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A Sonogel/L-Cystein and a SAM L-Cystein Modified Electrodes for Detection of Dopamine

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Summary: The Sonogel-Carbon electrode is a special class of sol-gel electrode that exhibits favourable mechanics and electric properties that can be used as electrochemical sensors. In this study, a Sonogel-Carbon modified with L-Cysteine was used to prepare a novel electrochemical sensor. The objective of this new electrode modification was to seek new electrochemical performances for thedetection of dopamine (DA). The influence of natural interferents such as Ascorbic Acid (AA) and Uric Acid (UA) was explored. The concentration of theses strong interferent was increased to a certain level in order to determine to what extends AA and UA may disturb the neurotransmitters electroanalysis. Our work showed that the modified electrode offers interesting analytical performances such as:
(a) Fast and linear responses towards the neurotransmitter dopamine: The differential pulse voltammetry current peak was linear with the DA concentration in the range $2 \cdot 10^{-7}$ M to 10^{-5} M with a detection limit of $4 \cdot 10^{-8}$ M (S/N = 3).
(b) Simultaneous detection and well-resolved signals between the DA, AA and UA: The new sensor could sensitively and separately determine DA in the presence of 1000 and 900 times higher concentrations of AA and UA respectively.
Optimization of parameters such as the amount of L-cysteine in the Sonogel-Carbon mixture, interference effect, perm-selectivity and mechanical stability of the sensor are discussed. A comparison with a SAM L-Cysteine/gold electrode was also made. On the other hand the new Sonogel modified electrode has been applied to the determination of dopamine in urine samples with satisfactory results.
With good selectivity and sensitivity, the proposed sensor is a simple tool for the selective detection of DA, AA and UA in biological samples.

Keywords: complexation; dopamine; L-cysteine; self-assembled systems; sonogel

Introduction

Dopamine (DA) is an important neurotransmitter and extracellular messenger in biological systems. Extreme abnormalities of DA concentration levels may lead to Parkinson's disease and in some cases to clinical manifestations of HIV infections.[1,2] Determination of DA and related catecholamine compounds is significant for neurochemistry and brain-science studies.[3] Thus, there is a continuing interest in the development of simple, sensitive and reliable methods for the determination of dopamine. The major problem of DA electrochemical determination is the interference from ascorbic acid (AA), which usually exists in real systems in large amount. Usually, the concentration of the DA varies from 10^{-8} to 10^{-6} mol L^{-1} while AA is as high as 10^{-4} mol L^{-1} in biological systems.[4] Considerable efforts have been devoted to overcome this problem. Moreover, at almost all electrode materials, DA

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and AA are oxidized at nearly the same potential, which results in overlapped voltammetric responses.[5] The successful route to overcome the problems of selectivity is to modify the electrode surface. Indeed, this procedure is able to decrease the overpotential, improve the mass transfer velocity and effectively enrich the substance.[6–8] A variety of modification methods have been discussed in the literature, it include modification of the surface by an ionic polymer film,[9–12] enzyme-based techniques,[13–15] adsorption/medium exchange methods,[16,17] electrochemically pretreated electrodes,[18,19] self-assembled monolayer modified electrode[20,25] or complex of metals with electroactive center modified electrodes[26,28] were employed.

Recently, some of us have developed a new type of graphite-based sol-gel electrode, 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 homogeneous. This is followed by the employment of an ultrasound bath for several minutes to promote the hydrolysis. One 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.[29,30]

These type of electrodes show good electroanalytical properties for their use as amperometric sensors and, further more, they can easily permit the incorporation of numerous receptor molecules at the sonogel-carbon materials,[31,32] which can notably improve the selectivity. In the present paper, we propose a new application of sonogel-carbon electrodes based on the incorporation of L-Cysteine (L-Cys) for dopamine.

Experimental Part

Reagents and Materials

Methyltrimethoxysilane (MTMOS) was from Merk (Darmstad, Germany), Chloridic (HCl) and Sulfuric (H_2SO_4) acids were from Panreac (Barcelona, Spain). L-Cysteine (>99%) was obtained from Fluka Chemical Company (Switzerland). Ascorbic acid (99%) was purchased from Sigma Chemical Company (Barcelona, Spain). Dopamine was purchased from Aldrich chemical company (Milwaukee, USA) and used as received. A phosphate buffer solution (PBS, 0.05 mol L^{-1}) was purchased from Merck (Darmstadt Germany). All reagents were of analytical grade or higher and used as received without further purification. Graphite powder (spectroscopic grade RBW) was from SGL Carbon (Ringsdorff, Germany). Nanopure water was obtained by passing twice-distilled water through a Milli-Q system (18 $M\Omega \cdot cm$, Millipore, Bedford, MA).

Glassy capillary tubes, i.d. 1.15 mm, were used as the bodies for the composite electrodes.

Instrumentation

All electrochemicall measurements were performed with an Autolab PGSTAT20 (Ecochemie, utrecht, the Netherlands). The experiments were carried out in a three-electrode cell at room temperature ($25 \pm 1\,^{\circ}C$) under nitrogen atmosphere. 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. Differential pulse voltammograms (DPV) and cyclic voltammetry (CV) were the electrochemical techniques applied to study the behaviour of the sonogel-carbon electrodes.

The instrumental parameters for DPV were as follows: deposition potential: 0 V, duration: 40 s modulation time: 0.06 s, interval time 0.6 s, scan rate: 0.01 V/s, modulation amplitude: 0.10005 V, standby potential: 0 V. Measurements were carried out under N_2 atmosphere when required.

Preparation of the Sonogel Electrode

To prepare the sonosol, the general procedure was as follows: 500 μl of MTMOS was mixed with various volumes of HCl solutions of suitable concentrations, according to the amounts of water required but maintaining the quantity of the catalyst (HCl). This mixture was then insonated for 5 s (energy dose 0.083 $kJ \cdot mL^{-1}$). In the next step, the adequate amounts of L-cysteine and graphite powder (until 0.5 g) 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 glassy tubes leaving a little extra mixture sticking out of the glass tube to make easy the subsequent polishing step. After 20–24 h, depending on the proportion of H_2O, the Sonogel-Carbon L-cysteine composite electrodes become hardened and, therefore, structured. Adherence between the developed material and the glass was excellent. Before use, the electrodes were polished with N°: 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 system.

Results and Discussion

Influence of the L-Cysteine in the Sonogel-Carbon Matrix

The effect of the L-cysteine percentage in the manufacturing of the L-cys SNGC electrode in several supporting electrolytes was investigated employing a $5 \cdot 10^{-4}$ M solution of dopamine as the analyte and comparing the response of an unmodified SNGC electrode with a L-cys SNCG modified electrodes containing successively 2.5%, 5%, 7.5% and 10% of L-cycteine in 0.1M H_2SO_4 as the supporting electrolyte. The accumulation time and the potential were 200 s and 0 V respectively. DPV was used in this study.

Our results clearly showed a negligible response for the unmodified SNGC electrode. The same experiment performed in the presence of L-cys in the composite shows a spectacular increase in the response of the modified electrode. Most of the response was obtained with a Sonogel electrode modified with 5% of L-Cys (Figure 1).

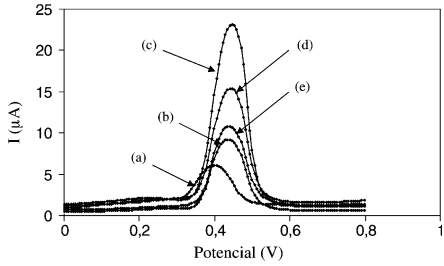

Figure 1.
DPV of $5 \cdot 10^{-4}$ M dopamine at unmodified electrode (a), and modified electrodes containing successively 2.5%, 5%, 7.5% and 10% of L-cysteine (b,c,d,e) in 0.05 M Tampon phosphate as supporting electrolyte.

For these reasons, 5% L-cys SNGC electrodes were choose as the optimal conditions for the subsequent experiments.

Sensing Performances of the L-Cysteine Modified Sonogel-Carbone

Figure 2 shows the cyclic voltammograms of $5 \cdot 10^{-4}$ M dopamine, on bare SNGC electrode (a) and 5% L-cys SNGC modified electrode (b).

The presence of the L-cys in the modified electrode can be summarized as following:

1– The potential for the dopamine oxidation (E_{pa}), is shifted to negative values.

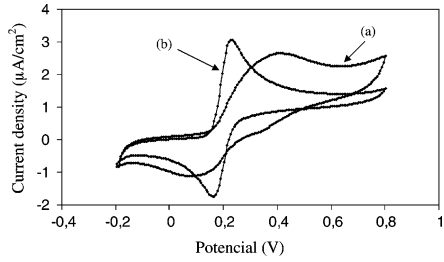

Figure 2.
Cyclic voltammograms obtained under the following conditions: $5 \cdot 10^{-4}$ M of dopamine at (a) unmodified electrode, (b) 5% L-cys SNGC modified electrode. Scan rate 50 mVs^{-1}, supporting electrolyte, 0.05 M phosphate buffer pH 6 and T = 20 °C.

2– There is an increase in the anodic and cathodic peak currents.
3– The kinetics process for dopamine is improved.

In conclusion, our results clearly demonstrate that the new film made of L-Cys SNGC shows improved catalytic and kinetic properties toward the dopamine electroanalytical analysis.

Analytical Determination

From a literature survey, it appears that the limit of detection for dopamine varies from work to work depending on the electrode used and the purpose of the sensing activity. Differential pulse Voltammetry (DPV) seems to be a suitable pulse technique to achieve good result, since it favours the measurement of the faradic over the non-faradic current, which improves sensitivity during the measurements. In this work, the anodic differential pulse peak was used for dopamine (Figure 3) determination in 0.05 M phosphate buffer pH6 solution. The calibration curves provided linear relationships for dopamine $I(\mu A) = 0.1135 + 0.156C(\mu M)$, $R^2 = 0.9995$ within the range of 10^{-7} to $4 \cdot 10^{-5}$ M. The detection limit of dopamine (S/N = 3) was $4 \cdot 10^{-8}$ M.

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Same study was made with a gold electrode modified with SAM's of L-Cysteine. Good performances are also obtained: linear relationships in the range of $2 \cdot 10^{-6}$ M and $5 \cdot 10^{-3}$ M and limits of detection of Dopamine (with S/N = 3) of $4 \cdot 10^{-7}$ M. These results show clearly the good performances of our novel sensor (5% L-Cys SNGC).

Interference Study

Interference of Ascorbic Acid

Figure 4a shows a DPV for a bare SNGC electrode immersed in a solution containing $5 \cdot 10^{-6}$ M DA and $5 \cdot 10^{-4}$ M AA. Under these conditions, we can see one large voltammetric peak due to a poor resolution between the two molecules. The situation is totally different at a 5%L-cys SNGC modified electrode (Figure 4b). The DPV shows indeed two well-resolved signals due to AA (−74 mV) and DA (170 mV). We can conclude from this result that the 5%L-cys SNGC modified electrode has the ability of selective determination of DA even in the presence of high amount of AA (100-fold higher).

The presence of a homogeneous catalytic oxidation of AA by the oxidation of the DA at carbon electrodes was proposed by Dayton et al.[33] Recently, the catalytic effect of DA on the AA oxidation at lipid modified GCE has also been observed. These observations support a strong interference from AA on the electrochemical determination of DA. Thus, the precise determination of DA in the presence of AA

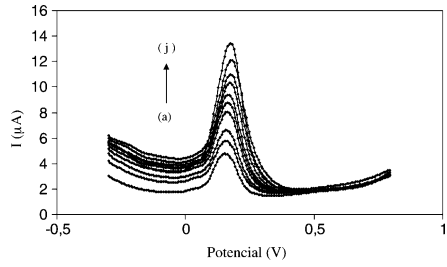

Figure 3.
DPV for 5% L-cys SNGC in 0.05 phosphate buffer pH 6 in different concentrations of DA (a-j: 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 μM). Scan rate: 50 mVs^{-1}, pulse amplitude: 25 mV, pulse rate: 0.5 s, pulse width: 60 ms.

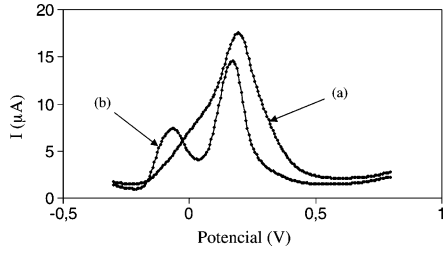

Figure 4.
DPV for mixture of $5 \cdot 10^{-6}$ M DA and $5 \cdot 10^{-4}$ M AA in 0.05 M phosphate buffer pH 6 at (a) unmodified SNGC electrode and (b) 5% L-cys SNGC modified electrode. Scan rate: 50 mVs^{-1}, pulse amplitude: 25 mV, pulse rate: 0.5 s, pulse width: 60 ms.

is disturbed. The oxidation peak current of DA increases in the presence of AA, especially at bare electrodes. As the AA concentration in extracellular fluid is usually high, this mediated oxidation would affect the accurate determination of DA. To explore this problem, we have carried out carried out a careful investigation of this catalytic effect, by DPV at a 5%L-Cys SNGC modified electrode in contact with a solution made of 10^{-4} M AA and $5 \cdot 10^{-6}$ M DA.

As it can be seen from Figure 5a, when varying the concentration of DA in the mixture from 1 to 9 μM, the DPV peak height of AA at (–74 mV) was constant, while DA peak increases linearly with concentration. On the last hand, when reversing the experiment by varying the concentration of AA from 1 to $5 \cdot 10^{-5}$ M, the DPV peak height of DA 170 mV remains constant (Figure 5b). These results demonstrate that there is no homogenous catalytic coupling between AA and DA at the modified 5%L-Cys SNGC electrode.

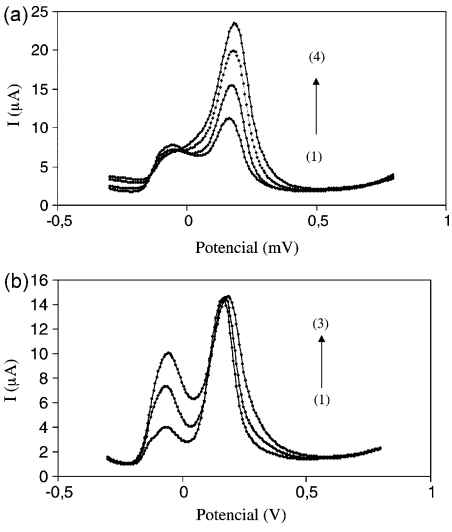

Figure 5.
DPV at 5% L-cys SNGC in 0.05 M phosphate buffer pH 6, (a) 10^{-4} M AA mixed with DA: (1) $1 \cdot 10^{-6}$ M, (2) $2 \cdot 10^{-6}$ M, (3) $3 \cdot 10^{-6}$ M, and (4) $4 \cdot 10^{-6}$ M. (b) $5 \cdot 10^{-6}$ M DA mixed with AA: (1) $1 \cdot 10^{-4}$ M, (2) $2 \cdot 10^{-4}$ M, (3) $3 \cdot 10^{-4}$ M. Scan rate: 50 mVs^{-1}, pulse amplitude: 25 mV, pulse rate: 0.5 s, pulse width: 60 ms.

Simultaneous Determination of AA, AU, and DA

It is well known that uric acid (UA) coexists with DA in the extracellular fluid of the central nervous system and its concentration is much higher than that of DA. Hence, UA and AA are the two important interfering substances for the electrochemical detection of DA. The interference effect of AA and UA was investigated. Figure 6 shows the DPV of $5 \cdot 10^{-4}$ M AA, $5 \cdot 10^{-6}$ M DA, and $5 \cdot 10^{-4}$ M UA in 0.05 M phosphate buffer pH6. Well defined anodic peaks at -2, 277 and 438 mV for the oxidation of AA, DA and UA respectively were observed at the 5% L-Cys SNGC. However, the oxidation of AA and UA does not modify or influence the current response of the oxidation of DA.

Our modified electrode has the ability for selective determinations of DA in the presence of large amount of AA and UA although, when 100-fold of AA and UA concentrations exists in the analyte solution.

Determination of Dopamine in Spiked Urine

Most of the previously reported modified electrodes were involved in the detection of DA in dopamine hydrochloride injection.[34–36]. Few reports are available for the determination of DA in urine sample.[37]. The applicability of the DPV to the determination of dopamine with a mixture of UA and AA in spiked urine was investigated here. Figure 7a shows a

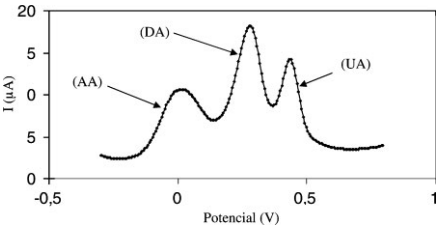

Figure 6.
DPV of $5 \cdot 10^{-4}$ M AA, $5 \cdot 10^{-6}$ M DA and $5 \cdot 10^{-4}$ M UA mixtures at 5% L-cys SNGC in 0.05 M phosphate buffer pH 6. Scan rate: 50 mVs^{-1}, pulse amplitude: 25 mV, pulse rate: 0.5 s, pulse width: 60 ms.

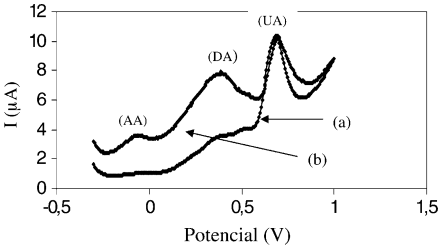

Figure 7.
DPV at 5% L-cys SNGC electrode. (a): urine sample diluted 50 fold. (b) Urine sample diluted 50 fold contained $5 \cdot 10^{-4}$ M of DA and 10^{-4} M of AA Scan rate: 50 mVs^{-1}, pulse amplitude: 25 mV, pulse rate: 0.5 s, pulse width: 60 ms.

voltammetric peaks obtained of a blank urine sample diluted 50 fold with phosphate buffer: A peak of UA is obtained. Surprisingly, when a mixture of (510^{-4}) of DA and (10^{-4}) of AA was added (Figure 7b), three resolved peaks where obtained corresponding of AA, DA and UA respectively. Again, this experiment shows the good analytical performance exhibited by our novel modified electrode.

Conclusion

In this work we have prepared a new modified Sonogel-carbon electrode based on the incorporation of L-Cysteine for the detection of dopamine either in phosphate buffer or in urine samples.

The resulting modified electrode exhibits high sensitivity and selectivity towards the electrochemical detection of Dopamine.

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