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Selective and sensitive detection of dopamine in the presence of ascorbic acid and uric acid at a Sonogel-Carbon L-Histidine modified electrode

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- ✓ modified electrode

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Abstract

The Sonogel–Carbon electrode is a special class of sol–gel electrode that exhibits efficient mechanical and electrical properties. In this study, Sonogel-Carbon modified with L-Histidine was used to prepare a novel electrochemical sensor. This new electrode showed excellent electrocatalytic behavior towards the oxidation of dopamine (DA) in PBS and human serum. The proposed sensor was applied to the simultaneous determination of dopamine, ascorbic acid (AA) and uric acid (UA) in pH 7.4 PBS and excellent results were obtained. A lower detection limit of 1×10^{-7} M was achieved. This work, illustrates, for first time, the use of L-histidine Sonogel-Carbon electrode as a simple and low-cost tool for the selective and sensitive determination of DA in the presence of AA and UA.

1. Introduction

Design and application of sensitive and selective electrochemical sensors for the determination of biologically important substances have gained considerable interest in recent years [1-5].

Dopamine (DA) is an important neurotransmitter molecule of catecholamines that is widely distributed in the mammalian central nervous system for message transfer. It plays a very important role in the function of central nervous, renal, hormonal, and cardio vascular systems [6]. Extreme abnormalities of DA levels can lead to brain disorders such as parkinsonism and schizophrenia [7, 8]. Therefore, developing simple and rapid methods for the sensitive determination of DA in routine analysis is always a challenge [9, 10].

In this context, electrochemical techniques have generated considerable attention, in recent years as good tools for the study of electrochemical mechanisms and for the detection and quantification of biological substances, in particular DA, AA and UA. However, AA and UA oxidize at similar potentials with DA and the result is an overlap of their voltammetric responses when using conventional electrodes. This overlap of potentials has an adverse effect on electrode selectivity and reproducibility [11, 12].

In literature, there are many papers reporting the manufacture process and analytical applications involving Sonogels. The Sonogel-Carbon materials were patented and described first by Hidalgo-Hidalgo de Cisneros et al [13, 14]. Recently a new type of graphite-based sol-gel electrode has been developed, the sonogel-carbon electrode, which was obtained using high-energy ultrasounds. In general, classical procedures for the synthesis of acid catalysed sol-gel-based electrode materials include the addition of an alcoholic solvent to the initial precursor mixture to make it homogenous. This is followed by the employment of an ultrasound bath for several minutes to promote the hydrolysis. in the other hand, by means of sonocatalysis, high-energy ultrasounds is applied directly to the precursors, and ultrasonic cavitation, sol-gel reactions occur in a unique environnement, leading to gels

with special characteristics. These so-called sonogels are mainly of high density, with a fine texture and homogeneous structure. The mix of sonogel with spectroscopic grade graphite leads to the sonogel-carbon electrode [14, 17].The Sonogel–Carbon electrodes show the general good properties of the other CCE's (Ceramic Carbon Electrodes). Besides, in comparison with other carbon electrodes, they exhibit especially favorable electrochemical properties, such as broad operational range of voltage and very low values of observed charging capacity (Cobs). These electrodes show very favorable electroanalytical properties for their use as amperometric sensors. Furthermore, they can easily permit the incorporation of numerous receptor molecules at the Sonogel–Carbon materials, and the deliberate chemical modification of the electrode surface with a suitable reagent results in the control of the rates and selectivity of electrochemical reactions at the solid/liquid interface [18, 22].
In the present work, we describe a new application of sonogel-carbone electrode based on the incorporation of L-Histidine (L-His) to detect the dopamine. Among various amino acids L-Histidine ($C_6H_9N_3O_2$), also known as (L)-2-Amino-3-(4-imidazolyl) contains an aromatic nitrogen and imidazole side chain. Due to its extreme biochemical importance, it is used in various biological process and medical applications [23].
Considerable attention has been focused on the use of histidine in biosensors. The imidazole group of histidine enables the covalent attachment of organic molecules and biomolecules of interest [24]. L-Histidine is a non conducting biomaterial that could serve as a good biomaterial for use in electrochemical sensors. With imidazole group (pKa = 6) on the side chain, L-His can easily undergo static interactions and form covalent bonds with a specific molecule [25]. In addition, imidazole group favor the immobilization of biomolecules on the electrode surface. And. In this article, we report for the first time the use of L-His to modify sonogel carbon electrode (SNCG) to investigate the electrochemical behavior of DA in the presence of AA and UA. Stability and reproducibility, response time and lifetime of the proposed sensor were also, investigated and results were very satisfactory.

2. Materials and methods

2.1. Materials and reagent

Methyltrimethoxysilane (MTMOS) was from Merk (Darmstad, Germany), Hydrochloric acid (HCl) and sulfuric acid (H_2SO_4) was from Panreac (Barcelona, Spain). L-Histidine (>99%) was obtained from Fluka Chemical Company (Switzerland). UA (99%) was purchased from Sigma (Barcelona, Spain), DA and AA were purchased from Aldrich (Milwaukee, USA), KH_2PO_4 and K_2HPO_4 for phosphate buffer were from Fluka. Graphite powder (spectroscopic grade RBW) was from SGL Carbon (Ringsdorff, Germany). All reagents were of analytical grade and used without a further purification. Nanopure water was by passing twice-distilled water through a Milli-Q system (18MΩ-cm, Millipore, Bedford, MA). Glassy capillary tubes, i.d. 1.15mm, were used as the bodies for the composite electrodes. Serum group (A) rhesus negative (blood transfusion center, Tetouan, Morocco).

2.2. Apparatus

All electrochemical measurements were performed with an Autolab PGZ301 DYNAMIC – EIS VOLTAMMETRY – France). The experiments were carried out in a three-electrode cell at a room temperature of (25±1 ◦C). The counter electrode was a platinum wire and a Ag/AgCl, 3M KCl electrode was used as the reference. The composite-filled capillary tubes were used as working electrode. Square wave voltammetry (SWV) and Cyclic Voltammetry (CV) were the electrochemical techniques applied to study the behaviour of the Sonogel–Carbon electrodes. Measurements were carried out under N_2 atmosphere when required. The synthesis of the Sonogel-Carbon was carried out sonicating with a high-power ultrasonic generator, SONICATOR 3000, from MISONIX (MISONIX Inc., Farmingdale, NY, USA) equipped with a 13mm titanium tip that provides a maximum power of 600W.
On the other hand, the impedance spectra were recorded using the same three-electrode cell setup described above. The initial frequency used was 100 kHz and the final frequency was 10 mHz, with an AC amplitude of 10 mV. A potential of 437 mV was chosen in order to insure the stability of the on the electrodes during the experiments.
Scanning electron microscopy (SEM) and energy dispersive spectroscopy spectrum (EDS) analysis were performed in a SH 4000M (BRUKER Company, HIROX, Oregon, JAPON).

2.3. Preparation of the Sonogel electrode

To prepare the Sonosol, the general procedure was as follows: 500µL of MTMOS and 100 µL of 0.2M HCl were mixed and then insonated during 5 s with the high-power ultrasonic processor, in this way the mixture is subjected to the phenomenon of ultrasonic cavitations, by which the sol–gel process begins, avoiding the use of alcoholic solvent and reducing drastically the time needed to get an unique phase. In the next step, the adequate amounts of L-histidine and graphite powder were added and homogenously dispersed in the obtained Sonosol.

After several minutes, the resulting material starts to acquire enough consistency thus it could fill easily the glass capillaries leaving a little extra mixture sticking out of the glass tube to facilitate the subsequent polishing step. After 24h, the Sonogel–Carbon L-histidine composite electrode becomes hardened and, therefore, structured. Adherence between the developed material and the glass was excellent. Before use, the electrodes were polished with No. 1200 emery paper to remove extra composite material and wiped gently with weighing paper. Electrical contact was established by inserting a cooper wire into the capillary.

3. Results and discussion

3.1. Characterization of the electrode

3.1.1. SEM and EDS studies

Fig. 1 shows SEM images of the surface of the 7% L-His Sonogel-Carbon electrode. The tip of the electrode was packed well with condensed graphite powder due to the chemical bonding of carbon in the graphite and silicon in MTMOS. Fig. 1(b) shows the smooth surface of the sonogel-carbon electrode incorporated with nanostructured L-Histidine. Even though it was difficult to identify L-Histidine inorganic spots in the SEM images as expected, it is believed that L-Histidine is uniformly coated and distributed on the graphite powders present at the tip of the Sonogel-Carbon electrode.

Fig. 2 shows the EDS composition analysis on the surface of the sonogel carbon electrode unmodified (a) and EDS composition analysis on the surface of the sonogel carbon electrode modifiied by L-Histidine. Five types of elements can be observed: carbon, oxygen, silicon, azote and aluminium. The main difference between both EDS is the presence of particles of L-Histidine on the surface of the material that is proven by the presence of azote in the elementary composition of the modified material.

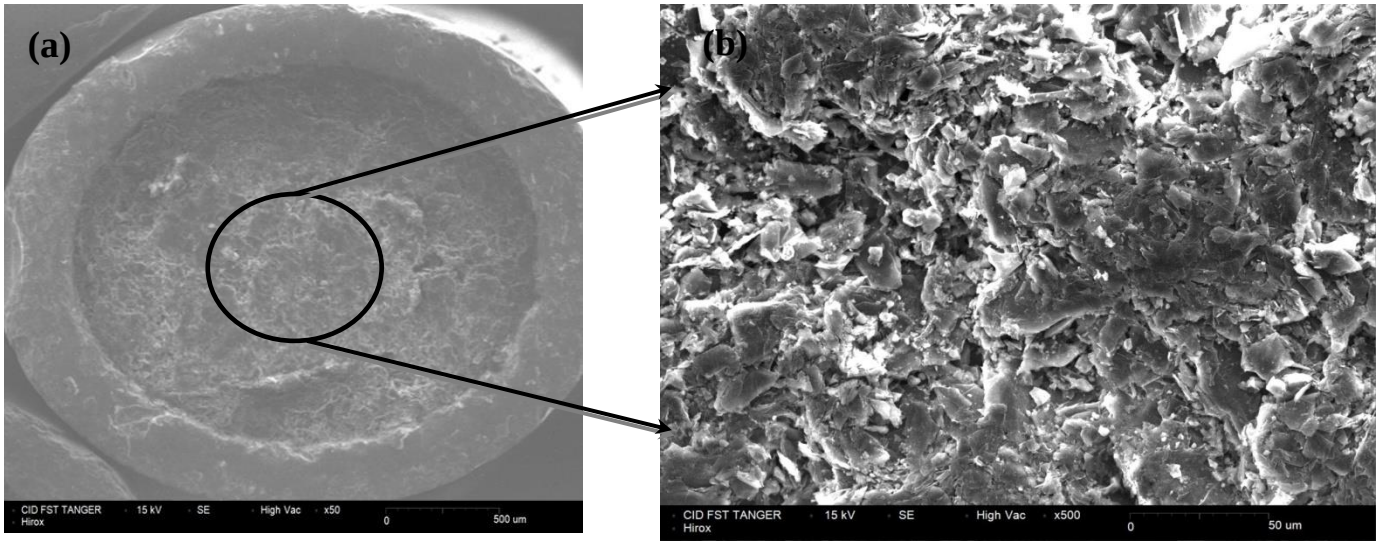

Fig. 1: (a) SEM images of 7% L-His Sonogel-Carbon electrode: (a) the tip (scale bar = 500µm) and (b) the surface of area high lighted in (a) (scale bar = 50µm).

3.1.2. Electrochemical impedance spectroscopy (EIS) of the L-His Sonogel-Carbon electrode

The EIS can give information on the impedance changes of the electrode surface during the modification process. In an EIS, the semicircle part at higher frequencies corresponds to the electron transfer limited process or the electron transfer resistance (Ret). This resistance controls the electron transfer kinetic process of the redox probe at the electrode interface. The linear segment at lower frequencies shows a controlled diffusion process.

Fig3 depicts the EIS of the bare 0% L-His Sonogel-Carbon electrode, showing very low electron transfer resistance (R=982.00ohm.cm^2) to the redox probe dissolved in the electrolyte solution. The results showed that the process is a controlled diffusion. The EIS of the 5% L-His Sonogel-Carbon modified electrode response shows a much lower interfacial electron transfer resistance (R=573.30ohm.cm^2) furthermore, the EIS of 7% L-His Sonogel-Carbon modified electrode response is very close to that of the 5% L-His Sonogel-Carbon electrode (R=465.80ohm.cm^2). We found that the EIS of the 7% L-His Sonogel-Carbon modified electrode has a lower electron transfer resistance than the bare sonogel-carbon electrode. This could be attributed to the facilitation of electron transfer by 7% L-His Sonogel-Carbon electrode. L-His play an important role as a conducting or electron transfer tunnel.

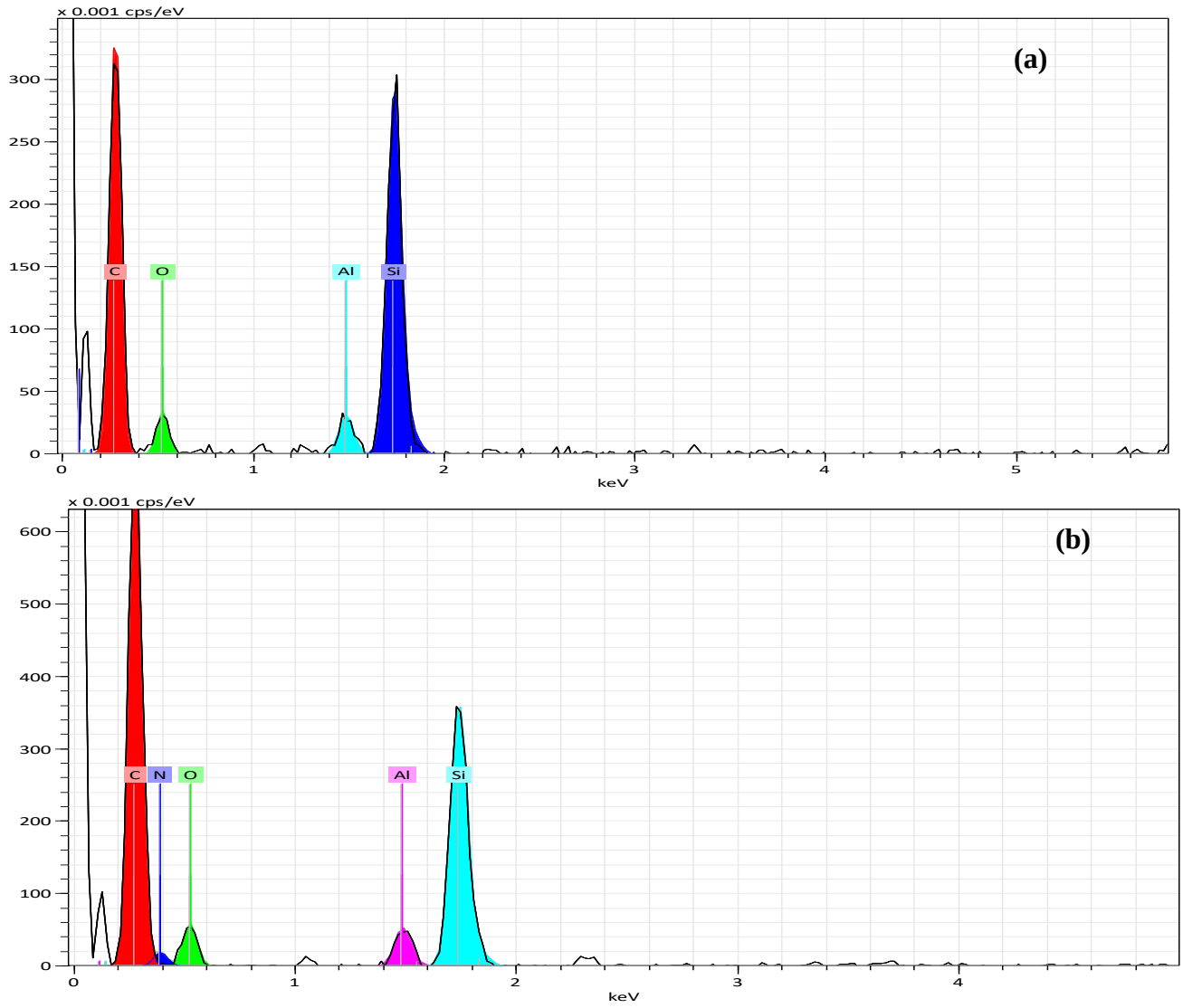

Fig. 2: (a) EDS spectrum of a unmodified Sonogel–Carbon electrode; (b) EDS spectrum of a 7% L-His Sonogel–Carbon modified electrode.

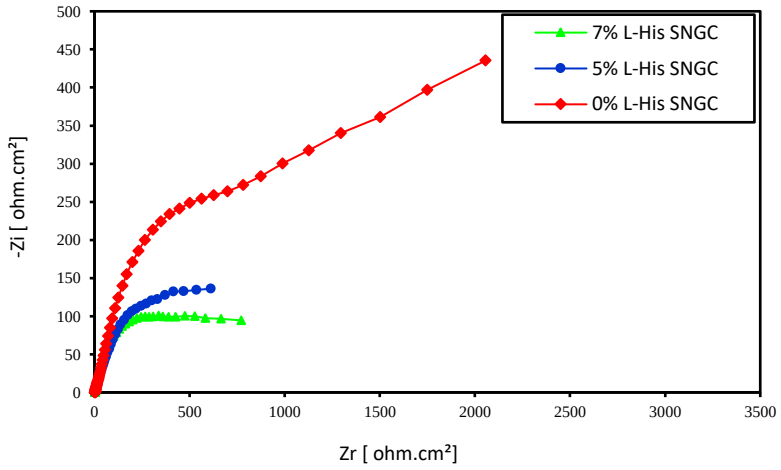

Fig.3: Nyquist diagram of 10^{-3}M DA 0.05M phosphate buffer of pH=2 using different electrodes at the oxidation of DA unmodified sonogel-carbon electrode, 5% L-His sonogel-carbon modified electrode and 7% L-His sonogel-carbon

3.2. Electrochemical behaviors of DA at the L-His sonogel-carbon modified electrode

Fig. 4 depicts the CVs of DA at the unmodified sonogel-carbon electrode, at the 5% and 7% L-His sonogel-carbon modified electrodes in 0.05M PBS (pH 2). The oxidation of DA has been documented to be a two-proton

and two-electron process [26]. At the unmodified sonogel-carbon electrode, the electrochemical response of DA is very low and the anodic peak potential is 510 mV, which is higher than the value reported in literature [27].
The 5%L-His sonogel-carbon electrode gives a letter response comparing with the unmodified sonogel-carbon electrode. The anodic peak potential of DA shifted to 456 mV and the peak current enhanced 3 times. Therefore, the anodic current value of DA at the 7% L-His sonogel-carbon modified electrode was 4 times higher than that obtained on the unmodified sonogel-carbon electrode.

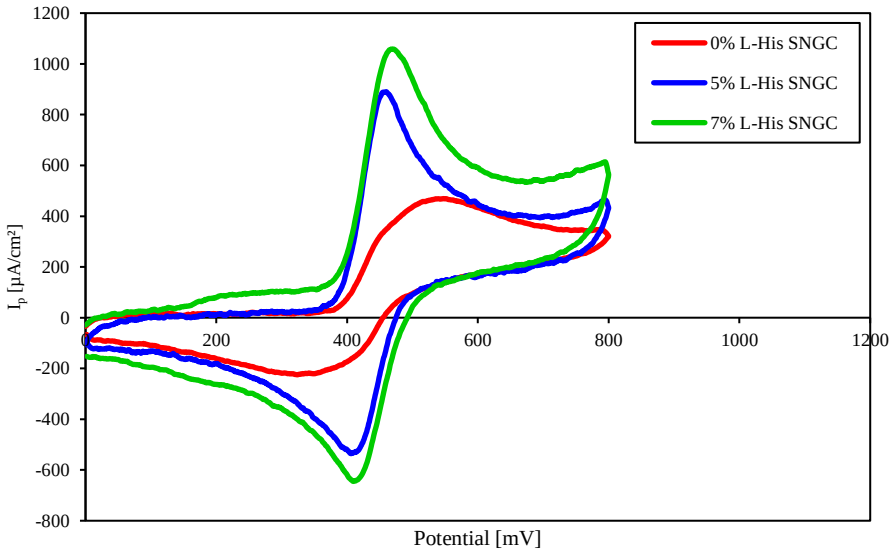

Fig.4: Cyclic voltammograms of 10^{-3}M DA in 0.05M phosphate buffer pH 2 at unmodified sonogel-carbon electrode, 5% L-His sonogel-carbon modified electrode and 7% L-His sonogel-carbon modified electrode. The scan rate is 100mV/s.

On the other hand, Table.1, which brings together the values of ΔE obtained on the three types of electrodes studied shows a visible improvement in reversibility on sonogel electrode modified by 7% histidine.

Table.1: Electrochemical characteristics of the DA on three types of electrodes. V = 100mV / s, 0.05M phosphate buffer of pH = 2.

Type of electrode	Ep_a (mV)	Ep_c (mV)	ΔEp (mV)
SNCG	510	372	138
SNCG 5% L-His	456	411	45
SNCG 7% L-His	457	417	40

These results clearly showed a negligible response for the unmodified sonogel-carbon electrode. The same experiment performed in the presence of L-His in the composite shows a spectacular increase in the response of the modified electrode. The best response was obtained with a sonogel electrode modified with 7% of L-His (Figure 4 and table 1).
For these reasons, 7% L-His sonogel-carbon electrodes were chosen as the optimal condition for the subsequent experiments.

3.3. Effect of scan rate on the peak current of DA oxydation
The influence of scan rate on the I_{pa} of DA was studied by cyclic voltammetry. Fig. 5A shows an increase of I_{pa} with increasing of the scan rate. The I_{pa} was directly proportional to the scan rate, which suggested a surface-controlled process of DA on the modified electrode surface [28]. The linear regression equation was got as I_{pa} (µA) = 75,436$V^{1/2}$ $(mv/s)^{1/2}$+ 117,01, r = 0.9997 (Fig. 5B). The good linear relationship between $v^{1/2}$ and I_{pa} at the scan rate range of 10mVs^{-1}- 100mVs^{-1} confirms a diffusion-controlled process.

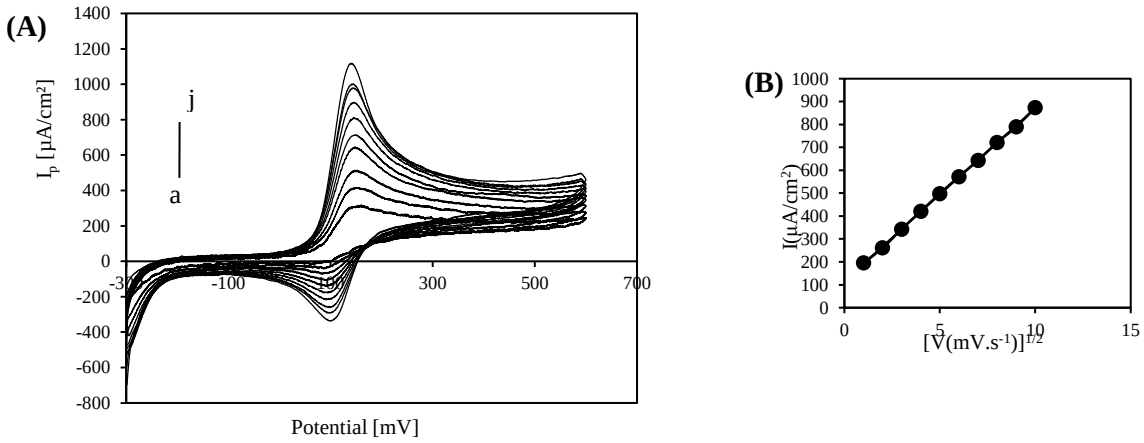

Fig.5 : (A) CVs of DA in pH 7.4 PBS at varois scan rates: (a) 10mVs^{-1}; (b) 20mVs^{-1}; (c) 30mVs^{-1}; (d) 40mVs^{-1}; (e) 50mVs^{-1}; (f) 60mVs^{-1}; (g) 70mVs^{-1}; (h) 80mVs^{-1}; (i) 90mVs^{-1}; (j) 100mVs^{-1}. (B) Plots of Ipa vs. Scan rates.

3.4. Effect of the concentration of the dopamine

In order to study the response of dopamine on L-His Sonogel-Carbon sensor, SWV was used as a sensitive electrochemical technique. Fig.6, illustrates an increase of the derivative of the current density at the L-His sonogel-carbon electrode surface with increasing concentration of dopamine.

The linear relationship between the current density and the concentration of dopamine in a range from 510^{-5}M to 210-4M was recorded The linear equation was found to be: I_p (µA/cm^2) = 360.2C (mM) – 0.006 with a correlation coefficient of R^2 = 0.998.

The detection limit (evaluated for S / N = 3 was found to be 1.010^{-7}M. This relatively low value indicates the good sensitivity of our electrode under the selected conditions.

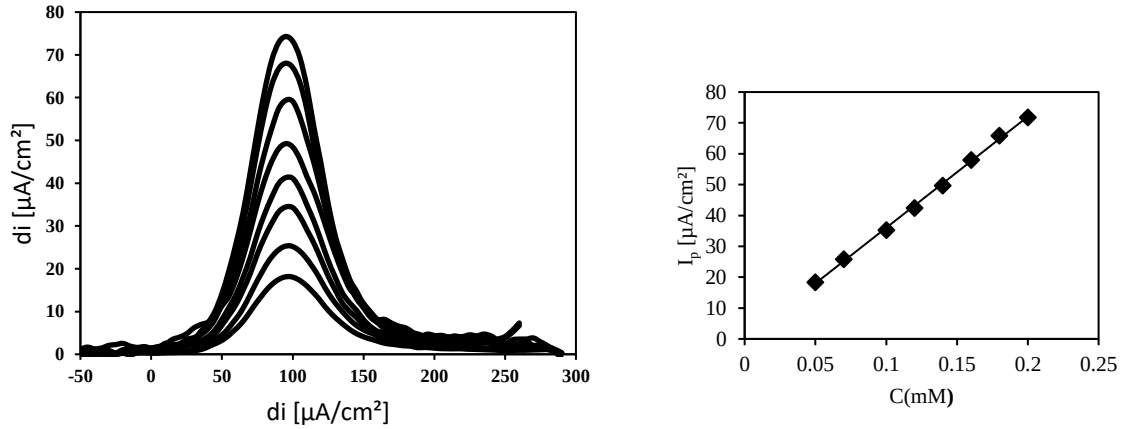

Fig. 6: Calibration curves of Dopamine on the surface of the L-His sonogel-carbon electrode. Phosphate buffer 0.05M at pH = 7.4.

3.5. Effect of pH on DA oxidation

The effect of buffer pH on the electrochemical response of the L-His sonogel-carbon modified electrode towards DA was studied. The variation of peak current and peak potential with buffer pH in the range from 2.0 to 8.0 are shown in Fig.7. The peak current decreased with pH from 2.0 to 5.0 and reached the maximum value at pH of 7, 4 approximately. In addition, the anodic peak potential shifts to negative values as pH increases over the range from 2 to 8. The slope was of −58.75mV/pH unit. The nearly Nernstian slope obtained here suggests two-proton, two-electron transfer process for dopamine oxidation [28–31].

3.6. Simultaneous determination of DA, AA and UA

3.6. 1. Differential pulse voltammetry of a mixture of AA and DA

It is well known that ascorbic acid (AA) coexists with dopamine (DA) in the extra cellular fluid of the central nervous system and its concentration is much higher than that of DA, so AA is the major interference for DA detection. The ability to determine these species has been a major goal of electro-analysis in the research.

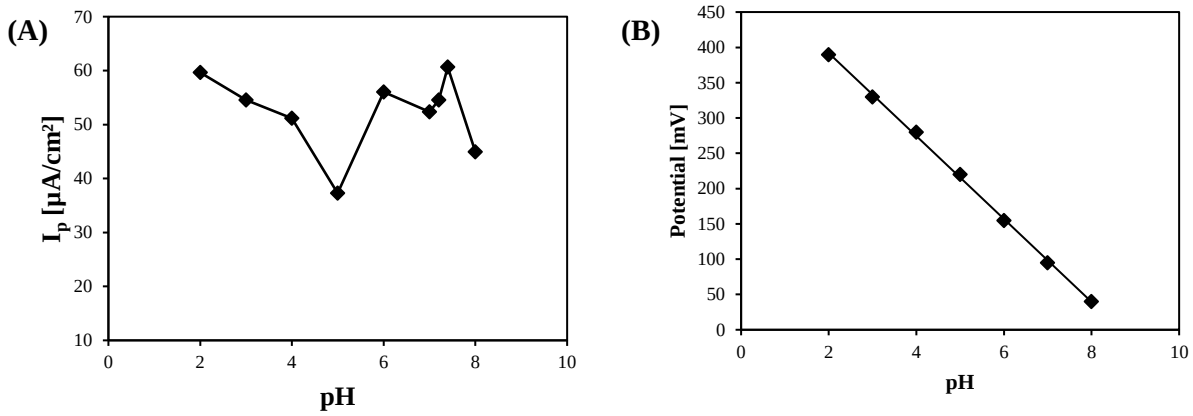

Fig.7: Effect of pH on the peak potential (B) and peak current (A) for the oxidation of DA

Fig. 8 shows the (SWV) of 510^{-5}M DA in the absence and presence of 510^{-4}M AA at L-His sonogel-carbon modified electrode in phosphate buffer solution (PBS) with the physiological pH of 7.4. Broad overlapped oxidation peak (not shown here) was obtained at bare sonogel-carbon electrode, but, at L-His sonogel-carbon modified electrode, two well-defined oxidation peaks were obtained at −120 and 65mV for AA and DA respectively. It was cited that for a given concentration of DA, a comparatively large oxidation current was noticed in the presence of AA at conventional electrodes [32]. This is due to the fact that oxidation product of DA, dopamine-o-quinone, catalytically reacts with AA and reduces the dopamine-o-quinone back to DA [32]. Therefore, the concentration of DA could not be determined accurately in the presence of AA. However, in the present investigation, the oxidation potential and peak currents for DA are almost the same in the presence or absence of AA. What's more, L-His sonogel-carbon modified electrode exhibited the bigger response for the oxidation of DA compared to AA, the oxidation of AA was effectively suppressed. AA had nearly no interference for the determination of DA.

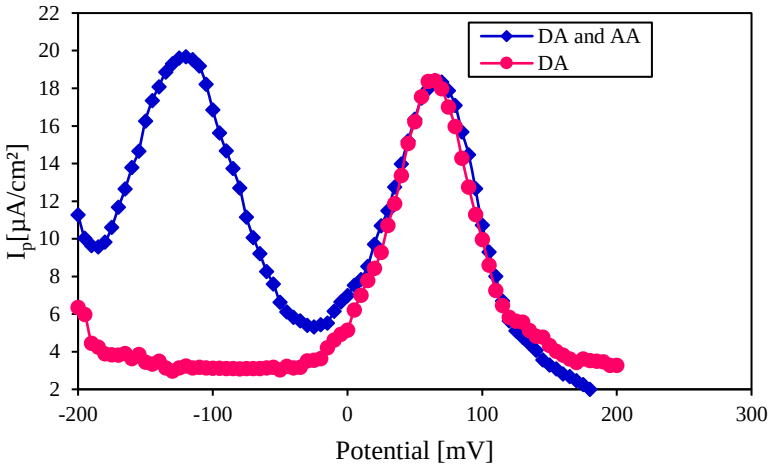

Fig. 8: SWVs for 5×10^{-5}M DA and 2×10^{-3}M AA at 7% L-His sonogel-carbon modified electrode in 0.05M pH 7.4 PBS.

3.5.2. Selective determination of AA in the presence of DA

The effect of the presence of DA on the determination of AA at different concentration was also studied, fig.9.A shows two separated current peaks corresponding to DA and AA with the intensity increasing of their concentration. This result indicates the accuracy of the proposed 7%L-His SNGC modified electrode for a simultaneous determination of DA and AA.

The peak current intensity of AA changes linearly with its concentration in the range 4×10^{-4} M– 1×10^{-3} M in presence of DA. The correlation coefficient (r^2) and the detection limit were of 0.9993 and 10^{-5} M respectively.

On the other hand, it appears that the peak corresponding to the oxidation of dopamine remains unaltered even if the concentration of AA is 250 times greater than that of dopamine. This shows once again that there is no electrocatalytic effect of AA on dopamine when these two molecules are present in solution.

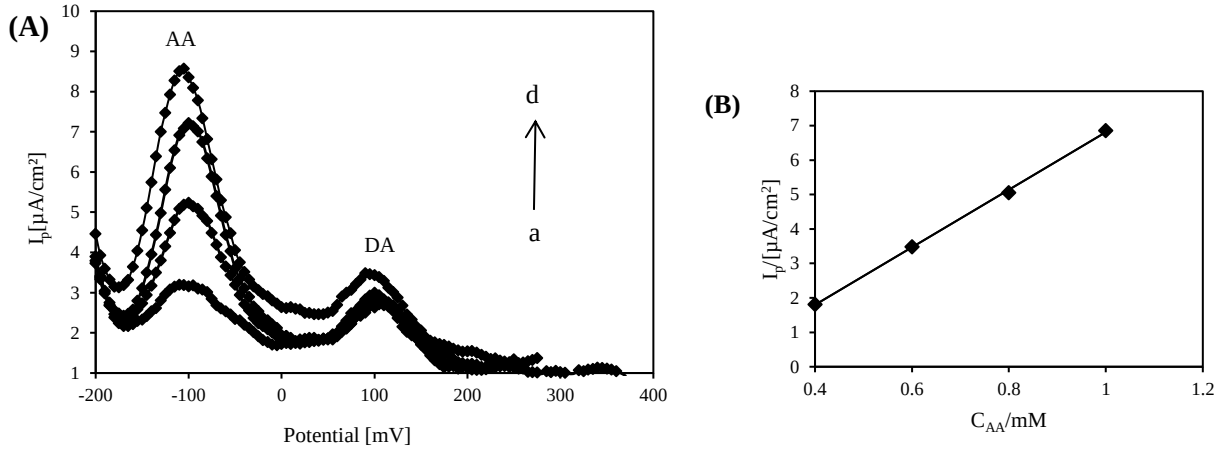

Fig. 9: (A) SWV at 7%L-histidine sonogel carbone modified electrode in pH 7.4 PBS containing 410^{-6}M DA in the presence of different concentrations of AA(a-d) :4×10^{-4},6×10^{-4},8×10^{-4} and 1×10^{-3}M. Scan rate: 100mVs^{-1}

3.6.2. Selective determination of UA in the presence of DA

The influence of DA on the oxidation of UA under the optimum conditions at a pH of 7.4 was also examined. As shown in Fig.10A, no significant change was observed in the DA peak currents while changing the UA concentration.

The oxidation peak current intensity of UA increased linearly with increasing of its concentration. The correlation coefficient was of 0.9984 as shown in figure Fig.10B. The detection limit of UA was found of 0.02µM. These results confirmed that the oxidation processes of DA and UA at L-His sonogel-carbon modified electrode are independent which suggests the absence of the electrocatalytical effect of this two components.

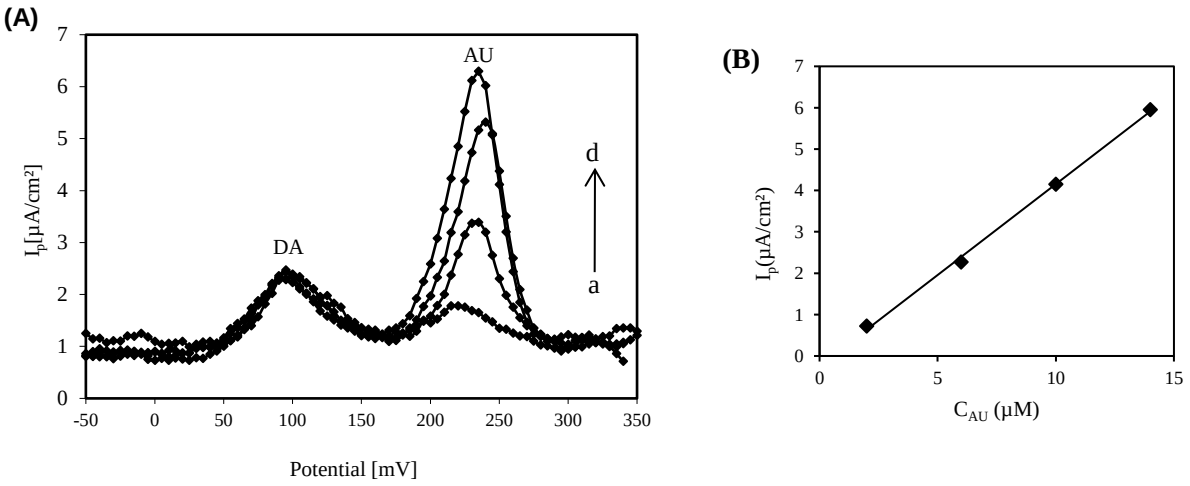

Fig.10: SWV voltammograms corresponding to the interference studies when dopamine (DA) is determined in the presence of uric acid (UA) at L-His sonogel carbone modified electrode. Concentration of DA is constant (410^{-6} M), and concentration of UA is varied (a-d): 2×10^{-6}, 6×10^{-6}, $10^{-5}$and 1.4×10^{-5}M. Scan rate: 100mVs^{-1}. pH7.4

3.6. 3.Electrochemical oxidation of DA, AA and UA at the L-His sonogel-carbon electrode

It is well known that DA, AA and UA coexist in the extra cellular fluid of the central nervous system and serum [33]. Since they have similar oxidation potentials at most solid electrodes, separate determination of these species is a great problem due to their over lapped signals.

Fig. 11 shows the CV curves recorded using a ternary mixture of 80µM UA 400µM AA, and 4µM DA at (a) unmodified electrode and (b) L-His Sonogel-Carbon modified electrode in pH 7,4 PBS. As is clear from the figure, at unmodified electrode, the anodic peaks of AA, DA and UA are fully merged to give a broad and overlapped peak (curve a). When L-His Sonogel-Carbon modified electrode was used under identical conditions, AA, DA and UA gave their oxidative peaks distinctly at –97mV, 81 mV and 206 mV respectively (curve b). The

anodic peak potential separations are calculated to be 118 mV between AA and DA, 125mV between DA and UA and 303 mV between AA and UA. Since SWV has a much higher current sensitivity and better resolution than CV, it was used in the oxidation of DA, UA and AA. (fig.12) shows the SWV curves recorded using a ternary mixture of 80 µM UA 400 µM AA, and 4 µM DA in pH 7,4 PBS both at bare and modified electrode. As it is evident from the figure, at the unmodified electrode, the oxidation peaks of AA, DA and UA are overlapped (curve a). Furthermore, the anodic peaks of the three analytes are well resolved at L-His Sonogel-Carbon modified electrode with peak potentials of -165 mV, 45 mV and 160 mV for AA, DA and UA respectively (curve b). The corresponding peak potential separations are 210 mV, 115 mV and 325 mV for AA–DA, DA–UA and AA–UA respectively. Therefore, the extent of separations between the anodic peaks of AA, DA and UA, achieved in CV and SWV modes is large enough for the selective and simultaneous determination of DA and UA even in the presence of high concentrations of AA.

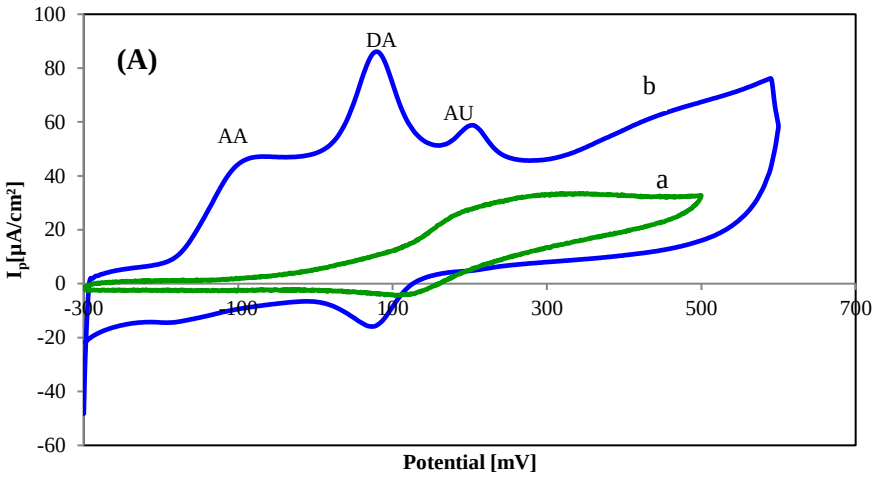

Fig. 11: Cyclic voltammograms for mixture of 8×10^{-7}M UA, 4×10^{-4} M AA and 4×10^{-6} M DA in 0.05M phosphate buffer pH7.4 at (a) unmodified electrode, (b) L-Histidine sonogel-carbone modified electrode. Scan rate of 100mVs^{-1} and T=25°C

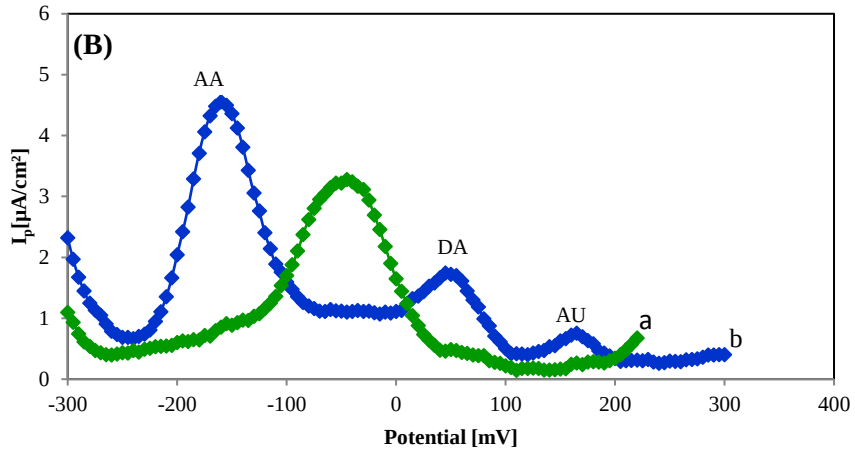

Fig.12: SWV of 4×10^{-6}M DA, 4×10^{-4}AA and 8×10^{-7}M UA mixtures at (a) unmodified electrode, (b) L-Histidine sonogel-carbon modified electrode in 0.05M phosphate buffer (pH 7.4). Pulse amplitude: 25mV

3.7. Determination of DA in human blood serum at the Sonogel modified electrode

As has been previously reported ascorbic acid, uric acid and dopamine usually coexist in several biological fluids. To overcome the problem of interference of these three analytes, we have studied the phenomenon in a serum diluted 60 times with a pH 7.4 phosphate buffer solution. Figure 13 (a) shows the rest of the electrode sonogel L-His in serum. One peak is obtained corresponding to the AT naturally present in serum. Then, a binary mixture of 8.10-6 and 2.10-4 M of DA and AA, respectively, was added to the serum. The result shown in Figure 13 (b) shows trios well separated peaks that correspond to the oxidation of AA, DA and the AU. This result shows that the L- His sonogel-carbon electrode could present a very promising alternative to a simultaneous determination for these three analytes in real biological samples.

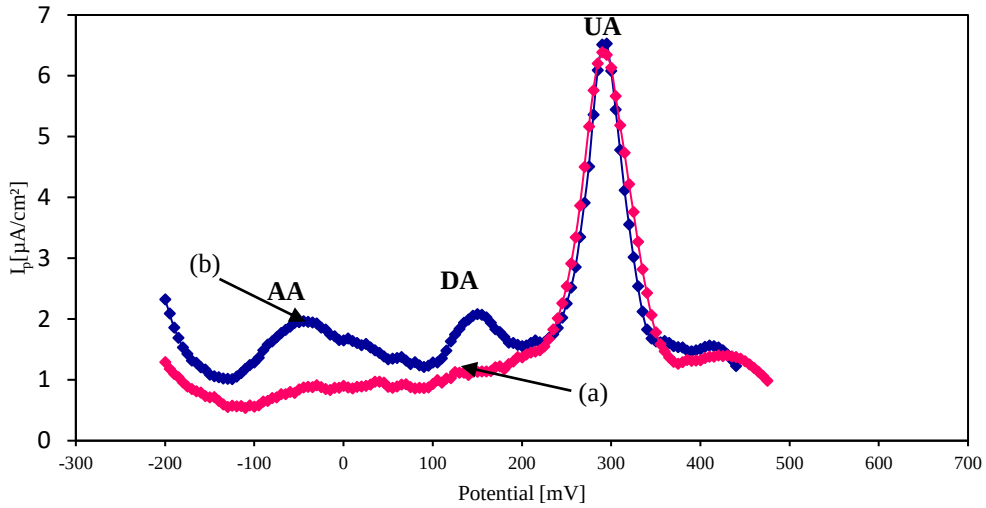

Fig.13.SWV at 7%L-His modified electrode (a): serum diluted 60 fold. (b): serum diluted 60 fold contained 8×10^{-6}M DA and 2×10^{-4} M AA.

Conclusions

In this work a new modified Sonogel-carbon electrode was synthesized by incorporation of L-Histidine. The modified electrode shows improved detection performances in term of sensitivity and selectivity for electrochemical determination of dopamine either in phosphate buffer and human serum. The interference of DA, AA and UA could be also eliminated. Moreover, the modified electrode showed good reproducibility and stability. The application of the proposed electrode should be extended to real samples of blood and urines in the frame of clinical and environmental analysis.

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