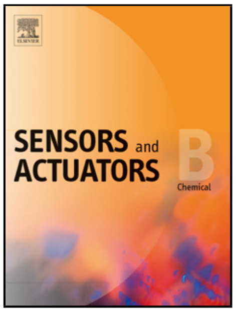

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# Simultaneous determination of pindolol, acebutolol and metoprolol in waters by differential-pulse voltammetry using an efficient sensor based on carbon paste electrode modified with amino-functionalized mesostructured silica

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Highlights

- Functionalized mesostructured silica helps in developing promising sensors
- Simultaneous determination of pindolol, acebutolol and metoprolol by DPV is reported
- Factors affecting the detection of the target β-blockers were investigated
- Portable sensor for *in situ* simultaneous analysis of β-blockers
- God recovery values for β-blockers analysis in drinking, environmental and wastewaters

## ABSTRACT

In this work, hexagonal mesoporous silica (HMS) and amino-functionalized HMS (HMS-$NH_2$) were prepared and incorporated into carbon paste electrodes (CPEs) to obtain electrochemical sensors for pindolol (Pin), acebutolol (Ace) and metoprolol (Met) determination in waters by voltammetric techniques. The materials were characterized and they showed high specific surface area, large pore volume and a quasi-spherical morphology. Firstly, the electrochemical behaviour of the modified CPEs was studied by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Then, the dependence of the current intensity with the electrolyte support (type and pH) and the accumulation time was investigated to optimize the experimental conditions for simultaneous β-blockers determination by DPV. Results showed that the sensor modified with functionalized silica ($NH_2$-HMS-CPE) exhibited strong adsorption activity, showing three well-defined peaks in the differential pulse voltammograms, that appeared at about +0.85 V (Pin), +1.11 V (Ace) and +1.45 V (Met). This fact can be attributed to the high surface area of the material, with a 3 D wormhole-like channel structure that favoured excellent pore accessibility, and the synergistic effect produced by the grafted amino groups producing hydrogen bond interactions with the oxygen atoms of the target β-blockers. The prepared sensor exhibits good reproducibility, wide linear ranges and very low detection limits (0.1, 0.046 and 0.23 μmol $L^{-1}$ for Pin, Ace and Met, respectively). Moreover, the $NH_2$-HMS-CPE was successfully applied for the simultaneous determination of Pin, Ace and Met in tap, mineral, river and sewage effluent waters with simple sample treatment (recoveries 97- 112%).

*Keywords:* Simultaneous determination; β-blockers; Mesostructured silica; Modified carbon paste electrodes; Drinking and environmental waters

## 1. Introduction

In recent years, the so-called emerging pollutants have aroused considerable interest. They are compounds of diverse origin and chemical nature, whose presence and consequences in the environment have gone unnoticed. They are found in waters in low concentrations and are considered very harmful to human health and for the environment [1]. Pharmaceuticals are an important group of emerging contaminants and β-blockers (β-adrenenergic receptor blockers) are one of the most frequently prescribed pharmaceutics all over the world. They are used for treatment of several cardiovascular diseases such as angina pectoris, cardiac arrhythmias, hypertension and associated pathologies [2]. Metoprolol (Met) is the world's most widely prescribed antihypertensive drug belonging to the β-blockers family [3], pindolol (Pin) is used to combat hypertension in pregnancy, because it is harmless to the fetus, and acebutolol (Ace) is used if a patient with obstructive pulmonary disease or asthma needs treatment with β-blockers [4].

Due to its extensive use, β-blockers residues can nowadays be detected in waters, in the ng $L^{-1}$ to μg $L^{-1}$ range, because are only partially eliminated by wastewater treatment plants, so that residual amounts can reach natural waters and cause environmental hazards [5, 6]. Surface water is probably the natural environment most impacted by these residues, but the presence of β-blockers is also found in drinking and groundwaters in ng $L^{-1}$ [2, 7 - 9]. Thus, it is very important to develop analytical methodologies for simple, sensitive, inexpensive and *in situ* simultaneous determination of β-blockers in drinking, environmental and wastewaters. Various techniques have been employed for the analysis of β-blockers, such as capillary electrophoresis [10], high-performance liquid chromatography with fluorescence detection [11] and high-performance liquid chromatography–tandem mass spectrometry [8, 12], among others. However, most of the reported methods suffer some disadvantages, as they require expensive instrumentation, high amount of reagents and time consumption and/or complex preliminary sample treatment procedures. Moreover, these analytical techniques cannot be applied for *in situ* determination of the target β-blockers in waters. Compared with these methodologies, electrochemical techniques are characterized by portability, minimal cost and reasonably short analysis time, combined with an excellent sensibility. Additionally, the development of modified electrodes is a growing field to improve the analytical parameters (in terms of sensitivity, selectivity, reproducibility, robustness, etc.) for the determination of several drugs by electrochemical techniques. For example, Svorc et al. [13] employed a bare boron-doped diamond electrode for simultaneous determination of paracetamol and penicillin V. More recently, Khairy et al. [14] used MgO nanoplatelet modified screen-printed electrode for simultaneous determination of nifedipine and atenolol.

In recent years, the use of advanced materials such as mesostructured silicas has appeared as a promising way to develop different modified carbon paste electrodes (CPEs) [15]. To achieve this goal, the mesostructured silicas are usually directly embedded into the paste of the electrode, which consists of an homogeneous mixture of graphite and mineral oil. Most of the mesostructured silicas exhibit monodisperse pore sizes that can be tuned typically in the 2–10 nm range. They have extremely high specific surface area that is fully accessible to the ordered mesostructure, allowing its easy functionalization with organic compounds. These characteristics are highly desired for their use in electrochemical sensors because they can contribute to improve the mass transport and charge transfer [15,16] , which are important limiting factors in common-electrochemical sensors, the long-term durability (due to the mechanical stability of the mesoporous framework), the preconcentration efficiency, selectivity and sensitivity of the detection [16]. Different research groups have developed CPEs modified with functionalized mesostructured silicas. For example, Morante-Zarcero et al. [17] used a CPE modified with 5-mercapto-1-methyltetrazol grafted on hexagonal mesoporous silica (HMS) to determine Pb(II) in natural waters. Bifunctional periodic mesoporous organosilica (PMO) has also been used to develop a modified CPE that was evaluated for the determination of Pb(II) in drinking waters [18]. Other mesostructured silicas (MCM-41 and FMS) functionalized with amine groups were also successfully used for determination of Cu(II) in tap and lake waters, rat serum and tea samples [19] and for determination of capsaicin in hot pepper samples [20].

In this work, the simultaneous determination of three β-blockers (Pin, Ace and Met) by differential-pulse voltammetry (DPV) using a CPE modified with mesostructured silica functionalized with amine groups (denoted $NH_2$-HMS) have been proposed for the first time. The electrochemical behaviour of the modified CPE (denoted $NH_2$-HMS-CPE) and factors affecting the detection of the target β-blockers were investigated. To evaluate the usefulness of the $NH_2$-HMS-CPE in real waters, it was applied for the simultaneous determination of Pin, Ace and Met in drinking, environmental and wastewaters, and it was found to be an excellent alternative for the simultaneous, rapid, simple, sensitive and *in situ* determination of these pollutants.

## 2. Experimental

### *2.1. Chemicals, reagents and standard solutions*

All chemicals were of analytical reagent grade and used as received. For HMS preparation, dimethyldodecylamine (DDA 97%, M = 213.4 g $mol^{-1}$), tetraethylorthosilicate (TEOS 98%, M = 208.33 g $mol^{-1}$, d = 0.934 kg $m^{-3}$) and 3-

aminopropyltriethoxysilane were purchased from Sigma-Aldrich (USA). Ethanol and diethyl ether were purchased from Scharlau (Spain). A Milli-Q system (Millipore Waters, USA) was employed to purify water. Phosphoric acid and mineral oil were obtained from Sigma-Aldrich (Spain). Sodium chloride (99%), sodium carbonate (99%), sodium nitrate (99-101%), sodium sulphate (99%), calcium nitrate (100%) and magnesium nitrate (99-102%) were obtained from Panreac (Spain). Graphite powder (Metrohm, No 6.2801.000) was obtained from Sugerlabor (Spain). Pindolol (1-(1*H*-indol-4-yloxy)-3-(isopropylamino)-2-propanol, ≥ 98%), acebutolol (N-(3-acetyl-4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl)butanamide, ≥ 98%) and metoprolol (1-isopropylamino)-3-[p-(β-methoxyethyl)phenoxy]-2-propanol, ≥ 98%), were purchased from Sigma–Aldrich (Spain).

Stock standard solutions of each β-blocker (10 mmol $L^{-1}$) were prepared in MeOH and stored at 4 ºC. For electrochemical studies a working solution of Pin (1 µmol $L^{-1}$), Ace (1 µmol $L^{-1}$) and Met (10 µmol $L^{-1}$) was prepared daily by diluting the standard stock solutions with the corresponding electrolyte support. For linearity determination, working solutions of Pin, Ace and Met were prepared directly in the electrochemical cell by adding successive aliquots of working solutions of each β-blocker.

### *2.2. Water samples*

Table 1 shows the origin and conductivity of different water samples analysed in this work. Drinking waters (tap and mineral waters) were stored in polyethylene bottles at -20 ºC prior to use. River waters and sewage effluent water were filtered through a 0.45 µm membrane filter (HA-type Millipore) to remove suspended particles, and stored in polyethylene bottles, at -20 ºC prior to use. Prior voltammetric determination of β-blockers in waters, under optimized conditions, the pH of the samples was adjusted to 4 with phosphoric acid (0.1 mol $L^{-1}$).

## 2.3. Instrumentation

For electrochemical studies with CPEs, cyclic voltammetry (CV) and DPV measurements were performed on a hand-held, battery-powered bipotentiostat model μSTAT200 (Dropsens, Spain) using a conventional voltammetry cell equipped with three electrodes: a CPE modified with mesoporous silica as working electrode, a saturated Ag/AgCl as reference electrode and a platinum wire (Metrohm, Spain) as auxiliary electrode. A computer with DropView software for Windows was used to control the instrument, plot the measurements and perform the analysis of results. All measurements were made at room temperature. A pH-meter model Basic 20 (Crison Instruments, Spain) was used to adjust the pH of electrolyte supporting. A conductivity-meter model CON 510 (Eutech Instruments, USA) was used to conductivity measurement of different water samples.

## 2.4. Synthesis and functionalization of mesostructured silicas

HMS mesoporous silica was synthesized following the method described by Pérez-Quintanilla et al. [21]. Briefly, 30 g of DDA were dissolved in 388.8 mL of Milli-Q water and 179.4 g of ethanol. The solution was stirred until its homogenization, and subsequently 124.8 g of TEOS were added drop by drop. The solution was stirred for 18 h, yielding a thick white suspension that was filtered and dried at 80 ºC for 1 h. The DDA was removed by heating the solid for 8 h at reflux in ethanol with a Soxhlet. Finally, the rest of surfactant was removed by calcination at 550 ºC for 18 h. To functionalize the material, it was previously activated by heating at 150 ºC for 12 h. Later, 1.5 g of HMS was suspended in 1.5 mL of 3-aminopropyltriethoxysilane dissolved in 40 mL of toluene. The mixture was heated for 24 h at reflux. After this time, the solid was washed with 50 mL of toluene, ethanol and diethyl ether. Finally, the material (HMS-$NH_2$) was dried at room temperature in vacuum for 12 h.

### *2.5. Characterization of ordered mesoporous silica*

$N_2$ gas adsorption–desorption isotherms were obtained using a Micromeritics ASAP 2020 analyzer, and pore size distributions were calculated using the Barrett–Joyner–Halenda (BJH) model on the adsorption branch. Adsorption isotherms were measured at -196 ºC over the interval of relative pressures from $10^{-4}$ to 0.993. Prior to each adsorption analysis the samples were out gassed at 90 ºC in vacuum during 10 h in the port of degasification of the instrument. Such temperature was chosen to avoid any degradation of the aminopropyl chains and to remove adsorbed species, solvents and water. Scanning electron (SEM) micrographs and morphological analysis were carried out on a XL30 ESEM Philips with an energy dispersive spectrometry system. The materials were treated with a sputtering method with these parameters: sputter time 100 s, sputter current 30 mA, and film thickness 20 nm a using sputter coater BAL-TEC SCD 005. Conventional transmission electron microscopy (TEM) was carried out on a TECNAI 20 Philips, operating at 200 kV. Elemental analysis (%C, %N, %H) was performed with a LECO CHNS-932 analyzer. The thermal stability of the modified mesoporous silicas was studied using a Setsys 18A (Setaram) thermogravimetric analyzer (from 25 to 800 ºC at 10 ºC $min^{-1}$).

### 2.6. Preparation of modified CPEs

CPEs modified with mesostructured silicas were prepared according to a previous work [17] by mixing 0.100 g (20%, w/w) of the materials (HMS or HMS-$NH_2$) with 0.300 g (60%, w/w) of carbon paste. Then, 0.100 g (20%, w/w) of mineral oil were added to the mixture and mixed until obtain a uniform paste. The obtained materials were packed into the end of a 5.0 cm long polyethylene tube (3 mm i.d.), with a metallic wire as inner electrical contact (HMS-CPE or $NH_2$-HMS-CPE). Appropriate packing was achieved by pushing the electrodes surfaces against a sheet of paper (this procedure also helps to eliminate the excess of mineral oil). For comparative purpose, an unmodified CPE was prepared according to the same procedure. Smoothing of the electrode surface was made by hand polishing on a filter paper. When necessary, new electrode surface was obtained by replacing the wetted paste by freshly prepared uniform paste and polishing them again on a filter paper. All freshly made electrodes were activated by performing 3 DPV measurements in 0.1 mol $L^{-1}$ phosphate buffer (from 0.0 to + 1.8 V) until the background was stable. This activation process was realized each day prior to use the electrode.

### 2.7. Voltammetric measurements

Electrochemical studies with CPEs were accomplished by CV and DPV. Unless otherwise stated, a 0.1 M phosphate buffer (pH 4) was used as electrolyte support for Pin, Ace and Met determination. Cyclic voltammograms were recorded from 0.0 to + 1.8 V at different scan rates without accumulation time. Differential pulse voltammograms were recorded from 0.0 to +1.8 V using a pulse amplitude of 10 mV with a potential step height of 5 mV, pulse width of 40 ms and a scan rate of 0.05 V $s^{-1}$ and after open-circuit accumulation of Pin, Ace and Met for 2 min with stirring at 300 rpm. After each measurement, to ensure that Pin, Ace and Met were not present on the electrode surface, regeneration of the electrode was performed by three DPV measurements in 0.1 mol $L^{-1}$ phosphate buffer (from 0.0 to + 1.8 V). Each measurement was performed in triplicate and the average value was reported.

## 3. Results and discussions

### *3.1. Characterization of the mesoporous materials*

Fig. 1A show the $N_2$ adsorption-desorption isotherms for mesostructured silicas. For HMS and HMS-$NH_2$ isotherms were type IV according to the I.U.P.A.C. classification with a H2 hysteresis loop, indicative of wormhole-like pores causing some bottleneck effect. In the HMS isotherm, the volume adsorbed increased at a relative pressure ($P/P_0$) of approximately 0.3, representing capillary condensation of nitrogen within the mesopores. The inflection position shifted slightly toward lower relative pressures and the volume of nitrogen adsorbed decreased with functionalization.

Table S1 (see supplementary material) shows the physical parameters of nitrogen isotherms, such the Brunauer-Emmett-Teller surface area ($S_{BET}$), total pore volume and BJH (Barrett, Joyner and Halenda) average pore diameter for both mesostructured silicas. HMS possessed very high $S_{BET}$ (962 $m^2$ $g^{-1}$), a pore volume of 0.88 $cm^3$ $g^{-1}$ and a BJH pore diameter of 25.8 Å, typical of surfactant-assembled mesostructures. After functionalization with the aminopropyl groups, a decrease in the $S_{BET}$ (821 $m^2$ $g^{-1}$), pore volume (0.51 $cm^3$ $g^-1$) and BJH average pore diameter (23.5 Å) was observed in the material. Thus, it can be inferred that the aminopropyl groups were grafted also inside the mesostructured pore channels. Fig. 1B shows the pore size distribution in the material after the functionalization procedure.

The morphology, shape and size of the HMS particles were studied by SEM. SEM micrographs (Fig. S1A) showed that materials had a quasi-spherical morphology, with mean diameters around 1.7 µm. On the other hand, TEM images (Fig. S1B) of this material show irregularly aligned mesopores throughout the materials with relatively uniform pore sizes (wormhole-like pore arrangement). These properties were maintained in the HMS-$NH_2$ material.

The amount of aminopropyl groups attached onto the HMS-$NH_2$ surface was calculated by elemental analysis from the percentage of nitrogen. Results obtained indicated that the HMS-$NH_2$ material had a functionalization degree of 1.38 mmol $g^{-1}$. Thermogravimetric curves for the HMS-$NH_2$ silica (see Fig. 1C) showed that exothermic degradation processes occur in the range of 200 – 600ºC, with weight loss of 12 % that demonstrated good thermal stability of the material.

*3.2. Electrochemical behaviour of Pin, Ace and Met on different CPEs*

In order to study the electrochemical behaviour of Pin, Ace and Met on the CPEs modified with mesostructured silicas, different CPEs were prepared: unmodified CPE, HMS-CPE and $NH_2$-HMS-CPE. The study was carried out by CV using 0.1 mol $L^{-1}$ phosphate buffer (pH 4) and standard solutions of 1 µmol $L^{-1}$ for Pin and Ace and 10 µmol $L^{-1}$ for Met. Fig 2 shows the cyclic voltammograms obtained at the scan rate of 0.1 V $s^{-1}$ in absence of the target analytes (Fig. 2A) and in presence of Pin, Ace and Met (Fig. 2B, C and D, respectively). As it is shown in Fig. 2A, peaks were not observed in buffer solution (without analytes), indicating that in the electrodes surface (unmodified CPE, HMS-CPE and $NH_2$-HMS-CPEs) there are not electroactive species in the potential region evaluated (from 0.0 to + 1.8 V). In addition, using the unmodified CPE oxidation peaks were not obtained for any of the target analytes under the assayed experimental conditions (Fig 2B, 2C and 2D). On the other hand, one oxidation anodic peak was obtained at about 0.8 for Pin (Fig. 2B), 0.95 V for Ace (Fig. 2C) and 1.45 V for Met (Fig. 2D) using HMS-CPE. The oxidation peak showed in presence of the β-blockers confirmed and irreversible electrochemical reaction, that could be due to a cleavage reaction of the aromatic ether of Pin, Ace and Met, respectively (process that involves transfer of two electrons) [22]. Comparing with unmodified CPE, the CV response observed using the HMS-CPE can be attributed to the regular structure and monodisperse pore dimension of the mesotructured silica, which ensure an easy and fast access to the analytes into the active centres, where an adsorption process occurs by electrostatic interaction between the sylanol groups of the HMS and the nitrogen atoms of the β-blockers (Fig. 3A) [23]. This effect was much more effective for Ace that showed a better-defined oxidation peak that can be explained as a consequence of its structure, that favours the adsorption process (see structure in Fig S2). It is noteworthy that the CV response of these three β-blockers was dramatically improved when amino groups were introduced in the surface of the HMS. This fact clearly indicates the synergistic effect between mesostructured silica and the functional $-NH_2$ groups introduced, as these amino groups make it possible an hydrogen bonding with the oxygen atoms of the β-blockers (Fig. 3B). This effect leads to a greater amount of Pin, Ace and Met adsorbed on the surface of the $NH_2$-HMS-CPE prior to voltammetric measure. These results are according with those previously reported by Ya et al. [20]. For Pin and Met one irreversible anodic oxidation peak was obtained about 0.85 and 1.45 V, respectively. However, for Ace two anodic peaks and one cathodic peak were obtained using $NH_2$-HMS-CPE (Fig. 2C). The first anodic peak ($E_{pa}$ = 0.58 V) and the cathodic peak ($E_{pc}$ = 0.41 V) were attributed to a quasi-reversible redox process with a peak-to-peak separation ($\Delta E = E_{pa} - E_{pc}$) of 0.17 V, which was greater than the 0.059 V expected for reversible process. A similar effect for Ace has been previously reported by Bagoji et al. [22]. For this quasi-reversible redox mechanism (Fig S3A), Ace first undergoes oxidation of the hydroxyl group with loss of two protons ($-2H^+$) and, in a reverse reaction, the addition of two electrons ($+2e^-$) to form the starting compound. The second peak is due to an irreversible redox reaction (Fig S3B) that leads to the breakdown of the molecule and subsequent oxidation. As it can be seen, the second anodic peak at 1.11 V was more intense than first one, so it was used for further experiments.

The effect of scan rate on the current response of Pin, Ace and Met on the $NH_2$-HMS-CPE was investigated by CV in 0.1 mol $L^{-1}$ phosphate buffer solution (pH 4) at different scan rates (from 0.05 to 0.45 V $s^{-1}$). The results obtained in all cases showed, that increasing the scan rate leads to an increase of peak current (Fig S4). In addition, there is a slight potential shift to more positive values with the scan rate typical for irreversible systems. In the study of voltammetric behaviour of the β-blockers on the $NH_2$-HMS-CPE it was found that the oxidation peak current (μA) showed a linear relationship with the scan rate in the range of 0.05-0.55 V $s^{-1}$ for Pin and Met ($y = 1.103x - 0.08$, $R^2 = 0.989$, and $y = 8.541x - 1.47$; $R^2 = 0.987$, respectively) and in the range of 0.05-0.45 V $s^{-1}$ for Ace ($y = 7.282x - 0.82$, $R^2 = 0.988$). This indicates that the electro-oxidation of Pin, Ace and Met on the $NH_2$-HMS-CPE surface was controlled by an adsorption process.

Finally, the electrochemical behaviour of Pin, Ace and Met on the unmodified CPE, HMS-CPE and $NH_2$-HMS-CPE was also studied by DPV after 2 min of accumulation time (Fig S5). From the comparison of differential pulse voltammograms, it was found that the oxidation peak current of the analytes increases at $NH_2$-HMS-CPE, indicating that the functionalization of HMS with amino groups is necessary to improve the sensitivity in the voltammetric determination of Pin, Ace and Met. In order to develop a voltammetric method for simultaneous determination of Pin, Ace and Met, DPV mode was selected, since the peaks obtained were sharp and better defined than those obtained by CV mode, with a lower background current, resulting in an improved resolution.

### *3.3. Optimization of the experimental conditions using $NH_2$-HMS-CPE by DPV*

#### *3.3.1. Effect of the type and pH of electrolyte support*

Two supporting electrolytes, 0.1 mol $L^{-1}$ acetate buffer and 0.1 mol $L^{-1}$ phosphate buffer (pH 4) have been tested by DPV. In this study, a multicomponent solution was used with a concentration of 1 μmol $L^{-1}$ for Pin and Ace and 10 μmol $L^{-1}$ for Met. The results showed that the oxidation peak current obtained, for the three analytes, was higher in phosphate buffer (data not shown). Thus, phosphate buffer was used for all the experiments. Furthermore, the influence of pH on the peak current of Pin, Ace and Met on the $NH_2$-HMS-CPE was also studied in the range from 3.0 to 8.0. As it can be seen in Fig. 4A, the peak current of β-blockers oxidation is influenced by the pH, and reaches its maximum at pH 4 for Pin and Ace and at pH 5 for Met.  These results can be attributed to the fact that at pH < 4 the nitrogen atom with the lone pair of electrons in the amino groups anchored to the HMS can get protonated, which makes the surface of the $NH_2$-HMS-CPE positively charged. In this situation, the hydrogen bonding between the amino groups and the β-blockers is negatively affected, reducing their accumulation in the electrode surface [24]. On the other hand, the adsorption also depends on the pKa of the silanols groups (which is 4.5) and the pH of the buffer solution. Thus, at higher pH values the acidity in the silica surface changes, due to the deprotonation of these silanol groups [25, 26], inhibiting the electrostatic interactions with the nitrogen atoms of the β-blockers and, consequently, the adsorption of the target analytes on the electrode surface. Finally, due to the pH value can affects the oxidation potential this effect was also studied. Fig 4B shows the potential *vs* pH value curves obtained for the three analytes. It can be found that oxidation peaks potential remains moderately stable for Met and Ace, whereas for Pin an important reduction in the peak potential was observed at pH 4. At this pH the peak potential difference of Pin and Ace reaches its maximum (400 mV). Thus, considering the best operational conditions for a simultaneous determination of Pin, Ace and Met, phosphate buffer pH 4 was selected for the subsequent analytical experiments.

### *3.3.2. Effect of accumulation time*

An accumulation time for the analytes, by immersion in a solution of Pin, Ace and Met, can improve the adsorption process on the $NH_2$-HMS-CPE surface, improving the sensitivity on the voltammetry step. Therefore, the effect of accumulation time (0, 2, 5 and 10 min, at 300 rpm) was studied in a multicomponent solution of 1 μmol $L^{-1}$ for Pin and Ace and 10 μmol $L^{-1}$ for Met in a 0.1 mol $L^{-1}$ phosphate buffer (pH 4). As shown in Fig. 4C, the peak currents of Ace, Pin and Met increased slightly with accumulation time up to 2 min, and after this time a platform appears for Pin and Met. This fact could be explained due to the oxidation rate of Ace, Met and Pin is low, therefore when the accumulation time is increased, the current response do not increase (Met and Pin) or increases slightly (Ace). On the other hand, it is noteworthy that the electrode surface was not saturated from 0 to 10 min of accumulation time, suggesting high content of amino groups and silanol groups in the $NH_2$-HMS-CPE surface. Then, for a rapid simultaneous analysis of Pin, Ace and Met, a short accumulation time (of 2 min) was found to be enough, which can be attributed to a fast adsorption rate, due to the structure, particle morphology and size of the HMS silica.

### *3.4. Analytical performance*

### *3.4.1. Linearity, sensitivity and selectivity*

Firstly, the linearity of each analyte was studied separately. Fig. S6 shows differential pulse voltammograms obtained for different concentrations of Pin, Ace and Met under the optimized conditions (0.1 mol $L^{-1}$ phosphate buffer, pH 4, and 2 min of accumulation time). As it can be seen, in all cases, the peak current increases when an increase of the analyte concentration takes place. The calibration curves obtained for Pin, Ace and Met indicated a linear relationship in the concentration range of 0.5 – 30 µmol $L^{-1}$, 0.5 - 50 µmol $L^{-1}$ and 1 - 60 µmol $L^{-1}$, with a regression equation of $y$ (µA) = 0.010$x$ (µmol $L^{-1}$) + 0.01 ($R^2$= 0.996), $y$ (µA) = 0.009$x$ (µmol $L^{-1}$) + 0.02 ($R^2$= 0.990) and $y$ (µA) = 0.010$x$ (µmol $L^{-1}$) - 0.0007 ($R^2$= 0.998), respectively. The limits of detection (LODs) and limits of quantification (LOQs) were calculated as 3 or 10 times the standard deviation (SD) of five measurements of the peak current of the smallest concentration of the calibration curves divided by the slope of the calibration curves according to [27]. The LODs obtained for Pin, Ace and Met were 0.077, 0.058 and 0.080 µmol $L^{-1}$, respectively, and the LOQs obtained were 0.25, 0.19 and 0.26 µmol $L^{-1}$ for Pin, Ace and Met, respectively.

Secondly, for simultaneous determination of Pin, Ace and Met, the linearity of one analyte in the presence of the other two was studied under the optimized conditions. Differential pulse voltammograms and calibration curves are shown in Fig 5. In Fig. 5A the concentration of Pin was increased from 0.5 to 40 µmol $L^{-1}$ in presence of 3 µmol $L^{-1}$ of Ace and 10 µmol $L^{-1}$ of Met, showing an increase in the peak current of Pin while its counterparts Ace and Met remained unchanged. The calibration curve for Pin exhibited a good linear relationship in the concentration range studied with a regression equation of: $y$ (µA) = 0.008$x$ (µmol $L^{-1}$) + 0.01 ($R^2$=0.990). Similarly, selective determination of Ace, keeping constant the concentration of Pin (3 µmol $L^{-1}$) and Met (10 µmol $L^{-1}$) was also performed (Fig. 5B). The calibration curves indicated a linear relationship in the concentration range of 0.5 - 70 µmol $L^{-1}$, with a regression equation of: $y$ (µA) = 0.019$x$ (µmol $L^{-1}$) + 0.02 ($R^2$=0.995). Finally, for the determination of Met, the concentration of Pin (3 µmol $L^{-1}$) and Ace (3 µmol $L^{-1}$) was kept constant, the analytical curve fits well with a linear equation of: $y$ (µA) = 0.003$x$ (µmol $L^{-1}$) + 0.01 ($R^2$=0.993) in a concentration range of 1 - 60 µmol $L^{-1}$ (Fig 5C). The LOD and LOQ obtained, calculated as indicated above, were 0.1 and 0.33 µmol $L^{-1}$ for Pin, 0.046 and 0.15 µmol $L^{-1}$ for Ace and 0.23 µmol $L^{-1}$ and 0.76 µmol $L^{-1}$ for Met, respectively. To the best of our knowledge, there are not published electroanalytical methods for simultaneous determination of Pin, Ace and Met.

Finally, the stripping performance of Pin, Ace and Met was recorded in simultaneous determination under the optimized conditions (Fig. 5D). Three well-defined peaks around +0.85 V, +1.11 V and +1.45 V for Pin, Ace and Met, respectively, are observed in Fig. 5D. As it can be seen, in all cases, the peak current increases when an increase of the analytes concentration takes place (Pin: $y$ (µA) = 0.007$x$ (µmol $L^{-1}$) + 0.01, $R^2$ = 0.992, 0.5 - 40 µmol $L^{-1}$; Ace: $y$ (µA) = 0.018$x$ (µmol $L^{-1}$) + 0.02, $R^2$ = 0.996), 0.5 to 70 µmol $L^{-1}$ and Met: $y$ (µA) = 0.003$x$ (µmol $L^{-1}$) + 0.02, $R^2$ = 0.993, 1 to 60 µmol $L^{-1}$). When comparing the analytical curves obtained for the analysis of the compounds separately and the analysis of one in the presence of another, it can be observed that there is not difference in the linear ranges and slopes, which indicated that the developed sensor was able to discriminate an analyte in the presence of the others with an adequate sensitivity.

Another important parameter for a sensor is its ability to discriminate between the interfering species, commonly present in the sample, and the analytes. For this purpose, the influence of some coexistent substances in water samples was examined. The voltammetric response of 1 µmol $L^{-1}$ of Pin, 1 µmol $L^{-1}$ of Ace and 10 µmol $L^{-1}$ of Met in the presence of 1000 µmol $L^{-1}$ of $Cl^-$, $CO_3^{2-}$, $NO_3^-$, $SO_4^{2-}$, $Ca^{2+}$, $Mg^{2+}$ under the optimized conditions were recorded. Based on the results obtained (Table S2) none of the interferences caused a decrease in the peak current greater than 15% for any analytes, these results show that the $NH_2$-HMS-CPE possess a high selectivity.

### *3.4.2. Repeatability, reproducibility and stability of $NH_2$-HMS-CPE*

The reproducibility and stability are two important parameters for the evaluation of the performance of a sensor. The main advantage of using the $NH_2$-HMS-CPE is the easy and quick surface remove when it is necessary. The peak currents on $NH_2$-HMS-CPE after six consecutive measurements of 1 µmol $L^{-1}$ of Pin, 1 µmol $L^{-1}$ of Ace and 10 µmol $L^{-1}$ of Met in a multicomponent solution were compared to evaluate the repeatability of the electrode. The average of the resulted values has relative standard deviation (RSD) of 3.8% for Pin, 4.0% for Ace and 4.2% for Met. The reproducibility of the $NH_2$-HMS-CPE was checked using six independent surface electrodes. The RSD was smaller than 4% for all analytes, showing that the voltammetric signals are highly reproducible between different electrodes. In addition, $NH_2$-HMS-CPE showed good long-term stability for more than 155 measures carried out in 5 consecutive days with several independent surface electrodes. These results indicate that the developed sensor have good repeatability, reproducibility and stability.

### *3.5. Accuracy in water samples analysis using $NH_2$-HMS-CPE*

To investigate the usefulness of the $NH_2$-HMS-CPE in real water samples, it was applied in the determination of Pin, Ace and Met in drinking, environmental and wastewaters (Table 1) under optimized conditions. For quantitative purposes the standard addition method was used, in order to avoid the matrix effect by using a multicomponent solution of the analytes that was added to the different waters samples (at increments of 1, 3 and 5 µmol $L^{-1}$ of Pin and Ace and 5,10 and 20 µmol $L^{-1}$ of Met). As it is shown in Table 1, there was no detectable concentration of Pin, Ace and Met in the tap and mineral waters analysed. The accuracy of the method has been evaluated in terms of the recovery values obtained at the concentrations of 1 µmol $L^{-1}$ of Pin, 1 µmol $L^{-1}$ of Ace and 5 µmol $L^{-1}$ of Met in a multicomponent solution. Recovery values between 99-109% for Pin, 99-108% for Ace and 100-104% for Met were achieved. For river and sewage water (effluent), Ace and Met were found in different samples. Ace was quantified in river water sample 2 with a concentration of 0.7 µmol $L^{-1}$ and it was also detected in river water sample 3 and sewage water sample at concentrations below LOQ. Met was quantified in sewage water with a concentration of 5.7 µmol $L^{-1}$. Recovery values between 94-101% for Pin, 106-112% for Ace and 92-100% for Met were achieved for river and sewage water samples. These results indicate that the developed $NH_2$-HMS-CPE is useful for a rapid and *in situ* determination of Pin, Ace and Met in drinking, environmental and wastewater samples at very low concentrations without any sample treatment.

### *3.6. A comparative study of Pin, Ace and Met determination with different sensors*

The analytical performance of the proposed $NH_2$-HMS-CPE was compared with different modified electrodes previously published (Table 2). As to date, there are no scientific articles in the literature, which describe the use of electrodes for the simultaneous analysis of these analytes. We have compared the $NH_2$-HMS-CPE with others proposed sensors used for the individual analysis of Pin, Ace or Met in water samples. The potentials obtained with this electrode for the Ate and Pin are similar to that obtained in other electrodes. In the case of Met the potential it is slightly higher which makes it possible to measure all three analytes simultaneously. Compared with other electrodes reported in the literature, the proposed $NH_2$-HMS-CPE displayed a significant improve in linear concentration range [22, 28-33] and shows similar or lower LODs than some modified electrodes [29,31,32], except for those based on graphene [28], graphite pencil electrode [22], boron-doped diamond electrode [33] and carbon nanotubes [34]. Additionally, with $NH_2$-HMS-CPE a good linear concentration range and very low LODs were obtained for simultaneous analysis employing portable analyzers for *in situ* monitorization of Pin, Ace and Met in waters.

## 4. Conclusions

A highly sensitive, fast, experimentally convenient and low-cost strategy for the simultaneous analysis and quantitative determination of Pin, Ace and Met based on a modified carbon paste electrode with HMS functionalized with amino groups ($NH_2$-HMS-CPE) was developed. Mesostructured silicas are an intelligent solution to prepare electrochemical sensors because, its high specific surface area, high porosity, controllable and narrowly distributed pore size and ordered pore arrangement. In this work, we have demonstrated that $NH_2$-HMS-CPE is an excellent option to simultaneous determination of Pin, Ace and Met in water samples by DPV. The described method has good linear ranges, recovery values and very low detection limits for the three analytes studied and it has been successfully applied for the determination of Pin, Ace and Met in drinking and environmental and wastewaters.

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## Appendix A. Supplementary data

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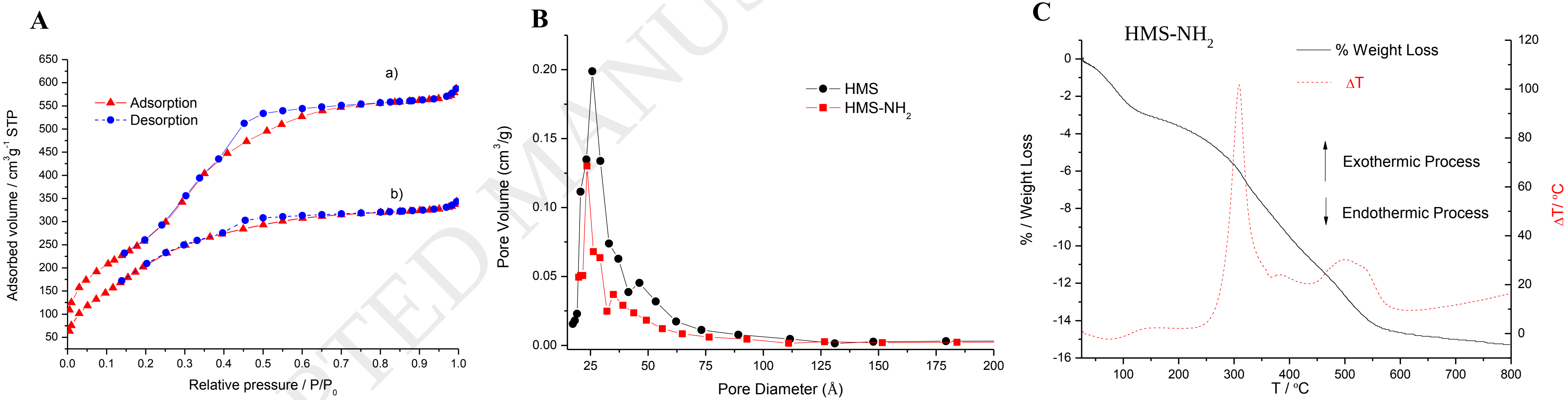

**Fig. 1.** (A) $N_2$ adsorption-desorption isotherms of a) HMS and b) $HMS\text{-}NH_2$; (B) Pore size distribution for HMS and $HMS\text{-}NH_2$ and (C) Thermogravimetric curves and heat flow of $HMS\text{-}NH_2$.

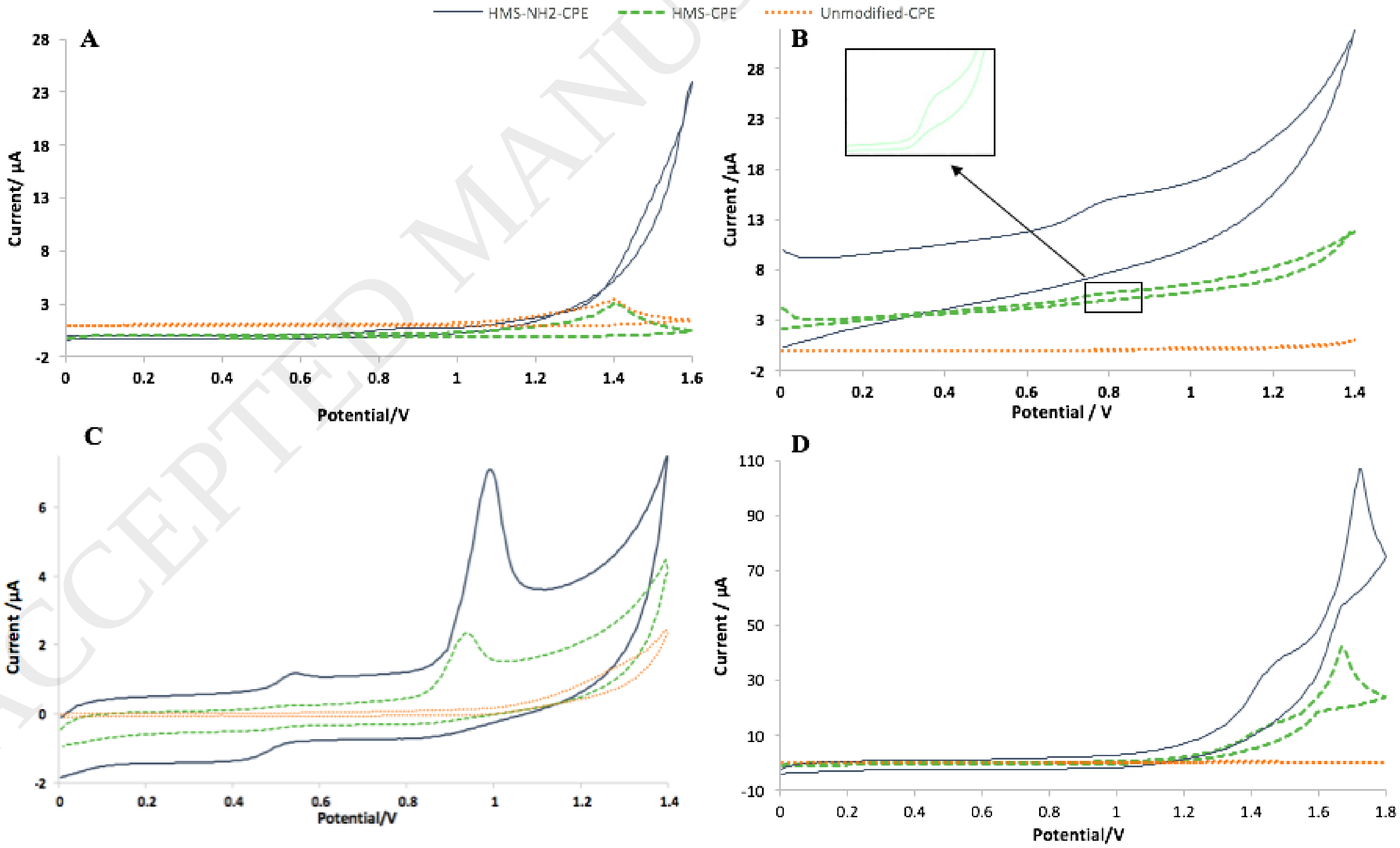

**Fig. 2.** Cyclic voltammograms obtained with unmodified CPE, HMS-CPE and $NH_2$-HMS-CPE at scan rate of 0.1 V $s^{-1}$ in 0.1 mol $L^{-1}$ phosphate buffer (pH 4): (A) without analytes, (B) 1 µmol $L^{-1}$ of pindolol, (C) 1 µmol $L^{-1}$ of acebutolol and (D ) 10 µmol $L^{-1}$ of metoprolol.

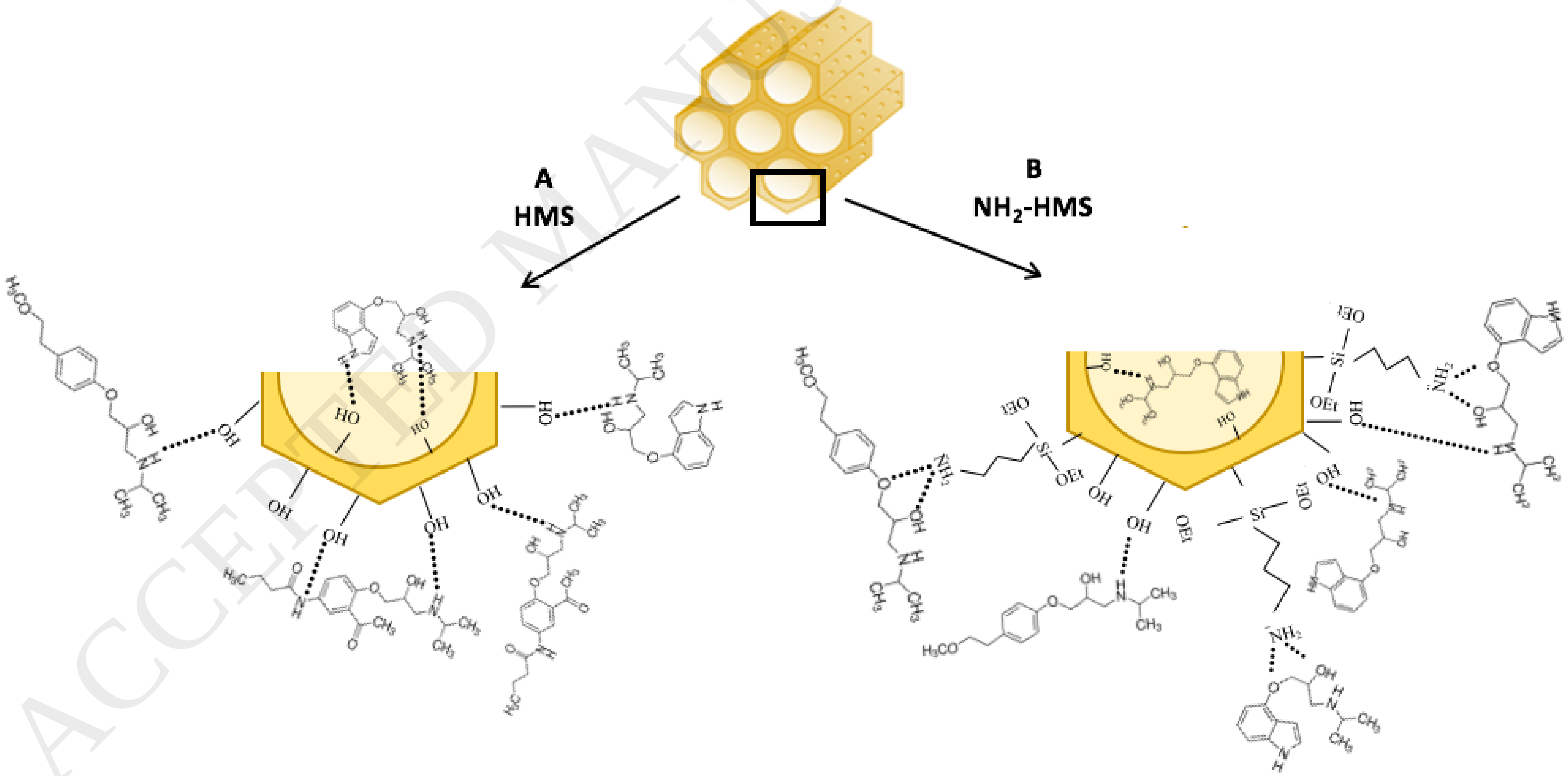

**Fig.3.** Adsorption mechanism proposed into CPE modified with mesoporous materials: (A) HMS-CPE, (B) $NH_2$-HMS-CPE.

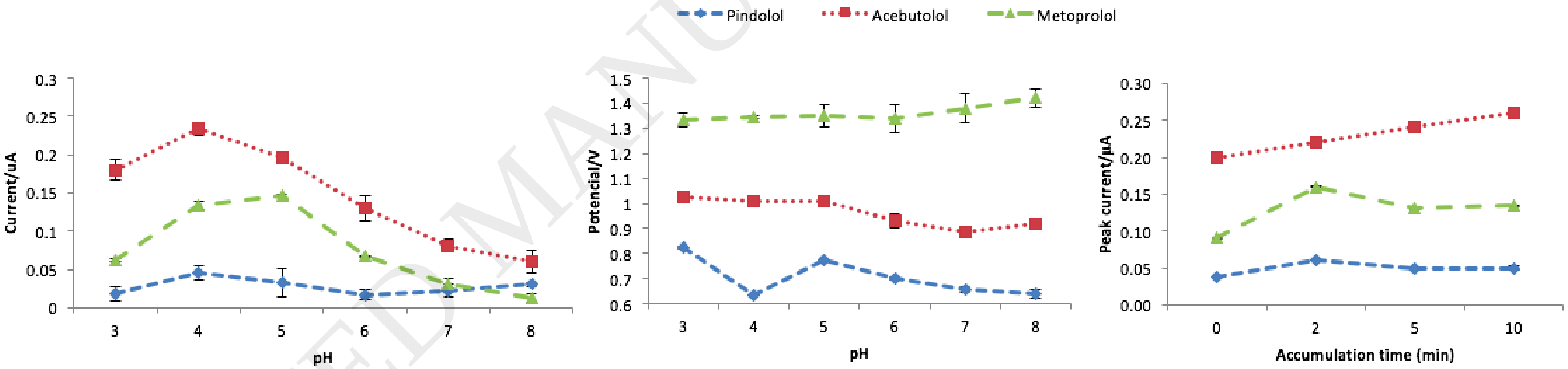

**Fig. 4.** Electrochemical behaviors of $NH_2$-HMS-CPE by DPV. (A) Peak current-pH value curves, (B) Potential-pH value curves, (C) Peak current-accumulation time curves. Conditions: 1 µmol $L^{-1}$ pindolol and acebutolol and 10 µmol $L^{-1}$ metoprolol in 0.1mol $L^{-1}$ phosphate buffer. Scan rate 0.05 V $s^{-1}$; $E_{step}$: 0.005 V; $E_{puls}$: 0.01 V; $t_{puls}$: 40ms (2 min of accumulation time in (A) and (B))

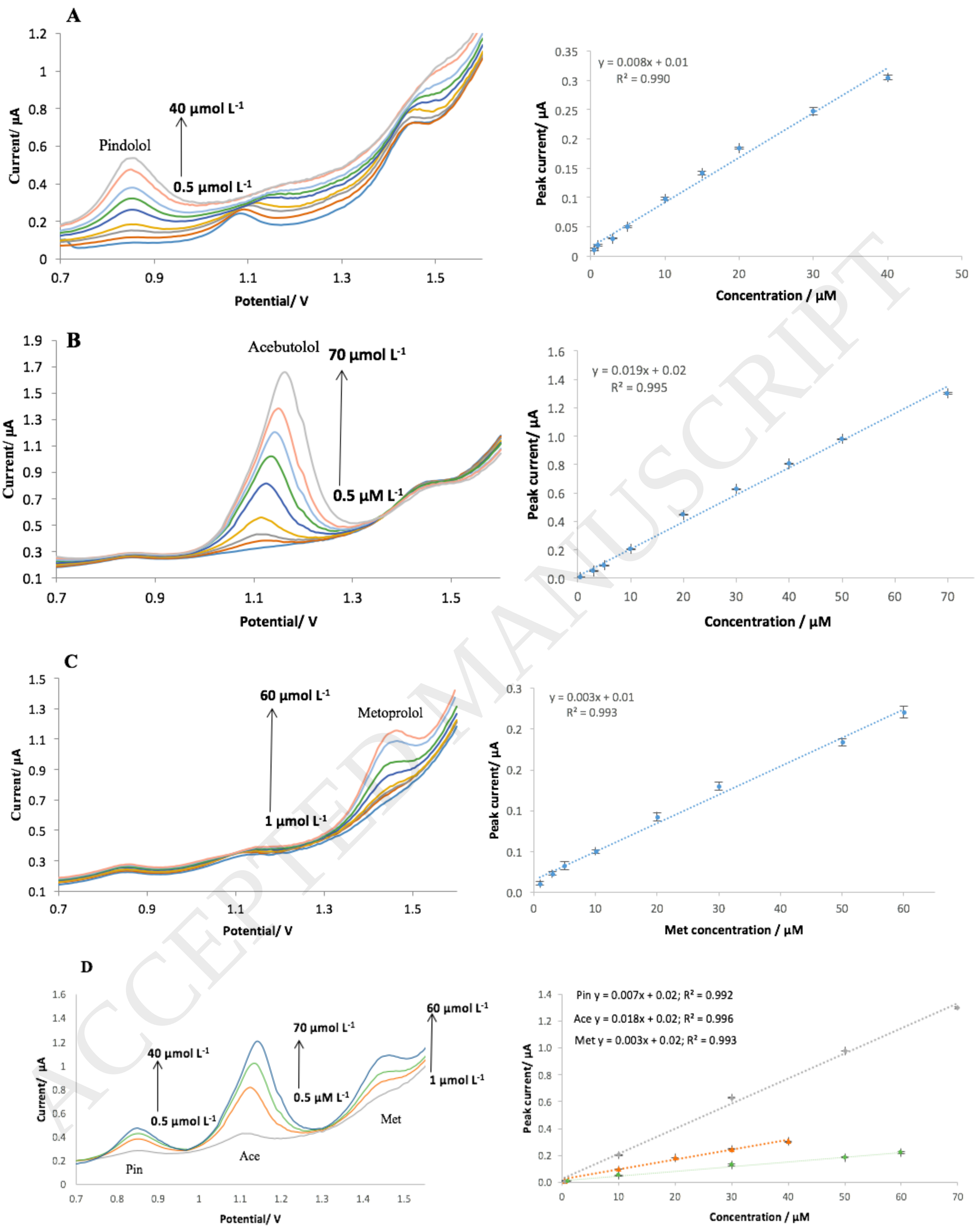

**Fig. 5.** Differential pulse voltammograms obtained for increased amounts of (A) pindolol;

(B) acebutolol (C) metoprolol and (D) pindolol, acebutolol and metoprolol using a $NH_2$-HMS-CPE and their respective calibration curves. Conditions: 0.1 mol $L^{-1}$ phosphate buffer, 2 min of accumulation time. Scan rate 0.05 V $s^{-1}$; $E_{step}$: 0.005 V; $E_{puls}$: 0.01 V; $t_{puls}$: 40ms.

**Table 1**

Determination of pindolol, acebutolol and metropolol in drinking, environmental and wastewaters and recoveries obtained under the optimized conditions (n = 3) using an electrode of $NH_2$-HMS-CPE.

| | Pindolol | | | | Acebutolol | | | | Metoprolol | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample* | Found ($\mu$mol $L^{-1}$) | Added ($\mu$mol $L^{-1}$) | Found after added ($\mu$mol $L^{-1}$) | Recovery ± SD (%) | Found ($\mu$mol $L^{-1}$) | Added ($\mu$mol $L^{-1}$) | Found after added ($\mu$mol $L^{-1}$) | Recovery ± SD (%) | Found ($\mu$mol $L^{-1}$) | Added ($\mu$mol $L^{-1}$) | Found after added ($\mu$mol $L^{-1}$) | Recovery ± SD (%) |
| Tap water 1 | - | 1 | 1.03 | 103±6 | - | 1 | 1.05 | 105±5 | - | 5 | 5.10 | 102±3 |
| Tap water 2 | - | 1 | 1.09 | 109±1 | - | 1 | 1.04 | 104±7 | - | 5 | 5.15 | 103±9 |
| Tap water 3 | - | 1 | 1.06 | 106±6 | - | 1 | 1.06 | 106±1 | - | 5 | 5.05 | 101±5 |
| Tap water 4 | - | 1 | 0.99 | 99±2 | - | 1 | 1.03 | 103±3 | - | 5 | 5.00 | 100±7 |
| Tap water 5 | - | 1 | 1.03 | 103±2 | - | 1 | 1.08 | 108±5 | - | 5 | 5.00 | 100±8 |
| Mineral water 1 | - | 1 | 1.02 | 102±4 | - | 1 | 1.07 | 107±5 | - | 5 | 5.20 | 104±4 |
| Mineral water 2 | - | 1 | 1.07 | 107±7 | - | 1 | 0.99 | 99±5 | - | 5 | 5.20 | 104±8 |
| River water 1 | - | 1 | 0.97 | 97±1 | - | 1 | 1.10 | 110±9 | - | 5 | 4.85 | 97±4 |
| River water 2 | - | 1 | 0.99 | 99±4 | 0.7 | 1 | 1.80 | 105±3 | - | 5 | 5.00 | 100±6 |
| River water 3 | - | 1 | 0.98 | 98±5 | < LOQ | 1 | 1.10 | 110±3 | - | 5 | 4.90 | 98±6 |
| River water 4 | - | 1 | 1.10 | 101±1 | - | 1 | 1.12 | 112±1 | - | 5 | 4.25 | 85±4 |
| Sewage water | - | 1 | 0.94 | 94±1 | < LOQ | 1 | 1.06 | 106±5 | 5.7 | 5 | 10.30 | 98±2 |

DPV analysis using 0.1 mol $L^{-1}$ phosphate buffer (pH 4) and 2 min of accumulation time. Scan rate 0.05 V $s^{-1}$; $E_{step}$: 0.005 V; $E_{puls}$: 0.01 V; $t_{puls}$: 40ms. - : not detected.

* Waters (origin and conductivity): Tap water 1 (Madrid, 315µS), Tap water 2 (Santiago de Compostela, 333µS), Tap water 3 (Rome, 140µS), Tap water 4 (Lisbon, 1527µS), Tap water 5 (Valencia, 3050µS), Mineral water 1 (Aquarel, 104µS), Mineral water 2 (Fuente Primavera, 797µS), River water 1 (Manzanares river, 477µS), River water 2 (Manzanares river near an hospital, 520µS), River water 3 (Tajo river, bathing site, 2670 µS), River water 4 (Tajo river, 2330 µS), Sewage water (WWT Móstoles, 1984 µS)

**Table 2**

Comparative study of reported methods for pindolol, acebutolol and metoprolol determination with different modified electrodes.

| Modified electrodes | Detection method | Accumulation Time (s) | Oxidation potential (V) | Linear range (µmol $L^{-1}$) | Limit of detection (µmol $L^{-1}$) | Ref |
|---|---|---|---|---|---|---|
| *Pindolol* | | | | | | |
| Reduced graphene oxide-GCE | SWV | 10 | + 1.0 | 0.1-10 | 0.026 | [28] |
| Bare screen-printed-GE | SWV | - | + 0.8 | 0.1-10 | 0.097 | [29] |
| MIP-GCE | AdS-DPV | 90 | + 0.8 | 0.1-50 | 0.05 | [30] |
| *Acebutolol* | | | | | | |
| Mercury drop electrode | AdS- SWV | 30 | - 1.25 | 0.5-6 | 0.5 | [31] |
| Unmodified-PGE | DPV | 2 | + 1.0 | 1.0-15 | 0.013 | [22] |
| *Metoprolol* | | | | | | |
| Mercury drop electrode | DPV | 15 | - 1.2 | 3.18-28.5 | 0.93 | [32] |
| Unmodified-GCE | DPV | 15 | + 0.8 | 1.62-101 | 0.51 | [32] |
| Boron-doped diamond electrode | DPV | - | + 1.32 | 0.38-22 | 0.034 | [33] |
| MIP-MWCN-PGE | DPV | 300 | + 1.25 | 0.06-490 | 0.003 | [34] |
| *Pindolol, acebutolol and metoprolol* | | | | | | |
| $NH_2$-HMS-CPE | DPV | 120 | + 0.85<br>+ 1.11<br>+ 1.45 | Pin 0.5-40<br>Ace 0.5-70<br>Met 1-60 | Pin 0.1<br>Ace 0.046<br>Met 0.23 | This work |

AdS-SWV: adsorptive stripping square wave voltammetry; AdS-DPV: adsorptive stripping differential pulse voltammetry; GCE: glassy carbon electrode; GE: graphite electrode; CPE: carbon paste electrode; DPV: differential pulse voltammetry; PGE: pencil graphite electrode; MIP: molecular imprinted polymer; MWCN: multi-walled carbon nanotubes; SWV: square wave voltammetry.