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Simultaneous Determination of AA, DA, And UA Based on Bipolymers by Electropolymerization of Luminol And 3,4-Ethylenedioxythiophene Monomers

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Simultaneous determination of ascorbic acid (AA), dopamine (DA), and uric acid (UA) based on the bipolymers of polyluminol and poly(3,4-ethylenedioxythiophene) has been successfully performed. The bipolymers can be easily deposited on electrode surface by electropolymerization of luminol and 3,4-ethylenedioxythiophene in acidic aqueous solution by repeatedly cyclic voltammetry. The film formation is dependent on their electro-oxidation potential so that two electropolymerized procedures were applied to avoid the disorder of polymer chains. Polyluminol was performed in the potential range of 0.2–0.9 V while poly(3,4-ethylenedioxythiophene) was performed in the potential range of 0.3–1.2 V. It was found surface-confined and stable in various scan rate and different pH conditions. The calculated surface coverage (Γ) was 6.72×10^{-9} mol cm^{-2}. AA, DA, and UA were simultaneously determined by this electrode at +0.16 V, +0.31 V, and 0.43 V, respectively. Compared with bare electrode, the bipolymers modified electrode exhibits lower over-potential and higher current response. It also shows more higher current response to AA, DA, and UA as compared with polyluminol and poly(3,4-ethylenedioxythiophene) modified electrodes. This bipolymers (polyluminol/poly(3,4-ethylenedioxythiophene)) modified electrode can be developed as an electrochemical biosensor to determine AA, DA, and UA.

Keywords: Ascorbic acid (AA), Dopamine (DA), Uric acid (UA), Luminol, 3,4-ethylenedioxythiophene (PEDOT)

1. INTRODUCTION

Dopamine (DA) plays an important role in the function of central nervous, renal, hormonal and cardiovascular systems [1]. It is of great clinical importance to measure the DA level in extracellular fluid to monitor neurotransmission processes and diagnose Parkinson's disease. There is an intense investigation in the development of methods for DA quantification in blood and biological fluids. Electrochemical methods have proven to be rapid, simple and sensitive in the determination of neurotransmitters. However, an overlapping voltammetric response has been observed because the oxidation of DA at bare electrodes occurs along with the oxidation of AA and UA in biological tissues [2–4]. Thus, it is a challenge to separate the oxidation peaks of AA, DA and UA from each other in electrochemical analysis. UA is an important analyte in clinical field. In a healthy human being, the typical concentration of UA in urine is in millimolar range (~2 mM), whereas in blood it is in the micro-molar range (120–450 μM) [5,6]. Abnormalities of UA level indicate symptoms of several diseases, such as gout, hyperuricaemia and Lesch-Nyhan syndrome [7]. Ascorbic acid (vitamin C) is a water-soluble substrate present in a wide number of foods such as, fruits and vegetables. AA is also added to foodstuffs as an antioxidant for stabilization of color and aroma, as well as prolonging the life of commercial products [8]. Due to the presence of ascorbate in the mammalian brain, it plays an important role in bioelectrochemistry, neurochemistry and clinical diagnostics applications. It is also necessary for the formation of collagen and has been used for prevention and treatment of common cold, scurvy and cancer [9].

AA, DA and UA are electroactive compounds with very similar electrochemical properties and they will be oxidized at nearly same potential with poor sensitivity at unmodified electrodes. Therefore simultaneous determination of AA, DA and UA is a major goal in modifying the electrodes. Various modified electrodes have been constructed. A working electrode coated with ion-exchange membrane such as nafion was proposed to avoid electrode surfaces from interferences [10,11]. However, this kind of modified electrodes suffers from slow response due to low diffusion coefficient of analytes through the films. Detection sensitivity of DA and UA in presence of high concentration of AA was improved by nafion coated clay-modified electrode [12]. The disadvantages of ion-exchange membrane modified electrodes include non-uniform thickness and poor reproducibility due to solvent evaporation method used in the film preparation. Electropolymerization of conducting polymers can be used to prepare polymer films with uniform and controllable thickness on the electrode surface. Due to their high selectivity, various polymer-modified electrodes have been used for determination of AA, DA and UA [13–15].

Among the organic conducting polymers, poly(3,4-ethylenedioxythiophene) (PEDOT) and its derivatives have attracted great interest, because of their high electrical conductivity, chemical stability, low band gap, good film forming ability, outstanding environmental stability and compatibility with aqueous media [16–18].

These properties have allowed the development of many applications of PEDOT including sensors, supercapacitors, organic solar cells, electrochromic devices and light emitting diodes [19–21]. Because of the interesting properties of PEDOT, different derivatives have been synthesized to tailor its properties [17, 18, 22–24]. The modification of the basic EDOT structure is only possible by substituents at the ethylenedioxy bridge. Thus, some authors have been recently engaged in the design and synthesis of functionalized PEDOT derivatives by using pre-polymerization [25–28] and post polymerization [29] strategies.

Luminol has been widely used in chemiluminescence detection [30], electrochemiluminescence [31, 32], as well as for immunoassays using a flow injection system and liquid chromatography [33, 34]. As the polyluminol film modified on the working electrode, it provides a reversible redox couple involving electron transfer. Such as our previous works, this modified electrode also can provide good electrocatalytic properties for biological molecules [35–37]. However, the electrocatalytic activity seems not majestic and it's necessary to be improved in neutral pH condition. It would be greatly interesting to further study the electrocatalytic enhancement using the hybrid composites of polyluminol and PEDOT.

In this work, we immobilized the bipolymers of polyluminol and PEDOT on electrode surface and studied the electrocatalytic property of this electrode for AA, DA, and UA. Different films formation related to the electropolymerization of luminol and EDOT monomers to form polyluminol, PEDOT, and polyluminol/PEDOT was discussed by the suitable potential range and procedures. The polyluminol/PEDOT modified electrode was electrochemically characterized with various scan rate and pH condition. The electrocatalytic oxidation of AA, DA, and UA was performed and compared by different electrodes including polyluminol, PEDOT, and polyluminol/PEDOT film modified electrodes.

2. MATERIALS AND METHODS

2.1. Reagents

Luminol, 3,4-ethylenedioxythiophene (EDOT), ascorbic acid (AA), dopamine (DA), uric acid (UA) were purchased from Sigma-Aldrich (USA). All other chemicals (Merck) used were of analytical grade (99%). Double distilled deionized water was used to prepare all the solutions. A phosphate buffer solution (PBS) of pH 7 was prepared using Na_2HPO_4 (0.05 M) and NaH_2PO_4 (0.05 M).

2.2. Apparatus

All electrochemical experiments were performed using CHI 1205a potentiostats (CH Instruments, USA). The working electrode was glassy carbon electrode (GCE) using BAS GCE (with diameter of 0.3 cm, geometric surface area of 0.07 cm^2, Bioanalytical Systems, Inc., USA). Electrochemical experiments carried out with a conventional three-electrode system which consisted of an Ag/AgCl (3M KCl) as a reference electrode, a GCE as a working electrode, and a platinum wire as a counter electrode.

The buffer solution was entirely altered by deaerating with nitrogen gas atmosphere. The electrochemical cells were kept properly sealed to avoid the oxygen interference from the atmosphere. Prior to modification, the GCE was mechanically polished with BAS polishing kit (Bioanalytical Systems, Inc., USA) and alumina powder (0.05 μm) to mirror finish and ultrasonicated in double distilled water for 3 min.

Prior to the electrochemical experiments, the buffer solution was deoxygenated with nitrogen for 10 min.

2.3. Electrochemical preparation of polyluminol, PEDOT, and polyluminol/PEDOT films

The polyluminol modified electrode was prepared by the electropolymerization of luminol monomers in sulfuric aqueous solution. The electro-active system was electro-generated in situ from luminol oxidation, the electrode applied in the potential range of 0.2–0.9 V (vs. Ag/AgCl.) with scan rate of 100 mV s^{-1} and 30 scan cycles in 0.1 M sulfuric aqueous solution (pH 1.5) containing 10^{-3} M luminol monomers.

The PEDOT modified electrode was prepared by the electropolymerization of EDOT monomers in sulfuric aqueous solution. The electro-active system was electro-generated in situ from luminol oxidation, the electrode applied in the potential range of 0.3–1.2 V (vs. Ag/AgCl.) with scan rate of 100 mV s^{-1} and 10 scan cycles in 0.1 M sulfuric aqueous solution (pH 1.5) containing 10^{-2} M EDOT monomers.

The polyluminol/PEDOT modified electrode was prepared by two electrochemical methods. One is the electropolymerization of luminol and EDOT monomers in sulfuric aqueous solution. The electro-active system was electro-generated in situ from oxidation of luminol and EDOT monomers, the electrode applied in the potential range of 0.2–1.2 V (vs. Ag/AgCl.) with scan rate of 100 mV s^{-1} and 20 scan cycles in 0.1 M sulfuric aqueous solution (pH 1.5) containing 10^{-3} M luminol and 10^{-2} M EDOT monomers. The second method is the electropolymerization of luminol using PEDOT modified electrode. The PEDOT/GCE was applied in the potential range of 0.2–0.9 V (vs. Ag/AgCl.) with scan rate of 100 mV s^{-1} and 10 scan cycles in 0.1 M sulfuric aqueous solution (pH 1.5) containing 10^{-3} M luminol. Then, the polyluminol/PEDOT/GCE modified electrode was performed.

3. RESULTS AND DISCUSSION

3.1. Electropolymerization of luminol and EDOT monomers

Preparation of polyluminol, PEDOT, and polyluminol/PEDOT involving the electropolymerization of luminol and EDOT monomers was individually investigated by cyclic voltammetry.

The electropolymerization of luminol and PEDOT monomers were studied. Fig. 1 displays the consecutive cyclic voltammograms of (A) polyluminol, (B) PEDOT, (C) polyluminol/PEDOT using GCE in sulfuric aqueous solution (pH 1.5) containing suitable amount of luminol and EDOT, respectively. Fig. 1A shows the voltammogram characterized by one well-defined redox couple, with the formal potential occurring at about +0.52 V (vs. Ag/AgCl). The single redox couple is attributed to the reduced and oxidized forms of polyluminol [35]. Fig. 1B shows the PEDOT electro-deposition voltammogram which is well known with an electro-oxidation potential of approximate 0.8 V (vs. Ag/AgCl.) in 0.1 M H_2SO_4 solution (pH 1.5) containing 10^{-2} M EDOT. Directly electropolymerization of luminol and EDOT monomers was carried out in Fig. 1C. However, the redox peaks seems not obviously and the peak current develops not very well as increasing the scan cycles in the potential range of 0.2–1.2 V (vs. Ag/AgCl.). This might be due to the disorder of polyluminol and PEDOT polymer chains when luminol and EDOT monomers kept in the prepared solution in the same time.

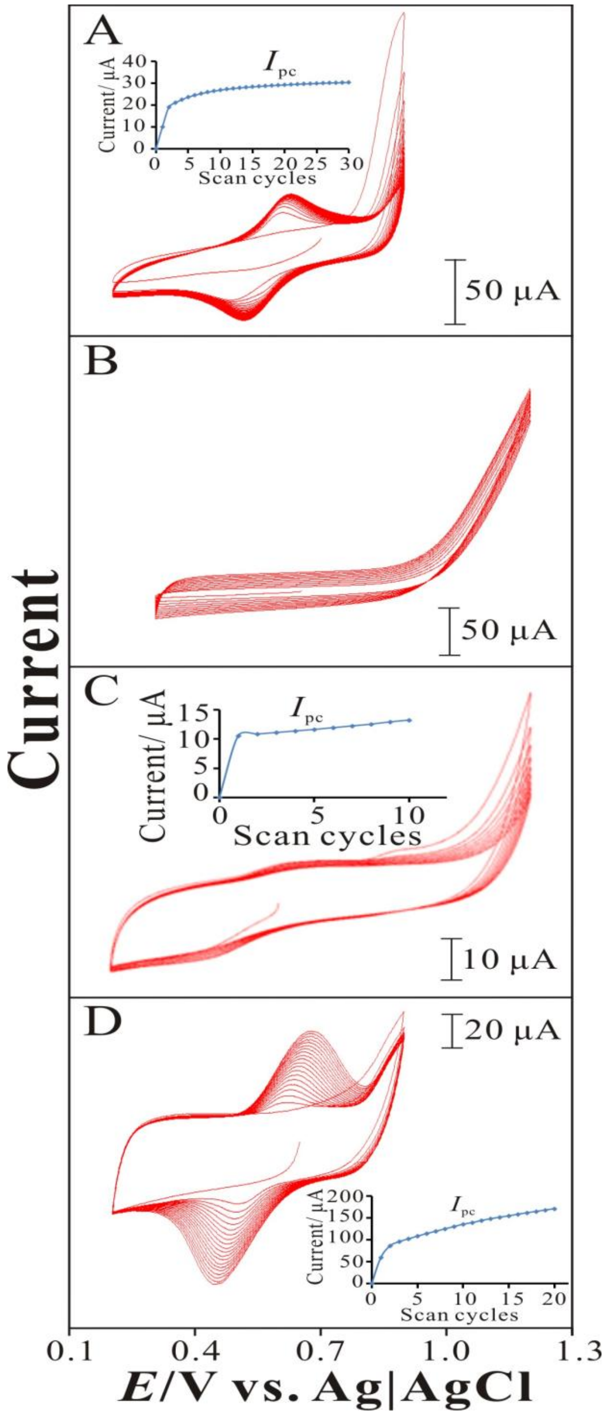

Figure 1. Cyclic voltammograms of (A) polyluminol, (B) PEDOT, and (C) polyluminol/PEDOT electro-deposition using glassy carbon electrode in sulfuric aqueous solution (pH 1.5) containing (A) 10^{-3} M luminol, (B) 10^{-2} M EDOT, and (C) 10^{-3} M luminol + 10^{-2} M EDOT, respectively. (D) Cyclic voltammogram of polyluminol/PEDOT sequential deposition using PEDOT modified glassy carbon electrode in sulfuric aqueous solution (pH 1.5) containing 10^{-3} M luminol. Scan rate = 0.1 V s^{-1}. Insets: the plots of cathodic peak current (I_{pc}) vs. scan cycles.

Considering the electropolymerization of luminol and PEDOT monomers in the same solution, the two different monomers might react and affect each other and the formed polyluminol and PEDOT might competitive to locate on electrode surface result in worse co-deposition voltammogram (Fig. 1C). Another preparation method of polyluminol/PEDOT was presented by two procedures. First, the PEDOT was deposited on electrode surface. Then, this PEDOT modified electrode was transferred in 0.1 M sulfuric aqueous solution (pH 1.5) containing 10^{-3} M luminol. Applied potential in the range of 0.2–0.9 V with 20 scan cycles, the current develops obviously as increasing scan cycles (as shown in Fig. 1D). The obvious redox peaks of polyluminol might provide the evidence of good electropolymerization result from the same kind of monomers in the solution. Compared the correlation between current response and scan cycles (insets of Fig. 1), the current develops very well when prepared the polyluminol/PEDOT by sequential electropolymerization of EDOT and luminol. One can conclude that the bipollymers of polyluminol and PEDOT can be well performed by sequential electropolymerization. Moreover, we suggest that the PEDOT must be deposited on electrode go first due to the obvious polyluminol redox peaks can be monitored for the final polyluminol/PEDOT film formation.

3.2. Electrochemical characteristics of polyluminol/PEDOT bipolymers

The polyluminol/PEDOT bipolymers formed by sequential electropolymerization was examined with various scan rate and pH condition by cyclic voltammetry.

Fig. 2 shows the cyclic voltammograms of the polyluminol/PEDOT/GCE examined with scan rate of 10–500 mV s^{-1} in 0.1 M H_2SO_4 solution (pH 1.5). The formal potential of the redox couple is found similar to the one of the polyluminol/GCE which has a formal potential at 0.52 V (Fig. 1A). Both anodic and cathodic peak currents are also directly proportional to scan rate up to 500 mV s^{-1} (insets of Fig. 2) as expected for surface confined process. This also means that this process is diffusion-less controlled and stable in the electrochemical system. The observation of well-defined and the persistent cyclic voltammetric peaks indicate that the polyluminol/PEDOT/GCE exhibits electrochemical response characteristics of redox species confined on the electrode surface.

This film modified electrode shows the linear regressing equation of peak currents (I_{pa} & I_{pc}) and scan rate (v) can be expressed as follows:

$$I_{pa}(\mu A) = 0.4462v(mV\ s^{-1}) + 4.3177\ (R^2 = 0.9963) \qquad (1)$$

$$I_{pc}(\mu A) = -0.4829v(mV\ s^{-1}) - 5.0892\ (R^2 = 0.9956) \qquad (2)$$

Moreover, the ratio of oxidation-to-reduction peak currents is nearly unity and formal potential is not change with increasing scan rate in this pH condition. This result reveals that the electron transfer kinetics is very fast on the electrode modified surface.

We have estimated, the apparent surface coverage (Γ), by using Eq. (3) [38]:

$$I_p = n^2F^2vA\Gamma/4RT \tag{3}$$

where, I_p is the peak current of the polyluminol/PEDOT composite electrode; n is the number of electron transfer; F is Faraday constant (96485 C mol^{-1}); v is the scan rate (V s^{-1}); A is the area of the electrode surface (0.07 cm^2); R is gas constant (8.314 J mol^{-1} K^{-1}); and T is the room temperature (298.15 K). In the present case, the calculated surface coverage (Γ) was 6.72×10^{-9} mol cm^{-2} for assuming a one-electron process.

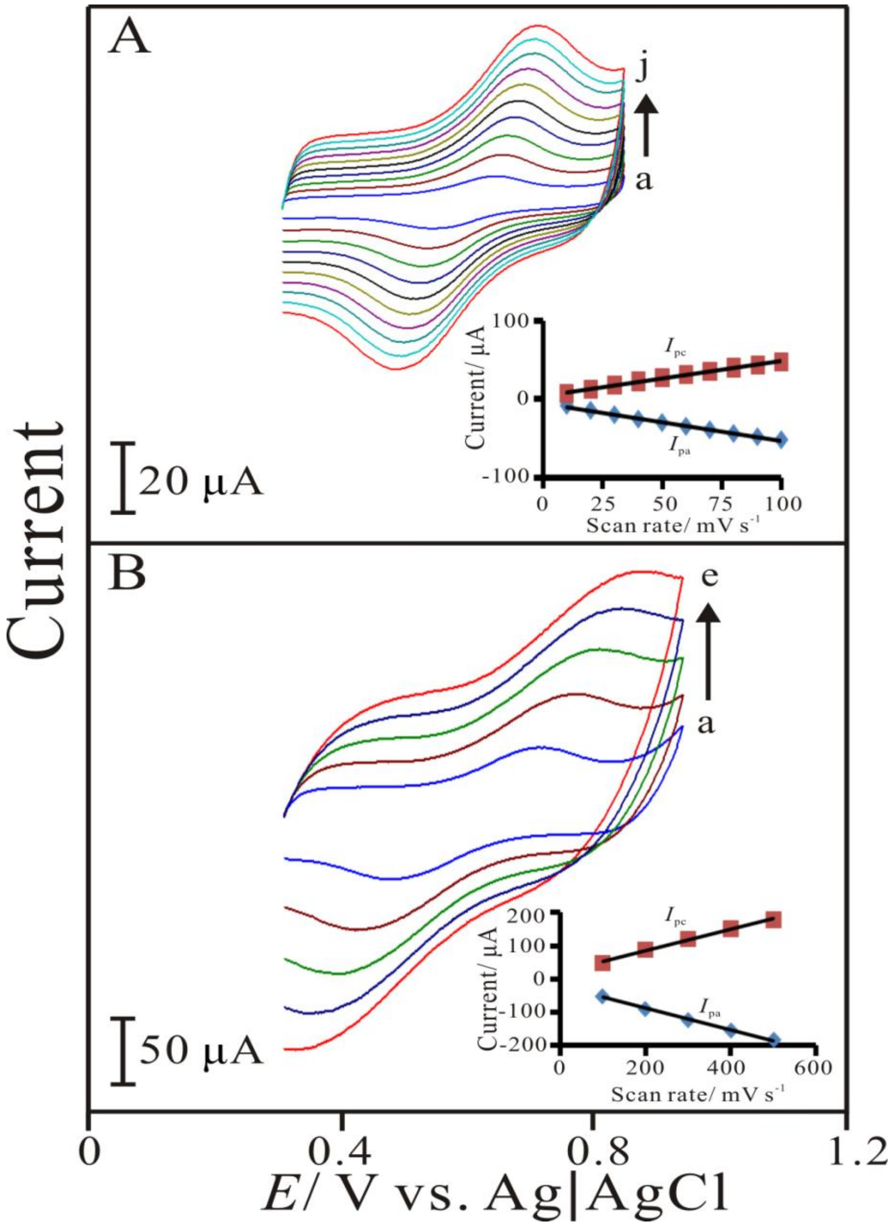

Figure2. Cyclic voltammograms of polyluminol/PEDOT/GCE examined in 0.1 M H_2SO_4 solution (pH 1.5) with different scan rate of (A) low scan rate: (a) 0.01 Vs^{-1}, (b) 0.02 Vs^{-1}, (c) 0.03 Vs^{-1}, (d) 0.04 Vs^{-1}, (e) 0.05 Vs^{-1}, (f) 0.06 Vs^{-1}, (g) 0.07 Vs^{-1}, (h) 0.08 Vs^{-1}, (i) 0.09 Vs^{-1}, and (j) 0.1 Vs^{-1}; and (B) high scan rate: (a) 0.1 Vs^{-1}, (b) 0.2 Vs^{-1}, (c) 0.3 Vs^{-1}, (d) 0.4 Vs^{-1}, and (e) 0.5 Vs^{-1}, respectively. Insets: the plots of anodic and cathodic peak current (I_{pa} & I_{pc}) vs. scan rate.

Fig. 3 displays the pH-dependent voltammetric response of polyluminol/PEDOT modified electrode. In order to ascertain this, the voltammetric responses of polyluminol/PEDOT electrode were obtained in the solutions of different pH values varying from 1 to 11. The formal potential of the redox couple is pH-dependent with negative shifting as increasing pH value of the buffer solution. The inset in Fig. 3 shows the formal potential ($E^{0'}$) of the polyluminol/PEDOT/GCE plotted over a pH range of 1－11. The response ($E^{0'}$) of polyluminol redox couple shows a slope of -53.1 mV pH^{-1}, which is close to that given by the Nernstian equation for equal number of electrons and protons transfer processes. The phenomenon indicates that the number of electrons and protons might be the same. The pH response result is due to the deprotonation of the –CO–NH–NH–CO– group of polyluminol. The possible chemical composition of the polyluminol film redox process is analogous to that of polyaniline. It exists in the form of reduced and oxidized segments, or as only oxidized dimeric segments. The above result shows that the polyluminol/PEDOT film is stable and electrochemically active in the aqueous buffer solutions.

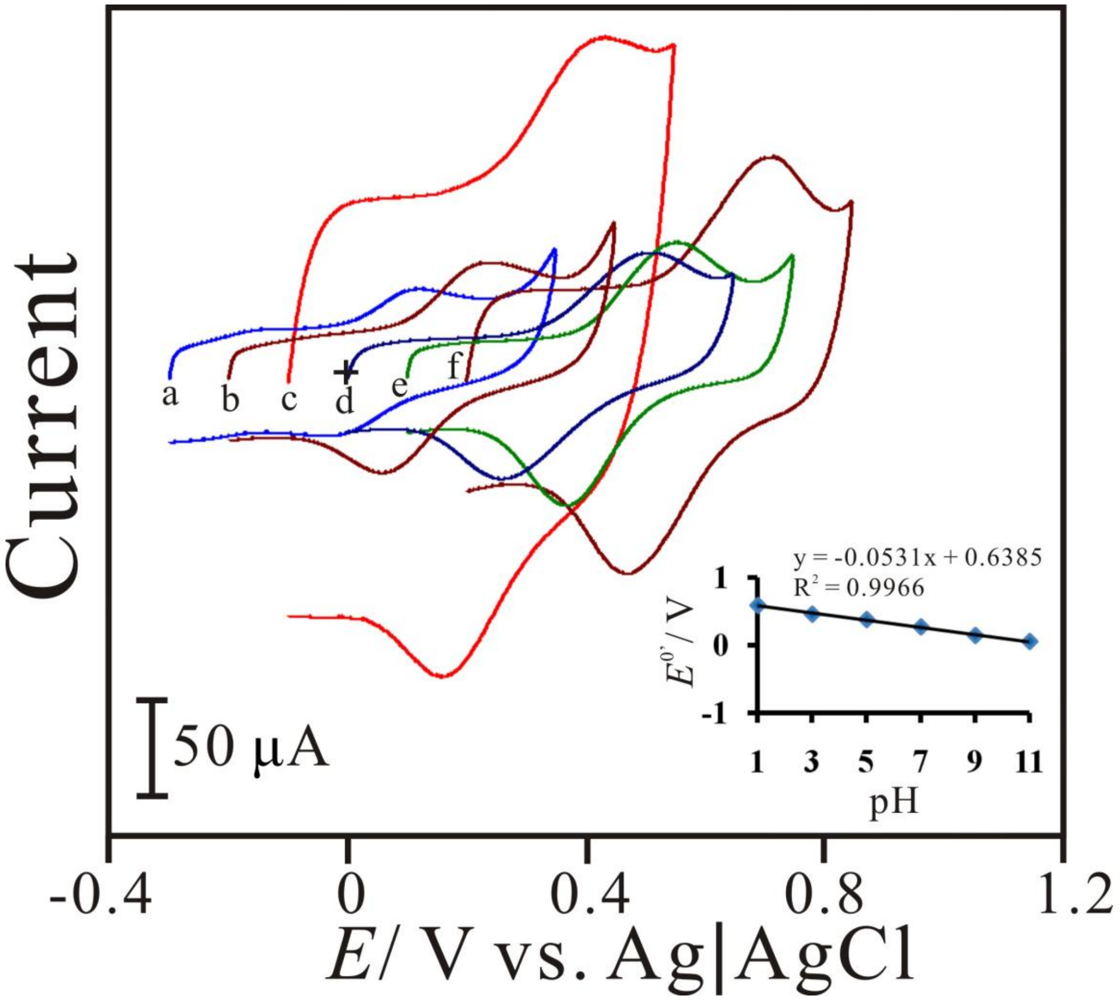

Figure 3. Cyclic voltammograms of polyluminol/PEDOT/GCE examined in various pH conditions of: (a) pH 11, (b) pH 9, (c) pH 7, (d) pH 5, (e) pH 3, and (f) pH 1, respectively, scan rate = 0.1 Vs^{-1}. Inset: the plot of formal potential ($E^{0'}$) vs. pH.

3.3. Electrocatalytic oxidation of AA, DA, UA by polyluminol, PEDOT, and polyluminol/PEDOT films

Electrocatalytic property of polyluminol/PEDOT film was studied with AA, DA, UA species and compared with the results of polyluminol and PEDOT materials in 0.1 M PBS (pH 7).

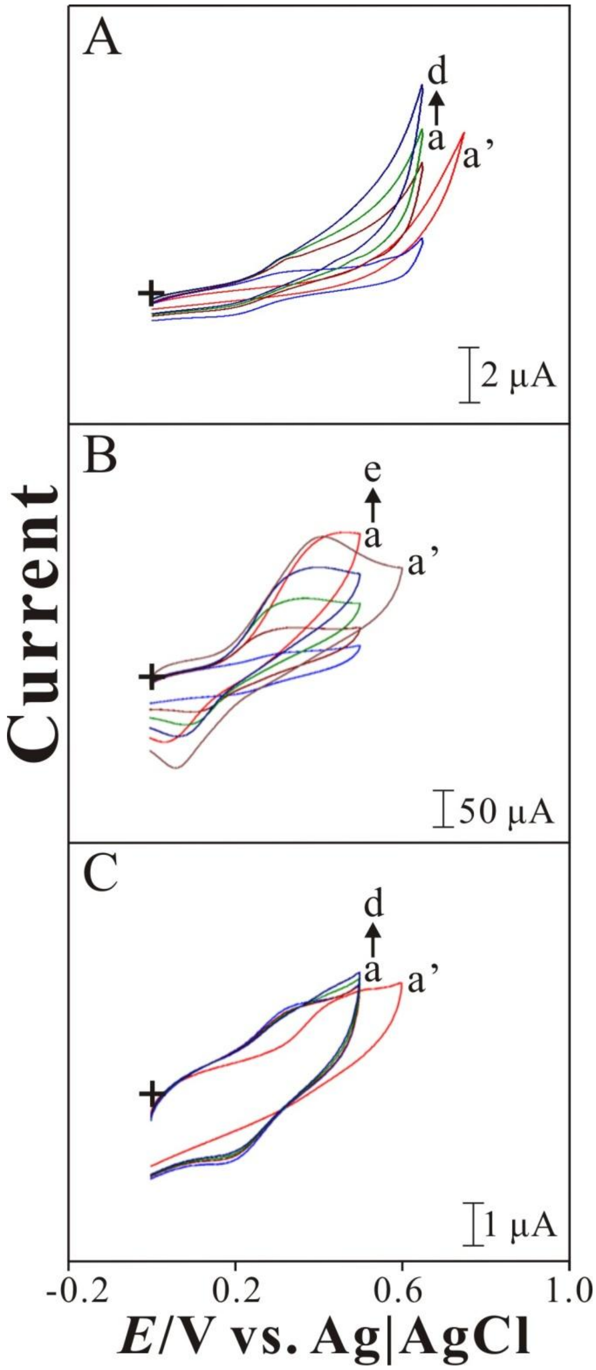

Figure 4. Cyclic voltammograms of polyluminol/GCE examined in 0.1 M PBS (pH 7) containing (A) [AA] = (a) 0 M, (b) 1×10^{-4} M, (c) 2×10^{-4} M, (d) 3×10^{-4} M; (B) [DA] = (a) 0 M, (b) 1×10^{-4} M, (c) 2×10^{-4} M, (d) 3×10^{-4} M, (e) 4×10^{-4} M; and (C) [UA] = (a) 0 M, (b) 5×10^{-4} M, (c) 1×10^{-3} M, (d) 1.5×10^{-3} M, respectively. (a') is the cyclic voltammogram of the bare GCE examined in the maximal concentration of species in each case, scan rate = 0.1 V s^{-1}.

Fig. 4 shows the cyclic voltammograms of polyluminol/GCE examined in 0.1 M PBS (pH 7) containing (A) AA, (B) DA, and (C) UA, respectively.

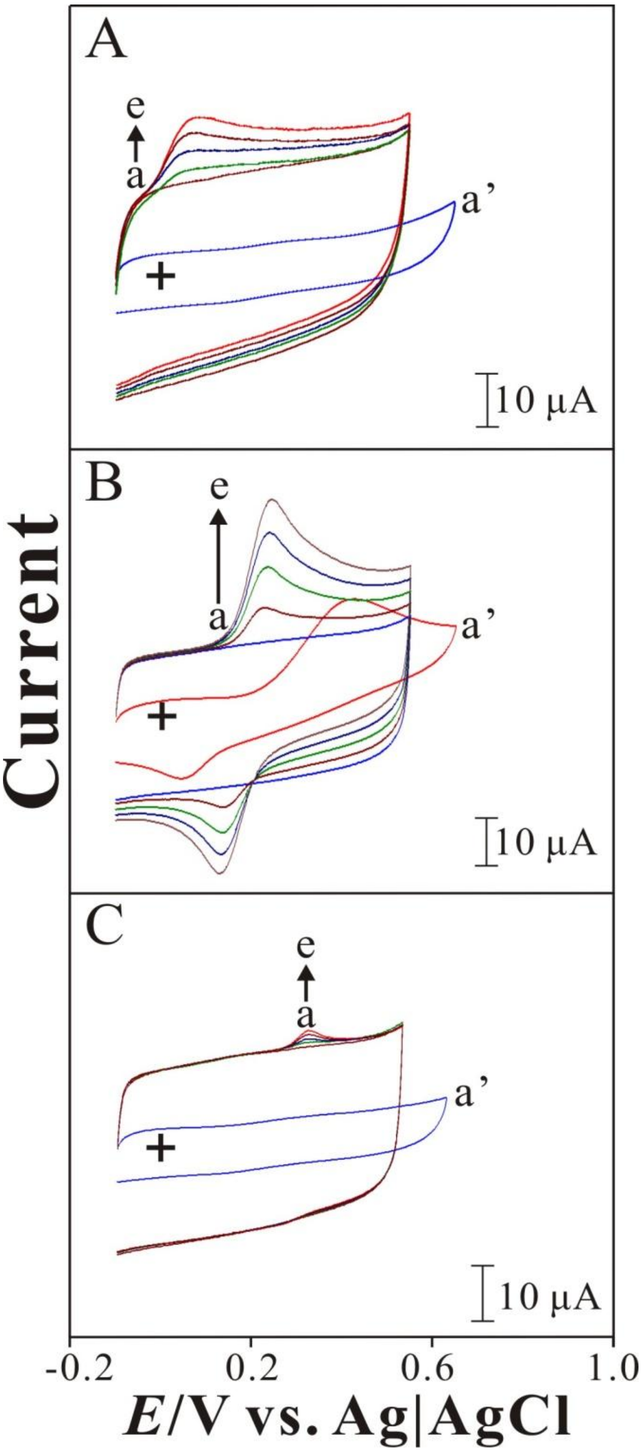

Figure 5. Cyclic voltammograms of PEDOT/GCE examined in 0.1 M PBS (pH 7) containing (A) [AA] = (a) 0 M, (b) 1×10^{-4} M, (c) 2×10^{-4} M, (d) 3×10^{-4} M, (e) 4×10^{-4} M; (B) [DA] = (a) 0 M, (b) 1×10^{-4} M, (c) 2×10^{-4} M, (d) 3×10^{-4} M, (e) 4×10^{-4} M; and (C) [UA] = (a) 0 M, (b) 5×10^{-4} M, (c) 1×10^{-3} M, (d) 1.5×10^{-3} M, (e) 2×10^{-3} M, respectively. (a') is the cyclic voltammogram of the bare GCE examined in the maximal concentration of species in each case, scan rate = 0.1 Vs^{-1}.

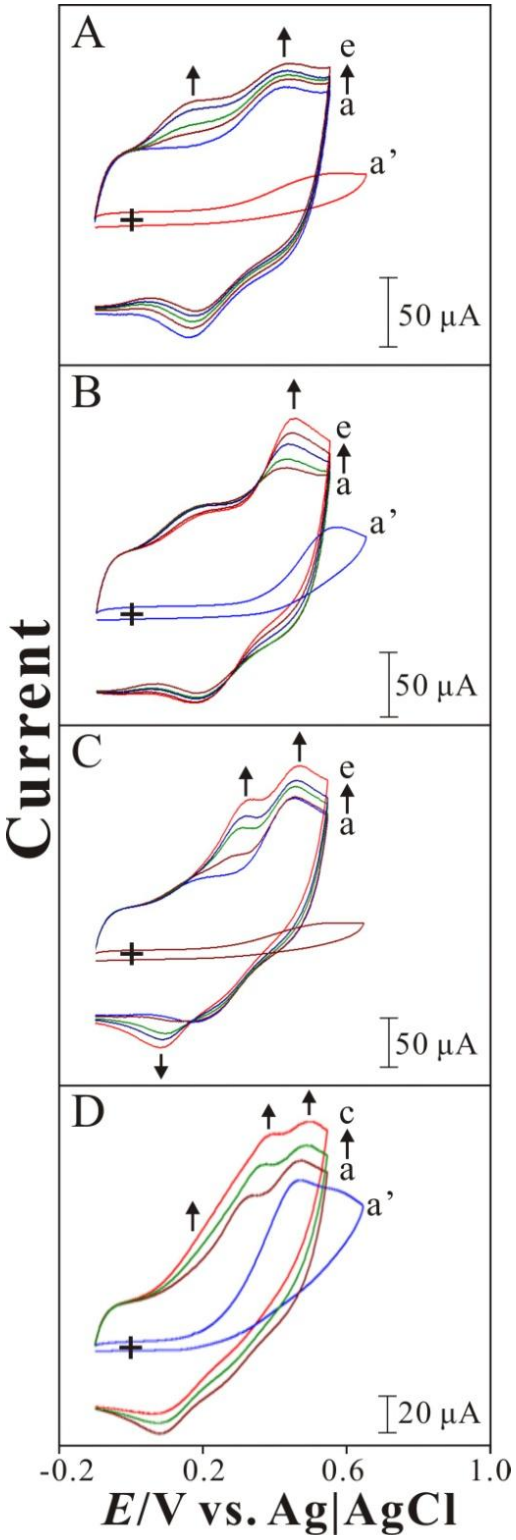

Figure 6. Cyclic voltammograms of polyluminol/PEDOT/GCE examined in 0.1 M PBS (pH 7) containing: (A) [AA] = (a) 0 M, (b) 1×10^{-4} M, (c) 2×10^{-4} M, (d) 3×10^{-4} M, (e) 4×10^{-4} M; (B) [UA] = (a) 0 M, (b) 5×10^{-4} M, (c) 1×10^{-3} M, (d) 1.5×10^{-3} M, (e) 2×10^{-3} M (in the presence of 4×10^{-4} M AA); and (C) [DA] = (a) 0 M, (b) 1×10^{-4} M, (c) 2×10^{-4} M, (d) 3×10^{-4} M, (e) 4×10^{-4} M (in the presence of 4×10^{-4} M AA and 2×10^{-3} M UA); (D) [AA] = (a) 4×10^{-4} M, (b) 5×10^{-4} M, (c) 6×10^{-4} M (in the presence of 4×10^{-4} M DA and 2×10^{-3} M UA), respectively. (a') is the cyclic voltammogram of the bare GCE examined in the maximal concentration of species in each case, scan rate = 0.1 Vs^{-1}.

The polyluminol/GCE exhibits electrocatalytic peaks at about +0.3 V, +0.35 V, and +0.45 V for AA, DA, and UA, respectively. It can be found that the anodic peak current is increasing as the increase of AA, DA, and UA concentration. However, the polyluminol/GCE does not show the excellent electrocatalytic current to AA, DA, and UA as compared with bare electrode. And the electrocatalytic peaks seems closed and need to improve.

Fig. 5 shows the cyclic voltammograms of PEDOT/GCE examined in 0.1 M PBS (pH 7) containing (A) AA, (B) DA, and (C) UA, respectively. The PEDOT/GCE exhibits electrocatalytic peaks at about +0.1 V, +0.25 V, and +0.32 V for AA, DA, and UA, respectively. It can be found that the anodic peak current is increasing as the increase of AA, DA, and UA concentration. Compared with bare electrode and polyluminol/GCE, the PEDOT/GCE shows good electrocatalytic result to AA, DA, and UA. It has lower over-potential and higher current response to these species.

In this work, we try to immobilize polyluminol and PEDOT on electrode surface and expect to have good electrocatalytic property to AA, DA, and UA. We applied the cumulative additions of AA, DA, UA in the same solution to investigate determination ability of the polyluminol/PEDOT/GCE.

Fig. 6 shows the cyclic voltammograms of polyluminol/PEDOT/GCE examined in 0.1 M PBS (pH 7) in the presence of (A) AA, (B) AA + DA, (C) AA + DA + UA, and (D) AA + DA + UA + AA, respectively. For the addition of AA species (Fig. 6A), the polyluminol/PEDOT/GCE exhibits two electrocatalytic peaks at about +0.16 V and +0.4 V. It has excellent electrocatalytic current response to AA as compared to bare electrode, polyluminol/GCE, and PEDOT/GCE. Adding UA species in the solution in the presence of 4×10^{-4} M AA (Fig. 6B), one anodic peak appears at about +0.43 V and the peak current is increasing as the increase of UA concentration. Further adding DA species in the solution in the presence of 4×10^{-4} M AA and 2×10^{-3} M UA (Fig. 6C), two anodic peaks at about +0.31 V and +0.43 V are found with current increasing as the increase of DA concentration. When adding AA in the solution in the presence of 4×10^{-4} M DA and 2×10^{-3} M UA (Fig. 6D), three anodic peaks at about +0.16 V, +0.31 V, and +0.43 V are also found with current increasing as the increase of AA concentration.

Fig. 7 shows the electrocatalytic peak current to AA, DA, and UA by polyluminol, PEDOT, and polyluminol/PEDOT modified electrodes. By comparison, the polyluminol/PEDOT modified electrode shows the largest slope of response current versus species concentration in each case. It means that the polyluminol/PEDOT has very well electrocatalytic property to AA, DA, and UA better than polyluminol and PEDOT.

This film modified electrode shows the linear regressing equation of electrocatalytic peak currents (I_p) and species concentration (C_i) can be expressed as follows:

$$I_{p,\,AA}\,(\mu A) = 7.92 C_{AA}(\mu M) + 44.09\ (R^2 = 0.9954) \qquad (4)$$

$$I_{p,\,DA}\,(\mu A) = 18.84 C_{DA}(\mu M) + 65.1\ (R^2 = 0.9789) \qquad (5)$$

$$I_{p,\,UA}\,(\mu A) = 9.76 C_{UA}(\mu M) + 100.72\ (R^2 = 0.997) \qquad (6)$$

By above results, one can conclude that the polyluminol/PEDOT can be a good material to determine AA, DA, and UA with lower over-potential, higher current response, and good selectivity.

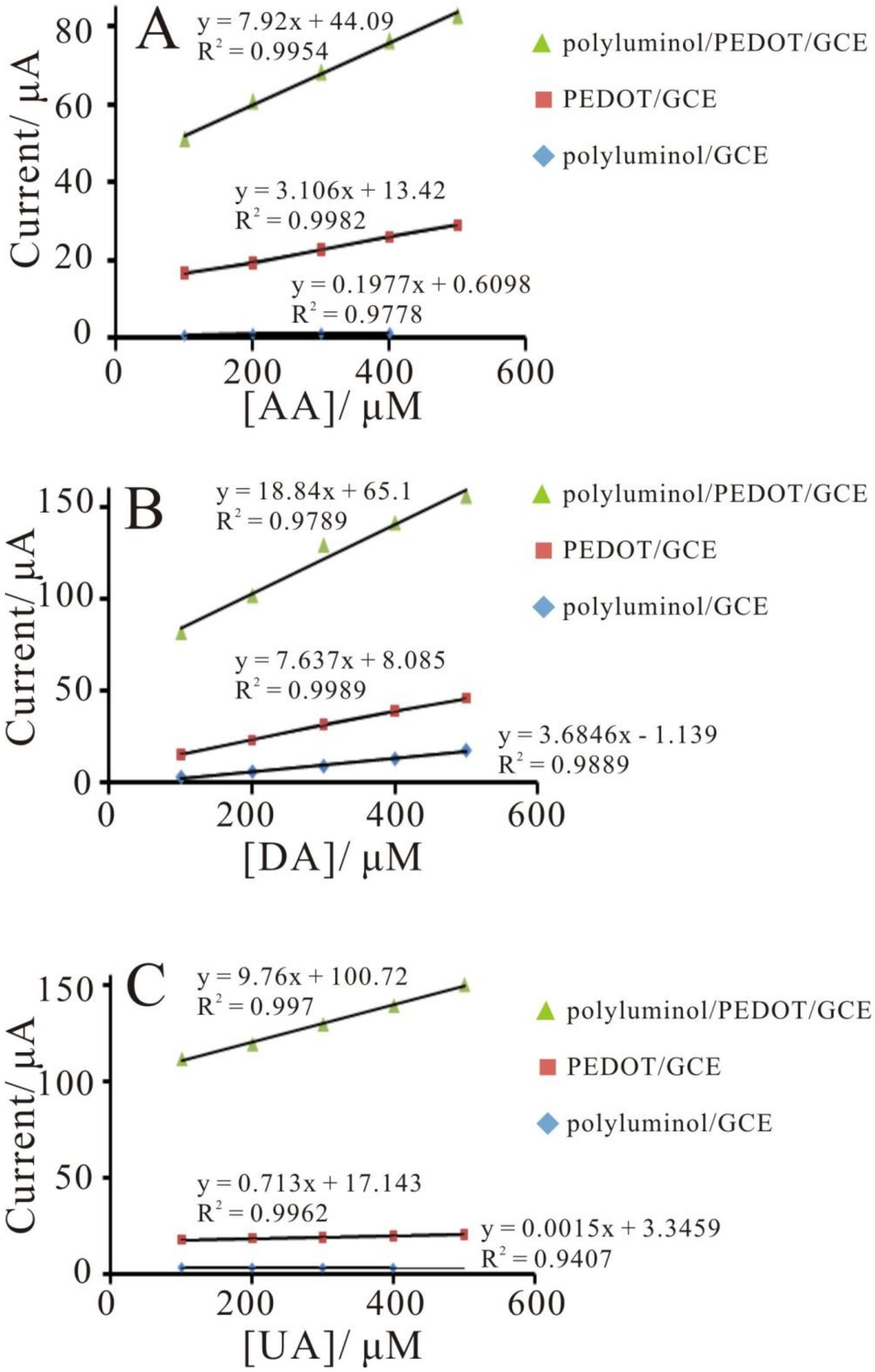

Figure 7. The plots of electrocatalytic current vs. various species concentration. (A) AA, (B) DA, and (C) UA were determined by different modified electrodes including polyluminol/PEDOT/GCE, PEDOT/GCE, and polyluminol/GCE, respectively.

4. CONCLUSIONS

The polyluminol/PEDOT bipolymers have been successfully prepared on electrode surface by the electropolymerization of luminol and EDOT monomers. The film formation is dependent on their electro-oxidation potential so that two electropolymerized procedures were applied to avoid the disorder of polymer chains. It was found surface-confined and stable in various scan rate and different pH conditions. As compared with bare electrode, polyluminol, and poly(3,4-ethylenedioxythiophene) modified electrodes, the bipolymers modified electrode shows lower over-potential and higher current response to AA, DA, and UA, respectively. AA, DA, and UA can also simultaneously determined by this electrode. This bipolymers (polyluminol/poly(3,4-ethylenedioxythiophene)) modified electrode can be used as an electrochemical biosensor to simultaneously determine AA, DA, and UA.

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