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Modification of Glassy Carbon Electrode by a Simple, Inexpensive and Fast Method Using an Ionic Liquid Based on Immidazolium as Working Electrode in Electrochemical Determination of Some Biological Compounds

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To achieve a simple, inexpensive and fast method for modification of glassy carbon electrode (GCE) by ionic liquids is the aim of this study. For this purpose, the surface of glassy carbon electrode was modified with 3-methyl-1-octylimidazolium hexaflourophosphate [omim][PF_6] and determination of dopamine, ascorbic acid and uric acid was done simultaneously. In this method, modification of surface electrode was done by pouring the droplet of dissolved ionic liquid in acetonitrile on surface of glassy carbon electrode. After this stage, the surface was stabled by taking cyclic voltammetry and electrochemical properties of dopamine, ascorbic acid and uric acid were investigated by cyclic voltammetry on glassy carbon electrode modified with [omim][PF_6] ionic liquids. Then, this species was measured by differential pulse voltammetry (DPV) in human serums. Under optimal conditions, the linear calibration curves in the ranges of 5×10^{-6} - 5×10^{-3} M, 1×10^{-5} - 5×10^{-3} M and 5×10^{-6} - 1×10^{-4} were obtained for dopamine, ascorbic acid and uric acid respectively. Detection limit of current of this technique was about 2×10^{-6}, 5×10^{-6} and 2×10^{-6} M for dopamine, ascorbic acid and uric acid respectively. The relative standard deviation for 10 measurements of dopamine (1×10^{-4}), ascorbic acid (5×10^{-4}) and uric acid (5×10^{-4}) were determined to be 3.21, 2.88 and 3.57 %, respectively. The simultaneous determination of these species in human serum was done successfully.

Key Words: Dopamine, 3-Methyl-1-octylimidazolium hexaflourophosphate, Ionic liquid, Glassy carbon electrode.

INTRODUCTION

Electrochemical study of dopamine, ascorbic acid and uric acid is a key in research topic of neurotransmitter in mammalian central nervous system. In the extra-cellular fluid of the central nervous system, the basal dopamine concentration is very low (0.01-1 M)[1]. *In vivo* detection of neurotransmitters in mammalian brain has been the subject of considerable interest by using modified electrodes and microelectrodes[2-5]. A major problem in its determination is the lack of resolution between dopamine and coexisting ascorbic acid and uric acid and concentrations of ascorbic acid and uric acid are generally much higher than dopamine. At traditional solid electrodes, ascorbic acid is oxidized at potentials close to that of dopamine, resulting in an overlapping voltammetric response[6-8]. Moreover, the solid electrodes very often suffer from the fouling effect due to the accumulation of oxidized products on the electrode surface, which results in rather poor selectivity and sensitivity. Various approaches have been made to overcome these difficulties[9-13]. For example, the voltammetric behaviour of dopamine was studied at an unmodified, exfoliated graphite electrode and also on the electrodes modified with organic polymers[12-16] and metal complexes[14-19].

Recently, room temperature ionic liquids (RTILs) have been suggested to be very interesting pasting binder in place of non conductive organic binders for the preparation of carbon ionic liquid electrodes (CILEs)[20,21]. These types of electrodes show some advantages *versus* traditional carbon paste electrodes (CPEs) such as high conductivity, provision of fast electron transfer and antifouling properties[20].

In this study, we constructed ionic liquid glassy carbon electrode (ILGCE) for the simultaneous determination of dopamine, ascorbic acid and uric acid, without any additional modification such as addition of electron transfer mediator or specific reagents. For this purpose, the surface of glassy carbon electrode was coated with only ionic liquid based on the immidazolium gel. Measurements were done and the electrochemical behaviour s of these species at the ionic liquid glassy carbon electrode were investigated. The differential pulse voltammetry (DPV) technique was used for the simultaneous

determination of ternary mixtures of dopamine, ascorbic acid and uric acid.

EXPERIMENTAL

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with an AUTOLAB PGSTAT30 electrochemical workstation (made by Echo chemie, Netherland). The working electrode was either a glassy carbon electrode (GCE) or a modified glassy carbon electrode. The auxiliary electrode was platinum wire and reference electrodes was saturated Ag/AgCl-KCl.

The ionic liquid of 3-methyl-1-octylimidazolium hexafluorophosphate [omim]PF_6 was purchased from Research Institute of Petroleum Industry (RIPI). Dopamine (3-hydroxytyramine hydrochloride) was purchased from Fluka. Ascorbic acid and uric acid were obtained from Merck. Double distilled water was used in present experiments. All other reagents used in this work were of analytical grade. The human blood serum was obtained from Milad Hospital of Iran University and was diluted 10 times with 0.1 M phosphate buffer (pH 7.08) before using. The buffer and sample solutions were purged with highly purified nitrogen for at least 5 min prior to the experiments. All experiments were carried out at room temperature (18 ± 2 ºC).

Fabrication of the modified electrode: For modification of glassy carbon electrode, a 20 % solution of [omim]PF_6 in acetonitrile was prepared. Then, several drops of this solution were poured on surface of glassy carbon electrode with 2 mm diameter and were put in front of the IR lamp for 2 min. After that, the electrode remained in laboratory atmosphere for 15 min until acetonitrile evaporated. Then, current of electrode was stabled via immersing it in phosphate buffer solution with pH = 4.5 and taking 20 in the range of 0-1 volt with the scan rate of 50 mV/s.

RESULTS AND DISCUSSION

Selection of the ionic liquid optimum value for modification: In this study, modification of glassy carbon electrode was done *via* coating the surface of electrode with an optimum layer of ionic liquid and voltammograms of dopamine, ascorbic acid and uric acid was studied. For this purpose, at first ionic liquid was dissolved in acetonitrile. Then, several w/w solutions of ionic liquid in acetonitrile were prepared. These solutions are 5, 10, 20 and 33 %. The current of cyclic voltammetry shows the optimum value of ionic liquid in acetonitrile. Fig. 1 shows the cyclic voltammogram of dopamine on glassy carbon electrode. The concentration of ionic liquid has great effect on the thickness of the modification layer. As shown in Fig. 1, the best current is appeared in 20 % w/w. In higher concentration, high density of ionic liquid is prevented the regulation of ionic liquid thickness.

Cyclic voltammogram of dopamine, ascorbic acid and uric acid on glassy carbon electrode and modified glassy carbon electrode: The obtained cyclic voltammograms of dopamine, ascorbic acid and uric acid on glassy carbon electrode and modified glassy carbon electrode with [omim]PF_6 are shown in Figs. 2 and 3. As it can be seen, the oxidation peak potential of dopamine, ascorbic acid and uric acid on glassy carbon electrode are very close to each other and electrochemical reactions of these species are quasireversible or irreversible. This probably shows that electron transfer rate in biomolecules is slow[1-3]. It is presumed that this slow rate is because of coating of electrode surface with oxidation product.

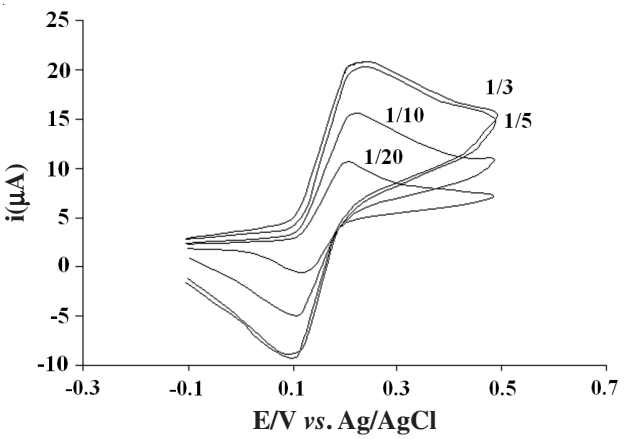


Fig. 1. Cyclic voltammogram of 2 μM solution of dopamine (DA) on modified glassy carbon electrode (GCE) in different w/w ratio of [omim]PF_6 in acetonitrile. Scan rate is 50 mV/s and glassy carbon electrode diameter is 2 mm

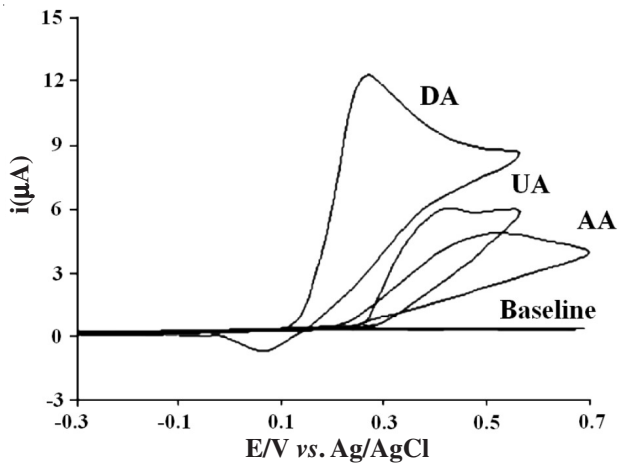


Fig. 2. Cyclic voltammograms of 4 mM of dopamine (DA), ascorbic acid (AA) and uric acid (UA) on glassy carbon electrode (GCE). All the peaks were overlaid. Scan rate is 50 mV/s and pH phosphate buffer is 7

For dopamine, anodic and catholic peaks on surface of glassy carbon electrode are appeared in 285 and 70 mV respectively. Nonetheless, anodic to catholic peak ratio is far from unit value. On glassy carbon electrode, dopamine is oxidizing before ascorbic acid and simultaneous electrocatalytic oxidation of ascorbic acid on surface of glassy carbon electrode is caused of interfering in determination of dopamine. It can be seen from Fig. 3 that increasing the current is probably due to the high rate of electron transfer on surface of ionic liquid glassy carbon electrode in comparison with glassy carbon electrode or may be this increasing can be related to preconcentration of these species to thin layer of ionic liquid on surface of glassy carbon electrode. This may be occurred due to decreasing in participant chemical reaction of dopamine which because of nonexistence of some material for reaction with dopamine products.

TABLE-1
ELECTROCHEMICAL CHARACTERIZATION OF DOPAMINE, ASCORBIC ACID AND URIC ACID ON ELECTRODES

Electrochemical methods	Parameters	Ionic liquid-Glassy carbon electrode			Glassy carbon electrode		
		Dopamine	Ascorbic acid	Uric acid	Dopamine	Ascorbic acid	Uric acid
Cyclic voltammetry	E_{pa} (mV)	310	85	410	285	500	430
	E_{pc} (mV)	95	-	280	70	-	-
	I_{pa} (μA)	13.9	11.8	13.9	12.3	5.5	5.1
	I_{pc} (μA)	-5.9	-	-2.2	-1	-	-
Differential pulse voltammetry	E_p (mV)	195	55	295	-	-	-
	I_p (μA)	23	12	30	-	-	-

In comparison with glassy carbon electrode, reversibility of dopamine in ionic liquid glassy carbon electrode is increased very much and anodic and cathodic peak potentials were separated from each other about 215 mV and $I_{p,a}/I_{p,c}$ are near to unit value. Peak potential of ascorbic acid is appeared in 85 mV, which is about 415 mV more negative than that of glassy carbon electrode. In uric acid, a sharp oxidation peak is appeared in 410 mV and a small reduction peak is appeared in 280 mV on ionic liquid glassy carbon electrode, which shows negative shift in comparison with glassy carbon electrode in anodic peak. This increase in the current peak shows that a high increment in electron transfer kinetics of ascorbic acid and uric acid is occurred. Ascorbic acid is in ascorbate form in basic and neutral solutions. So, the increase of electron transfer kinetics on surface of ionic liquid glassy carbon electrode is probably due to the electrostatic interaction between ascorbate anion and surface of ionic liquid[1,20,21]. Another probability is that increasing of current can be related to preconcentration of ascorbate anion in ionic liquid film. Also, increasing the electron transfer kinetics near the surface of ionic liquid glassy carbon electrode is another probability which can be due to a change in nature of surface electrode from glassy carbon electrode to ionic liquid glassy carbon electrode.

Increasing of current peaks for dopamine, ascorbic acid and uric acid on ionic liquid glassy carbon electrode in comparison with glassy carbon electrode is due to the high reaction rates of species on surface electrode with ionic liquid. Table-1 shows a comparison between potentials and currents of glassy carbon electrode and ionic liquid glassy carbon electrode. Fig. 3 shows cyclic voltammograms of uric acid on glassy carbon electrode and ionic liquid glassy carbon electrode. As shown, voltammetric peak on ionic liquid glassy carbon electrode is appeared more negative than glassy carbon electrode. This means that electron transfer rate is increased on ionic liquid glassy carbon electrode. In anodic oxidation, uric acid is oxidized to quinonoid and the resulting product reacts with H_2O and produces tertiary alcohol or carboxylic acid. Therefore the mechanism of electrochemical reaction of uric acid on ionic liquid glassy carbon electrode[10, 21] is EC.

Effect of scan rate: The cyclical voltammograms of dopamine, ascorbic acid and uric acid taken in different scan rates are shown in Fig. 4. The results show that there is a linear correlation between peak current (I_p) and scan rate (ν) ranging from 20 to 100 mv/s. There is also a linear correlation between current (I_p) and scan rate ($\nu^{1/2}$) for dopamine, ascorbic acid and uric acid in the range of 100-800, demonstrating diffusion control mechanism dominates the surface control one in higher scan rates. The changing from surface control to diffusion control, for reaction mechanism in higher scan rates shows

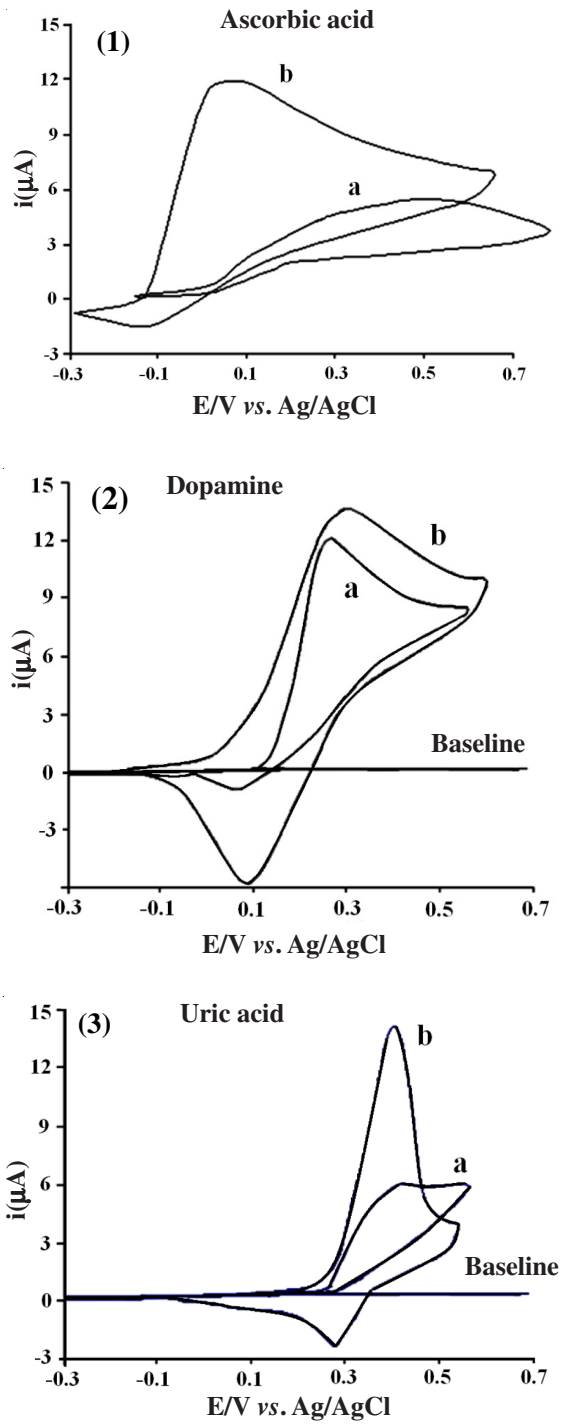


Fig. 3. Cyclic voltammograms of 4 mM of dopamine (DA), ascorbic acid (AA) and uric acid (UA) on (a) glassy carbon electrode (GCE) and (b) Ionic liquid glassy carbon electrode (ILGCE). Scan rate is 50 mV/s and pH of phosphate buffer is 7

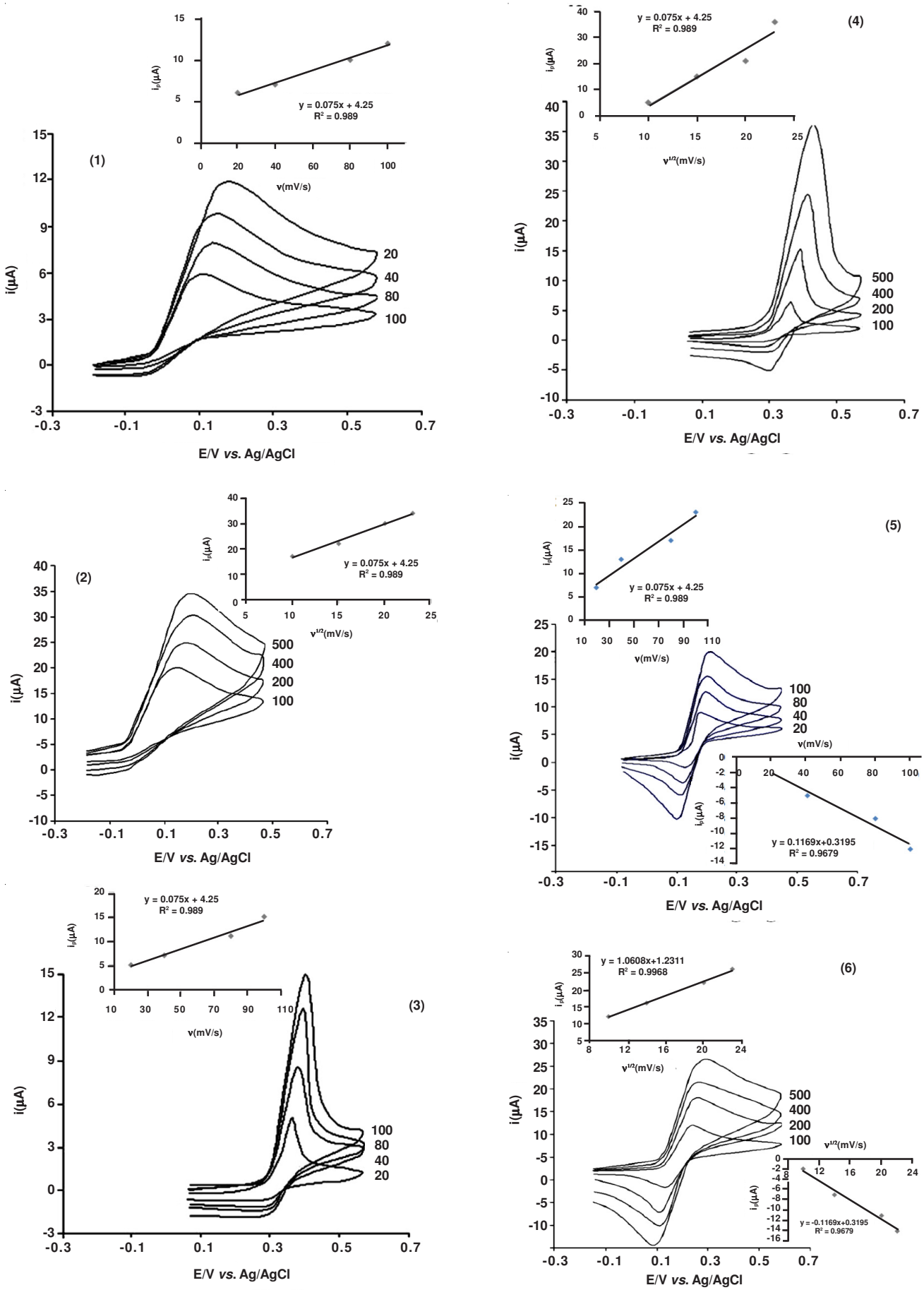
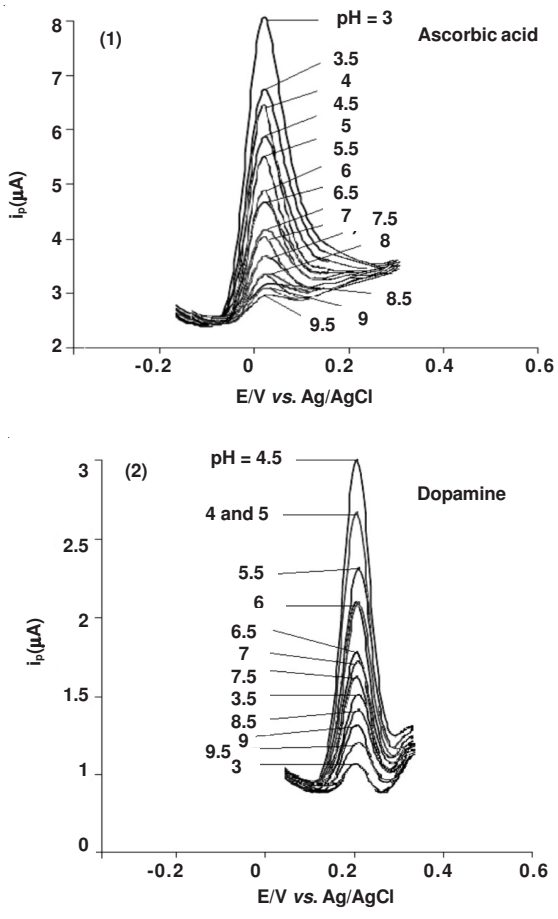


Fig. 4. Scan rate effect and it's calibration curve for ascorbic acid, uric acid and dopamine in 2 mM concentration in two ranges 20-100 and 100-500 is shown. Buffer is phosphate with pH = 4.5

that ionic liquid glassy carbon electrode has a faster electron transfer and follows a higher scan rate. As the scan rate increases, peak potential shifts to positive potentials for dopamine, ascorbic acid and uric acid [Fig. 4 (1-6)]. In order to analyze this situation, it should be stated that species react quickly in high scan rate and the surface is empties from these species. Hence they have to diffuse from solution bulk to the electrode surface and probably inside the ionic liquid film; therefore the mechanism is of diffuse control. This may be refers to the linear correlation between peak current (I_p) and scan rate (ν) in lower rates that it is due to the diffusion of species into the ionic liquid film.

Investigation of pH effect: Variation of peak current value of differential pulse voltammetry in different pHs for oxidation is shown in Fig. 5. Effect of pH on the current value of cyclic voltammogram corresponding to the mixture of these three species in pH 4.5 and 7 are observed in Figs. 6 and 7. The best pH for simultaneous determination of these species is detected in pH = 4.5. In this pH, the maximum of peak current in mixture of three species is obtained by differential pulse voltammetry. As it can be seen, with reduce of pH, peak current of ascorbic acid is appeared and is separated from dopamine peak. Also with reduce of pH, the cyclic voltammograms of three species are shifted to more positive values.

Differential pulse voltametry: Differential pulse voltametry was used for simultaneous detection of dopamine, ascorbic acid and uric acid (Fig. 8). The mixtures of dopamine, ascorbic acid and uric acid were measured on the glassy carbon electrode and ionic liquid glassy carbon electrode. A good separation was observed between the peaks on the modified electrode in



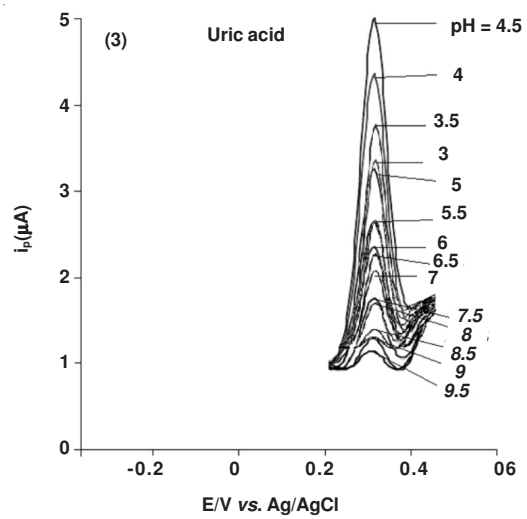


Fig. 5 Variation of peak current value of differential pulse voltammetry in different pH for oxidation of 3 mM ascorbic acid (AA), 100 μM dopamine (DA) and 200 μM uric acid (UA). Scan rate is 50 mV/s in room temperature

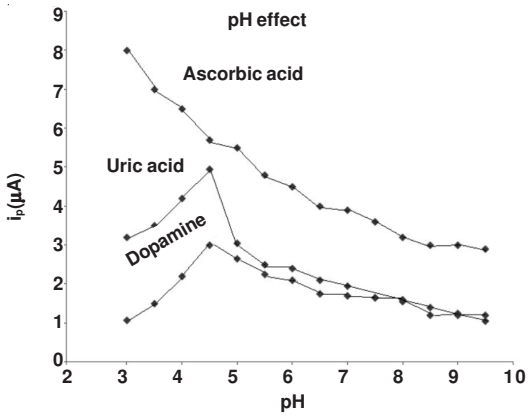


Fig. 6. Investigation of variation of peak current value with pH for oxidation of 3 mM ascorbic acid (AA), 100 μM dopamine (DA) and 200 μM uric acid (UA). Scan rate is 50 mV/s in room temperature

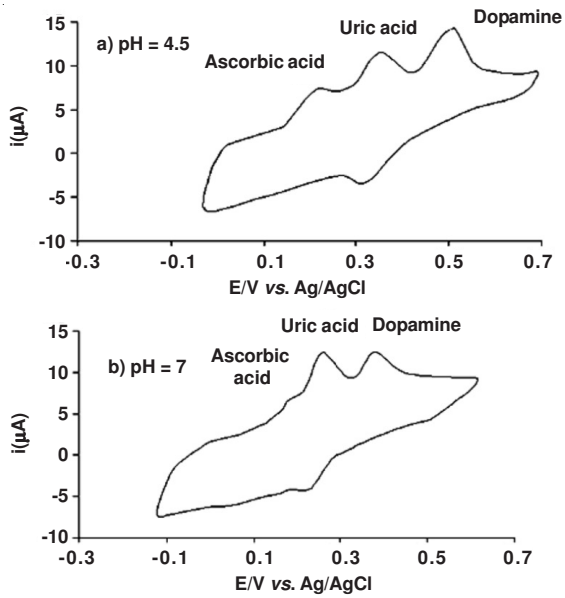


Fig. 7. Cyclic voltammogram of mixture of dopamine (DA), uric acid (UA) and ascorbic acid (AA) in 2 mM concentration and 10 mM phosphate buffer in pH 4.5 and 7. Scan rate is 50 mV/s and temperature is 18 ºC

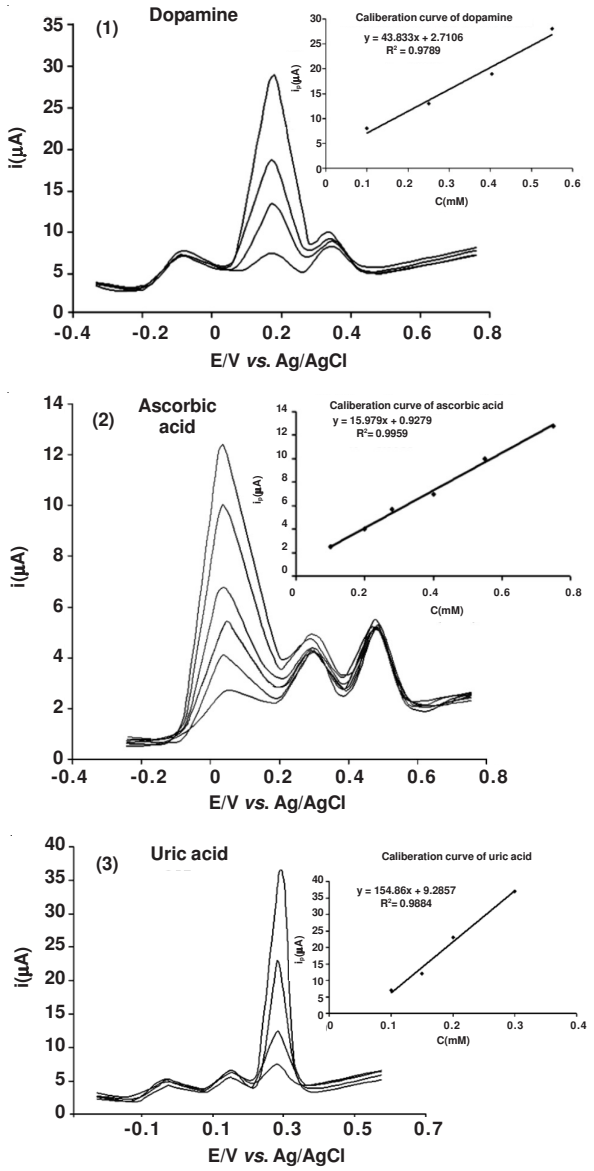


Fig. 8. Differential pulse voltammetry (DPV) of dopamine (DA), ascorbic acid (AA) and uric acid (UA) in different concentration on glassy carbon electrode (GCE) and ionic liquid glassy carbon electrode in phosphate buffer in room temperature and 2 mm electrode diameter. Other species in each measuring is 0.1 mM

comparison to bare glassy carbon electrode. As observed, the peaks of both ascorbic acid and dopamine were completely overlaid on the glassy carbon electrode. The obvious peaks were observed in 55, 195 and 295 mV in comparison with reference electrode Ag/AgCl. It is related to differential pulse voltammetry of dopamine, ascorbic acid and uric acid. There is a peak difference of 140, 100 and 240 mV between dopamine, ascorbic acid and uric acid that allows us to measure the three species simultaneously. Relative standard deviation (RSD) for 10 measurements of dopamine (1×10^{-4}), ascorbic acid (5×10^{-4}) and uric acid (5×10^{-4}) were determined to be 3.21, 2.88 and 3.57 % respectively. Using differential pulse voltametry, the detected ranges for dopamine, ascorbic acid and uric acid were 5×10^{-6} - 5×10^{-3} M, 1×10^{-5} - 5×10^{-3} M and 5×10^{-6} - 1×10^{-4} respectively. Theoretical detection limits, which are defined as σ, were 2×10^{-6}, 5×10^{-6} and 2×10^{-6} for dopamine, ascorbic acid and uric acid, respectively.

Real sample analysis: A real human serum was examined to analyse dopamine, ascorbic acid and uric acid using standard addition. Human serum was centrifuged before the examination. All the samples were diluted by using BPS (4.5) and then a suitable amount was transferred to electrochemical cell for differential pulse voltammetry measuring. The results are shown in Table-2.

TABLE-2
DETERMINATION OF DOPAMINE, ASCORBIC ACID AND URIC ACID BY DPV IN HUMAN SERUM (ALL SAMPLES WERE DETERMINED BY STANDARD ADDITION THREE TIMES)

Sample	Found (mM)	Added (mM)	Found after adding (mM)	Recovery (%)
Human serum	7.4 (± 0.3)	30.0	37.7 (± 0.5)	101
	-	100.0	98.2 (± 1.4)	98.2
	-	50.0	52.3 (± 1.7)	104

Conclusion

By this electrochemical investigation, we can determine dopamine in the presence of uric acid and large quantities of ascorbic acid, based on the application of the ionic liquid glassy carbon electrode by a simple and fast method. This technique has been used in the determination of dopamine in human blood serum with satisfactory result. The rates of the electrode reactions were considerably improved. Electrode fouling wasn't appeared upon redox reactions of dopamine, ascorbic acid and uric acid on ionic liquid glassy carbon electrode.

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