Novel electrochemical sensor based on a glassy carbon electrode modified with carbon black/poly(allylamine hydrochloride) composite for multianalyte determination

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Abstract

We present a novel electrochemical sensor that was developed by modification of a glassy carbon electrode (GCE) with carbon black (CB) using poly(allylamine hydrochloride) (PAH) as a dispersant agent. The sensor (CB-PAH/GCE) was electrochemically characterized by cyclic voltammetry and electrochemical impedance spectroscopy, and the experiments demonstrated that the presence of CB contributed to the decrease in charge transfer resistance of the sensor. The CB-PAH/GCE was applied for voltammetric determination of dopamine (DA), paracetamol (PAR), amlodipine (AML) and rosuvastatin (RSV). Under the optimized experimental conditions in linear sweep voltammetry, DA, PAR, AML, and RSV presented a linear dependence to the anodic peak current within the range of 1.0 – 22; 2.4 – 27; 12 – 90; 7.8 – 40 μM, with a limit of detection of 0.17; 0.13; 1.02; 0.82 μM, respectively. The sensor was successfully applied to the simultaneous determination of DA, PAR, AML and RSV in pharmaceutical and environment samples, and DA, PAR in biological sample.

Keywords: simultaneous determination, carbon-based electrode, signal amplification, polymer, pharmaceuticals.

Introduction

Electrochemical chemical sensing offersThe modification of the electrode surface makes possible to control surface/solution interaction and solve problems such as slow electron transfer in electrochemical processes, and high overpotential of the redox process.[1,2] Carbon black (CB) is a nanomaterial with amorphous carbon in its structure, and it has attracted a lot of attention because of its advantageous properties, such as high superficial area, excellent conductivity, chemical and physical stability, and its low cost in comparison with other carbon materials.[3] According to Vicentini and collaborators, CB Vulcan XC-72R has produced enhanced electrochemical performance and presented better surface coating.[4]

For the immobilization of CB on the surface of the glassy carbon electrode (GCE), several materials have been reported in the literature, such as Nafion®,[5] dihexadecylphosphate[6] and carboxymethylated polysaccharide.[7] Another material that can be used for the immobilization of CB on the GCE surface is poly(allylamine hydrochloride) (PAH), however, it has not previously been described for this purpose. PAH is a weak cationic polyelectrolyte, which comprises of many ionizable amine groups. Thus, when in acidic or basic solution it is fully protonated, while in neutral solutions it is partially deprotonated.[8]

Rosuvastatin calcium (RSV) is a member of the statin family used to treat high cholesterol. The rate controlling enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase controls the production of cholesterol. High cholesterol can lead to a build up of low-density lipoproteins (LDL) in arteries, restricting blood flow. RSV is a selective and competitive inhibitor of HMG-CoA reductase, which results in a marked reduction in serum low-density lipoprotein (LDL) levels.[9,10] The majority of hyperlipidemic patients may also have hypertension problems, thus antihypertensive and antihyperlipidemic agents are commonly administered in combination.[11] Amlodipine besylate (AML) is a calcium channel blocker, and it is generally used in the management of hypertension and angina pectoris.[12] Both drugs are co-administered for the simultaneous treatment of hypertension and high cholesterol.Recent studies show that pre-existing hypertension may be a risk factor for Parkinson's disease diagnosis,[11] where low levels of dopamine are present in the human body. Dopamine (DA) is a neurotransmitter in mammalian brain tissue that plays a major physiological role in the functioning of the central nervous, renal, hormonal and cardiovascular systems as an extracellular chemical messenger.[13] Paracetamol (PAR) or acetaminophen is among the most extensively employed drugs in the world, it is used to relieve moderate pain and the symptoms

of fevers.[14] Furthermore, 50% of the patients with Parkinson disease manifest significant pain,[15] thereby it is common practice to prescribe PAR to relieve chronic pain caused by loss of motor function. Recent published data suggests that low concentrations of PAR significantly reduced DA neurodegeneration while a high concentration of PAR did not protect DA neurons 6-hydroxydopamine-induced degeneration.[15]

Thereby, the determination of drugs of different therapeutic classes is very important for human health, to monitor their concentration in plasma, and for environmental protection, due to inappropriate disposal protocols. Several publications describe methods for individual and simultaneous determination of DA, PAR, AML, and RSV, among them, chromatography,[16–20] and spectrophotometry.[21–23] However, these techniques present some disadvantages such as high instrumentation cost, long analysis time, and extensive sample preparation. In these circumstances, electroanalytical techniques appear as an alternative method. They are simple, rapid, portable, highly sensitive, selective, and environmentally friendly. In the literature, there are reports of the voltammetric determination of DA, PAR, AML and RSV,[7,24–28] although there is not for this quaternary determination.

This paper describes for the first time a new electrochemical platform based on the immobilization of CB on the GCE surface employing PAH as a dispersant agent. The new sensor has been applied for the simultaneous determination of DA, PAR, AML, and RSV in commercial pharmaceutical formulations, synthetic plasma, river, and tap water samples.

Experimental

Reagents and solutions

All chemicals used were of analytical grade and used as received without any further purification. CB VXC72R was kindly provided by Cabot Corporation (São Paulo – SP, Brazil). All solutions were prepared with ultra-purified water (resistivity ≥ 18.2 MΩ cm) supplied by a Milli-Q system (Millipore®, USA).

DA, PAR, AML, and RSV (purity ≥ 99.5%) were obtained from Sigma-Aldrich (USA). Potassium monobasic phosphate, potassium dibasic phosphate, acetic acid, boric acid, phosphoric acid, ascorbic acid, citric acid, sodium hydroxide, potassium chloride, and sodium chloride were purchased from Anidrol (Brazil). For the simulated blood serum: bovine serum albumin (BSA), creatine, creatinine, glycine, lysine, uric acid, and glucose were obtained from Sigma-Aldrich (USA).

Pharmaceutical samples were purchased from a drugstore, in Londrina, Brazil. The tablets were labelled 5 mg mL^{-1} and 500 mg mL^{-1} for DA and PAR, respectively. The besylate amlodipine tablet was labelled 3.48; 6.93; 13.9 mg mL^{-1} (which, for amlodipine, the concentrations were 2.5; 5.0; 10 mg mL^{-1}), and the calcium rosuvastatin tablet was labelled 5.20; 10.4; 20.8 mg mL^{-1} (which, for rosuvastatin the concentrations were 5.0; 10.0; 20.0 mg mL^{-1}).

The tap water sample was collected from a tap at Universidade Estadual de Londrina, Londrina, Brazil. The river water sample was collected from a local river (Ribeirão Cambé, Brazil).

The electrolyte supporting solutions were: BR buffer solutions (pH 2.0 – 5.0), phosphate buffer solution (pH 2.0), and KCl 0.5 mol L^{-1}. The BR buffer solutions were prepared with acetic, phosphoric and boric acids, and the pH adjusted with a 2.0 mol L^{-1} NaOH solution. The phosphate buffer solution was prepared with a 0.1 mol L^{-1} potassium monobasic phosphate solution and the pH was adjusted with 0.1 mol L^{-1} potassium dibasic phosphate.

Stock solutions of DA and PAR were prepared in deionized water at 10 mmol L^{-1} concentration. AML was dissolved in ethyl alcohol and RSV in propanone both at 10 mmol L^{-1} concentration. Their working solutions (1 mmol L^{-1}) were made by appropriate dilution of stock solutions with phosphate buffer solution (pH 3.0).

Apparatus

Voltammetric measurements were carried out using a PGSTAT-101 potentiostat/galvanostat (Metrohm Autolab B. V., Netherlands) controlled by NOVA 2.1 software. This was coupled to a three-electrode single-compartment glass cell containing an Ag/AgCl (3.0 mol L^{-1} KCl) as the reference electrode, a platinum plate as an auxiliary electrode, and bare GCE or modified-GCE as working electrodes. GCE was obtained from Tokay Carbon Co., Japan (3 mm, diameter).

Electrochemical impedance spectroscopy (EIS) measurements were performed on a FRAII μAutoLab type III potentiostat/galvanostat (Metrohm Autolab B. V., Netherlands) controlled by NOVA 1.0 software. These experiments were performed using 1.0 mmol L^{-1} hexaammineruthenium(III) chloride in 0.1 mol L^{-1} KCl in the range from 0.1 Hz to 100 kHz (10 points per decade) and with a 10 mV (r.m.s) ac perturbation.

Microscopy images were obtained by a scanning electron microscope (SEM) FEI Quanta 200 FEG operated at 5 kV.

Individual spectrophotometric determination of DA, PAR, AML, and RSV were conducted on a PerkinElmer lambda 40 UV-Vis spectrometer coupled at a computer, a 1-cm quartz cell was used.

Preparation of CB-PAH/GCE

The preparation of CB-PAH/GCE was realized according to the following steps (Fig. 1). First, the bare GCE was carefully polished with diamond spray 1-µm particle size, and in sequence with ¼-µm particle size (Kemet, UK) on a polishing cloth. Afterward, a CB-PAH dispersion was prepared by weighing 2 mg of CB and 1 mg of PAH, which was dispersed in 1 mL of ultra-purified water with the aid of an ultrasonic bath. The CB-PAH modified GCE was produced *via* a drop-cast process, 8 µL of CB-PAH dispersion was immobilized on the bare GCE surface. The electrode was left to dry for 1 hour at room temperature.

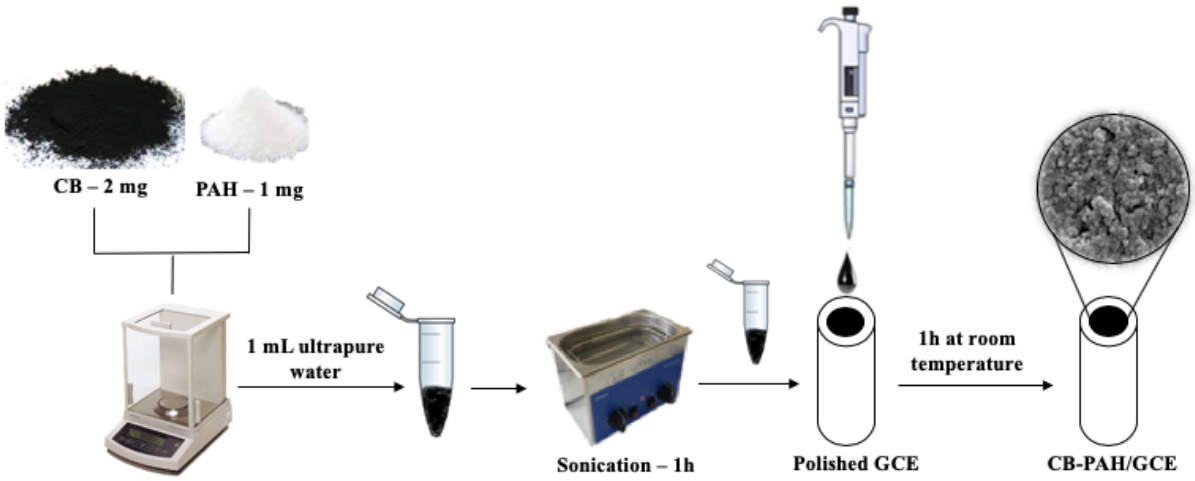

Fig. 1 Schematic representation of experimental steps followed for preparation of CB-PAH/GCE.

Measurement Procedures

Cyclic voltammetry (CV) was used for the electrochemical characterization of the electrode surfaces and the voltammetric behaviour of DA, PAR, AML, and RSV. The simultaneous determination of the analytes in pharmaceutical, biological and environmental samples was performed using linear sweep voltammetry (LSV). After optimizing the experimental parameters, the analytical curve was constructed by the addition of aliquots of a

standard solution of DA, PAR, AML, and RSV into the electrochemical cell containing phosphate buffer solution (pH 2.0). All measurements were carried out in triplicate (n = 3) for each concentration. The limit of detection (LOD) was calculated according to three times the standard deviation of the blank solution divided by the slope of the analytical curve.

Preparation of the pharmaceutical samples

The pharmaceutical samples were prepared in four combinations, namely sample A, sample B, sample C, and sample D, with different concentrations of each analyte, as shown in Table 1. To prepare the injectable-DA sample, an aliquot was transferred into a 20 mL volumetric flask containing phosphate buffer solution (pH 2.0); then an aliquot of 120 μmol L^{-1} was transferred to the electrochemical cell. For PAR, AML and RSV ten tablets of each respective pharmaceutical formulation were weighed and reduced to a homogeneous fine powder in a mortar. An amount of these powders corresponding to one simple tablet was weighed and transferred to calibrated volumetric flasks. PAR was transferred to 25 mL of phosphate buffer solution (pH 2.0), AML to 5 mL of ethyl alcohol, and RSV to 5 mL of acetone. Afterward, aliquots of each sample solution were directly transferred to the electrochemical cell containing phosphate buffer solution (pH 2.0). The concentration of each sample solution was determined directly by the interpolation of the previously obtained analytical curve. These measurements were carried out in triplicate and the relative standard deviation (RSD) was calculated.

Table 1 Preparation of the pharmaceutical samples and the respective concentration of DA, PAR, AML and RSV

	Amount			
	DA / mg mL^{-1}	PAR / mg $tablet^{-1}$	AML / mg $tablet^{-1}$	RSV / mg $tablet^{-1}$
Sample A	5.0	500	3.48	5.20
Sample B	5.0	500	6.93	10.4
Sample C	5.0	500	13.9	20.8
Sample D	5.0	500	13.9	5.20

Preparation of the biological and water samples

A solution of simulated blood serum was prepared by mixing the following reagents (adapted from Krebs):[29] ascorbic acid (10 mg L^{-1}), BSA (40 mg L^{-1}), citric acid (25 mg L^{-1}), creatine (10.7 mg L^{-1}), creatinine (4.2 mg L^{-1}), glycine (17.7 mg L^{-1}), glucose (200 mg L^{-1}) lysine (29.5 mg L^{-1}), and uric acid (10 mg L^{-1}) in phosphate buffer saline (PBS) solution 0.1 mol L^{-1} (pH 7.4).

The river and tap water were spiked with a standard solution of DA, PAR, AML, and RSV of known concentration, and then 1.0 mL was transferred to the electrochemical cell containing 9.0 mL of PBS (pH 2.0). Recovery tests were performed to evaluate the accuracy of the method.

Results and discussion

Morphological and electrochemical characterization of the proposed carbon-based sensor

The surface characterization of the GCE and CB-PAH/GCE sensors were performed by SEM analysis. As shown in Fig. 2B, the surface of the GCE modified with the CB-PAH composite presented a significant difference when compared to the unmodified GCE surface (Fig. 2A), indicating the formation of a homogeneous film of the CB particles covered with the PAH polymer.

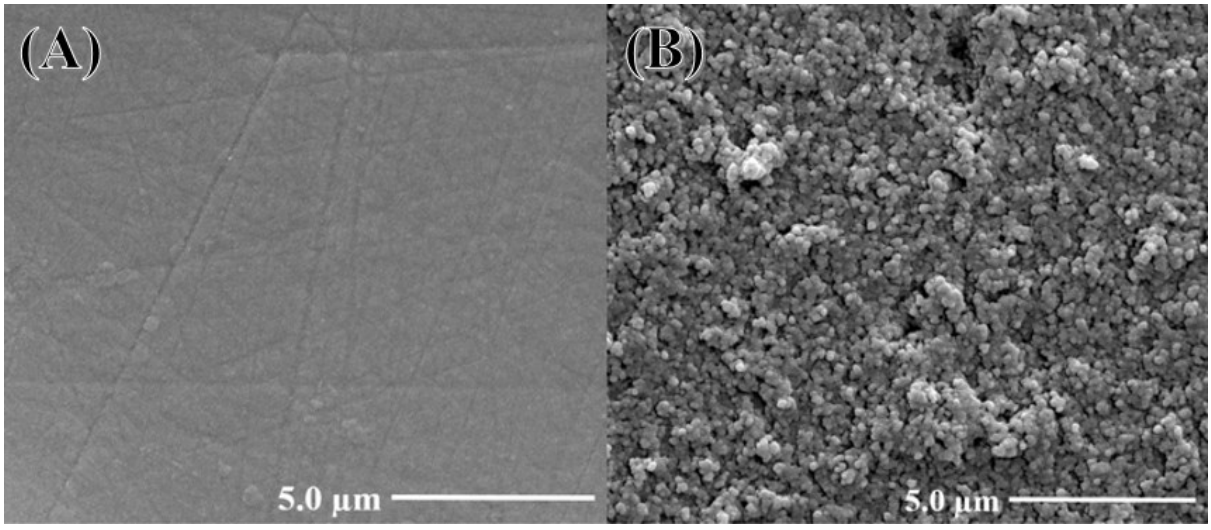

Fig. 2 SEM images of the surfaces of (A) bare GCE and (B) CB-PAH/GCE.

Considering that carbon black particles are produced by incomplete combustion or thermal decomposition of gas or liquid hydrocarbons, the miscibility in water is not favored due to the non-polar character of this nanomaterial. On the other hand, PAH is a cationic polyelectrolyte based on a hydrophobic chain with some ionizable amine groups along with the structure. The composite CB-PAH is based on the hydrophobic interaction between the CB particles and the carbon chain on the PAH structure. The degree of ionization of poly(allylamine hydrochloride), at pH 7.0 (experimental condition of this study), is approximately 95% for the amine groups.[8] As seen in Figure 3A, the miscibility of CB particles in water is highly favored in the presence of PAH polymer. The ion-dipole interaction between the ionized amino groups and the dipole from the water molecules ensures a more stable and homogeneous dispersion of CB particles in water (Fig. 3B).

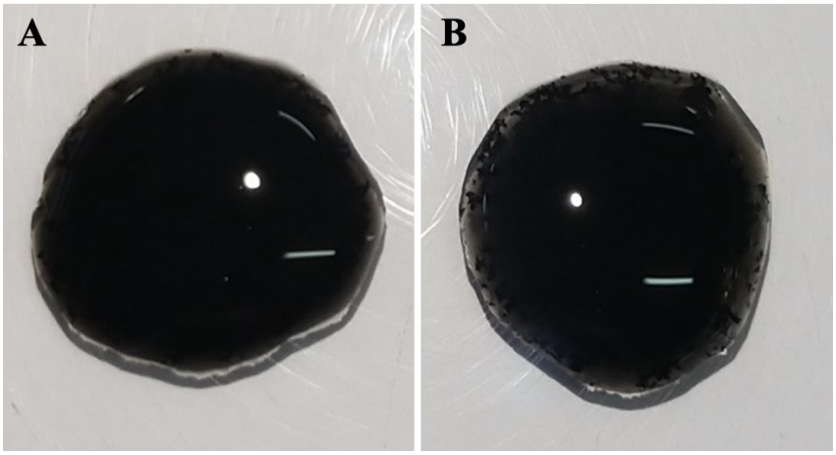

Fig. 3 Photographs of (A) CB-PAH composite dispersion and (B) CB particles dispersion in water.

Electrochemical characterization was performed by cyclic voltammetry and EIS. For both techniques, the electrochemical behaviour of bare GCE, PAH/GCE, and CB-PAH/GCE were studied using 1.0 mmol L^{-1} hexaammineruthenium(III) chloride in 0.1 mol L^{-1} KCl. Fig. 4 shows the cyclic voltammograms (50 mV s^{-1}) for GCE, PAH/GCE, and CB-PAH/GCE, it is possible to observe a peak-to-peak separation (ΔEp) of 0.0696, 0.0801 and 0.0571 V, respectively. Thereby, the introduction of CB on the PAH film improved the electron transfer when compared with the bare GCE and PAH-GCE.

Electroactive area of the electrodes was calculated using Randles-Sevcik equation[30] and a diffusion coefficient for hexaammineruthenium(III) chloride of 9.1×10^{-6} cm^2 s^{-2}. The electroactive area of bare GCE, PAH/GCE, and CB-PAH/GCE was calculated as 0.057, 0.044

and 0.133 cm^2, respectively. The results show that CB increases the electroactive area of the sensor, as expected when using a nanomaterial.

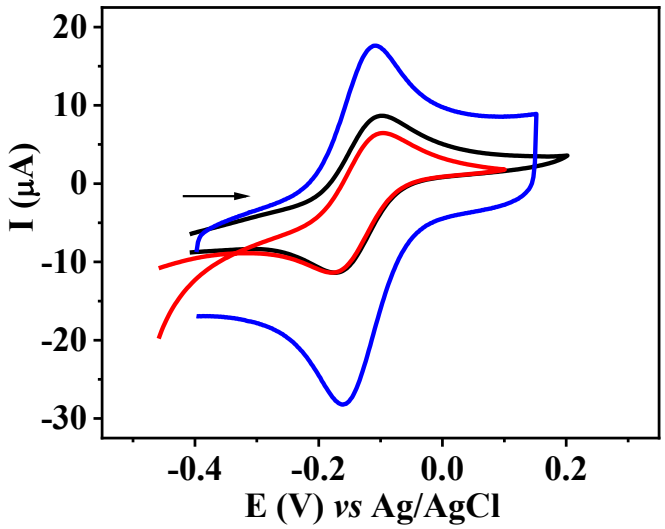

Fig. 4 Cyclic voltammograms (50 mV s^{-1}) in KCl 0.1 mol L^{-1} in the presence of 1.0 mmol L^{-1} hexaammineruthenium(III) chloride, using as working electrode: (—) bare GCE, (—) PAH/GCE, and (—) CB-PAH/GCE.

Modified electrochemical devices can be studied by methods based on impedance measurements, associated with electronic transfer kinetics. The electrochemical characterization of the GCE, PAH/GCE and CB-PAH/GCE sensors was performed based upon the electrochemical impedance spectroscopy (EIS) using the well-know electrochemical probe, hexacyanoferrate system $[Fe(CN)_6]^{3-/4-}$. The Randles circuit is the most common model used for impedance measurements, including only solution resistance (R_{SOL}), a parallel combination of a double-layer capacitor (CPE – constant phase element) and a charge transfer or polarization resistance (R_{CT}), as represented in the circuit shown in Fig. 5 (inset).

Nyquist's plot of a Randles cell is a semicircle and the diameter represents the charge transfer resistance (at higher frequencies), while the straight line at an angle of 45° to the real axis at lower frequencies represents the diffusion process (mass transfer control). As can be seen in Fig. 5, the addition of the polyelectrolyte (PAH) caused an increase in R_{CT} (1.2 kΩ) compared to the bare GCE (R_{CT} = 0.32 kΩ). This behaviour can be attributed to the organic macromolecular film (PAH) that can block the diffusion of electroactive species on the electrode surface. The introduction of the composite (CB-PAH/GCE) caused a

physicochemical alteration at the electrode/solution interface, decreasing the semi-circle radius (R_{CT} 0.05 kΩ) compared to the other sensors. This result suggests that the nanomaterial increased the electron transfer rate in the electrochemical system, as expected.

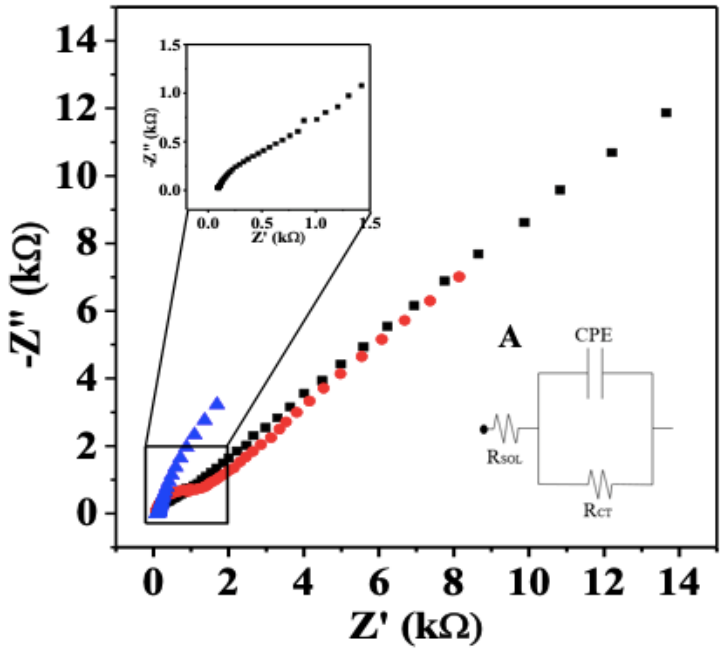

Fig. 5 Nyquist diagram to (■) bare GCE, (●) PAH/GCE and (▲) CB-PAH/GCE in 0.1 mol L^{-1} KCl containing 1.0 mmol L^{-1} hexaammineruthenium (III) chloride, open circuit mode (inset A), 10 mV amplitude and frequency range of 10 Hz to 100 kHz. *Insert:* magnifications of the high-frequency part of the impedance spectra for the bare GCE.

Application of CB-PAH/GCE for multianalyte determination of DA, PAR, AML and RSV

The electrochemical behaviour of DA, PAR, AML, and RSV were compared by cyclic voltammetry (50 mV s^{-1}) using, as working electrode, a bare GCE, PAH-GCE, and CB-PAH/GCE in BR buffer (pH 3.0) in the presence of 0.4 mmol L^{-1} of each analyte. In Fig. 6, it is possible to observe that DA, PAR, AML, and RSV presented a well-defined oxidation peak at 0.44, 0.63, 0.90 and 1.32 V, respectively *vs.* Ag/AgCl (KCl 3.0 mol L^{-1}). DA and PAR show characteristic quasi reversible processes, presenting reduction peak on the reverse scan. On the other hand, no reduction peaks were observed to AML and RSV, indicating that the oxidation process is irreversible. When the CB is incorporated on the PAH film it is possible to observe an improvement in the electrochemical response of all analytes. This is due to the nanometre

scales of the CB that provide an increased electroactive area and faster charge transfer, as previously mentioned in the electrochemical characterization of the sensor.

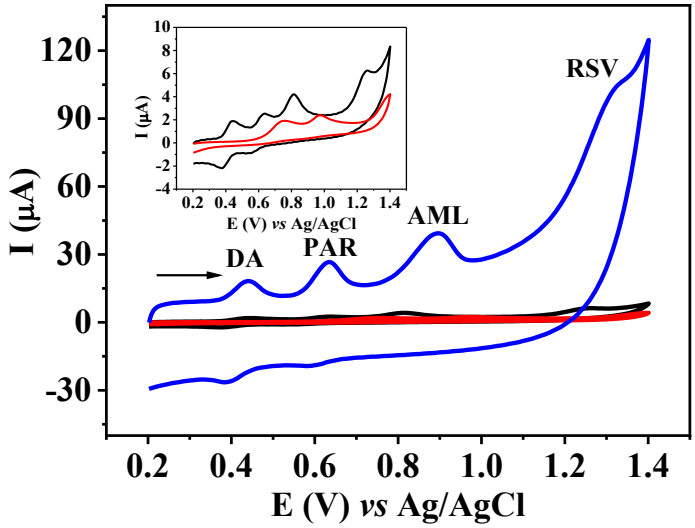

Fig. 6 Cyclic voltammograms (50 mV s^{-1}) recorded in BR buffer solution (pH 3.0) in the presence of DA, PAR, AML and RSV at 0.4 mmol L^{-1} concentration with the working electrode (—) bare GCE, (—) PAH/GCE, and (—) CB-PAH/GCE. *Inset*: magnifications of the cyclic voltammograms using (—) bare GCE, (—) PAH/GCE as working electrodes, under the same conditions.

The effect of the amounts of CB and PAH on the analytical responses was studied using BR buffer solution (pH 3.0) in the presence of DA, PAR, AML, and RSV at 0.4 mmol L^{-1} concentration by CV (data not shown). In this study, the ratio between CB and PAH was 1:1, 1:2, 2:1, 3:1 (m/m; CB:PAH). The dispersion of 2:1 (m/m; CB:PAH) showed a higher current magnitude and better repeatability. Thereby, this dispersion was chosen for all further electrochemical experiments.

The fabrication reproducibility of the CB-PAH/GCE was studied by CV and using three different sensors, which were constructed using the same and then three different dispersions of CB-PAH (2:1; m/m). The measurements were performed in the presence of DA, PAR, AML and RSV at 0.4 mmol L^{-1} concentration in BR buffer solution (pH 3.0). For the same dispersion, the RSD values were 1.99%, 1.33%, 1.78% and 1.49% for DA, PAR, AML and RSV, respectively, and for the three different dispersions the RSD values were 2.55%, 0.78%, 1.37% and 0.85% for DA, PAR, AML and RSV, respectively. The results demonstrated the viability

and high reproducibility of the fabrication of CB-PAH/GCE. Under the same conditions, repetitive current measurements were performed in order to investigate the CB-PAH/GCE stability. After 35 measurements, the current decreased only 5.0% of its original value, indicating good stability of the film of CB-PAH formed on the GCE surface.

Study of pH, supporting electrolyte and effect of scan rate

The pH of the supporting electrolyte was studied between 2.0 to 5.0 using a BR buffer solution. Above pH 6.0, the peak current for RSV vanishes. pH 2.0 was chosen for the simultaneous determination of DA, PAR, AML, and RSV, due to the highest magnitude and repeatability. In sequence, the effect of the supporting electrolyte on the oxidation peak of DA, PAR, AML, and RSV was comparatively investigated for BR and phosphate buffer solutions (0.1 mol L^{-1}) at pH 2.0. Better repeatability and higher peak current were obtained employing a phosphate buffer solution, which was selected.

The effect of scan rate on the oxidation peak of DA, PAR, AML, and RSV at 0.3 mmol L^{-1} concentration using CB-PAH/GCE in phosphate buffer solution (pH 2.0) was investigated by CV at different scan rates from 5 – 350 mV s^{-1}. Fig. 7 shows all the voltammograms and plots of the logarithm of the oxidation peak current (log I_{ap}) *vs.* logarithm of the scan rate (log v). As can be seen, the plots log I_{ap} *vs.* log v are linear with slope values around the theoretical value to 1.0, characteristic of a system controlled by adsorption. The corresponding equations are:

DA: $\log I_{ap} = -1.19 + 1.22 \log v$ (r = 0.994) (Eq. 1)

PAR: $\log I_{ap} = -0.61 + 1.16 \log v$ (r = 0.996) (Eq. 2)

AML: $\log I_{ap} = -0.26 + 0.97 \log v$ (r = 0.999) (Eq. 3)

RSV $\log I_{ap} = -0.16 + 0.85 \log v$ (r = 0.995) (Eq. 4)

Additionally, the oxidation peak current (I_{ap}) *vs.* scan rate (v) were plotted, and a linear behaviour was obtained for DA, PAR, AML and RSV, reaffirming that the process is predominantly controlled by adsorption on the CB-PAH/GCE surface. In the voltammograms,

it was possible to observe, for all analytes, an increase of potential to more positive potentials, which is a characteristic of quasi reversible and irreversible processes.

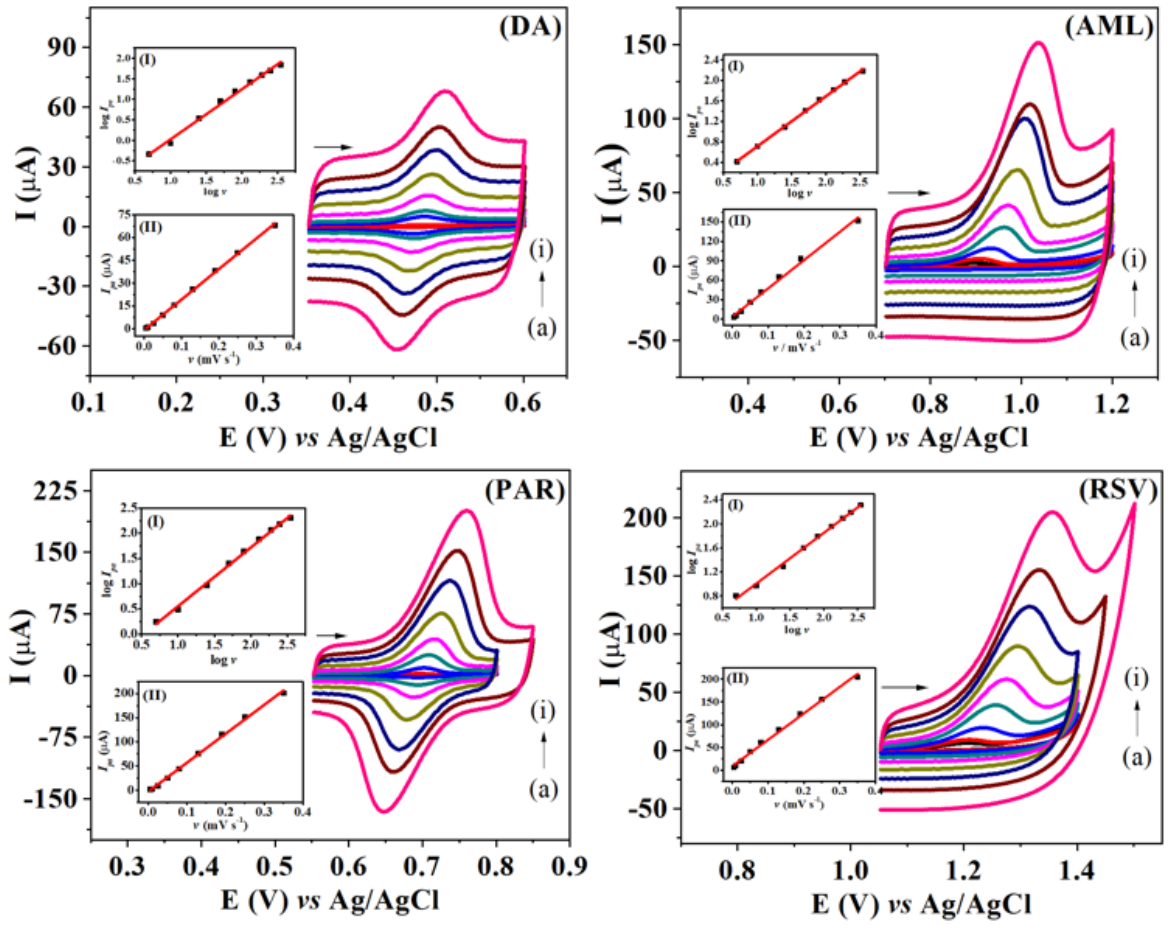

Fig. 7 Cyclic voltammograms obtained at different scan rates (a – i: 5 – 350 mV s^{-1}) for DA, PAR, AML and RSV at 0.3 mmol L^{-1} concentration in phosphate buffer solution (pH 2.0). *Inset*: Plots of (I) log I_{pa} *vs.* log *v* and (II) I_{pa} *vs.* $v^{1/2}$.

The number of electrons involved in the electrochemical oxidation of DA, PAR, AML, and RSV were estimated using the cyclic voltammograms obtained at 50 mV s^{-1} and applying the equation $E_{ap} - E_{ap/2} = 0.047/\alpha n$.[30] The value of E_{ap} and $E_{ap/2}$ were 0.495 and 0.457 for DA; 0.715 and 0.676 V for PAR; 0.962 and 0.906 for AML; 1.257 and 1.188 V for RSV, respectively. Assuming α value as 0.5 (commonly employed),[30] the number of electrons involved in the oxidation of DA, PAR and AML are 2, and for RSV is 1 employing the CB-PAH/GCE. These values are according to the literature.[7,31] The electrochemical oxidation of DA happens in both phenol groups and 2 electrons and 2 H^+ are released, for PAR the oxidations are in the phenol and amine groups with the release of 2 electrons and 2 H^+.[33] The

electrochemical oxidation of AML occurs in the amine group and release 2 electrons and for the RSV the oxidation occurs in the alcohol group, and 1 electron is released.[34,35]

Simultaneous determination of DA, PAR, AML and RSV employing the CB-PAH/GCE

Prior to the analytical curve construction, the influence of the scan rate on the sensitivity of LSV was investigated. For this purpose, analytical curves were constructed for each evaluated value in a scan rate range of 10 to 100 mV s^{-1}. Based on these curves, due to a better sensitivity and linearity, the scan rate of 75 mV s^{-1} was chosen for the analytical curve construction.

Under these chosen conditions, the analytical curve of each analyte in the presence of the ternary mixture of other analytes was performed by changing the concentrations of one analyte and maintaining the concentration of each of the three analytes constant. All results obtained employing the CB-PAH/GCE were summarized in Table 2. The peak oxidation currents of the analytes that remained constant present RSD values smaller than 8.65%, indicating that the change of concentration of one studied analyte did not have a significant influence on the voltammetric response of the other one.

Table 2 Slope and RSD values obtained from LSV technique for the simultaneous determination of one analyte in the presence of the ternary mixture of other analytes employing the CB-PAH/GCE for DA, PAR, AML and RSV

Analyte	Slope / μA μmol L^{-1}	Correlation coefficient	RSD (%)			
			DA	PAR	AML	RSV
Dopamine	0.362	0.999	–	3.69	8.65	4.61
Paracetamol	0.205	0.999	2.43	–	4.90	1.94
Amlodipine	1.103	0.995	5.73	4.49	–	5.12
Rosuvastatin	0.471	0.997	3.85	4.92	7.07	–

Then, the analytical curves were constructed by successive additions of a standard solution of DA, PAR, AML and RSV by LSV employing the CB-PAH/GCE in phosphate buffer solution (pH 2.0). Fig. 8 shows the linear sweep voltammograms obtained for the solution containing DA, PAR, AML and RSV, and the respective analytical curves for each

analyte. The analytical parameters obtained to the simultaneous determination of DA, PAR, AML, and RSV are listed in Table 3.

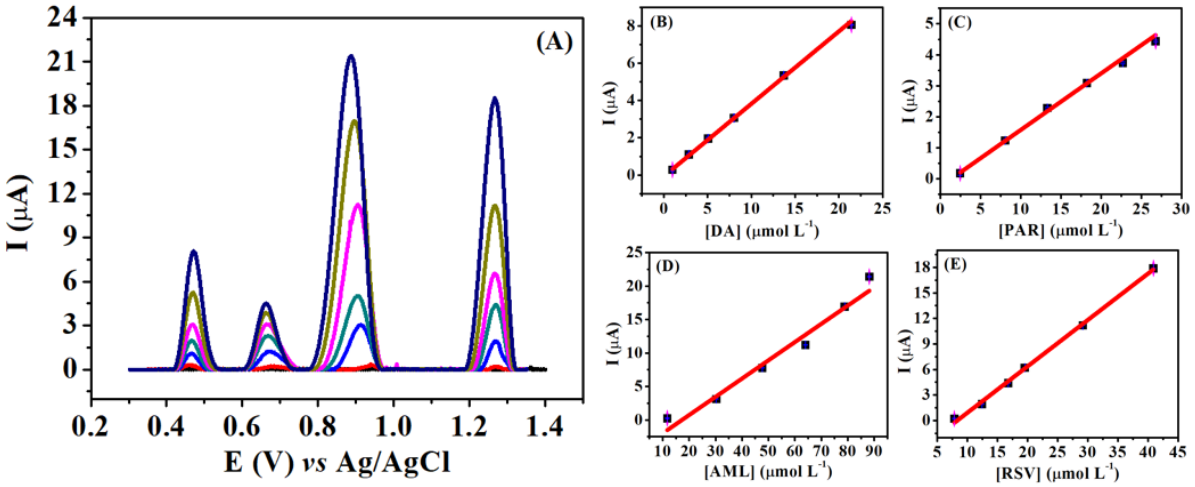

Fig. 8 (A) Linear sweep voltammograms obtained employing the CB-PAH/GCE for various concentrations of (2-7) DA (1.0 – 22 μmol L^{-1}), PAR (2.4 – 27 μmol L^{-1}), AML (12 – 90 μmol L^{-1}), and RSV (7.8 – 40 μmol L^{-1}). Respective analytical curves for (B) DA, (C) PAR, (D) AML, (E) RSV.

Table 3 Analytical parameters for simultaneous determination of DA, PAR, AML and RSV by LSV in phosphate buffer solution (pH 2.0) employing the CB-PAH/GCE

Analytical parameters	**Analytes**			
	DA	**PAR**	**AML**	**RSV**
Peak potential (V)	0.47	0.66	0.90	1.27
Linear range (μmol L^{-1})	1.0 – 22	2.4 – 27	12 – 90	7.8 – 40
Correlation coefficient	0.999	0.999	0.992	0.998
Slope (μA μmol L^{-1})	0.38	0.18	0.27	0.54
LOD (μmol L^{-1})	0.17	0.13	1.02	0.82

The *intra*- and *inter*-day repeatability studies were performed using a concentration of 15 μmol L^{-1} for DA and PAR, and 30 μmol L^{-1} for AML and RSV in phosphate buffer solution (pH 2,00) employing the CB-PAH/GCE. The *intra*-day repeatability was determined by successive measurements (n = 10), and the RSD values obtained were 1.99% (DA), 1.86% (PAR), 4.07% (AML) and 1.82% (RSV). The *inter*-day repeatability was assessed by measuring the peak current over a period of 5 days, and the RSV values obtained were 5.03%

(DA), 4.82% (PAR), 3.36% (AML) and 5.16% (RSV). The RSD values obtained indicated that the proposed method employing the CB-PAH/GCE provides good repeatability for the determination of these four analytes.

Table 4 compares the linear range obtained by CB-PAH/GCE employing LSV with those in the literature obtained by different electrodes and techniques. Although the electrodes described in the literature present a better linear concentration range, with lower concentrations, they cannot perform the simultaneous determination for DA, PAR, AML, and RSV, consequently, the CB-PAH/CB has its advantages, because it enables the simultaneous determination. Moreover, CB-PAH/GCE is easy to prepare and demonstrates that PAH disperses CB very well in an aqueous medium.

Table 4 Comparations of linear concentration ranges for the electrochemical determination of DA, PAR, AML and RSV using differents electrodes

Electrode	Technical	Linear concentration range (μmol L^{-1})				Ref.
		DA	PAR	AML	RSV	
Platinum	SWV	–	–	9.0 – 70	5.0 – 40	[24]
Bi/Nafion/BDDE	DPV	–	0.2 – 500	–	–	[25]
RGO-GCE	DPV	0.1 – 100	–	–	–	[28]
MOFs/MWCNT-Au@Ag/GCE	DPV	0.6 – 70	1.0 – 40	–	–	[32]
CB-PAH/GCE	LSV	1.0 – 22	2.4 – 27	12 – 90	7.8 – 40	This work

Abbreviations. SWV: square-wave voltammetry; DPV: differential pulse voltammetry; Bi/Nafion/BDDE: bismuth particles Nafion covered boron-doped diamond electrode; MOFs/MWCNT-Au@Ag/GCE: metal-organic framework/multiwalled carbon nanotubes-Au@Ag core shell nanoparticles on GCE.

Selectivity and analytical application of the CB-PAH/GCE for the simultaneous determination of DA, PAR, AML and RSV

To evaluate the selectivity of the CB-PAH/GCE by LSV, different chemical species were selected, such as sodium bisulfite, starch, microcrystalline cellulose, silicon dioxide, creatine, creatinine, glucose, and inorganic ions as Na^{+}, Fe^{2+}, Zn^{2+}, Ca^{2+}, NO_3^{-} and SO_4^{2-}. For this, DA, PAR, AML, and RSV at 30 μmol L^{-1} concentration were used in the absence and presence of possible interfering agents at the same concentration of the analytes (30 μmol L^{-1})

in the ratio of 1:1 and 1:10. The results show that the current intensity for all analytes does not suffer interference from any of these chemical species with RSD lower than 7%.

Thereby, the CB-PAH/GCE was applied for simultaneous voltammetric determination of DA, PAR, AML, and RSV in pharmaceutical samples in different concentrations (described in the experimental section). Table 5 presents the results obtained for this determination. As can be seen, there are no significant differences between the values found for DA, PAR, AML, and RSV in pharmaceutical samples employing LSV and spectrophotometric method.[33,34] The paired Student *t*-test was applied to the statistical comparison between the voltammetric method and the spectrophotometric method at a confidence level of 95%. The $t_{experimental}$ values calculated were smaller the $t_{critical}$ value (3.18). Additionally, the *F*-test provided information about the precision of both methods. As can be noted, all calculated values were lower than the $F_{critical}$ (19.0; 95% confidence interval). Thus, it is possible to conclude that there is no significant difference between the methods.

Table 5 Results obtained to the simultaneous determination of DA, PAR, AML and RSV in combined forms using the LSV method compared with spectrophotometric method

Sample	Analyte	Amount[a]			E (%)[b]	F_{calc}[c]
		Label value	LSV method	Reference method		
A	DA	5 mg mL^{-1}	4.97 ± 0.13	5.02 ± 0.12	–0.99	1.17
	PAR	500 mg $tablet^{-1}$	490 ± 4.7	495 ± 5.8	–1.02	0.66
	AML	3.47 mg $tablet^{-1}$	3.42 ± 0.13	3.58 ± 0.11	–4.68	1.40
	RSV	5.2 mg $tablet^{-1}$	5.04 ± 0.10	5.15 ± 0.08	–2.18	1.56
B	DA	5 mg mL^{-1}	5.15 ± 0.12	5.06 ± 0.10	1.78	1.44
	PAR	500 mg $tablet^{-1}$	514 ± 5.5	491± 6.9	4.47	0.64
	AML	6.93 mg $tablet^{-1}$	6.71 ± 0.21	7.15 ± 0.18	–6.70	1.36
	RSV	10.4 mg $tablet^{-1}$	10.2 ± 0.20	9.98 ± 0.27	2.07	0.55
C	DA	5 mg mL^{-1}	4.90 ± 0.10	5.12 ± 0.11	–4.29	0.83
	PAR	500 mg $tablet^{-1}$	508 ± 5.1	516 ± 6.1	–1.57	0.70
	AML	13.87 mg $tablet^{-1}$	13.55 ± 0.67	13.84 ± 0.34	–2.14	3.88
	RSV	20.8 mg $tablet^{-1}$	21.1 ± 0.35	20.5 ± 0.44	2.84	0.63
D	DA	5 mg mL^{-1}	5.11 ± 0.13	4.99 ± 0.15	2.40	0.75
	PAR	500 mg $tablet^{-1}$	492 ± 5.0	507 ± 4.5	–3.05	1.23
	AML	13.87 mg $tablet^{-1}$	13.95 ± 0.56	13.66 ± 0.26	2.07	4.64
	RSV	5.2 mg $tablet^{-1}$	5.15 ± 0.08	5.4 ± 0.14	–3.30	0.33

[a]Average of 3 measurements.
[b](LSV method – spectrophotometric method / spectrophotometric method) × 100.
[c]Critical *F*-value = 19.0 (95% confidence level).

The CB-PAH/GCE was also employed for simultaneous determination of DA, PAR, AML, and RSV in environmental samples (river and tap water) and for the simultaneous determination of DA and PAR in biological samples (simulated blood serum). Environmental samples were spiked with two different concentration of each analyte: 2.0 and 5.0 µmol L^{-1} for DA; 5.0 and 10 µmol L^{-1} for PAR; 20 and 30 µmol L^{-1} for AML; 10 and 20 µmol L^{-1} for RSV. As shown in Table 6, excellent recovery percentages were obtained, demonstrating the applicability of the CB-PAH/GCE in environmental sensing.

Table 6 Results obtained from the analysis of environmental samples

Analyte	River Water			Tap water		
	Added (μmol L^{-1})	Found[a] (μmol L^{-1})	Recovery[b] (%)	Added (μmol L^{-1})	Found[a] (μmol L^{-1})	Recovery[b] (%)
DA	2.0	1.95 ± 0.3	97.5	2.0	1.97 ± 0.4	98.5
	5.0	4.98 ± 0.2	99.6	5.0	5.04 ± 0.3	100.8
PAR	5.0	4.91 ± 0.6	98.2	5.0	4.93 ± 0.6	98.6
	10	10.08 ± 0.5	100.8	10	9.96 ± 0.4	99.6
AML	20	21.01 ± 0.9	105.05	20	21.03 ± 0.8	105.15
	30	28.98 ± 1.1	96.6	30	30.87 ± 1.1	102.9
RSV	10	9.92 ± 0.7	99.2	10	9.88 ± 0.9	98.8
	20	20.51 ± 0.9	102.55	20	19.85 ± 0.8	99.25

[a]Average of 3 measurements.
[b]Recovery percentage = (Found/Added) × 100.

Another applicability of the CB-PAH/GCE was in the simulated blood serum. This sample was spiked with two different concentrations of each analyte, 2.0 and 5.0 μmol L^{-1} for DA, and 5.0 and 10 μmol L^{-1} for PAR. The recovery percentages obtained were in the range of 97.1 to 101.2% and 98.3 to 102.4% for 2.0 and 5.0 μmol L^{-1} DA and 99.6 to 103.2% and 98.6 to 102.8% for 5.0 and 10.0 μmol L^{-1} PAR, respectively, indicating that the matrix analysed did not cause any interference. The excellent recovery percentages indicate that the CB-PAH/GCE can be applied as sensing for the simultaneous determination of DOP and PAR in biological samples.

Conclusions

The proposed electrochemical sensor (CB-PAH/GCE) presented a good analytical performance for the simultaneous determination of DA, PAR, AML, and RSV, providing high sensitivity and low limits of detection. The promising results obtained can be attributed to the nanomaterial used for the modification of the GCE surface, which increased the electroactive surface area and facilitated the electronic transfer on the electrode/solution interface, as proved by EIS data. The simultaneous determination of DA, PAR, AML, and RSV was successfully demonstrated in pharmaceutical, river and tap water samples, without any significant matrix

interference. Additionally, the developed sensor was applied for the simultaneous determination of DA and PAR in biological samples (simulated blood serum). The sensing platform presented in this study appears to have wide applications in the multianalyte determination, with fast response time, low cost for fabrication and applicability to real and complexes samples.

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