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Origonal Research Article

Preparation of Acridine Orange-MWCNT Modified Electrode for Voltammetric Detection of Dopamine in the Presence of Ascorbic Acid

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Abstract

Acridine orange supported on multi-walled carbon nanotube (MWCNT) is used for modification of carbon-paste electrode. The studies show that acridine orange efficaciously immobilized in the matrix of the electrode by applying nafion/MWCNT composite under the ultrasonic condition. The results of voltammetric experiments demonstrate that the prepared electrode has an effective response to DA and AA and a relatively big anodic peak separation (nearly 368 mV) is obtained for these compounds. Good sensitivity and selectivity and very low detection limit (0.03 μ M) makes the modified electrode very effective in the manufacture of simple devices for the concurrent detection of dopamine in the presence of ascorbic acid in clinical and pharmaceutical preparations.

Keywords

Acridine Orange; Carbon Nanotube; Dopamine; Voltammetry; Electrocatalysis.

1. INTRODUCTION

In recent years, the modification of electrodes using new modifiers and their application have been of substantial interest [1-4]. Specially detection of different samples using modified electrodes received many interests. For instance modified carbon based electrodes have used for detection of paracetamol [5], rGO/β-cyclodextrinmodified electrode has been applied in determination of catechol [6], TiO2 nanoparticles modified carbon paste electrode has been prepared for detection of paracetamol in presence of Adrenaline [7], MWCNT-COOH/ covalent triazine framework -modified glassy carbon electrode has been used for determination of hydroquinone [8], Ag decorated C nanosphere modified fluorine-doped tin oxide electrode has been prepared for determination of procaine [9], Fe₂O₃@Ni/Al-LDH modified electrode has been applied in sensing of mercury [10]. AuNPs/Graphene modified GCE has been prepared for determination of chlorzoxazone [11], MoO3-graphitic carbon nitride sheets modified electrode has been applied for caffeine detection [12], laser-induced graphene modified electrode has been used for detection of hydrogen peroxide [13], La₂(MoO₄)₃ modified electrode has been prepared for detection of antibiotic drug [14], NiO nanocrystal modified electrodes has been used for 1,2-Benzenediol detection [15], Zn-mMOF/Cu ions modified glassy carbon electrode has been prepared for detection of amoxicillin [16], pyrogallol red modified electrode has been applied for paracetamol detection [17], poly(p-

modified electrodes have been applied for illicit drugs detection [19], imprinted polymer/solgel/polyoxometalate/ reduced graphene oxide modified electrode has been used for detection of sumatriptan and paroxetine [20], pd nanoparticles modified electrode has been used for detection of direct yellow 50, carbendazim and caffeine [21], Au nanoparticles modified sensor has been used for As(III) detection [22], Fe₃O₄@polypyrrole-Pt core-shell nanoparticles modified electrode has been applied for sensing 6-mercaptopurine and 6thioguanine [23], carbon black modified electrode has been used for detection of dissolved iron [24]. Among the biological compound, determination of neurotransmitters, e.g., dopamine (DA; a significant neurotransmitter) and ascorbic acid (AA; vitamin C) using modified electrodes, received many interests. An earnest drawback in detection of DA or AA is the lack of the enough resolution between the anodic peaks of AA and coexisting DA at common solid electrodes. Various techniques have been used for prevail this disadvantage. For instance, the electrochemical response to DA was studied at a carbon black nanoparticles modified porous boron-doped diamond electrode [25], SU-8 modified carbon nanograss electrodes [26], TiO₂/Stainless steel modified electrode [27], cerium phosphate nanorods/rGO modified glassy $ZnO@MnO_2$ carbon electrode [28], nanocomposite modified carbon paste electrode [29] and Hydrogenated conical-tip carbon

coumaric acid) modified electrodes has been used

for detection of cadmium and lead [18], different

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electrode [30]. CuCo₂O₄ rods [31], Capsaicin/MWCNT [32], electropolymerized safranin [33], Molecularly imprinted/MXene [34] and cetylpyridinium [35] were used as modifier in preparation of modified electrode for determination of ascorbic acid.

In this work, acridine orange-MWCNT (immobilized via ultrasonic agitation in aqueous solutions) is described. This composite applied in preparation of modified electrode. The modified electrode is used for determination of DA in the presence of AA.

2. EXPERIMENTAL

2.1. Chemicals

Multi-walled carbon nanotubes were purchased from Nanostructured & Amorphous Materials (USA). Spectroscopic mineral oil, Graphite powder, acridine orange, dopamine and ascorbic acid were prepared from Merck.

2.2. Apparatus

A conventional three-electrode system (a carbonpaste working electrode, a saturated Ag/AgCl reference electrode and a Pt wire as the counter electrode) was applied in voltammetric experiments using a Metrohm Computrace Voltammetric Analyzer model 757 VA.

2.3. immobilization of acridine orange on carbon nanotubes

For preparation of acridine orange-MWCNT, a 100 mg portion of pretreated MWCNT was dispersed in a 3 mL methanol solution containing 15 mg acridine orange. For immobilization of acridine orange on the surface of MWCNT, a 200 mg portion of pretreated nanotube was dispersed in a 5 mL methanol solution containing 20 mg acridine orange. In order to better dispersion and also, more efficient immobilization of acridine orange in matrix of the modifier composite, a portion of 2.0 mL of Nafion solution (5%) was added to the above mentioned mixture and sonicated for 2 h. The prepared suspension was filtered and washed thoroughly with doubled distilled water and methanol. The mixture was dried under the IR lamp.

2.4. Preparation of the modified electrode

The unmodified carbon-paste electrode (UCPE) was prepared by mixing graphite powder with appropriate amount of mineral oil (Nujol) and through hand mixing in a mortar and pestle (\sim 75:25 w/w%). A portion of the composite mixture was packed into the end of a Teflon tube (ca. 2.5 mm i.d.). Electrical contact was made by forcing a copper pin down into the Teflon and into the back of the composite. To prepare nafion-

incorporated CPE (NCPE), a mixture of nafion solution, Nujol and graphite powder (~10:15:75 w/w %) with a total weight of 1.00g was transferred to the mortar and pestle and then homogenized by adding 2.0 ml of dichloromethane. The solvent was evaporated in room temperature (for 24h) and the resulting composite was applied as NCPE. To prepare the acridine orange/MWCNT-modified electrode (CNT-AOCPE), an appropriate amount of the MWCNT/acridine orange composite (in a ratio of 10% w/w) was transferred to 3.5 ml of dichloromethane, and mixed with graphite powder and silicon oil. The solvent was evaporated in room temperature for 24h.

3. RESULT AND DISCUSSION

3.1. Voltammetric studies

The electrochemical behavior of dopamine and ascorbic acid at different prepared electrodes was studied with cyclic voltammetry technique. The results showed that the presence of acridine orange at the surface of AOCPE, caused a remarkable improvement of anodic current of AA. Fig. 1 shows the CVs obtained in buffered solution of AA and DA at the surface of different prepared electrodes.



Fig. 1. Cyclic voltammograms for a mixture of 1 mM AA and 1mM DA in acetate buffer solution (0.1 M, pH = 4.0) on the surface of different prepared electrodes; (black) UCPE, (red) NCPE, (green) OACPE and (blue) CNT-OACPE. Sweep rate was 100 mV s⁻¹.

As observed, at the surface of unmodified CPE the voltammetric peaks of two compounds is overlapped, which manifested that this electrode is not appropriate in order to simultaneous detection in mixture samples. By introduction of acridine orange at the surface of the modified electrode, a remarkable negative shift in the anodic peak of AA (due to catalytic effect of acridine orange) together with a significant improvement in peak current are obtained. Also, positive shift in DA peak potential caused to increase the peak separation of AA and DA. Using AOCPE, a peak separation of 321 mV is resulted for mixture solution of AA and DA. The anodic peak separation for these compounds at the surface of CNT-AOCPE is increased to 368 mV, which is better than previously reported separation of AA and DA peaks using other prepared electrodes.

A superior explanation of the performance of the modified electrodes in simultaneous determination of AA and DA can be obtained using differential pulse voltammetry. Fig. 2 shows the DPVs obtained in mixture solution of ascorbic acid and dopamine in buffered solution (pH 4.0) at the above-mentioned electrodes.



Fig. 2. Differential pulse voltammograms for a mixture of 0.1 mM AA and 0.1mM DA in acetate buffer solution (0.1 M, pH = 4.0) on the surface of different prepared electrodes; (black) UCPE, (red) NCPE, (green) OACPE and (blue) CNT-OACPE. Pulse amplitude was 50 mV.

The peak resolution for ascorbic acid and dopamine at the UCPE, NCPE, AOCPE, and CNT-AOCPE was 139, 133, 332 and 368 mV, respectively. By applying CNT-AOCPE, the deterioration of the anodic peak current (between AA and DA peaks) reach to the background current. On the other hand, a complete peak separation of AA and DA is obtained by using of CNT-AOCPE in mixture samples. The obtained peak separation for AA and DA with this prepared electrode is extremely better than those of the reported modified electrodes [32].

3.2. Effects of pH and potential sweep rate

For optimization of pH and acquire the maximum separation between the anodic peaks of AA and DA $(\Delta E_{p,a})$ and good sensitivity in measurements, voltammetric studies were performed in various pHs of the buffered solutions. In these experiments, 0.1 M phosphate was applied for pHs 3.0, 6.0 and 7.0, and 0.1M acetate for pHs 4.0 and 5.0. These results demonstrate the oxidation peak current of AA is increased from pH 3 to 4 and then decreased by increasing the pH. The anodic peak current of DA, is diminished gradually by increasing the pH of the buffer solution. Results show that, the best resolution is resulted in pH 4.0. As a result, the acetate buffered solution with pH 4.0 was applied as supporting electrolyte in all voltammetric experiments.

The results showed that the anodic peak currents $(i_{p,a})$ vary linearly with the square root of the scan rate $(\upsilon^{1/2})$. These results manifested that the electrochemical reactions of AA and DA at the CNT-AOCPE are diffusion controlled.

3.3. Analytical characterization

The Differential pulse voltammetry using CNT-AOCPE was applied as an electrochemical technique with very low detection limits in detection of AA and DA in a wide concentration range. Some typical results of these experiments are displayed in Fig. 3. The calibration curve of the oxidation current versus the concentration, exhibit a linear range of 0.1 to 90 µM for both AA and DA (Fig. 4). The slope of the calibration line for AA and DA was 0.0201 and 0.0209 µA/µM, respectively. The correlation coefficient (R) for the linear calibration curve of AA and DA was 0.9989 and 0.9988, respectively. The voltammetric detection limit (based on 3σ) using the modified electrode for both AA and DA was 0.03 uM. The relative standard deviation (RSD) for the slope variations, based on the 25 calibration measurements during six months, was 3.2% and 3.4% for AA and DA respectively.



Fig. 3. Differential pulse voltammograms of 0.1 acetate buffer solution (pH 4.0) containing various concentrations of (A) AA and (B) DA. The analyte concentrations in both curves is, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4.0, 8.0, 10.0 μ M (down to up). Pulse amplitude in all measurements was 50 mV.



Fig. 4. Linear calibration curves of anodic peak currents versus AA and DA concentration in the range 0.1 to $80.0 \ \mu M$.

The substantial advantages of this work are the complete separation of anodic peaks of AA and DA, and the low detection limits. These results make the CNT-AOCPE quite appropriate in simultaneous voltammetric determination of the trace amounts of AA and DA. Fig. 5A displays DPV waves of different concentrations of AA (from 5 to 50 μ M) in the presence of a constant concentration of 10 µM of DA. Fig. 5B displays DPVs of different DA concentrations in the presence of 10 µM of AA at the CNT-AOCPE. The CNT-AOCPE in this investigation displayed complete separation of anodic peaks of AA and DA, which is better than previous reports. As demonstrated in Figs. 1 and 2, a peak separation of 315 and 368 mV is resulted in cyclic and differential voltammetric experiments, respectively. The obtained detection limit for AA and DA in this work is better in comparison with other reports (Table 1).



Fig. 5. Differential pulse voltammograms for solutions containing (A) 10.0 μ M DA (constant) and various concentrations of AA: 5.0, 8.0 20.0 and 50.0 μ M. (B) 10.0 μ M AA (constant) and various concentrations of DA: 5.0, 8.0, 20.0 and 50.0 μ M. Supporting electrolyte in these experiments was 0.1 M acetate buffer (pH = 4.0) and pulse amplitude was 50 mV.

 Table 1. Various modified electrodes: DA and AA analytical properties.

Electrode	Experimental condition	Method	ΔE_p (mV)	Linear range (µM)		DL (µM)		Ref.
				DA	AA	DA	AA	-
SDS/rGO/	pH 7.4	DPV	178	0.5 - 5.0	-	0.26	-	[36]
CPE	PBS							
MWCNT/IL/	pH 4.5	DPV	180	0.1 - 151	0.6 - 112	0.03	0.2	[37]
Pd-NP/CPE	PBS							
PSNSB/CPE	pH 4.6	DPV	222	0.05 - 1200	2.5 - 1003	0.02	0.18	[38]
	PBS							
AgNPs/CPE	рН 7.5	DPV	181	1.0 - 50.0	-	0.08	-	[39]
	PBS							
SrPdO ₃ /CPE	pH 7.4	DPV	168	7 - 70	-	0.02	-	[40]
	PBS			90 - 160				
CPE modified	pH 5.0	DPV	200	0.05 - 30.0	0.05 - 30.0	0.04	0.02	[41]
with	PBS							
$[Cu(bp)(H_2O)_2]_n$								
GCE modified	pH 4.0	DPV	150	0.1-130	0.2-1.0	0.04	25.0	[42]
with	BR solution							
ZnO-Cu _x O-PPy								
GCE modified	pH 7.0	DPV	133	0.1-600	_	0.02	_	[43]
with	PBS							
γ-WO3								
CNT-OACPE	pH 4.0	DPV	368	0.1-90.0	0.1-90.0	0.03	0.03	This
	0.1M acetate							work
	buffer							

Note: EBT, Eriochrome Black T; CPE, carbon paste electrode; SDS, sodium dodecyl sulphate; RGO, reduced graphene oxide; MWCNTs, multiwalled carbon nanotubes; IL, ionic liquid;Pd-NP, Pd nanoparticle; PSNSB, PbS nanoparticles Schiff base; AgNPs, silver nanoparticles; Pd-CNF/CPE, Palladium nanoparticle-loaded carbon nanofibers; $[Fe(II)TSPc]^4$, ron(II)tetrasulfophthalocyanine; GCE, glassy carbon electrode; $[Cu(bp)(H_2O)_2]_n$, polymer of 4,4'-dicyanamidobiphenyl Cu(II) complex; PPy, polypyrrole; γ -WO₃, monoclinic structure of Tungsten trioxide nanoparticles.

4. CONCLUSION

In this work acridine orange/MWCNT is used in preparation of modified-CPE. The immobilization of acridine orange on carbon nanotubes demonstrated to be useful, and shows very good long-time stability and reproducibility of the electrode response. The prepared electrode demonstrated the complete separation of anodic peaks of AA and DA (368 mV), because of a negative shift in the anodic response of AA and concurrent positive shift in the DA peak. Good sensitivity and selectivity and very low detection limit (0.03 μ M) makes the modified electrode very effective in the manufacture of simple devices for the concurrent detection of AA and DA in clinical and pharmaceutical preparations.

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حمیدرضا زارع مهرجردی بخش شیمی، دانشکده علوم، دانشگاه پیام نور، تهران، ایران تاریخ دریافت: ۲٦ دیماه ۱۳۹۹ تاریخ پذیرش: ۱ فروردین ۱٤٠٠

چکیدہ

در این کار تحقیقاتی، الکترود خمیر کربن اصلاح شده با استفاده از آکریدین اورانژ تثبیت شده بر روی MWCNT و پخش شده در نفیون تهیه شده و رفتار الکتروشیمیایی دوپامین و آسکوربیک اسید در سطح آن با استفاده از روش ولتامتری چرخهای مطالعه شد. نتایج این مطالعه نشان می دهد که استفاده از مخلوط MWCNT-نفیون باعث تثبیت آکریدین اورانژ در بافت خمیر کربن می شود. به عبارت دیگر، حضور نفیون پایداری آکریدین اورانژ تثبیت شده روی MWCNT را در بافت الکترود افزایش داده و تکرارپذیری سطح الکترود اصلاح شده را بهبود می بخشد. نتایج مطالعات ولتامتری چرخهای و پایداری آکریدین اورانژ تثبیت شده روی MWCNT را در بافت الکترود افزایش داده و تکرارپذیری سطح الکترود اصلاح شده را بهبود می بخشد. نتایج مطالعات ولتامتری چرخهای و پالس تفاضلی نشان می دهند که الکترود اصلاح شده فعالیت الکتروکاتالیتیکی موثری برای اکسایش الکتروشیمیایی دوپامین و آسکوربیک اسید فراهم می کند و جدایی پیک دو ترکیب حدود ۲۶۸ میلی ولت به دست می آید. خواص الکتروکاتالیتیکی موثر، جدایی بسیار زیاد بین پیک ها و حذف پاسخ ولتامتری سایر ترکیبات کاهنده بیولوژیکی، الکترود اصلاح شده را برای اندازه گیری همزمان مقادیر زیر میکرومولار دوپامین و آسکوربیک اسید فراهم می کند و جدایی پیک دو ترکیب حدود ۲۶۸

واژههای کلیدی

آكريدين اورانژ؛ نانولولههاى كربنى؛ دوپامين؛ ولتامترى چرخهاى؛ پالس تفاضلى.