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Full Paper

Zerovalent Nano Iron Electrochemical Sensor for the Determination of Ascorbic Acid in Presence of Dopamine using Sodium Dodecyl Sulphate

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Abstract- An important electro-chemical sensor was developed for the simultaneous determination of biologically electro-active compounds like ascorbic acid (AA) and dopamine (DA) which coexist in the body as an extra-cellular fluid with high oxidation potentials. Zerovalent nano iron (ZVNI) particles were synthesized by chemical method and the same particles were incorporated into a carbon paste electrode. ZVNI/ZVNISDS modified electrode was characterized by using electro-analytical techniques like SEM, cyclic voltammetric (CV) and differential pulse voltammetric (DPV) techniques in 1 mM AA with 0.1 M PBS (pH 7.0) at a scan rate of 50 mVs^{-1}. This chemically modified electrode showed a fivefold enhancement in the oxidation peak current when compared to the bare carbon paste electrode (BCPE). Scan rate was linearly proportional to the anodic peak current with a correlation coefficient of 0.9932 indicating that it is a diffusion controlled reaction. The oxidation peak current obtained for AA and DA with a linear range of 0.01 mM to 0.06 mM and 0.01 µM to 0.08 mM with a correlation coefficient of 0.9955 and 0.99562 respectively. The interference of AA and DA was successfully separated into two well resolved peaks with a potential difference of 0.0863 by using the ZVNI modified electrode. So, the proposed electro-chemical method was used to analyze the AA in clinical and pharmaceutical samples. The developed method was successfully evaluated with analytical merits like detection level, fast response, sensitivity, selectivity, stability, reproducibility and reliability.

Keywords- Ascorbic acid (AA), Dopamine (DA), Zerovalent nano iron (ZVNI) particle, Sodium Dodecyl Sulphate (SDS), Cyclic voltammetry (CV) and Differential pulse voltammetry (DPV)

1. INTRODUCTION

Now-a-days, many efforts have been exerted for the development of electrochemical sensors with good selectivity, sensitivity, fast response, stability, low back ground current, short analysis time, renewability and low cost. The study on the interaction of biologically electro-active compounds like ascorbic acid (AA) and dopamine (DA) play a vital and challenging role in human metabolism. Zonghua Wang group was the first to use a modified graphite electrode for the simultaneous determination of ascorbic acid and dopamine [1]. L-Ascorbic acid (vitamin C) is a naturally occurring water-soluble organic compound present in animal and plant kingdoms. It is chemically ((5R)-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one) and it is a vital compound in human diet. It is rich in green leafy vegetables, fresh fruits and animal organs like liver, anterior pituitary lobe etc., AA (L-threo-hex-2-enono-1,4-lactone) is diacid (p^{K1}=4.2 and p^{K2}=11.8), that exists as a monoanion at physiological pH values [2,3]. It plays a vital role in neuronal physiology, neuro protection, free radical scavenger, collagen biosynthesis, iron absorption and immune response activation [4] and also acts as effective neuro modulator for dopamine in glutamate mediated transmissions. It acts as a protein, structure to the cartilage bones, muscles and blood vessels. It is present in mammalian brain along with several neurotransmitter amines. Normally, AA in the human body ranges from 0.2–0.4 mM. The metabolic products and excess concentration of AA leads to gastric irritation and renal problems [5]. Human milk provides enough AA to prevent scurvy disease in breast-fed infants. It is also used in prevention and treatment of common cold, mental illness, cancer and AIDS [6]. It also acts as a powerful antioxidant which fights against free-radical induced diseases [7–11]. It also plays another important role as a labile substance and is also easily degraded by the enzymes and atmospheric oxygen. Its oxidation can be accelerated by excessive heat, light and heavy metal cations [3]. Because of this, AA content in foodstuffs and beverages represents a relevant indicator of quality and it has to be carefully monitored, regarding its variation during manufacturing and storage.

Dopamine is a naturally occurring catecholamine which plays a vital role in function of the central nervous, cardiovascular, hormonal and renal systems [12,13]. In human blood plasma DA level normally ranges from 0.04–4.50 nM [14]. It is an easily oxidizable compound and it can be easily detectable by electrochemistry methods based on anodic oxidation [15]. Abnormal levels of DA in the body leads to various neurodegenerative diseases such as Huntington's, burning mouth syndrome [16], epilepsy, Senile dementia, fibromyalgia [17,18] Schizophrenia, restless leg syndrome [19], Alzheimer's and HIV [20-22].

Consequently DA and AA have overlapping oxidation potentials at bare carbon paste electrodes and often cause a surface fouling effect, reproducibility and poor selectivity. To overcome the problems, electrochemists are focusing on the "Rising-Star" of different

precious metal nano particles often using in biochemical and chemical sensing due to their physical and chemical properties as low-cost, high sensitivity, stable, reproducibility and non-cytotoxic [23]. Among them ZVNI particles play a key role in different fields due to their physical and chemical properties, it is electro active in nature with good stability and also used in magnetic resonance image [24]. Now-a-days electrochemical researchers are developing different nano modified electrodes like palladium nanoparticle-loaded carbon nanofibers modified electrode [25], zinc oxide composite film [26] silver doped poly(L-valine) modified GCE [27], carbon nano tubes [28], electrospun carbon nanofibers [29], mesoporous carbon materials [30], ferricyanide-doped Tosflex [31], other nano metal oxides [32,33], Carbon nano-onion[34] and Pd nanoparticles modified indium tin oxide electrode [35], etc., and all these electrodes have exhibited excellent results.

Hu's group [36-38] was the first group to study the surfactants use in electro analytical chemistry for the improvement of detection limit, surface modification and sensitivity. Surfactant is an organic compound with an amphilic molecular structure that contains both polar hydrophilic head at one side and a long hydrophobic tail on the other side. It is widely used in electro analytical chemistry due to its special properties like (a) aggregation into supramolecular structures and (b) adsorption at interfaces [39]. Jianbin Zheng and Xiaoli Zhau [40] explained that SDS forms a compact monolayer on the electrode surface with high density of negative charge. It is due to the critical micellar concentration of solid-liquid interface resulting in the hemi-micellar structure resulting in the adsorption on the electrode surface. Ramirez-Silva and coworkers [41] studied the simultaneous determination of AA in the presence of DA using SDS as masking agent.

The present work is aimed to develop a chemically nano-modified electro chemical sensor with an SDS surfactant, which shows high stability, good electro catalytic activity and excellent conductivity for the simultaneous determination of AA in the presence of DA. M.G. Hosseini et al were the first scientists to determine AA concentration in pharmaceutical tablets by using titanium dioxide nanotube containing gold nanoparticles [42].

2. MATERIALS AND METHODS

2.1. Chemicals

Analytical grade Ascorbic Acid (AA), Dopamine (DA) sodium hydrogen orthophosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4) and silicon oil were procured from Himedia chemicals. Fine graphite powder (particle size <20 µicrons) were supplied from Sigma-Aldrich. Phosphate buffer (pH 7.0) was prepared with 0.2M NaH_2PO_4 and Na_2HPO_4 solution in distilled water.

2.2. Apparatus

Electrochemical experiments including cyclic voltammetry (CV) and Differential Pulse Voltammetry (DPV) experiments were performed with a CH-Instrument Model no. CHI 610D attached to a personal computer. A conventional three-electrode electrochemical system was used for all the electrochemical experiments, which includes the saturated calomel electrode (SCE) as a reference electrode, platinum electrode as a counter electrode and zerovalent nano iron embedded in the carbon paste as a working electrode with a cavity of 3.0 mm diameter.

2.3. Synthesis of Green Zero Valent Nano Iron (ZVNI) particles

The zero valent nano iron particles were synthesized according to the literature. The mixtures of sodium borohydride ($NaBH_4$), ferrous nitrate (($Fe(NO_3)_3$) and ethanol were taken. ZVNI was synthesized by the reduction of 1 M ferrous nitrate with an equal volume of 1.6 M sodium borohydride (6 mL min−1) and to this ethanol, was added which provides a protection layer for nano iron particles. Here, the control of dropping rate is very important because quick addition may cause aggregation of ZVNI precipitates in contrast very slow addition may cause the oxidation of nanoparticles which are formed sequentially and the reaction was carried out at room temperature for about 30 minutes until the formation of black particles. Ferrous iron was reduced and the zerovalent iron particles were precipitated according to the equation [43],

$$Fe(H_2O)_6^{2+} + 2BH_4^- \longrightarrow Fe^0 \downarrow + 2B(OH)_3 + 7H_2 \uparrow$$

When all the nanoparticles were precipitated on the bottom of the beaker, the supernatant was removed and then the solid iron nano particles were washed with ethanol. After thorough drying the nano iron, air was allowed to slowly bleed over the iron for a period of approximately 8 h to passivate the iron. The resulting black clusters of iron were grinded and fine ZVNI powder was stored under nitrogen environment for later use [44].

2.4. Procedures

2.4.1. Preparation of bare CPE

A mixture of optimized ratio of 70:30 (w/w) graphite powder and silicone oil taken in an agate mortar was grinded using a pestle until a homogeneous carbon paste was obtained. A portion of the resulted homogeneous paste was packed into 3.0 mm diameter of a Teflon cavity current collector and it was polished on a soft paper before the measurement. The electrical contact was provided by copper wire connected to the paste at the end of the tube [45].

2.4.2. Fabrication of ZVNI CPE

The optimized ratio of 70:30 (w/w) graphite powder and silicone oil, in an agate mortor was grinded, by taking different weights of ZVNI particles (10,20,30,40 and 50mg). This mixture was thoroughly hand mixed in an agate mortar for about 45 min to get a homogenous paste and it was incorporated into 3.0 mm diameter of Teflon cavity current collector and polished on a piece of soft paper before application.

2.4.3. Commercial Ascorbic Acid tablet test sample

For formulation analysis of AA (labelled 500 mg AA, prasanna pharmaceutical, Tirupathi, India), one tablet was completely ground and homogenized. Then 0.1100 gm of it was accurately weighed and dissolved in 25 ml of distilled water in a standard flask. Finally, a suitable volume of the resultant solution plus 24 ml of 0.1 M phosphate buffer solution (pH 7.0) was placed in an electro chemical cell for the analysis of AA.

3. RESULTS AND DISCUSSION

3.1. SEM Analysis

Figure 1 shows the scanning electron microscope image (SEM) of the synthesized zero valent nano iron particles. These nano particles were homogeneously distributed with a size of 2 nm with an interlaced network, which consists of irregular pores.

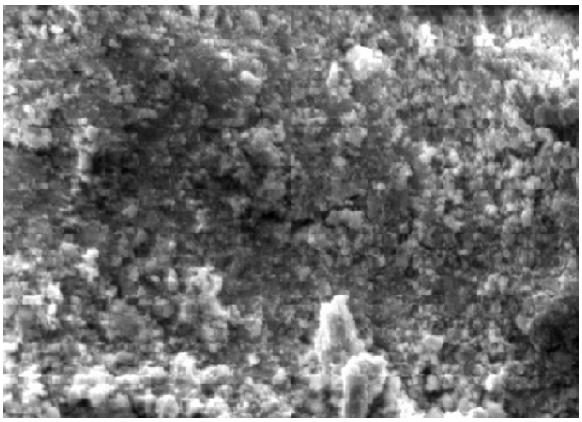

Fig. 1. SEM image of synthesized ZVNI particles

3.2. Electrochemical response of AA at the ZVNI particles at modified CPE (ZVNI MCPE)

Figure 2 depicts the study on the concentration effect of ZVNI particles in the presence of 1 mM AA with a supporting electrolyte of 0.1 M PBS at (pH 7.0) at a scan rate of 50 mVs^{-1}. This nano modified carbon paste electrode showed a good enhancement in the anodic peak current at 30 mg when compared with bare carbon paste electrode. It is due to substantial

increase in the peak current and decrement in the peak potential with an irreversible electron transfer process, indicating that AA is efficiently oxidized at ZVNI MCPE.

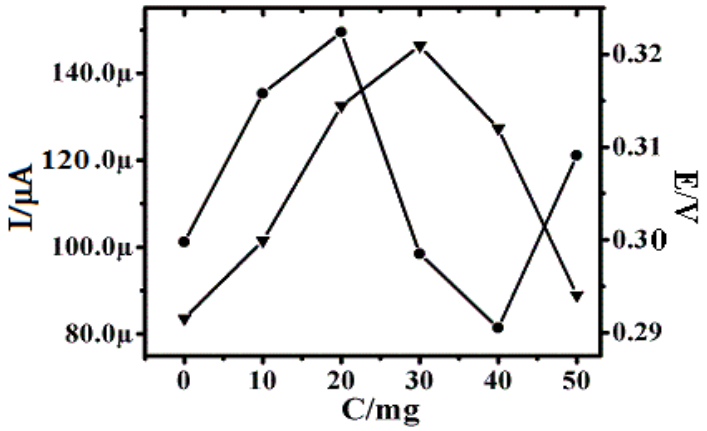

Fig. 2. Relationship between anodic peak current (I_{pa}) *vs.* Concentration of ZVNI and anodic peak potential (E_{pa}) in 1 mM AA with carbon paste electrode

3.3. Electrocatalytic oxidation of AA at the ZVNI particles

The electrocatalytic response of bare carbon paste electrode (dotted line) and ZVNI (solid line) modified electrode in 1 mM AA with 0.1 M phosphate buffer solution (pH 7.0) is shown in Figure 3. The bare CPE showed a poor oxidation peak potential (0.3105 V) with a low current response, irreversible reaction and requires high over potential due to the fouling of the electrode by the adsorption of oxidized product of AA [40]. Under the same conditions, the ZVNI MCPE exhibited low anodic peak potential (0.2998 V) with high current response. This ZVNI MCPE prevents the electrode fouling on the surface and accelerates faster electron transfer kinetics which enhances the irreversible oxidation of AA.

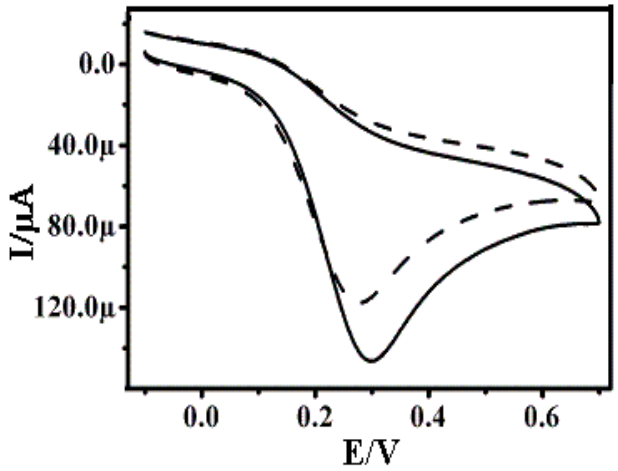

Fig. 3. Cyclic voltammogramms of AA at the bare carbon paste electrode and ZVNI in presence of 0.1 M PBS

3.4. Effect of scan rate on the peak current of AA

The variations in the scan rate i.e., 50-450 mVs^{-1} was carried out in 1 mM AA using ZVNI MCPE in a physiological 0.1 M phosphate buffer solution (pH 7.0) is shown in Figure 4. As the square root of scan rate increases linearly, the oxidation peak currents were gradually shifted towards the positive values with a correlation co-efficient of 0.9932. The above results indicate that it is an electron transfer reaction with a diffusion controlled process.

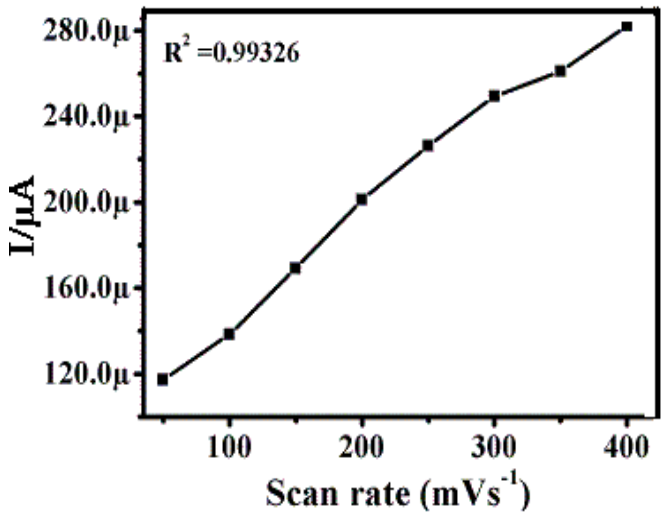

Fig. 4. Anodic peak current *vs.* square root of scan rate for 1 mM AA at ZVNI in presence of 0.1 M PBS at pH-7.0

3.5. Effect of pH on the oxidation of AA

In most of the cases, pH is considered as an important factor for the electrochemical reactions. The voltammetric response for the ZVNI modified electrode was studied in physiological 0.1 M phosphate buffer solution in 1 mM AA over a pH range of 5.5 to 8.0 with a scan rate of 50 mVs^{-1}. The maximum anodic peak current was obtained at pH 7.0, by increasing the pH greater than 7, the stability and sensitivity of the voltammetric response was decreased due to the deprotination as shown in Figure 5. Owing to this pH variations AA exerts a repulsive electro-static interactions at the surface of the ZVNI modified electrode. Hence 0.1 M phosphate buffer solution of pH 7.0 was selected as a supporting electrolyte and it was sparingly close to the physiological conditions.

3.6. Electrochemical response of AA at ZVNI MCPE with SDS surfactant

The main task of our work was to determine the ability of the ZVNI modified electrode without the interference of their oxidation peak potentials of AA and DA. To determine the ability of these selective species, cyclic voltammograms were recorded with a scan rate of 50

mVs^{-1} in a physiological 0.1 M phosphate buffer solution at pH 7.0. In Figure 6 the electrochemical response of AA and DA were carefully studied by using bare CPE, ZVNI MCPE without SDS surfactant and ZVNI MCPE in the presence of immobilized SDS surfactant (solid lines a,b). In the absence of anionic surfactant the ZVNI MCPE could not resolve the separate peaks of AA and DA because of its complex properties, fouling of electrode by the adsorption of the oxidized product and high potentials.

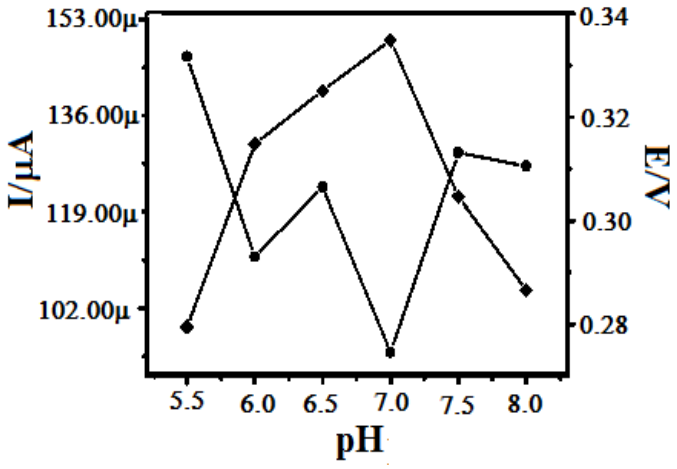

Fig. 5. Effect of pH on the peak current (A) and peak potentials for the oxidation of 0.1 mM AA with a scan rate of 50 mVs^{-1} in presence of 0.1 M PBS

However, ZVNI MCPE with anionic surfactant SDS exhibited very good enhancement and successfully resolved the two peaks of AA and DA in presence of 50 µL SDS with a potential difference of 0.0411 V and it reflected largely with anodic peak current (ip_a) due to the diffusion of SDS molecule into the carbon paste electrode. Therefore, ZVNI SDSMCPE successfully prevented the fouling of the electrode and faster electron transfer reaction of AA. Hence, the oxidation peak of AA has shifted towards less positive potential, without interfering in the measurement of DA.

3.7. Analytical Application to Resolve AA with DA

Differential pulse voltammetry is, an important electrochemical technique with high current sensitivity and low charging contribution to the back ground current was used to estimate the lower limit of detection and limit of quantification. Figure 7 shows the differential pulse voltammogramms recorded with different concentrations of AA and DA in the presence of ZVNI SDS MCPE. The voltammetric currents corresponding to the oxidation of AA was increased linearly with the increase of DA concentration. The results showed that the current was proportional to the concentration of AA in the range of 0.01 mM to 0.05 mM and DA 0.01 µM to 0.07 µM in PBS at pH 7.0 The linear regression equations for AA and

DA were expressed as Ipa(μA)=2.283Cm ML^{-1}+0.9 and Ipa(μA)=1.055 Cm ML^{-1}+0.7 respectively. The co-relation co-efficient of these two linear graphs was 0.9955 and 0.99562 and is shown in Figure 8. The graphs showed that the interference of AA and DA peaks were resolved with a potential difference of 0.0863 V individually. The voltammetric response of the ZVNISDSMCPE is an immobilized electrode, which act as a good electrochemical sensor for the simultaneous determination of Vitamin-C (or) AA, an important neurotransmitter i.e DA.

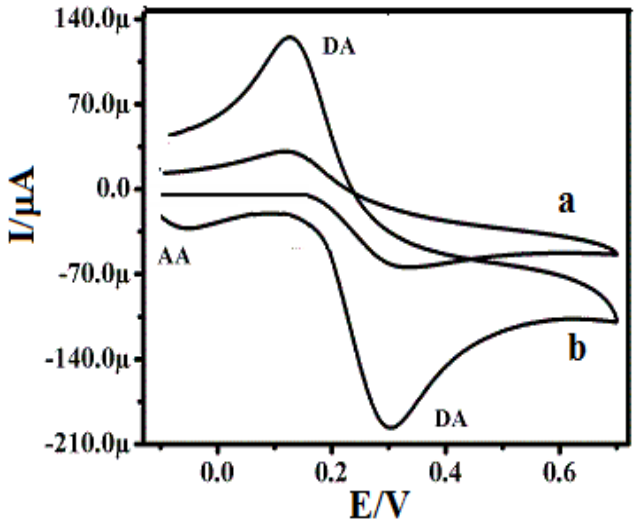

Fig. 6. Cyclic voltammograms for simultaneous determination of AA in presence of DA with 0.1 M phosphate buffer solution of pH 7.0 at (a) ZVNI MCPE and (b) ZVNISDSMCPE with the scan rate of 50 mVs^{-1}

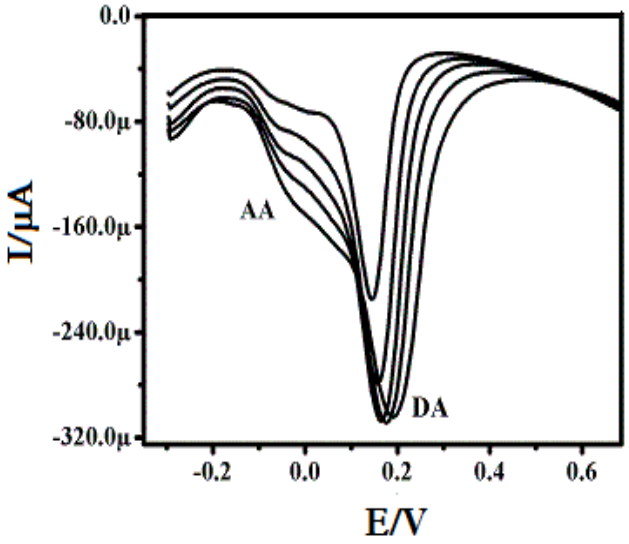

Fig. 7. Differential pulse voltammogram for simultaneous detection of AA and DA in 0.1 M phosphate buffer solution of pH 7.0 at ZVNISDSMCPE with the scan rate of 50 mV s^{-1}

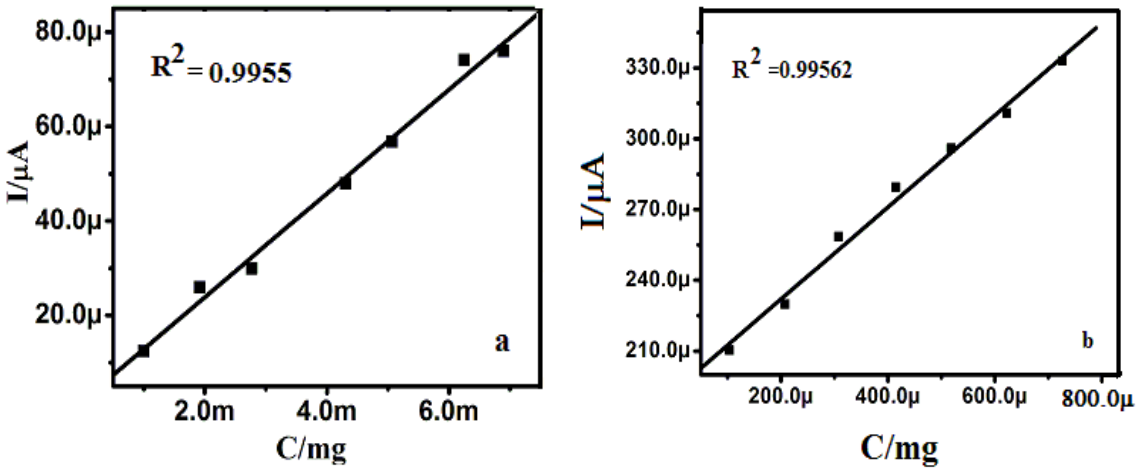

Fig. 8. (a) Differential pulse voltammogram for simultaneous detection of DA and AA with different concentrations in presence of 0.1 M PBS at pH7.0 with a scan rate of 50 mVs^{-1} of DA concentration from 1.04 mM to 7.27 mM; **(b)** graph of peak current Vs AA concentration from 1 mM to 6.85 mM in pH7.0 with the scan rate of 50 mVs^{-1}

3.8. Validity of the method developed

In order to evaluate the applicability and feasibility of the modified electrochemical sensor for the analysis of AA in human serum and pharmaceutical products (obtained from the Health Centre, Sri Venkateswara University, Tirupati, Andhra Pradesh, India). ZVNIMCPE was applied for the determination of AA in tablet sample which was procured from the local medical shop; Tirupati with a specified content of 500 mg. Procedure is as follows: 2 mL of human serum sample without any pretreatment was diluted to 100 mL with pH 7.0 in 0.1 M phosphate buffer solution. Different volumes of this solution were mixed with a known concentration of AA solution and also for known concentration, different concentrations of AA were spiked. In the same way 500 mg of the tablet sample was ground to a fine powder in an agate mortar and this powder was weighed from that weighed quantity of 0.1100 gm of ground tablet powder was taken and diluted in a 25 ml of standard flask with a concentration of 25 mM. Different volumes of this solution were mixed with a known concentration of AA obtained by spiking different concentrations of AA. 5 mL of the stock solution was diluted by the sterilized water to provide different known concentrations of AA, and then analyzed by DPV using the ZVNI MCPE as shown in Table 1. The Vitamin-C tablet was analyzed by using a calibration plot. The recovery and relative standard deviation (R.S.D) were acceptable (n=5), in the range of 89-98% showing that the developed method is very efficient, reliable and sensitive for the determination of AA in pharmaceutical samples.

Table 1. Determination of ascorbic acid in human serum and vitamin-C tablet (n=5)

Samples	Spiked (mM)	Found (mM)	Recovery (%)	RSD (%)
Human Serum	0.1	0.092	91%	5.7
	0.2	0.18	93%	3.3
	0.3	0.30	100%	1.4
Vitamin-C tablet	0.1	0.089	90%	5.3
	0.2	0.19	98%	2.5
	0.3	0.029	100%	1.5

4. CONCLUSIONS

A novel electrochemical method is developed and successfully applied for the selective and simultaneous determination of AA in the presence of DA by using immobilized SDS surfactant for the surface modification of ZVNI. The modified electrode was greatly catalyzed the electrode reactions of AA and DA in the optimized conditions with well-separated voltammetric peaks in the presence of 0.1 M PBS at pH 7.0. The proposed method could be successfully applied for the clinical and pharmaceutical samples with the satisfactory results.

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