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Determination of some β-Blockers by Electrochemical Detection on Polycrstalline Gold Electrode after Solid Phase Extraction (SPE)

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Dedicated to professor Pier Giorgio Zambonin on the occasion of his 80th birthday.

Abstract: The electroanalytical characterization and determination of three selected β-blocker agents, namely propranolol, atenolol and nadolol using cyclic voltammetry and differential pulse voltammetry (DPV) in phosphate buffer solution (pH 2.5) plus 22% acetonitrile (ACN), was described. The analytes were characterized through their electrooxidation processes on polycrystalline gold electrodes. The analytical determination of the selected molecules was performed using the differential pulse voltammetry (DPV) at pH 2.5. Under DPV conditions, the detection limits (LODs) ranged between 5 μM and 20 μM for propranolol and atenolol, respectively. For all investigated molecules, two well-defined ranges of linearity Ip vs analyte concentration have been identified which correspond to specific calibration parameters. Calibration graphs (Ip vs concentration) considered in the first interval of linearity, shown correlation coefficients >0.99. A solid phase extraction (SPE) procedure using a polymeric mixed-mode cationic sorbent (Strata-X-C), was studied and optimized. The proposed DPV-SPE method was successfully applied for the determination of propranolol in several pharmaceutical formulations and urine sample, with results in close agreement with those obtained using traditional liquid chromatography technique coupled with spectrophotometric detection.

Keywords: Propranolol · Gold electrode · Differential pulse voltammetry · Solid-phase extraction · Real matrices

1 Introduction

β-blockers, also called β-adrenergic antagonists such as: propranolol (Pp), atenolol (At), nadolol (Nd), bisoprolol, etc. are of therapeutic interest in the treatment of various cardiovascular disorders, angina pectoris, cardiac arrhythmia, hypertension and associated pathologies, etc. [1–3]. The propranolol, 1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol, is considered the prototype drug of β-adrenergic blockers. Its main medical use has changed, in the last years, from therapy of cardiovascular diseases to therapy of infantile haemangiomas in pediatric patients. In fact, Pp orally administered, has a consistent and rapid therapeutic effect, leading to considerable shortening of the natural course of infantile haemangiomas with good clinical tolerance. In addition, this therapeutic drug could also be used in some sports requiring intense concentration as a doping agent [4]. In this respect, the International Olympic Committee has classified the Pp as doping specie and has included in a list of forbidden substances.

Although these β-blockers are widely used in many therapeutic contexts, high plasma concentration of the Pp, Nd, At, etc. can cause severe respiratory problems such as asthma, adult respiratory distress syndrome and heart failure [5]. In particular, atenolol can be associated with an increased risk of diabetes mellitus and increased risk of stroke [6]. Thus, these drugs require continuous monitoring in biological fluids and/or pharmaceutical formulations for adjusting the corresponding dose in order to prevent hazardous effects on health.

Substantial amounts of these pharmaceuticals and their metabolites get into the wastewater after excretion and finally in surface water due to an incomplete elimination in wastewater treatments. In this respect, recent ecotoxicological studies show that several aquatic organisms are sensitive to the presence of some β-blockers and/or their derivatives [7]. In particular, the contact of hospital pollutants with aquatic ecosystems leads to a severe risk on biological balance of natural environments. On the basis of these considerations, simple, sensitive, accurate and inexpensive analytical methodologies for quantification of these compounds in biological fluids, environmental contexts and pharmaceutical formulations is required.

Numerous analytical methods proposed for the determination of the common β-blockers in various contexts are mainly based on reverse-phase liquid chromatography generally coupled with spectrophotometric UV-Vis detection, fluorescence or mass spectrometry devices used as on-line detectors [8–13]. Other analytical methodologies based on chemiluminescence measurements [14], diffuse

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reflectance spectroscopy [15] or near-infrared spectrometry [16], have also been developed. The voltammetric methods are generally sensitive, rapid, inexpensive and can be efficiently used as analytical techniques [17–20], as alternative to traditional methods based on chromatographic procedures for the determination of these drugs. In this respect, several electrochemical sensitive methods, using traditional, or in particular, modified electrodes based on composite graphite nanotubes or boron-doped diamond modified surfaces were successfully proposed for the direct determination of several β-blockers in synthetic and biological matrices [21–28]. Although gold electrode and/or modified electrodes based on the gold electrocatalyst are widely characterized and proposed as amperometric probes for the detection of several important classes of molecules, such as alcohols, carbohydrates, amines, DNA, antibiotics, etc. [29–32], studies regarding the electroanalytical determination of β-adrenergic blockers on this electrode material are lacking in the literature. In addition, data concerning the electrochemical behaviour, and in particular, the electrochemical characterization of propranolol at Au substrate electrode are scarce.

The aim of this study is focused on the electroanalytical characterization of a polycrystalline gold electrode toward the detection of some β-blockers (Pp, At and Nd) in acid medium by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. At the same time, a liquid chromatography method based on the strong cation exchanger mode was optimized and compared with the DPV technique toward the analytical determination of the selected β-blockers in real matrices such as pharmaceutical formulations and biological samples after the use of an optimized solid phase extraction (SPE) procedure. In fact, these molecules having similar pKa values (≅9.6–9.4), under acid conditions should be charged under cationic form and can be extracted with cation-exchange active phases.

2 Experimental

2.1 Reagents

All selected β-blockers were of analytical-reagent grade and were purchased from Sigma-Aldrich (Steinheim, Germany). Stock standard solutions of individual Pp, At and Nd at 2.5 mM were prepared in aqueous solutions using ultrapure water supplied by a Milli-Q RG unit from Millipore (Bedford, MA, USA). Solutions were prepared daily and used without further purification. LC grade acetonitrile (ACN), KH_2PO_4, H_3PO_4 and other chemicals were also purchased from Sigma-Aldrich.

2.2 Apparatus

Voltammetric measurements were performed with an Autolab PGSTAT 30 Potentiostat/Galvanostat (Eco Chemie, Utrecht, The Netherlands) and the relevant data were acquired using an Autolab GPES software package version 4.9. CV and DPV experiments were done in a three-electrode cell using a working gold polycrystalline disk electrode (Ø 2 mm) with a geometric area of ca. 0.03 cm^2, a saturated calomel electrode (SCE, 4 M KCl) reference electrode and a platinum counter-electrode (Amel, Italy).

The working electrodes before the CV or DPV experiments were polished with 0.05 μm α-alumina powder on a polishing micro-cloth, washed with ultrapure water and cycled in phosphate buffer solution composed of 45 mM KH_2PO_4 plus 100 mM H_3PO_4 (buffer pH 2.5) containing 22% ACN. All % compositions of solutions are expressed as volume percent (vol%). The working electrodes before of the experimental measurements, were cycled between −0.8 V and 2.0 V vs. SCE (100 mV s^{-1}) for 5 min.

The optimized operating conditions to obtain the differential pulse voltammograms were: modulation time, 0.1 s; modulation amplitude, 55.05 mV; interval time, 0.8 s; step potential, 5.1 mV. All current densities in this paper are quoted in terms of mA cm^{-2} of apparent geometric area of substrate electrode. All experiments were carried out at ambient temperature 20–22 °C). Dissolved oxygen does not interfere with the CV and DPV experiments, then they were carried out in not deaerated solutions.

All chromatographic experiments were performed using a metal-free pump Mod. PU-1580i (Jasco Corporation, Tokyo, Japan) equipped with a metal-free rotary injection valve Mod. 7725i (Rheodyne, Cotati, CA, USA) with a 100 μL sample loop. Analytical separations of selected β-blockers were achieved with a Supelcosil LC-SCX HPLC cation exchange column, 5 μm 25 cm × 4.6 mm I.D., (Supelco, Bellefonte, PA, USA). Detection by UV absorption was performed using an UV-VIS programmable-wavelength detector series 200 (Perkin Elmer, Shelton, CT, USA), operating at a fixed wavelength of 220 nm. A personal computer equipped with an in-house software allowed acquisition and processing of chromatograms. The mobile phase was protected from oxygen and other gaseous dissolved species by an on-line degasser system Series 1050 (Hewlett Packard, Avondale, PA, USA). Occasionally, the column was washed and reconditioned using about 10 column volumes (0.6 mL min^{-1}) each of 1) water, 2) 95%/5% water/acetonitrile, 3) acetonitrile, 4) 95%/5% acetonitrile/water, 5) water.

2.3 Solid Phase Extraction Procedure (SPE)

An effective sample pretreatment procedure is required in order to enhance the analyte concentration above the detection limits, to achieve acceptable selectivity and to remove interfering compounds present in complex real matrices. The SPE experiments were carried out using the Strata-X-C polymeric strong cation cartridges (33 μm, 200 mg/3 mL) purchased from Phenomenex (Torrance, CA, USA). In addition, the extraction experiments were carried out using a dedicated extraction system mod. VIS-

IPREP™ SPE Vacuum Manifold 57030-U (Supelco, Bellefonte, PA, USA). The cartridges before use were activated and conditioned by drawing 3 mL of methanol through followed by 3 mL of water. The aqueous solutions containing the selected β-blockers, conditioning and extracting solvents were passed through the cartridges at about 1 mL/min.

2.4 Sample Preparation

Commercial tablets of propranolol, Inderal 40 mg from AstraZeneca Reims (France) and the galenic form for oral administration of Pp were purchased from a local pharmacy. Urine sample was obtained from a normal volunteer subject (female, aged about 35–36 years) following oral administration of Pp capsule. A tablet of the pharmaceutical formulation (0.20 g) was thoroughly ground until a fine powder was obtained, was accurately weighted, dissolved in 25 mL of solution 45 mM KH_2PO_4, 100 mM H_3PO_4 and 22 % ACN (vol %) (pH≅2.5) and sonicated at ambient temperature for 20 min. The resulting solution was filtered through a Whatman filter (0.45 μm) diluted at 100 mL and loaded on SPE cartridges. Similarly, the galenic form of the Pp was directly diluted with the phosphate buffer solution (pH≅2.5) plus 22 % ACN (100 mL) (vol %) and loaded on SPE cartridges. The spiked urine samples (10 mL), were diluted at 100 mL with the phosphate buffer solution (45 mM KH_2PO_4, 100 mM H_3PO_4 pH≅2.5) plus 22 % ACN (vol %), filtered with Whatman filter (0.45 μm) and the relevant solutions were loaded on SPE cartridges. After the loading procedure of the cartridges, the analyte was eluted with 10 mL ACN containing 4 % NH_3 (28–30 wt %), adequately diluted with phosphate buffer and then analysed using DPV and chromatographic technique (Strata-X-C-HPLC cation exchange column). The concentration of the Pp was determined by a linear-square regression procedure using the method of standard addition, adding five consecutive (n = 5) adequate volumes of a standard solution containing 300 μM Pp.

3 Results and Discussion

3.1 Electroanalytical Characterization

Cyclic voltammetry was used to show the electrochemical behavior and activity of studied β-blockers on a polycrystalline gold electrode in buffer phosphate medium. A typical cyclic voltammogram of the Au electrode is shown in Fig. 1 (black curve). The main electrochemical profiles of the gold polycrystalline electrodes in buffer phosphate medium show a large anodic peak (Ia) observed during the anodic sweep at about 1.2 V vs. SCE corresponding to the hydroxide formation of both Au(I) and Au(III) species. A massive oxygen evolution process is observed for applied potentials higher than 1.5 V. During the negative scan of potentials, a well defined cathodic peak (Ic) was observed at about 0.75 V and corresponds to reduction of

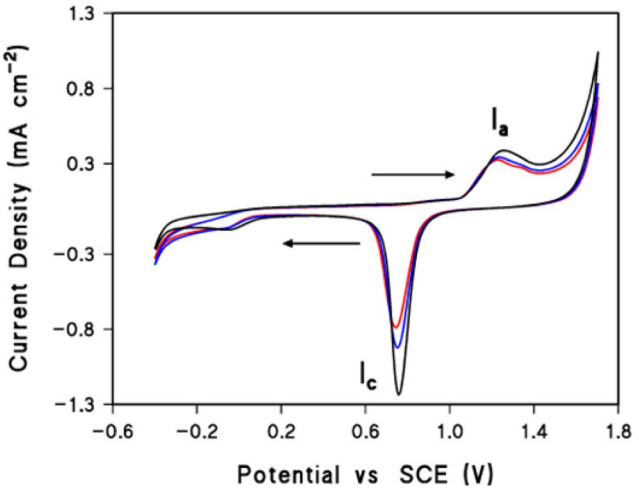

Fig. 1. Cyclic voltammograms (5th cycle) at a polycrystalline Au electrode; black curve: phosphate buffer solution pH = 2.5 (0.45 mM KH_2PO_4, 100 mM H_3PO_4); blue curve: after addition at the phosphate buffer medium 10 % ACN; red curve: as black curve, plus 20 % ACN; scan rate: 50 mV s^{-1}.

the gold oxide species formed during the preceding anodic scans. The blue and red curves of Fig. 1 show the electrochemical profile of the Au polycrystalline electrode in buffer phosphate medium containing increasing concentration of acetonitrile (ACN), a common organic modifier used in liquid chromatography for the separation of complexes organic matrices. As can be seen, the presence of the organic modifier induces a partial attenuation of the peak Ic, and Ia, indicating a strong adsorption process of ACN on the active sites of the Au electrode. Thus, the ACN specie partially inhibits the formation of gold oxide species (i.e., AuOH, AuO, Au_2O_3, $Au(OH)_3$, etc.) and its Au metal reformation [29]. A similar behavior was observed previously on polycrystalline Au electrodes in phosphate solutions containing 10 % ACN [30]. Figure 2 shows the voltammetric behavior of the propranolol specie on the Au electrode obtained in absence of ACN. After increasing addition of 3 mM Pp a large and complex anodic wave is observed on the anodic scan beginning at about 0.8 V vs. SCE, which completely overlaps the gold oxide formation and oxygen evolution reaction, while the cathodic peak Ic was significantly decreased in presence of propranolol. It is interesting to underline that, the complex oxidation wave decreased markedly during subsequent cycles of the applied potentials. This result suggests that, the considered β-blocker or its intermediate reaction preferentially adsorbs on the catalytic sites of the electrode surface and consequently induces the formation of passivation layers with subsequent deactivation of the active gold catalyst. A similar electrochemical behavior was observed when At and Nd were tested. In sharp contrast to the electrochemical behavior of the Pp observed in absence of ACN, enhancement and temporal reproducibility of the oxidation currents were observed in presence of 20 % ACN in phos-

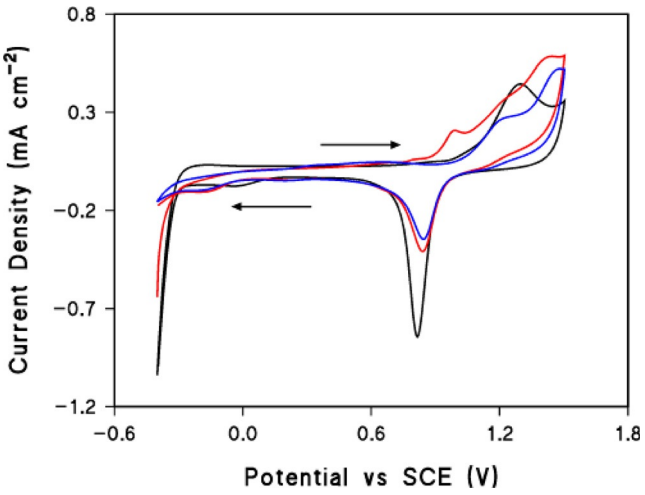

Fig. 2. Behaviour of Au electrode in phosphate buffer solution (pH = 2.5) in absence of ACN (black curve, 5th cycle). Red curve: as black curve in presence of 3 mM Pp: (1th cycle); blue curve: as red curve, 5th cycle; scan rate: 50 mV s^{-1}.

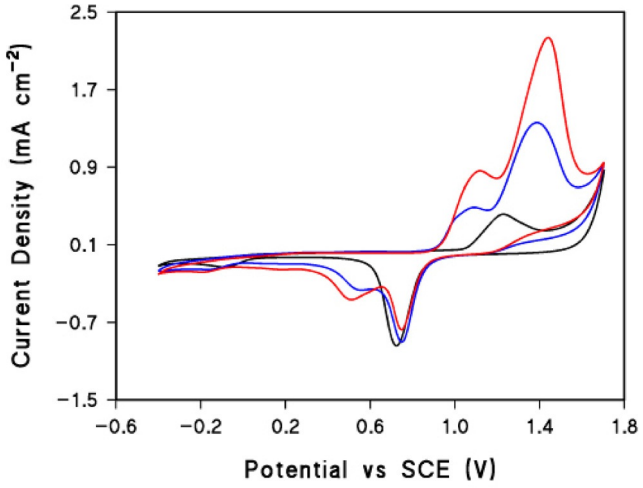

Fig. 3. Voltammograms at Au electrode in phosphate buffer solution at pH = 2.5 plus 22 % ACN (black curve, 5th cycle), in presence of 2 mM Pp (blue curve, 5th cycle) and 4 mM Pp (red curve, 5th cycle). Scan rate, 50 mV s^{-1}.

phate buffer solutions. Figure 3 shows the relevant results. As can be seen, the electrode responds rapidly and reproducibly to millimolar concentration changes of Pp and the resulting calibration plot appears linear over the range comprised between 1.0 mM and 6.0 mM with a correlation coefficient better than 0.99. Other investigated molecules such as nadolol and atenolol show similar voltammetric profiles; differences are related to the magnitude of the oxidation currents. The sensitivity evaluated in terms of the anodic peak current (i.e., between 1.2 V and 1.5 V vs SCE), decreases in the order: Pp > Nd > At. The effect of the pH on the voltammetric response of Pp was investigated in the range comprised between 2 and 5. The peak potential shifted to more cathodic potentials as the pH was increased, and a well resolved anodic peak was obtained at values comprised between 2.5 and 3.2.

The effect of the scan rate (ν) on the oxidation peak currents (10–400 mV s^{-1}) relevant to propanolol, nadolol and atenolol was evaluated. The resulting log/log plots display linearity, with slopes ranging between 0.7 and 0.9. These results certainly mean that the overall electrooxidation process of the analytes can be controlled by a rate determining step involving surface reactions. In addition, the experiments suggest that, analyte preferentially adsorbs on the electrode surface and consequently displaces the ACN molecules. As already observed (see Fig. 2 and 3), the ACN induces a significant contribution in order to prevent fouling steps during the entire electrooxidation process of the selected β-blockers. In other words, the presence of ACN perturbs the kinetics of Au oxide formation, inducing a favorable adsorption/desorption process of analyte species. Thus, although the specific reaction mechanism which involves ACN in the overall electroxidation process does not seem clear and requires further specific investigations, it is clear that ACN plays a significant role in terms of sensitivity and temporal stability for the electrooxidation of the Pp, At and Nd on the gold electrode surface.

In order to verify the real analytical performance of the electrochemical strategies for the determination of the electroactive molecules, the high-resolution and high-sensitivity electrochemical technique such as the differential pulse voltammetry (DPV), square wave voltammetry (SWV), pulsed amperometric detection (PAD), etc., should necessarily be used. In this respect, various chemically modified electrodes (CMEs) were characterized as electroanalytical probes for the determination of some β-blockers using these pulsed amperometric techniques (18–21,27,28). Figure 4 shows a typical set of optimized DPVs obtained in buffer phosphate medium at pH 2.5

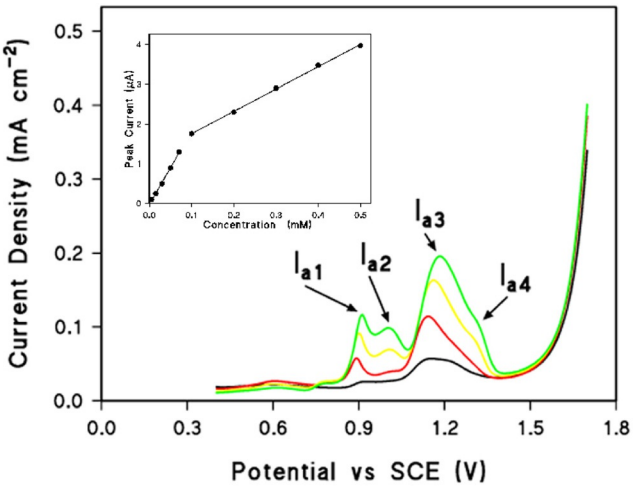

Fig. 4. Typical set of DPVs obtained at a Au polycrystalline electrode in phosphate buffer solution at pH 2.5 containing 22 % ACN (black curve); 20 μM Pp (red curve), 40 μM Pp (yellow curve) and 60 μM Pp (green curve); DPV conditions: modulation time, 0.1 s; modulation amplitude, 55.05 mV; interval time, 0.8 s; step potential, 5.1 mV. Inset: the relevant calibration graphs of peak current (I_{p1}) vs. Pp concentration.

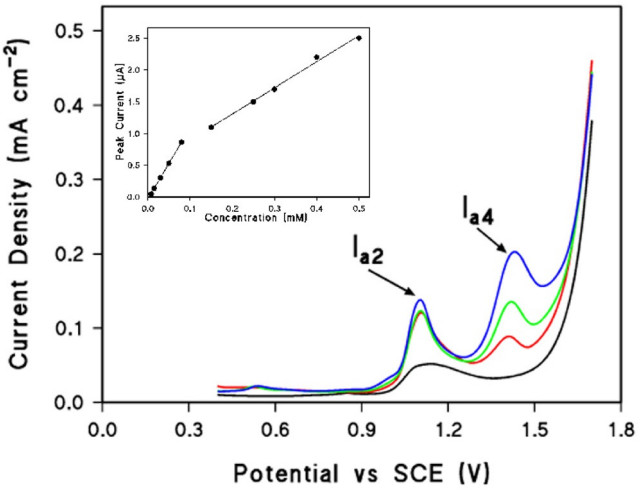

Fig. 5. Set of DPVs obtained at a Au polycrystalline electrode in phosphate buffer solution at pH 2.5 containing 22% ACN (black curve); 20 μM Nd (red curve), 40 μM Nd (green curve) and 80 μM Nd (Blue curve); DPV conditions: as reported in Fig. 4. Inset: the relevant calibration graphs of peak current (I_{p4}) vs. Nd concentration.

(45 mM KH_2PO_4, 100 mM H_3PO_4, 22% ACN) containing increasing concentration of Pp. The DPV parameters such as: modulation potential, interval time, frequency of measurements, etc. were experimentally defined in order to optimize the better signal/noise ratio of the measurements. As can be seen, a reproducible complex oxidation wave, in the range of potential comprised between 0.7 V and 1.4 V, was observed. Four anodic peaks, defined as Ia_1, Ia_2, Ia_3 and Ia_4 centered at 0.85 V, 1.0 V, 1.15 V and 1.35 V, respectively, were evidenced. These peaks current slightly increase (ca 10%–15%) with increasing time of accumulation (up 30 s) considering the range of potentials comprised between 0.0 and 0.4 V vs SCE. Quantitative evaluation of the compound was based on the dependence of peak current (Ia_1), vs. Pp concentration. The inset in Fig. 4 shows the relevant calibration graph for the analyzed molecule. As can be seen, the fitted curve shows a representative behavior of reaction kinetically limited at the electrode surface by adsorption processes of reactants and/or desorption steps of intermediate or reaction products. As consequence, the calibration curve of Pp under DPV conditions shows two distinct ranges of linearity Ip vs. concentration. Figure 5 shows the relevant I-E curves of the nadolol obtained under DPV conditions in buffer phosphate medium at pH 2.5 containing 22% ACN. In presence of Nd a broad anodic peak at applied potentials higher than 0.9 V vs SCE was observed. In particular, the voltammograms show a large peak centered at about 1 V (Ia_2) which is not suitable for analytical determinations. In fact, after the initial addition of 20 μM Nd, the further increases in analyte concentration was not accompanied with an increase in Ia_2 peak current due to surface saturation phenomenons. These findings indicate that the oxidation processes are surface controlled reactions associated at complex adsorption steps between analytes and surface catalytic sites. The Ia_4 anodic peak observed at about 1.4 V increases with the analyte concentration and the calibration curve, in the same manner of the Pp, was described by two distinct regression lines (see the relevant inset figure). A similar current-potential profile, obtained under DPV conditions was observed for atenolol, the only difference is related to the lower sensitivity of the At compared to Nd. The results including the first and second linear ranges, sensitivities, detection limits (LODs), coefficient of correlations (r^2), precision (% RSD), etc. are summarized in Table 1. As can be seen, two well-defined ranges of linearity Ip vs analyte concentration have been identified which correspond to specific calibration parameters. The LODs determined at a signal-to-noise ratio equal to 3 at the lowest added concentration of analytes, are comprised between 5 μM and 20 μM for propranolol and atenolol, respectively. Calibration graphs (Ip vs concentration) in the considered intervals, were all linear with correlation coefficients >0.99. The precision, estimated as percent relative standard deviation (% RSD) of ten consecutive DPV experiments of test solution containing 10 μM of each analyte ranged between 5% and 6%. The LODs obtained in this study, although encouraging and appropriate for routine applications, are generally higher than those specified in other studies based on the spectrophotometric or mass spectrometry procedures [8–13, 33–36]. On the other hand,

Table 1. Quantitative analytical results of the investigated β-blockers by DPV technique.

	1° linear range (r^2)	2° linear range (r^2)	LOD	RSD%
Propranolol (Pp)	5 μM–100 μM (>0.99) y=18.6(x)−0.02	120 μM–500 μM (>0.99) y=5.6(x)+1.2	5 μM	5
Nadolol (Nd)	8 μM–100 μM (>0.98) y=11.2(x)−0.03	120 μM–500 μM (>0.99) y=4.4(x)+0.34	8 μM	6
Atenolol (At)	20 μM–150 μM (>0.99) y=4.3(x)−0.03	160 μM–500 μM (>0.98) y=2.4(x)+0.24	20 μM	6

Experimental conditions: Phosphate buffer solution at pH 2.5 containing 22% ACN; DPV conditions: modulation time, 0.1 s; modulation amplitude, 55.05 mV; interval time, 0.8 s; step potential, 5.1 mV; The regression parameters of the relevant linear plots were evaluated as Ip (μA) vs. conc. (mM); The peak current was evaluated at 0.9 V (I_{a1}) for Pp, while for Nd and At the peak currents were evaluated at 1.4 V (I_{a4}); The percent relative standard deviation (% RSD) was estimated on ten consecutive DPV experiments of test solution containing 10 μM of each analyte. y indicates the peak current (I), while x indicates the analyte concentration (μM).

DPV techniques based on the chemically modified electrodes show LODs equal or less than 0.5 μM [21–28]. However, the use of robust and stable polycrystalline Au electrode coupled with an efficient extraction/concentration procedure can modulate effectively the desired sensitivity of the present analytical methodology for routine determination of these molecules in real matrices.

The lower limit of quantifications (LOQs), evaluated at a signal-to-noise ratio of 10 were 12 μM for Pp, 15 μM for Nd and 40 μM for At and they can be considered acceptable for potential analytical applications.

3.2 Solid Phase Extraction (SPE) of β-Blockers

Solid phase extraction (SPE) has been proven to be an effective tool for the sample pretreatment of biological fluids or wastewaters in order to determine β-adrenergic antagonists in traces levels [10,12,22,33–37]. In particular, considering that these molecules should be present under the cationic form at low pH values, a cation-exchange active phases can be proposed for the pretreatment procedure of these molecules [37]. Although the most common extraction or chromatographic treatments of these compounds are based on the use of C_{18} stationary phases, in this study we have tested the potential applicability of the cation-exchange phases in moderate acid solution (pH 2.5) for the extraction/concentration of the considered β-blockers. Thus, cation mixed-mode polymeric sorbent (Strata-X-C) based on copolymer styrene-divinylbenzene backbone with sulfonic active groups as well as other polar moieties capable of hydrogen bonding and dipolar interactions, were tested for the SPE pretreatment of Pp, Nd and At. A standard mixture of 100 mL of a phosphate buffer solution (45 mM KH_2PO_4 plus 100 mM H_3PO_4) containing 200 μM of Pp, Nd and At was passed at about 1 mL min^{-1} through the cartridges and successively eluted with 10 mL of ACN solvent containing NH_3. The resulting solutions were diluted 1:10 with mobile phase and analyzed by liquid chromatography (LC) coupled with a UV detector operating at 220 nm. Figure 6 shows a typical comparison between the chromatogram of a 200 μM of standard mixture of analytes and the relevant extracts from Strata-X-C cartridge eluted with 10 mL of ACN solution containing 2% or 4% NH_3. As can be seen, under moderate acidic conditions (pH 2.5), the studied molecules exist in the protonated form, and consequently, a strong interaction with the anionic sulfonic active sites of the Strata-X-C cartridge can be hypothesized, which provided good loading capacity for all investigated analytes. After the strong adsorption process of analytes, the cartridge must be washed with a suitable solvent that neutralizes ionic interaction between the analyte and the sites of the stationary phase. Thus, an extracting eluent having an sufficiently high pH value and chemical affinity with neutralized analytes can be proposed to elute effectively these species. In this study ACN was used at various concentrations of ammonia as a extracting solvent. After the loading process,

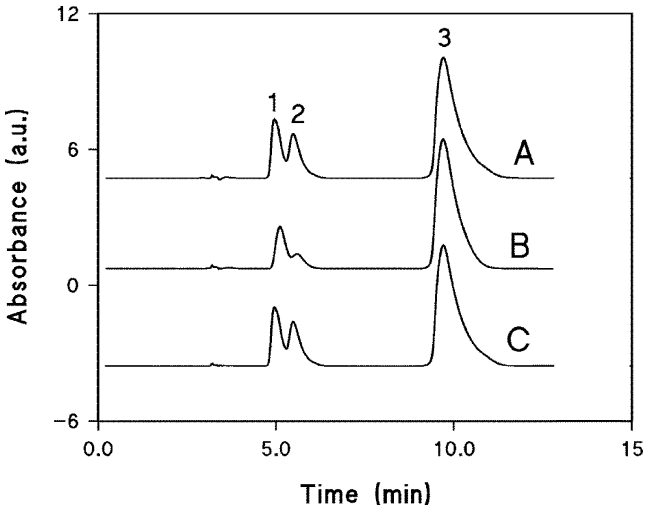

Fig. 6. Chromatograms (LC) of the selected β-blockers: 1) At, 2) Nd, 3) Pp. Conditions: Supelcosil LC-SCX HPLC cation-exchange column; UV detector operating at 220 nm; mobile phase: phosphate buffer solution (45 mM KH_2PO_4 plus 100 mM H_3PO_4) containing 22% ACN. A) standard mixture of the β-blockers 200 μM of each; B) 100 mL of standard solution of analytes passed at 1 mL min^{-1} through the SPE cartridges and successively eluted with 10 mL of ACN solvent containing 2% NH_3. The resulting solution was diluted 1:10 with mobile phase; C) as B), but the elution process was carried out with 10 mL of ACN solvent containing 4% NH_3.

the retained Pp was efficiently eluted with ACN solution containing 2% NH_3, while Nd and At were eluted together using the same organic eluent containing 4% NH_3. The relevant results, expressed as percent recoveries of the considered analytes are summarized in Table 2. The recovery efficiency was determined by comparison of chromatographic peak heights of the respective compounds following extraction to those obtained for unextracted 200 μM of standard mixture of analytes. In ab-

Table 2. Recoveries (%) for the tested β-Blockers after SPE pretreatment using the Strata-X-C cartridges and ACN as extracting solvent containing various NH_3 concentrations.

	[$NH_{3\%}$]	Recovery %
Propranolol	2	90
	4	>95
Nadolol	2	14
	4	89
Atenolol	2	62
	4	98

SPE conditions: Strata-X-C cartridge (200 mg/3 mL); 100 mL of phosphate buffer solution at pH 2.5 containing 200 μM of Pp, Nd and At were passed at 1 mL min^{-1} through the cartridges. The retained molecules were eluted with 10 mL of ACN solvent containing NH_3; The percent of recoveries were evaluated by comparing the chromatographic peaks of the standard solution of the analytes before and after the SPE process. The eluted solutions were analyzed by liquid chromatography (LC) using a Supelcosil LC-SCX HPLC cation-exchange column coupled with a UV detector operating at 220 nm.

sence of At, the calculated recoveries of Pp and Nd in presence of ACN containing 2% NH_3 were found to be 90% and 14%, respectively, indicating a good degree of separation between them. Consequently, these molecules were expected to be efficiently detected with good accuracy using DPV technique coupled with SPE procedure. In addition, the high loading capacity (or retention capacity) of the tested Strata-X-C cartridge, (i.e., 100 mL of a phosphate buffer solution containing 200 μM each of the analytes: Pp, Nd and At), combined with an high extraction efficiency (i.e, recoveries of 89%–98% using 10 mL ACN containing 4% NH_3 as extracting solvent), is able to achieve an effective method of pre-concentration of analytes and matrix cleaning. In this respect, the adopted SPE strategy allows easily to lower sensibly the detection limits of the studied analytes.

3.3 Analytical Determination of Pp by DPV-SPE Technique in Selected Contexts of Pharmaceutical Interest

Some selected real matrices having significant pharmaceutical interest such as commercial tablets of propranolol, a galenic form of Pp and urine samples were chosen as example for the determination of Pp under DPV conditions. Typical voltammograms of the pretreated urine sample are shown in Fig. 7. The concentration were calculated by a linear-square regression procedure using the method of the standard additions (five additions). Quantitative evaluation of the compound was based on the dependence of peak current (Ip_1), vs. Pp concentration, considering the first range of linearity signal vs. concentration (see paragraph 3.1). The correlation coefficients of the regression plots for the standard additions are generally >0.98. The recoveries were determined as the percent difference between the observed original amount experimentally determined and the first standard addition of Pp. Similar DPVs were obtained when tablet and galenic preparation of Pp were subjected to analysis and the experimental data were similarly treated as the analysis of the urine sample. In order to verify the analytical accuracy of the Pp determinations obtained by DPV proposed in this study, the same treated samples were analyzed by using a cation exchange chromatography coupled with

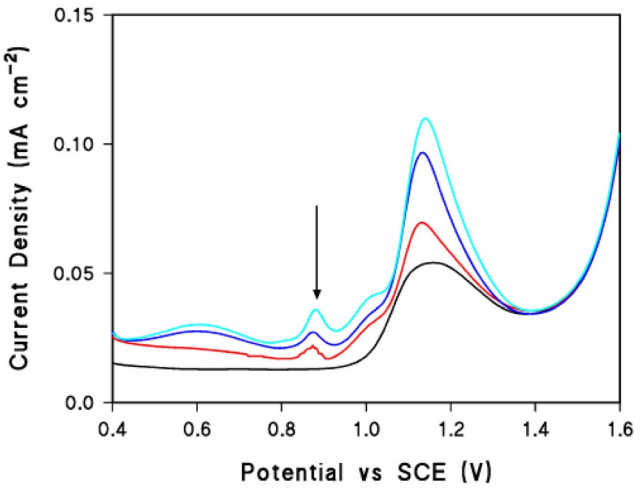

Fig. 7. DPV analysis of Pp in original urine sample spiked with 0.019 μg mL^{-1} Pp after SPE treatment using phosphate buffer solution (50 mL) at pH 2.5 containing 22% ACN (black curve); plus 1.0 ml of urine after SPE treatment (red curve), plus 1.0 mL of standard mixture 300 μM of analytes (blue curve); as red curve, plus 1.5 mL of standard anaytes (cyan curve). DPV conditions such as in Fig. 4. The real samples were treated as reported in the experimental section.

UV detection operating at a fixed wavelength of 220 nm and under isocratic chromatographic conditions. In this respect liquid chromatographic methods with UV detection scheme were already previously developed and used for Pp determination biological matrices or pharmaceutical preparations [11,33,38]. The relevant experimental results regarding the Pp determination in the selected real matrices are summarized in Table 3. As shown in this table, the differences between the DPV-SPE procedure proposed here and the more traditional chromatographic procedure coupled with UV detection yielded quite comparable results. In addition, the mean recoveries obtained by triplicate DPV measurements of the considered real samples ranged from 93% to 109%. The percent recoveries of the tablet and galenic form were calculated as differences between the nominal and the measured concentration. The percent recovery of the urine sample was calculated considering original urine free of Pp spiked with 0.064 nmol mL^{-1} Pp. All these results confirm the substan-

Table 3. Analytical determination of propranolol in some pharmaceutical formulations and urine samples by DPV-SPE and the cation-exchange chromatography coupled with UV detection procedure (LC-UV).

	DPV-SPE (mg)	Recovery (%)	LC-UV (mg)	Δ%
Tablet	33.4 ± 0.6 $y = 4\times10^{-7}(x) + 6\times10^{-8}$	94	35.2 ± 0.5	5
Galenic form	0.26 ± 0.04 $y = 1\times10^{-6}(x) + 7\times10^{-7}$	93	0.28 ± 0.09	8
Urine	0.21 ± 0.03 $y = 0.9\times10^{-6}(x) + 2\times10^{-7}$	109	0.19 ± 0.06	9

Samples treatment: as reported in the section 2.4. The determined amounts of Pp were referred to 1 tablet (≅0.2 g), 50 mL of galenic form and 10 mL of urine, respectively. The recoveries were determined as the percent difference between the observed original amount experimentally determined and the first standard addition of Pp. The term "x" indicates the analyte concentration (mM).

tial absence of interfering electroactive compounds present in the selected real matrices and/or time-dependent variations of the electrode properties in terms of catalytic activity and electrode resistance.

4 Conclusions

A rapid, accurate and inexpensive DPV based on the use of polycrystalline gold electrode as sensing probe for the quantitative determination of some β-blockers in buffer phosphate medium containing ACN solvent was developed. An efficient extraction procedure using a SPE technique based on the cation mixed-mode (Strata-X-C), has been optimized in order to obtain better results in terms of pre-concentration efficiency and solvent extraction capability of propanolol, adenolol and nadolol compounds. In this respect, acetonitrile (ACN) containing 2% or 4% NH_3, was successfully proposed as an efficient eluent for the quantitative elution of the considered β-blockers. The combined DPV-SPE technique was tested and advantageously proposed for the analytical determination of some β-blockers in real matrices such as pharmaceutical preparations (i.e., commercial tablets and galenic form of propranolol), and urine samples. In order to establish the real applicability of the proposed DPV-SPE method, the analytical results of the determination of the propranolol in real matrices were successfully compared with those obtained using a chromatographic procedure SCX-HPLC (strong cation-exchange chromatography), coupled with UV detection operating at a fixed wavelength of 220 nm.

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