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The Synergic Effect of Conducting Polymer/Ionic Liquid Composite Electrodes on the Voltammetric Sensing of Biomolecules

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A novel modified screen-printed carbon electrode (SPCE) was constructed by spin-coating room temperature ionic liquid, 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]), into electropolymerized conducting polymer matrix of poly(3,4-ethylenedioxythiophene) (PEDOT). The resulting surface morphology was characterized by scanning electron microscopy. X-ray photoelectron spectroscopy was employed to confirm the successful surface modification. Cyclic voltammograms have shown that the composite film-modified electrode combined the advantages of conducting polymer and ionic liquids, and exhibited good electrocatalytic activity for biomolecules. The results showed lowering the overpotentials and enhancing current responses, without showing any fouling effect. The proposed method was applied to the simultaneous determination of dopamine (DA), ascorbic acid (AA) and uric acid (UA) in pH 7.0 PBS.

Keywords: poly(3,4-ethylenedioxythiophene), ionic liquid, dopamine, uric acid, ascorbic acid, synergic effect.

1. INTRODUCTION

The development of a convenient, accurate and reliable method for the determination of biomolecules in the biological samples has attracted much attention, especially in biochemical research, diagnostic study and clinical medication. Dopamine (DA) belongs to catecholamine family, and is an important neurotransmitter for message transfer in the central nervous system [1]. The amounts in the extracellular fluid of caudate nucleus of mammalian brain were found to be 50 nmol/g [2]. Patients with neurological disorders such as schizophrenia and Parkinson’s disease showed abnormal low concentration of DA. In the reverse, high catecholamine levels in the body fluids are cardiotoxic, and cause possible death of the heart muscles [3].

Table 1. Comparison of the analytical performance at electrodes modified with polymer, carbon materials and composites for the determination of ascorbic acid, dopamine and uric acid.

No.	Electrode	Analyte	Peak potential, V	Linear range, µM	Detection limit, µM	Sensitivity µA/µM	Ref.
1.	SPCE/PEDOT/IL	AA	0.06	60-1000	1.15	0.07	This work
		DA	0.23	8-200	0.39	0.28	
		UA	0.35	8-200	0.27	0.43	
2.	GCE/PImox /AuNPs[a]	AA	0.136	210-1010	2.0	0.0158	[7]
		DA	0.320	5-268	0.08	0.0788	
		UA	0.485	6-486	0.5	0.0322	
3.	Au/PEDOT[b]	AA	-0.094	5–300	2.5	1.7x10^{-5}	[8]
		DA	------	---------	------	------	
		UA	0.38	2 –600	1.5	7.9x10^{-5}	
4.	GCE/PEDOT	AA	0.08	300-1500	------	0.012	[9]
		DA	0.21	100-500	------	0.022	
		UA	------	---------	------	------	
5.	GCE/ PEDOT/ MWCNT[c]	AA	0.1	100–2000	100	0.00016	[10]
		DA	0.3	10–330	10	0.00121	
		UA	0.4	10–250	10	0.00328	
6.	Pt/PMT/PdNPs[d]	AA	-0.128	20-120	6.0	0.0131	[11]
		DA	0.115	0.05-1	0.009	1.37	
		UA	0.400	---------	------	------	
7.	Pt/PMPy/PdNC[e]	AA	-0.025	50–1000	7.0	0.0056	[12]
		DA	0.175	0.1–10	0.012	0.71	
		UA	0.373	0.5–20	0.027	0.28	
8.	GCE/PAH-HCNTs[f]	AA	-0.162	7.5–180	0.92	0.31	[13]
		DA	0.083	2.5–105	0.8	0.69	
		UA	0.185	6.7–65	1.5	0.95	
9.	SPGNE[g]	AA	-0.006	4.0–4500	0.95	0.0314	[14]
		DA	0.215	0.5–2000	0.12	0.0867	
		UA	0.388	0.8–2500	0.20	0.0613	
10.	GCE/SWCNH[h]	AA	0.013	30-400	5	0.020	[15]
		DA	0.224	0.2-3.8	0.06	3.49	
		UA	0.376	0.06-10	0.02	5.95	
11.	GCE/SGNF/IL/ CS[i]	AA	-0.160	30–350	14.8	0.07	[16]
		DA	0.053	0.05–240	0.05	0.13	
		UA	0.204	0.12–260	0.10	0.12	

a. Overoxidized polyimidazole/gold nanoparticles
b. Gold microelectrodes modified with PEDOT (assume surface area = 1.96 x 10^{-5} cm^{2})
c. PEDOT/multi-walled carbon nanotubes
d. Pt disc modified with poly(3-methylthiophene)/palladium nanoparticles
e. Poly(N-methylpyrrole)/palladium nanoclusters
f. Poly(allylamine hydrochloride)-functionalized-helical carbon nanotubes
g. Screen-printed graphene electrode
h. Single-walled carbon nanohorn
i. Stacked graphene platelet nanofibers (SGNF)/ionic liquid (IL)/chitosan (CS)

Uric acid (UA) is the primary end product of purine matabolism in human body, and is widely distributed in urine and serum [4]. High UA levels in the physiological fluids have been suggested a risk factor for several diseases such as hyperuricaemia, gout, leukemia and pneumonia [5]. Ascorbic acid (AA) is widely present in vegetables, fruits, and is a vital vitamin in the human diet. AA functions as antioxidants and free-radical scavengers [6], and has been used for the prevention and treatment of common cold, scurvy and mental illness [7]. The level of the oxidative stress could be assessed by the determination of the AA concentration in the body fluids [6]. High AA levels can cause gastric irritation and renal problems [8].

Electroanalytical methods have been used to determine DA, UA and AA due to their high sensitivity, easy operation and low cost [7-16]. A major problem for their simultaneous determination is that the three molecules usually undergo oxidation at the same potentials at conventional electrodes, resulting in poor resolution of the voltammetric responses. Another disadvantage is the surface fouling effects by the accumulation of oxidized and polymerized products on the solid electrode, leading to poor reproducibility and stability [3]. Moreover, the basal DA concentration is very low (0.01-1 μM) compared to the high concentration of interfering AA (100-500 μM) [2].

To overcome these problems, various modified electrodes have been prepared in order to achieve a sensitive and selective method. Table 1 lists the present study and literatures on the electroanalytical performance for the simultaneous determination of AA, DA and UA using different sensing configurations [7-16]. The electrode modifiers include polymer film [8,9], carbon materials [13-15] and some hybrid materials [7,10-12,16].

Conducting polymers are promising materials as electrode modifiers in the farbrication of chemical and biochemical sensors due to their high electrical conductivity, electrochemical reversibility and high chemical stability in aqueous solutions [17]. Polyaniline, polypyrrole and polythiophene have been used as electrocatalysts for detecting organic and biological molecules [17]. Due to the ability to promote electron transfer reactions, electroanalysis using conducting film poly(3,4-ethylenedioxythiophene) (PEDOT) have been reported. Chen *et al*. used PEDOT-modified glassy carbon electrodes for detecting dopamine and ascorbic acid [9]. Manisankar *et al.* used PEDOT-modified electrodes for determining pesticides [18]. Ho *et al* reported detection of nitrite at PEDOT-modified screen-printed carbon electrode (SPCE/PEDOT) [19]. Our previous studies reported the sensitive and selective determination of cysteine at screen-printed electrode modified with PEDOT [20]. Modification of conducting polymer is a facial technique, but has the drawbacks of low sensitivity [8,9]. Improved sensitivity can be achieved by the addition of nanomaterials, such as gold nanoparticles [7,21] and Pd nanoparticles [11,12].

Ionic liquids (ILs) are promising materials in analytical chemistry; they belongs to the class of liquid electrolytes comprised of asymmetric heterocyclic cations (typically, immidazolium- or pyridinium-based cations) and various types of small anions. The use of ILs in electroanalysis is feasible because of their wide potential window, high ionic conductivity and good electrochemical stability [2]; they also have the advantages in the modulation of surface properties such as wettability, adhesion and biocompatibility [22]. Researchers have shown that these ILs-based electrodes possess striking electrochemical properties. For example, carbon (graphite or multi-walled carbon nanotubes) paste electrodes using ILs as pasting binders have been fabricated to detect certain biomolecules, such as DA, UA, AA and dihydronicotinamide adenine dinucleotide [23,24]. The results showed low background current, improved electroactivity and antifouling features. Direct modification of electrode surface by ILs film was also achieved [22]. The modified electrode demonstrated enhanced electron-transfer kinetics for AA and horseradish peroxidase. Extensive investigations on the preparation and analytical applications modified electrodes with ionic liquids and their composite materials have been reviewed [25-28].

The developments of polymer composites have large impacts on the fundamental academic researches and potential industrial applications. Conducting polymer composites have been exploited for chemical or biological sensing, using conjugated polymer and additives of inorganic, organic and biological materials [29]. However, the electroanalytical protocols based on composite film of conducting polymer and ionic liquid are few. The aim of the present study was to fabricate electrochemical sensors for DA, UA and AA determination based on conducting polymer and ionic liquids in hope for combining their unique advantages. The composite film-modified electrodes were prepared by the electropolymerization of a thin PEDOT film onto the screen-printed carbon electrode (SPCE), and subsequently cast a very thin film of ionic liquid, 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]), by drop and spin coating. The obtained composite film is expected to extract more electroactive molecules from aqueous solution, and provide enhanced electrochemical responses. The results showed that such hybrid materials showed advantages of good resolution and improved sensitivities for the simultaneous determination of DA, AA and UA.

2. EXPERIMENTAL

2.1. Reagents

1-Butyl-3-methylimidazolium chloride ([BMIM][Cl]), 3,4-ethylenedioxythio- phene (EDOT), hydroxypropyl-β-cyclodexdrine (HP-β-CD), dopamine, uric acid, ascorbic acid and *N*,*N*-dimethylformamide (DMF) were obtained from Acros. All of these chemicals were of the highest grade available and used without further purification. The other chemicals were of analytical reagent grade and also obtained from Acros. All of the solutions were prepared using de-ionized water from a Milli-Q ultrapure water system with a resistivity of 18 MΩcm.

2.2. Instruments

Cyclic voltammetry was performed using a voltammetric analyzer (CH Instruments, model CH I-621C). A screen-printed carbon electrode (0.2 cm^2) from Zensor R&D (Taiwan) was used as the working electrode, and was washed with de-ionized water before use. A platinum wire was used as the counter electrode. A home-made Ag|AgCl|KCl (sat.) electrode was used as the reference electrode. All potentials were reported with respect to this reference electrode. A Hitachi S-4700I high-resolution scanning electron microscopy (FE-SEM) was used to characterize the electrode surface. The composition of the modified film was investigated using a PHI 5000 VersaProbe/PHI Quantera SXM (XPS/ESCA) instrument with an AlKαX source.

2.3. Modified electrode preparations

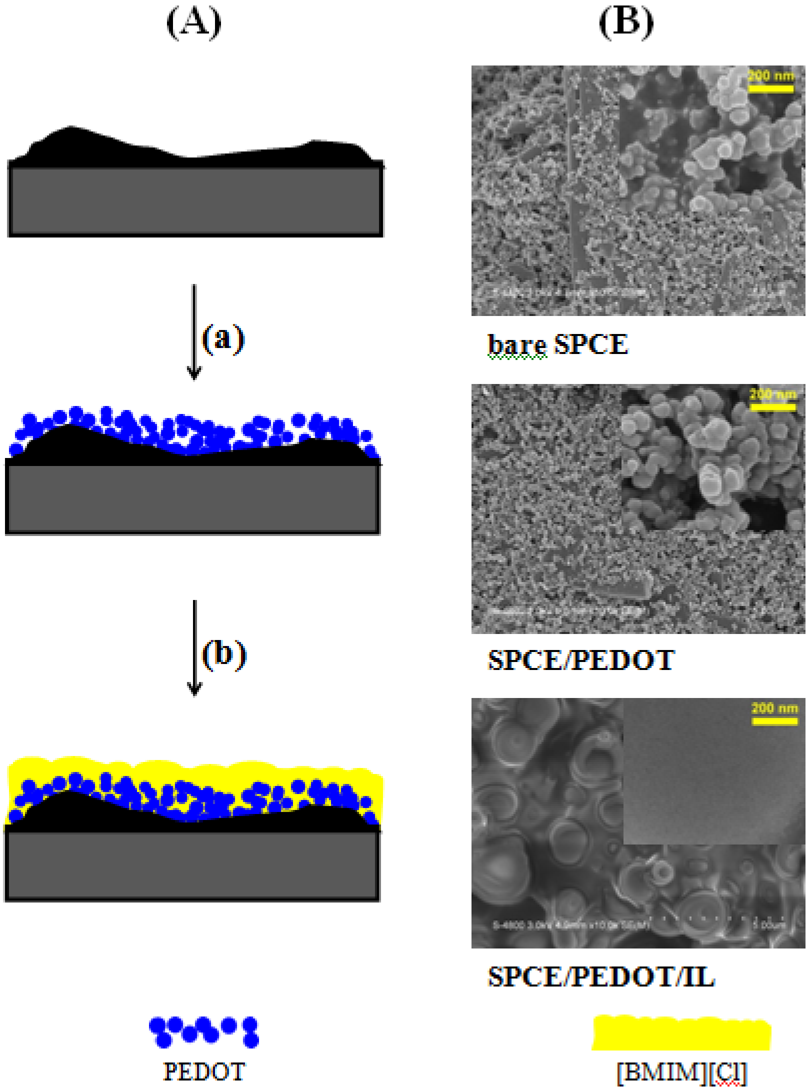


Figure 1. (A) The two-step procedures of the modified electrode: (a) electropolymerization in EDOT solutions, (b) deposition of [BMIM][Cl]. (B) FE-SEM micrographs of the modified electrode.

As shown in Fig. 1A, the modified electrodes were obtained by a two-step strategy. First, a mixed solution of 0.01 M EDOT, 0.1 M $LiClO_4$ and 5 mM HP-β-CD was used as the electrolyte solution. The PEDOT film-modified electrode (SPCE/PEDOT) was achieved by cyclic potential scanning from -0.6 to +1.0 V at a scan rate of 0.1 V/s for 3 cyclic numbers; then the electrodes were treated by repeated cycling (from -0.2 to +0.8 V, scan rate 0.1 V/s) in pH 7 buffer solution until a stable background current was obtained. Secondly, 0.0170 g of [BMIM][Cl] and 100 μL of DMF were mixed and stirred violently; 3 μL of the mixture were deposited onto the SPCE/PEDOT, allowed to cover the whole electrode surface area, stood for 12 h in the air, and then spun at 3000 rpm for 5 min. The composite film-modified electrodes (SPCE/PEDOT/IL) were then treated by repeated cycling (from -0.3 to +0.8 V, scan rate 0.1 V/s) in pH 7 buffer solution, and kept in air before use.

3. RESULT AND DISCUSSION

3.1. Surface characterization of SPCE/PEDOT/IL

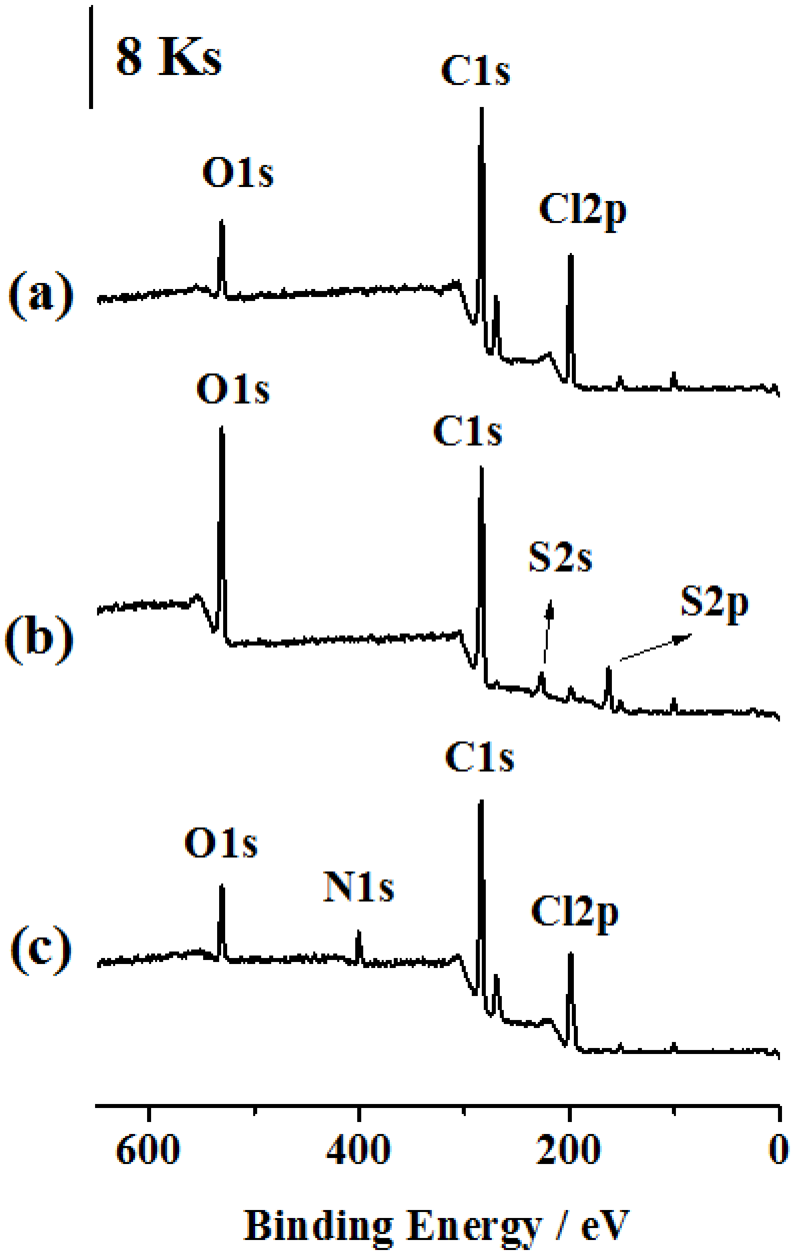


Figure 2. XPS spectra of (a) bare SPCE (b) SPCE/PEDOT (c) SPCE/PEDOT/IL.

Fig. 1B shows the corresponding electrode surface morphologies investigated with 10,000X and 50,000X magnifications by scanning electron micrographs (SEM) techniques to gain insights into the nature of the modified layers. The bare SPCE surface contained graphite pieces of varying dimensions and a high content of aggregated carbon particles. After electropolymerization in EDOT solution, the carbon surface was incompletely covered by packed PEDOT particles [30]. Upon spin-coating a thin layer of ionic liquid [BMIM][Cl], the SEM images changed significantly. The composite film-modified electrode (SPCE/PEDOT/IL) showed a very uniform, smooth surface topography. The plain structure indicated that ionic liquid filled the void spaces between the PEDOT particles and graphite flakes, and lead to the fine adherence of ionic liquid on the electrode surface. This was consistent with our previous reports where smooth surface morphologies were observed for ionic liquid-modified electrodes [31]. The embedded ionic liquids was bridged in the carbon layers and PEDOT conducting polymer, and acted as ion carriers to transport charges between them [32].

To gain a better understanding of the modified layers, the surface compositions were studied using XPS techniques. Fig. 2 showed the wide-scan spectra, focusing on the C(1s), S(2p) and N(1s) spectral regions for the characteristic elements of the bare SPCE, PEDOT film and ionic liquid film, respectively. The binding energy of the C(1s) peak was 283.9 eV (Fig. 2a), corresponding to abundant graphite component on the bare SPCE. The binding energy at 162.9 eV was associated with S(2p) in the thiophene ring (Fig. 2b) [21], evidenced that PEDOT film has been modified on the SPCE. The dramatic reduction in intensity of the sulfur peaks after covering an ionic liquid film is interesting. As showed in Fig. 2c, the S(2s) and S(2p) peaks were not present. It suggested that the ionic liquid film produced by spin coating completely covered and thus obscured the underlying PEDOT film. This inference is consistent with the smooth surface images obtained by the SEM technique. A characteristic single peak appeared at 400.0 eV, and was attributed to the N (1s) in imidazole ring of ionic liquid [33]. These results confirmed the successful modification of the composite film on the electrode surfaces.

3.2. Electrocatalytic oxidation of DA, AA, and UA

Fig. 3A shows the cyclic voltammograms of DA in pH 7.0 buffer solution. The electrochemical response to DA at bare SPCE was very poor; an oxidation peak occurred at E_{pa} = 0.43 V and a small reduction peak occurred at E_{pc} = 0.05 V (Fig.3A, curve a). Improved responses were obtained at the ionic liquid-, PEDOT- and composite film-modified electrodes. A well-defined redox couple (E_{pa} = 0.21 V, E_{pc} = 0.15 V) was recorded for the SPCE/IL modified electrodes (Fig.3A, curve b). The use of ionic liquids to improve electrochemical characteristics for organic molecules has been reported. Safavi and co-workers developed carbon paste electrodes using 1-octyl-pyridinium hexafluorophosphate as a pasting binder of graphite powder; the sensors showed a quasi-reversible redox couple for DA [2]. Another example was reported by Chang et al., where 1-butyl-3-methylimidazolium hexafluorophosphate was screen-printed at preanodized carbon electrodes [34]. In their case, both adsorption- and diffusion-controlled redox peaks for DA were observed. In the present study, the electrochemical oxidation of DA at SPCE/IL also showed an incensement in the rate of electron-transfer and a marked decrease in the overvoltage.

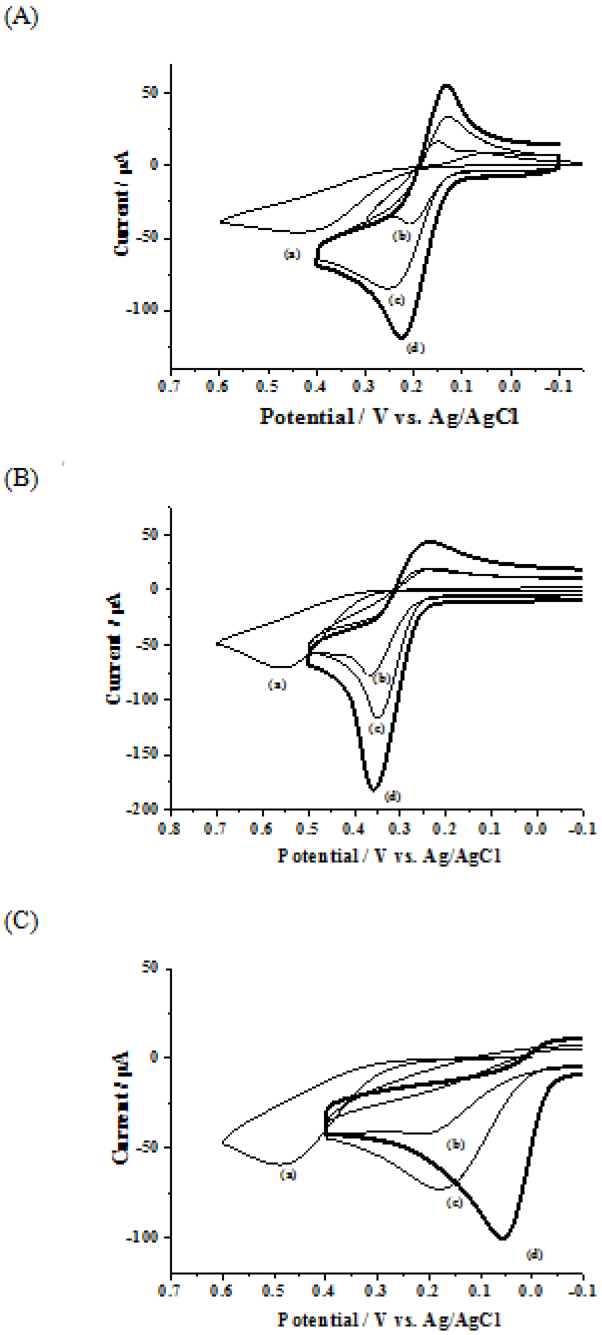


Figure 3. Cyclic voltammograms of 1.0 mM (A) DA (B) UA (C) AA at (a) bare SPCE (b) SPCE/IL (c) SPCE/PEDOT (d) SPCE/IL/PEDOT in pH 7.0 buffer solution. Scan rate = 0.1 Vs^{-1}.

The reason for observation of only one redox couple is suggested to be the thin ionic liquid layer obtained by spin-coating. At SPCE/PEDOT modified electrode, the anodic potential and cathodic potential for DA occurred at 0.25 and 0.13 V, respectively (Fig.3A, curve c). As it has been mentioned previously, the electropolymerized PEDOT has a strong electrocatalytic oxidation acitivity of DA, due to the hydrophobic interaction between the aromatic part of DA and the PEDOT film [9]. At the composite film-modified electrode SPCE/PEDOT /IL (Fig.3A, curve d), the peak potentials remained the same (E_{pa} = 0.23 V, E_{pc} = 0.13 V), and the current responses were enlarged. The anodic current value for SPCE/PEDOT/IL (116.0 μA) was very close to the sum of the other two modified electrodes, 44.5 μA for SPCE/IL and 82.2 μA for SPCE/PEDOT. The formation of the PEDOT film followed by the deposition of a thin layer of [BMIM][Cl] film have combined the unique properties of the individual materials, and exerted a strong impact on the characteristics of the polymer/ionic liquid composite. Thus, the synergic effects between the PEDOT film and [BMIM][Cl] have improved the detection of DA.

The synergic effect of polymer/ionic liquid composite was evidenced for the electrochemical oxidation of UA in pH 7.0 buffer solution as shown in Fig. 3B. An irreversible oxidation peak occurred at 0.56 V at bare SPCE, while the oxidation peak shifted to 0.35 V at all the three modified electrodes. Furthermore, the oxidation current responses of SPCE/PEDOT/IL (174.1 μA) is very close to the sum of SPCE/IL (67.9 μA) and SPCE/PEDOT (116.4 μA). The improved voltammetric responses at SPCE/PEDOT/IL are explained in the following aspects. First, the presence of [BMIM][Cl] provides a good extraction effect and a remarkable electrostatic attraction to negatively charged UA in pH 7.0 buffer solution (pKa = 5.4 for UA). Second, due to the hydrophobic interaction between the heterocyclic aromatic ring of UA and PEDOT film, the UA is concentrated and then, catalytically oxidized at the PEDOT/IL interfaces. The combined effects are expected to be responsible for the improved voltammetric responses at SPCE/PEDOT/IL in comparison with bare SPCE and the other two modified electrodes.

The electrocatalytic activity of the modified electrodes towards AA was also observed as shown in Fig. 3C. It is noted that AA oxidation occurred irreversibly at 0.48 V at bare SPCE in pH 7.0 buffer solution. The large overpotential indicated the slow electron transfer because of the electrode fouling caused by the adsorption of oxidation product, dehydroascorbic acid. The oxidation potential shifted to 0.21 V at SPCE/IL (I_{pa} = 35.96 μA). The electrocatalytic oxidation of AA at SPCE/IL resembled to the case of ionic liquid-fabricated carbon (graphite or multi-walled carbon nanotubes) paste electrodes, which have shown improved electroactivity due to the facile access of negatively charged ascorbate to the cationic ionic liquid films [22-24]. At SPCE/PEDOT, the electrocatalytic oxidation of AA occurred at 0.18 V (I_{pa} = 69.08 μA). The electrocatalytic oxidation of AA at SPCE/PEDOT is originated from the electrostatic attraction between the ascorbate and the cationic sites of PEDOT film [9]. Surprisingly, the anodic peak potential of AA at SPCE/PEDOT/IL further negatively moved to 0.06 V. The potential shifts were not observed in the case of DA and UA at SPCE/PEDOT/IL. Besides, obviously higher oxidation current for AA was obtained at the composite film-electrode, SPCE/PEDOT/IL (98.6 μA). In the literatures concerning the oxidation of DA, UA and AA at PEDOT/MWCNT composite film-modified electrode, the potential shifts were not observed [10]. Ionic liquid covalently bonded to silicate matrix was reported to be effective accumulation of electroactive anions [35]. AA is a hydrophilic species because the molecule contains many oxygen-containing functional groups. Therefore, the presence of PEDOT/IL composite film at electrode surface developed a strong synergic effect, and benefited not only in the lowering of oxidation overpotential but also in the dramatic enlargement of the current responses.

In conclusion, due to the combination of unique properties of each material, the composite-film modified electrode SPCE/PEDOT/IL had a better electroactivity than SPCE/IL and SPCE/PEDOT. Besides, the outer layer of ionic liquids actually showed advantages in the reservation of electroactivity. The investigated biomolecules were electrochemically oxidized to their oxidized products, which moved away from the electrode surface without fouling effects. This can be beneficial to the continuous, simultaneous determination of DA, AA, and UA at SPCE/PEDOT/IL with high sensitivity and selectivity.

3.3. Effect of solution pH and scan rates

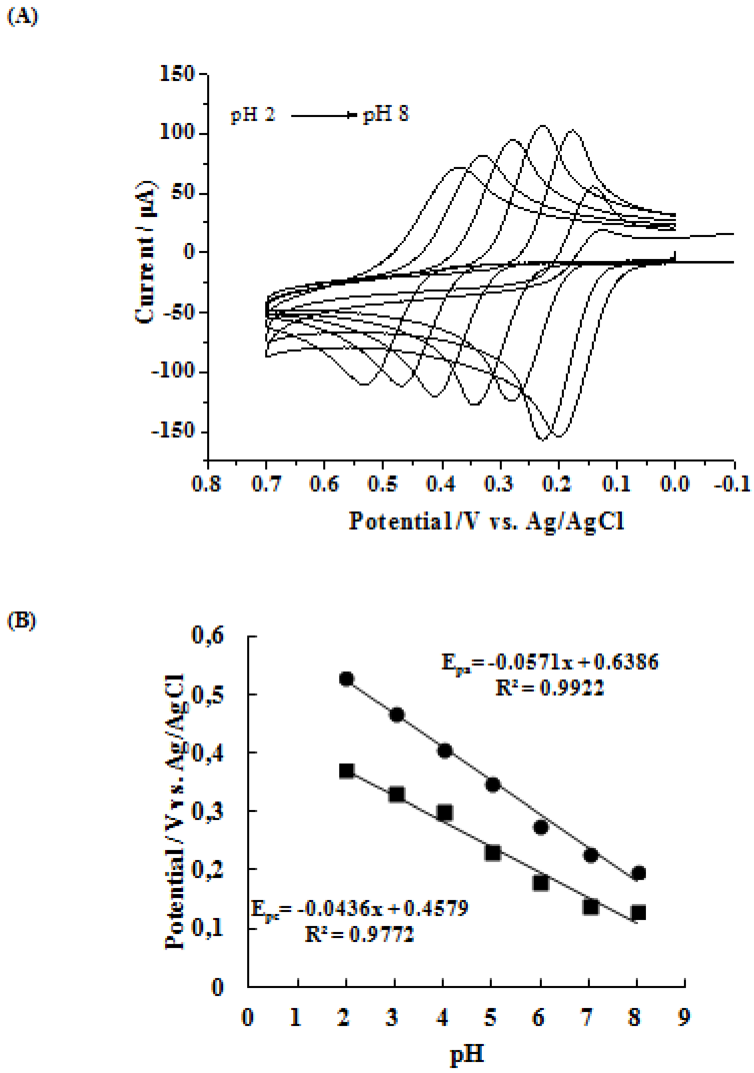
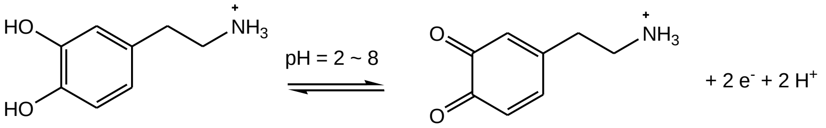


Figure 4. (A) Cyclic voltammograms of 1.0 mM DA at SPCE/PEDOT/IL electrodes in various buffer solutions (pH 2-8). Scan rate = 0.1 Vs^{-1}. (B) Plot of potential vs. pH. E_{pa} (●), E_{pc} (■).

Fig. 4A showed the effect of solution pH values on the cyclic voltammograms of 1.0 mM DA at SPCE/PEDOT/IL modified electrode. It was observed that both the anodic and cathodic peak currents change with the pH of solutions, which means different electrochemical activity for DA redox couple at the electrode/solution interfaces. A redox couple with high peak current ratio (I_{pc}/I_{pa}) was observed in acidic buffer solutions. The peak current ratios decreased to 0.25 in pH 8.0 buffer solutions. The low current ratio is due to the instability of the oxidation product, *o*-dopaminoquinone in alkaline solutions. The amine group of *o*-dopaminoquinone easily undergoes intramolecular reaction to form leucodopaminochrome [36], and the fast chemical reaction leads to the low current reversibility.

The relationship between the peak potentials and the solution pH values (over the pH range 2.0-8.0) was shown in Fig. 4B. The peak potentials shifted negatively with the increase of solution pHs, indicating that the electrocatalytic oxidation of DA at the SPCE/PEDOT/IL modified electrode is a pH-dependent reaction. The anodic peak potentials (E_{pa}) shifted negatively with a slope of 57.1 mV/pH, which is very close to the theoretical Nernstian value (-59.2 mV/pH). It is consistent to the literature results, revealing that the oxidation processes involved the 2-electrons and 2-protons transferred reactions, as shown in eq. 1 [36]. The cathodic peak potential (E_{pc}) shifted with a slope of -43.6 mV/pH. The deviation from the theoretical value indicated that the following chemical reaction in alkaline solutions affected the observed peak positions.



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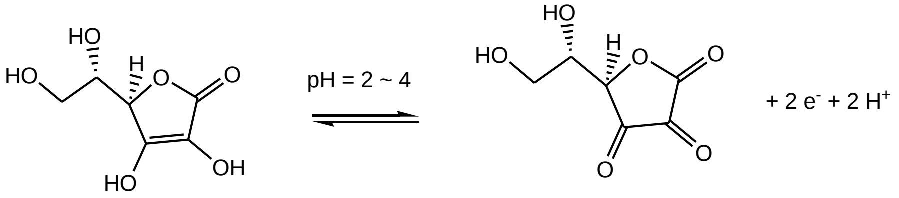
The pH effect on the oxidation potential of AA and UA was also investigated by cyclic voltammetry (figures not shown). The E_{pa} versus pH clearly indicated two regions for AA; the slopes were -0.066 V/pH and -0.036 V/pH in the pH 2-4 and pH 5-10, respectively. According to the Nernst values, the proportion of the electron and proton involved in the oxidation reaction is related to the acid dissociation constants (pK_a) values. The two pK_a values of AA are 4.1 and 11.6. According to the obtained slopes, the electrochemical oxidation of AA involved 2-proton, 2-electron process (eq. 2) and 1-proton, 2-electron process (eq. 3) depending on the solution pH values. The two pK_a values of UA are 5.4 and 10.3. Due to the limited solubility of UA in acidic solution, the pH effect on the oxidation potential of UA was investigated in pH 6-10 buffer solutions. It was observed that the oxidation potential shifted linearly with solution pH values, and the slope was -0.064 V/pH. The obtained slope suggested 2-proton, 2-electron process for UA oxidation, and the reaction was shown in eq. 4.



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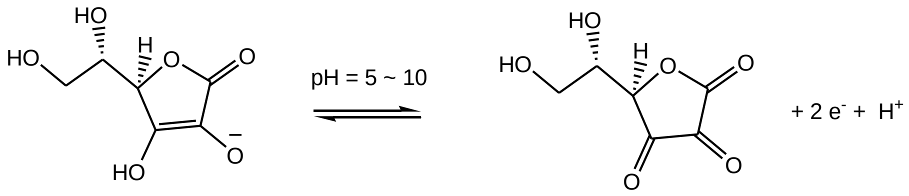

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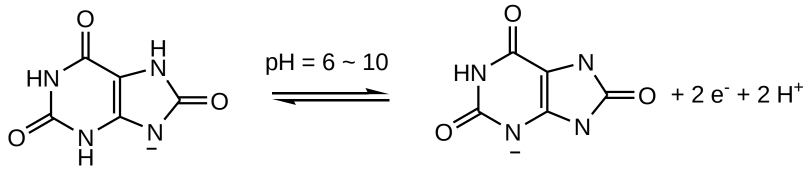

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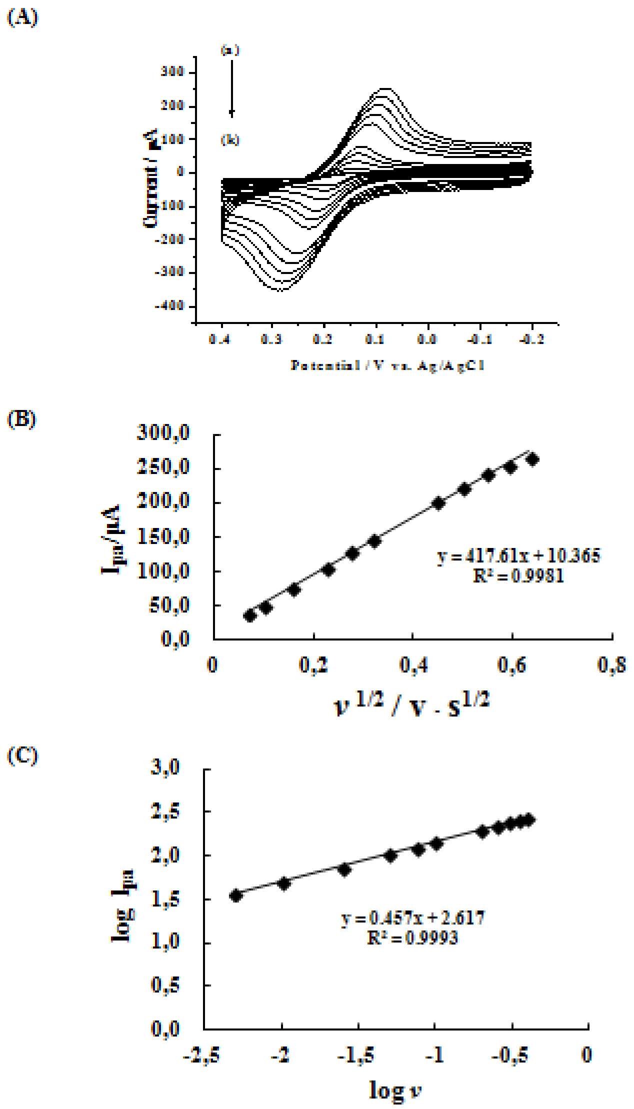

Figure 5. (A) Cyclic voltammograms of 1.0 mM DA at SPCE/PEDOT/IL electrodes in buffer solutions with various scan rates. Scan rate = (a) 0.005 (b) 0.01 (c) 0.025(d) 0.05(e) 0.075(f) 0.1(g) 0.2(h) 0.25(i) 0.3(j) 0.35(k) 0.4 Vs^{-1}. (B) Plot of I_{pa} vs. $v^{1/2}$. (C) Plot of log I_{pa} vs. log v.

The information of electrode kinetics at SPCE/PEDOT/IL with 1.0 mM DA in pH 7.0 PBS can be obtained by the examination of the influence of scan rates (0.005-0.4 V/s) on the cyclic voltammograms. As shown in Fig. 5A, the anodic peak currents increased as the scan rates increased. Meanwhile, the anodic peak potentials shifted positively and the cathodic potentials shifted negatively. The oxidation peak currents were linearly proportional to the square root of the scan rate (υ), yielding a slope of 417.61 $\mu As^{1/2}V^{-1/2}$ and $R^2 = 0.9981$ (Fig. 5B). The plot of log I_{pa} vs. log υ showed a good linearity with a slope of 0.46 (Fig. 5 C). The phenomena indicated that the electrochemical oxidation reaction is a diffusion-controlled process. The oxidation current followed the diffusion-controlled reaction mechanism at high scan rates also demonstrated that the PEDOT/IL composite film possesses a fast electron transfer kinetics. The scan rate effects on the electrochemical oxidation of AA and UA were also obtained by the same way. The results indicated diffusion-controlled reactions at the composite film-modified electrodes.

3.4. Analytical applications

For the simultaneous determination of AA, DA and UA, differential pulse voltammetry (DPV) in the range of -0.2 to 0.5 V was carried out in pH 7.0 buffer solutions. The optimized values of the DPV parameters were amplitude of 90 mV, potential increment of 7 mV, pulse width of 50 ms and sampling width of 0.0333 s. Three distinguishable oxidation peaks were observed at 0.0, 0.15 and 0.25 V in ternary mixture solution, corresponding to the oxidation potentials of AA, DA and UA, respectively.

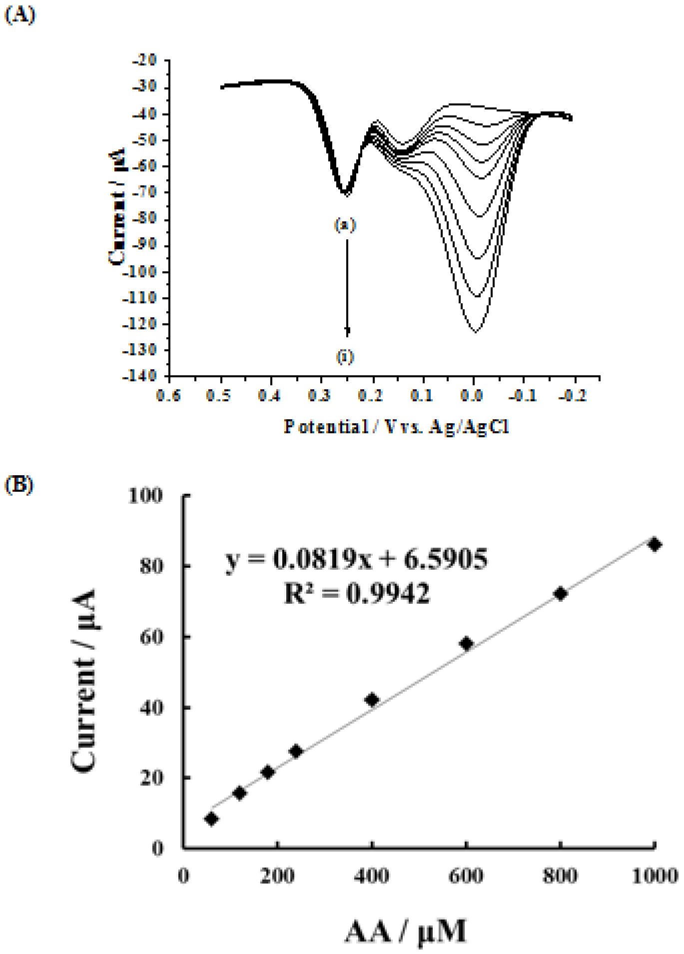

Figure 6. (A) DPVs of SPCE/PEDOT/IL modified electrode in pH 7.0 buffer solution containing 40 µM UA, 40 µM DA and various concentration of AA. [AA] = (a) 0, (b) 60, (c) 120, (d) 180, (e) 240, (f) 400 ,(g) 600, (h)800, (i) 1000 µM, The DPV parameters were an amplitude of 0.09 V, pulse width of 0.05 s, pulse period of 0.3 s, sampling width of 0.0333 s and potential increment of 0.007 mV. (B) The plot of I_{pa} vs. [AA].

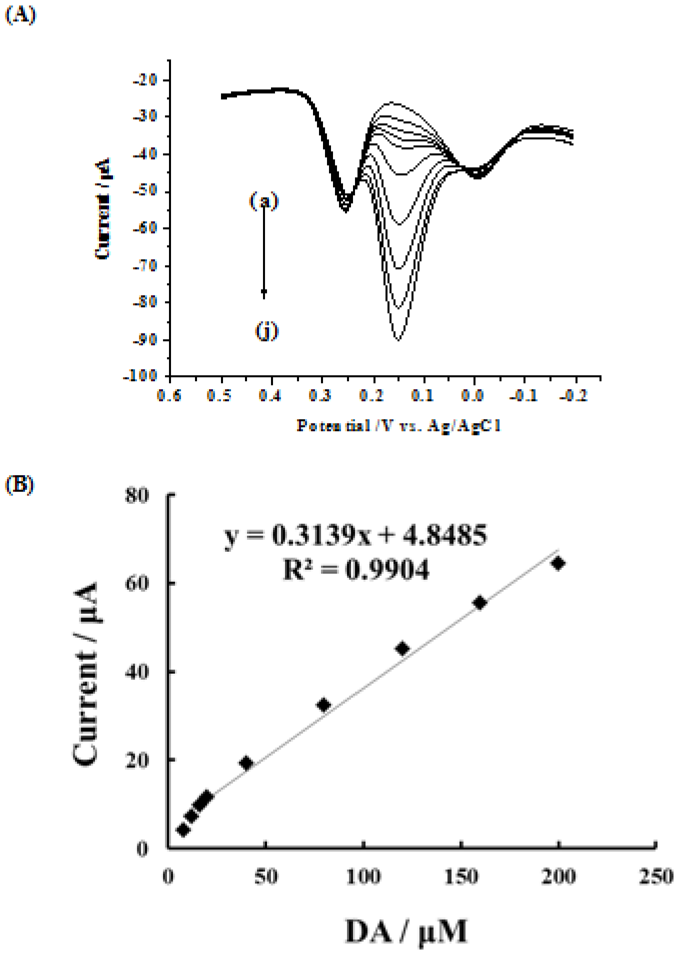

Figure 7. (A) DPVs of SPCE/PEDOT/IL modified electrode in pH 7.0 buffer solution containing 40 μM UA, 200 μM AA and various concentration of DA. [DA] = (a) 0, (b) 8, (c) 12, (d) 16, (e) 20, (f) 40, (g)80, (h)120 (i) 160, (j) 200 μM. The DPV parameters were an amplitude of 0.09 V, pulse width of 0.05 s, pulse period of 0.3 s, sampling width of 0.0333 s and potential increment of 0.007 mV. (B) The plot of I_{pa} vs. [DA].

The appropriate peak potential separation allows the simultaneous determination of the three compounds in their mixture where one species increased concentration and the other two species remained constant.

As shown in Fig. 6A, the oxidation peak current of AA increases linearly with their concentrations increase, while keeping the other two peaks constant. The results also indicate the stability and efficiency of the electrocatalytic activity of SPCE/PEDOT/IL towards AA. The resulting calibration curve exhibited a linear variation of the peak current as a function of the concentration of AA as shown in Fig. 6B. The linear regression equation is calibrated as I_{AA} (μA) = 0.0819 C_{AA} + 6.5905 (μM) (C_{AA}: 60–1000 μM) with the correlation coefficient of $R^2 = 0.9942$. The linear ranges for determination of DA and UA were also obtained by the same way. For DA, the linear regression equation is expressed as I_{DA} (μA) = 0.3139 C_{DA} + 4.8485 (μM) (C_{DA}: 8–200 μM) with the correlation coefficient of $R^2 = 0.9904$ (Fig. 7). In the case of UA, the linear regression equation is calculated as I_{UA}

(μA) = 0.4291 C_{UA} + 5.2599 (μM) (C_{UA}: 8–200 μM) with the correlation coefficient of $R^2 = 0.9928$ (Fig. 8). The detection limits (S/N = 3) for the determination of AA, DA, and UA are evaluated as 1.10 μM, 0.19 μM, and 0.14 μM, respectively. The relative standard deviation (RSD) for fifteen successive determinations of a mixture solution containing AA (400 μM), DA (10 μM) and UA (10 μM) was investigated. The relative standard deviations obtained were 4.44%, 4.22% and 4.12% for AA, DA and UA, respectively. The results indicated low susceptibility to electrode surface fouling, and high reproducibility of the proposed methods. It was noted that the above analytical characteristics for the determination of AA, DA, and UA at the SPCE/PEDOT/IL are nearly the same with those obtained in solution only containing one species, confirming no interference between each other in the measurements.

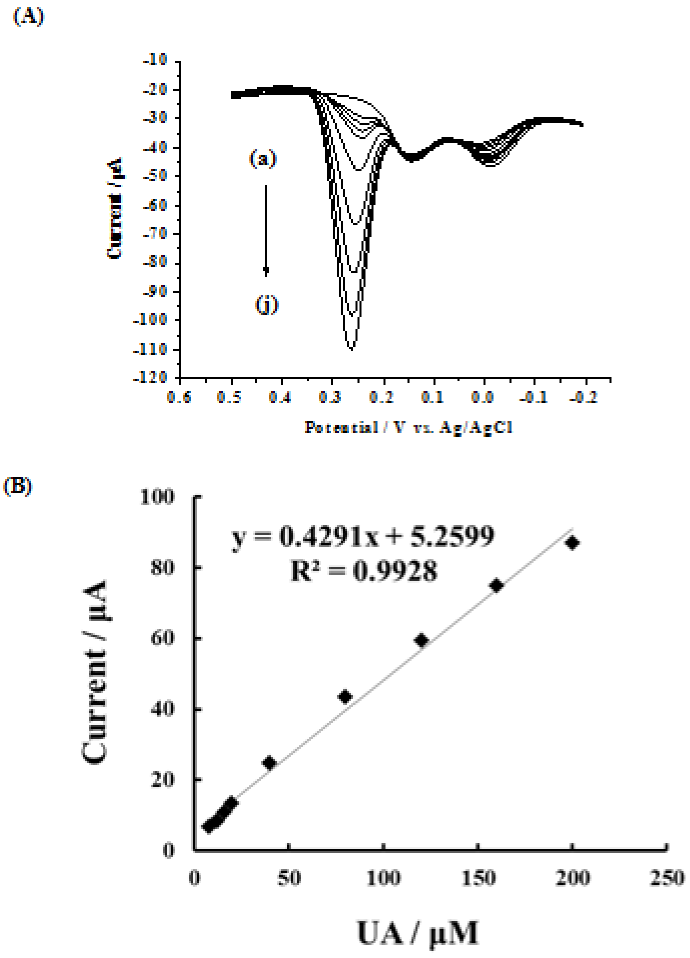

Figure 8. (A) DPVs of SPCE/PEDOT/IL modified electrode in pH 7.0 buffer solution containing 200 μM DA, 40 μM AA and various concentration of UA. [UA] = (a) 0, (b) 8, (c) 12, (d) 16, (e) 20, (f) 40, (g)80, (h)120 (i) 160, (j) 200 μM .The DPV parameters were an amplitude of 0.09 V, pulse width of 0.05 s, pulse period of 0.3 s, sampling width of 0.0333 s and potential increment of 0.007 mV. (B) The plot of I_{pa} vs. [UA].

The analytical performance of the proposed method was compared to those obtained on different modified electrodes reported in the recent literatures. It can be found in Table 1 that

SPCE/PEDOT/IL possesses improved or comparable performance for the simultaneous determination of AA, DA and UA. The sensitivity of 0.28 μA/μM for DA and 0.43 μA/μM for UA showed relatively high sensitivities of DA and UA assays compared with the other studies. An obvious advantage in this protocol is the modified materials are inexpensive, not involving expensive metal nanoparticles and synthesized carbon nanomaterials; the preparation of the modified electrodes is also easily handled and handed.

4. CONCLUSION

In the present study, a composite film of conducting polymer and ionic liquid film was immobilized step by step onto a screen-printed carbon electrode. Due to the synergic effects of the two modified layers, SPCE/PEDOT/IL have resolved the voltammetric oxidation potentials of AA, DA and UA with enhanced peak currents in pH 7.0 buffer solution. It is expected that the positively charged backbone of PEDOT and cationic sites of ionic liquids display electrostatic attraction for anionic AA and UA, while the hydrophobicity of PEDOT film and imidazole ring of ionic liquid has a π-π interaction with DA. The extraction ability of ionic liquid also plays an important role in discriminating the three compounds. Thus, a significant enhancement in the peak current and the negative shift in the peak potential was observed for AA, DA and UA. The composite film-modified electrode showed good analytical characteristics, including wide linear working ranges, high sensitivity, good selectivity, good reproducibity and long term stability.

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