; $\nu = 1$ V s^{-1} for CV; pulse amplitude = 0.010 V, pulse width = 10 ms, interval time = 1 s and $\nu = 0.002$ V s^{-1} for DPP.

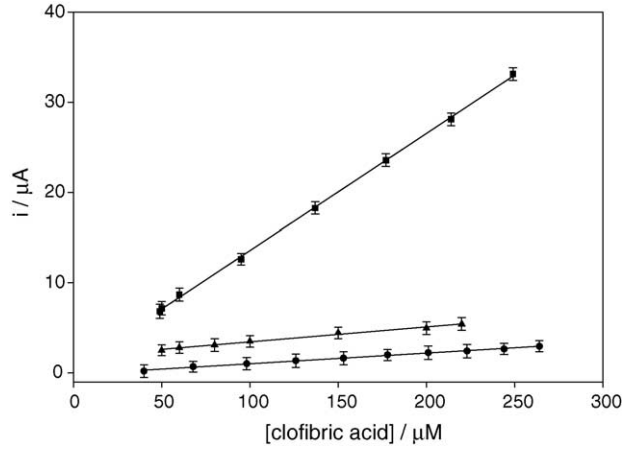

Fig. 2. Calibration curves of clofibric acid obtained with DPP (■), DPV (▲) and CV (●). Current values recorded at $E = -1.5$ V (vs. Ag/AgCl) for CV and $E = -1.3$ V (vs. Ag/AgCl) for DPP and DPV. Experimental conditions: see Fig. 1; error bars: standard deviations of three replicates.

Ofloxacin was studied with DPV and DPP, as the cyclic voltammograms showed quite broad cathodic peaks, not suitable for analytical determination. However, the dependence of I_p on $\nu^{1/2}$ was always linear, indicating that the current is semi-infinite linear diffusion controlled. DPV measurements showed well-shaped peaks at a potential of −1160 mV (versus Ag/AgCl) (Fig. 3). The relative calibration curve is shown in Fig. 4, together with those obtained with DPP. Once more, DPV turned out to be the best electrochemical technique, in terms of sensitivity and linearity range.

3.1.2. Anodic field experiments

Normal pulse voltammetry was used for quantification of diclofenac and propranolol, since it gives better-defined voltammograms than those obtained by cyclic voltammetry. Fig. 5 shows normal pulse voltammograms at varying

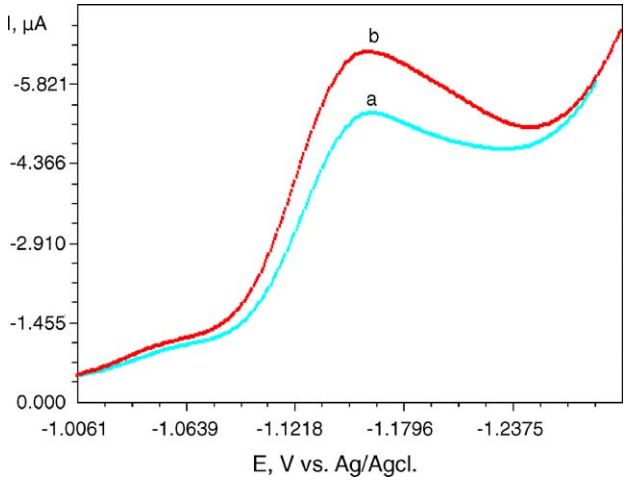

Fig. 3. Differential pulse voltammograms of 8×10^{-5} mol/L (a) and 1×10^{-4} mol/L (b) ofloxacin solutions at SMDE. Experimental conditions: 0.2 M $NaClO_4$; pulse amplitude = 0.010 V, pulse width = 10 ms and $\nu = 0.002$ mV s^{-1}.

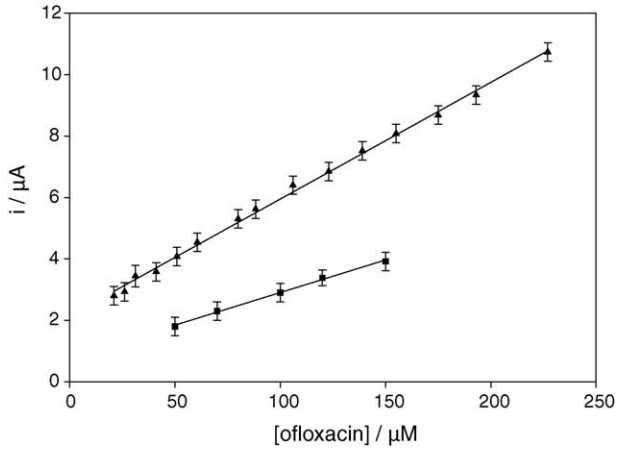

Fig. 4. Calibration curves of ofloxacin obtained with DPV (▲) and DPP (■). Current values recorded at $E = -1.17$ V (vs. Ag/AgCl). Experimental conditions: see Fig. 1; error bars: see Fig. 2.

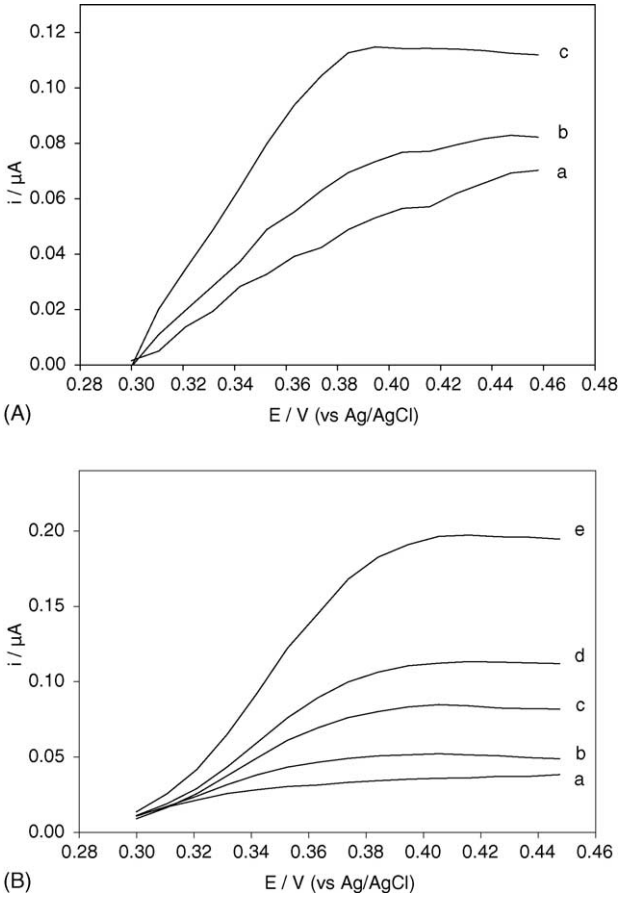

Fig. 5. Normal pulse voltammograms of different concentrations of diclofenac: (A) 1×10^{-6} mol/L (a); 1.4×10^{-6} mol/L (b); 2×10^{-6} mol/L (c) at CNTP electrode. (B) 1×10^{-6} mol/L (a); 2×10^{-6} mol/L (b); 3×10^{-6} mol/L (c); 4×10^{-6} mol/L (d); 5×10^{-6} mol/L (e) at CP electrode. Experimental conditions: 0.2 M $NaClO_4$; electrode diameter = 3 mm; pulse width = 50 ms; interval time = 5 s; $\nu = 0.002$ V s^{-1}. Voltammograms recorded after correction for base current.

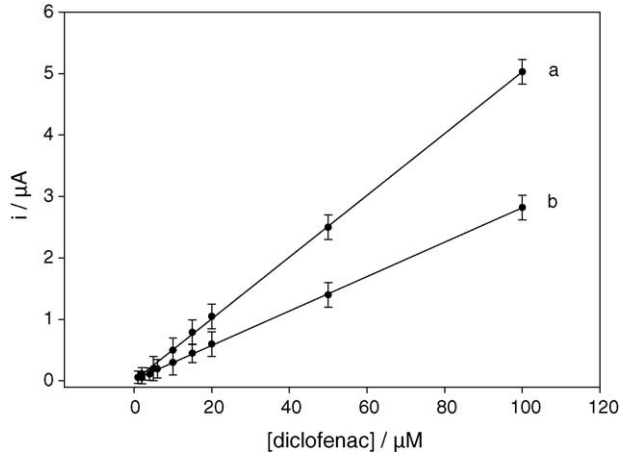

Fig. 6. Calibration curves of diclofenac obtained with NPV at CNTP (a) and CP electrodes (b). Current values recorded at $E = 0.4$ V (vs. Ag/AgCl). Experimental conditions: see Fig. 1; error bars: see Fig. 2.

concentrations of diclofenac obtained with CNTP (Fig. 5A) and CP electrodes (Fig. 5B). The relative calibration lines are shown in Fig. 6. The CNTP electrodes clearly show higher sensitivity, whereas the linearity interval is about the same for both paste electrodes. This result was expected, because of the high surface area of carbon nanotubes compared with normal graphite. However, both CNTP and CP electrodes gave better results than those obtained with the conventional GC electrode (not shown), because of the very low capacitance of the paste electrodes [21], which allows lower detection limits to be achieved.

Fig. 7 shows normal pulse voltammograms obtained at varying propranolol concentrations in 0.1 M NaOH at a gold electrode. The effect of different pH on peak sensitivity and morphology was investigated by CV and indicated 0.1 M NaOH as the pH of choice. Cyclic voltammograms carried out in 0.1 M NaOH showed a broad oxidation peak at about +400 mV (versus Ag/AgCl) (not shown), whereas

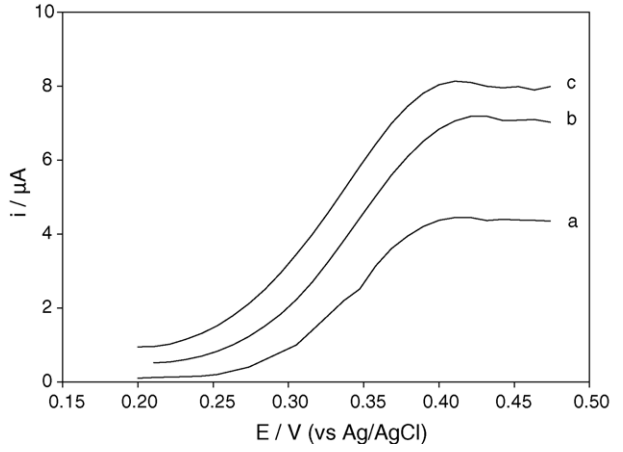

Fig. 7. Normal pulse voltammograms of 1×10^{-6} mol/L (a); 5×10^{-6} mol/L (b); 1×10^{-5} mol/L (c) propranolol solution at Au electrode. Experimental conditions: 0.1 M NaOH; electrode diameter = 2 mm; pulse width = 50 ms; interval time = 5 s; $\nu = 0.002$ V s^{-1}.

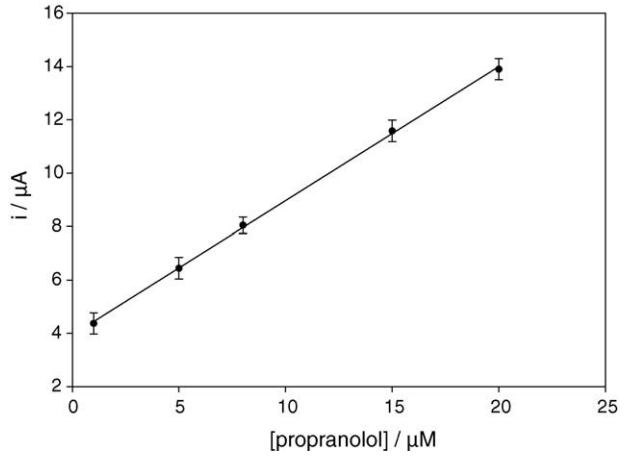

Fig. 8. Calibration curves of propranolol obtained with NPV at Au electrode. Current values recorded at $E = 0.4$ V (vs. Ag/AgCl). Experimental conditions: see Fig. 5; error bars: see Fig. 2.

no oxidation signal was obtained in 0.2 M $NaClO_4$. This was probably due to partial deprotonation of the hydroxy group, which occurs at pH 13. It seems reasonable to presume that this group could now undergo an oxidation process, which was responsible for the oxidation peak observed in the forward scan.

The relative calibration curve is shown in Fig. 8. Also for propranolol, NPV showed good sensitivity, although the linearity interval was not very large.

3.2. Calibration curves

Quantitative evaluation of the pharmaceutical compounds was based on the dependence of peak current (in CV, DPP and DPV) or plateau current (in NPV) on drug concentration. As already shown, the calibration lines for all the compounds were found to be linear over quite large intervals. Table 1 lists the linear regression equations obtained for all drugs by the various techniques and electrodes.

3.2.1. Limits of detection and quantification

Limits of detection (LOD) and quantification (LOQ) were calculated using the relation kS.D.$_{a/b}$ [23] where $k = 3$ for LOD and 10 for LOQ, S.D.$_a$ is the absolute standard deviation of the intercept and b the slope of the calibration curve. LOD and LOQ values are listed in Table 1. It is interesting to note that the pulse techniques showed the lowest LOD values for all drugs. In particular, DPV revealed the lowest detection limits for ofloxacin and DPP for clofibric acid. As for diclofenac and propranolol, NPV showed very high sensitivity, allowing the determination of both drugs at detection limits 10 times lower than those of all the other compounds.

3.2.2. Reproducibility

The inter-day reproducibility of the methods with the lowest LOD was evaluated by making six independent measurements on six consecutive days on a solution containing

Table 1
Analytical parameters obtained with various voltammetric techniques for pharmaceutical active compounds

PhACs	CV				DPP				DPV			
	Linear regression equation[a]	Linearity range (µM)	LOD (µM)	LOQ (µM	Linear regression equation[a]	Linearity range (µM)	LOD (µM)	LOQ (µM)	Linear regression equation[a]	Linearity range (µM)	LOD (µM)	LOQ (µM)
Clofibric acid	$y = (0.0117 \pm 0.0002)x - (0.14 \pm 0.04)$	35–260	9.0	30.0	$y = (0.1292 \pm 0.0014)x + (0.6 \pm 0.2)$	50–250	4.7	15.7	$y = (0.0166 \pm 0.0007)x + (1.78 \pm 0.09)$	50–220	16.4	54.6
Ofloxacin	–	–	–		$y = (0.0211 \pm 0.0005)x + (0.78 \pm 0.06)$ CP electrode	50–150	7.7	25.6	$y = (0.0385 \pm 0.0006)x + (2.08 \pm 0.07)$ Au electrode	20–230	5.2	17.2
	CNTP electrode											
	Linear regression equation[a]	Linearity range (µM)	LOD (µM)	LOQ ((M)	Linear regression equation[a]	Linearity range (µM)	LOD (µM)	LOQ ((M)	Linear regression equation[a]	Linearity range (µM)	LOD (µM)	LOQ (µM)
NPV												
Diclofenac	$y = (0.0503 \pm 0.0007)x + (0.00 \pm 0.01)$	1–100	0.9	2.96	$y = (0.0281 \pm 0.0004)x + (0.013 \pm 0.007)$	2–100	0.8	2.7	–	–		
Propranolol	–	–			–	–			$y = (0.503 \pm 0.007)x + (3.93 \pm 0.08)$	1–20	0.5	1.6

[a] y (µA) = $(a \pm$ S.D.$_a) + (b \pm$ S.D.$_b) \times$ (µM) where a is intercept, b slope and S.D.$_a$ and S.D.$_b$ standard deviations of intercept and slope, respectively.

Table 2
Recovery of pharmaceutical active compounds in distilled water samples at various pH after the SPE step

PhACs	Technique	Recoveries (%)		
		pH 3	pH 5	pH 7
Clofibric acid	DPP	91.2	84.4	47.3
Ofloxacin	DPV	87.1	78.7	72.6
Diclofenac	NPV[a]	92.5	75.3	54.5
Propranolol	NPV[b]	61.2	84.2	90.3

[a] With CNTP electrode.
[b] With Au electrode.

1×10^{-4} mol/L of each drug. Measurements were carried out on fresh electrode surfaces. DME and SMDE were automatically renewed, CNTP and CP electrodes were freshly prepared every day, and the Au electrode was carefully cleaned before use.

Inter-day reproducibility was quite good for all methods, although NPV showed slightly higher R.S.D. values (1.5 for diclofenac, 1.8 for propranolol) compared with DPV (1.1 for ofloxacin) and DPP (0.9 for clofibric acid). This may be ascribed to the different nature of the electrodes: DPV and DPP were performed with mercury electrodes, which allow perfectly constant renewal of their surface, as mentioned above, whereas this was not possible with the CNTP and Au electrodes used for NPV.

3.3. Recovery studies

The proposed methods were applied to the determination of the four drugs in spiked river water samples from river Tiber. An SPE step was performed as an enrichment factor. Following the procedure (see Section 2.4), an enrichment factor of 100 was achieved for all drugs with the exception of propranolol, for which an enrichment factor of only 20 was obtained, always assuming 100% recovery.

Unspiked samples (blanks) were previously analysed with the electrochemical method after the SPE step, and no amounts of the tested drugs were detectable.

In order to optimise the pH of the extraction step, distilled water samples containing a concentration of 5×10^{-7} mol/L of all drugs at different pH values were used. The extracts at different pH were analysed by DPV, DPP and NPV, and drug concentrations were determined using the previously established calibration plots. Results are shown in Table 2, and allowed us to choose the optimum pre-concentration pH for each drug. In particular, this value was 3 for clofibric acid, diclofenac and ofloxacin, and 7 for propranolol.

Two simulated real samples – that is, surface water samples from the same sampling location, spiked with 5×10^{-7} mol/L of the four drugs – were then analysed by DPV, DPP and NPV. Results obtained are listed in Table 3. The extract at pH 3 gave very good recovery for clofibric acid, quite good recoveries for ofloxacin and diclofenac, and excellent recovery for propranolol.

Table 3
Recovery of pharmaceutical active compounds in river water samples after SPE step

	Technique	Initial concentration (μM)	Final concentration (μM)	Recoveries (%)
Extract pH 3				
Clofibric acid	DPP	0.5	38.2[a]	76.3
Ofloxacin	DPV	0.5	27.6[a]	55.3
Diclofenac	NPV[b]	0.5	44.2[a]	88.4
Extract pH 7				
Propranolol	NPV[c]	0.5	9.04[d]	90.4

[a] After an enrichment factor of 100.
[b] With CNTP electrode.
[c] With Au electrode.
[d] After an enrichment factor of 20.

4. Conclusions

Four important pharmaceutical active compounds were electrochemically characterized using various voltammetric techniques and electrodes. Pulse techniques such as DPV, DPP and NPV gave the best results with all drugs in terms of sensitivity, linearity range and detection limits.

These techniques were then applied to the determination of the same drugs in spiked river water samples, after a preliminary enrichment step of two orders of magnitude based on solid-phase extraction.

The main advantages of the method based on SPE/pulse voltammetry are that it can be applied directly to analysis of surface waters without any separation or derivatization of samples, and it is simple, rapid and inexpensive.

Unfortunately, the method cannot be applied to analysis of real environmental samples, because its sensitivity does not allow determination of the drugs at their actual concentrations in surface waters (10^{-7} g/L), even after the enrichment step. Nevertheless, as the concentrations of drugs most frequently found in surface waters have been increasing at a dramatic rate – from 10^{-9} to 10^{-7} g/L in the last 20 years – it will be possible, in principle, to use this SPE/pulse voltammetric method as a good alternative to high-cost and time-consuming chromatographic methods.

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