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Enhanced Electrochemical Determination of Riboflavin in Biological and Pharmaceutical samples at Poly (Arginine) Modified Carbon Paste Electrode

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An electrogenerated Polyarginine modified carbon paste electrode (PAMCPE) was fabricated through a simple electropolymerization procedure. The devised electrode was characterized by cyclic voltammetry (CV) and Field Emission Scanning Electron Microscopy (FESEM). This electrode was utilized for electrocatalytic estimation of Riboflavin (RF) and its instantaneous resolution with ascorbic acid (AA) and folic acid (FA) in phosphate buffer solution (PBS) of pH 6.0 by differential pulse voltammetry (DPV). It was observed to be a very responsive electrode for the electrochemical detection and quantification of RF. It was revealed that PAMCPE generates higher current response towards RF contrast to the bare carbon paste electrode (BCPE). Under optimized condition, the RF oxidation current values were linearly reliant on the RF concentration increment with a limit of detection (LOD) of $9.3 \cdot 10^{-8}$ M using DPV. The stable PAMCPE was effectively applied for estimation of RF in B-complex pill and complex human blood serum samples.

Keywords: electrochemical sensor, poly(arginine), carbon paste electrode, vitamin B_2, human blood serum, B-complex capsule

Vitamins are bioactive species which play a substantial role in human metabolic pathways. RF (vitamin B_2) is water-soluble and is the major constituent of flavoenzymes which play a crucial role in the biochemistry of the human body. It cannot be formed in the human body; thus, it has to be obtained through diet from food sources such as milk, egg, vegetables, mushrooms, almonds and meat. Its inadequacy causes eye and skin problems [1-4]. AA is a water-soluble vitamin (Vitamin C), and it is found in foodstuffs, fruits, and is used as a dietary supplement. Its deficiency is associated with a disease called scurvy. AA is frequently used as an antioxidant in food, pharmaceutical formulations, and cosmetic applications and it is also useful in cancer prevention and immunity enrichment [5-9]. FA also known as folate (vitamin B_9) is found in leafy vegetables, pasta, bread and liver. It's deficiency causes anemia. It is utilized in the treatment of stroke, ischemic heart attack, cancer and liver diseases [10-13].

Vitamins were determined concurrently and independently by dissimilar methods such as Fluorescence [14], spectrophotometry [15], spectro-flurometry [16], high performance - diode array detection [17], reverse phase HPLC [18]. These are expensive and time-consuming approaches. A few other electrochemical methods were reported for simultaneous and individual determination of vitamins such as conducting polymer modified glassy carbon electrode [19], Metallic Bulk Annular Band Electrodes [20], silver doped poly (arginine) modified glassy carbon electrode [21], hydroquinone derivative multiwall carbon nanotube paste electrode [22], screen printed carbon sensor [23] etc.

Electroanalytical practices are flexible and superior tools for biomolecular and clinical diagnosis. Electrochemical Procedures are popular due to their effortless preparation, sensitivity, selectivity, and low cost, swift response time [24-30]. Surface-modification of electrodes is a strategy for enhancing the sensing efficiency of electrodes. The variety of functional molecules are employed as a modifier for the production of biosensors. Carbon materials have exceptional electrical and mechanical features, hence, they are used repeatedly in the construction of biosensors [31-33]. Modifiers such as TX-100, SDS, CTAB, CPC etc, (surfactants), conducting polymeric films, nanomaterials, ionic liquids etc, were used to regulate over potential and to accelerate the sensing property of electrode [34-35]. The goal of present work is the fabrication of a responsive and selective voltammetric sensor based on poly (arginine) modified carbon paste for voltammetric determination of RF in the presence of AA and FA.

Experimental part

Apparatus. The Electrochemical experimentations were performed by employing CHI-6038E (electrochemical work station - USA) in connection with a tri-electrode system and a private computer for information storage and control. PAMCPE is used as the working electrode, saturated calomel electrode (SCE) and a platinum wire were adopted as a reference electrode and auxiliary electrode correspondingly. All the tests were carried out at room temperature.

Chemicals and preparations. RF, AA, FA, and arginine were procured from Molychem, India. Graphite powder and silicone oil were brought from Nice chemicals, India. Other chemicals were A.R grade utilized as received. RF stock solution of 2.5 mM was prepared before to testing with distilled water. AA, FA, and arginine solution of 25 mM were prepared by dissolving in distilled water. 0.1 M Phosphate buffer solution (PBS) with different pH was prepared by mixing suitable quantity of 0.1 M monosodium dihydrogen phosphate and 0.1 M disodium hydrogen phosphate and was employed as supporting electrolyte.

Preparation of BCPE. BCPE was developed by hand mixing of 70% graphite and 30% silicone oil thoroughly in a mortar and grinded well for 20 min to get uniform paste; the obtained paste was filled into the hollow of Teflon tube, and it was polished by a smooth tissue paper. The electrical link was given through a copper wire coupled to the terminal of the tube.

Results and Interpretations

Electrochemical Polymerization of Arginine on BCPE. Cyclic voltammetry is an effortless and convenient method to formulate a thin amino acid polymer film on the surface of BCPE. The PAMCPE was made by placing a 1 mM arginine solution in 0.1 M PBS (pH 5.5) in the voltammetric cell. The potential domain was from -0.2 V to 1.5 V with a potential sweep rate of 0.1 V/s for 10 multiple cycles, the voltammograms have progressively inclined with the rise of cyclic time as shown Fig. 1.

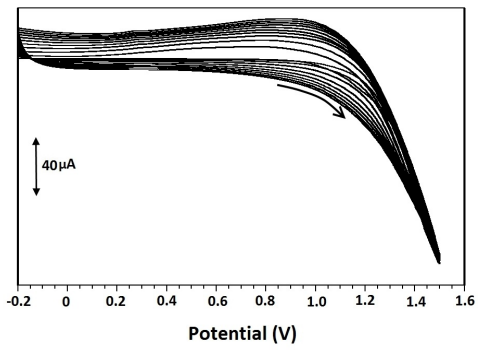


Fig. 1 Electropolymerization of L - arginine (1 mM) on the surface of the carbon paste electrode at 0.1 M PBS, pH 5.5 at sweep rate 0.1 V/s.

This indicates that the poly (arginine) film was formed on the surface of BCPE. The Poly (arginine) modified electrode was rinsed with small amount of distilled water to discard the unreacted arginine and it is applied for concurrent electrochemical determination of RF, AA, and FA. Electro-polymerization parameters were optimized to carry out the entire experiment.

Electrochemical characterization of PAMCPE and BCPE. The electrochemical presentation of BCPE and PAMCPE were analyzed by CV in 0.1 M KCl solution comprising 1 mM $K_4[Fe(CN)_6]$ as shown in Fig. 2 At BCPE, oxidation and reduction peaks of $K_4[Fe(CN)_6]$ were detected at 0.324 V, and 0.071 V with current responses 11.2 µA and 7.68 µA. At PAMCPE sharp oxidation and reduction peaks of 1 mM $K_4[Fe(CN)_6]$ were observed at 0.201 V, and 0.069 V with current responses 14.5 µA and 14.17 µA. By comparing the outcomes, we can say that the PAMCPE yields raised current responses with less ΔEp because, the positively charged poly(arginine) surface strongly interacts with negative charged $[Fe(CN)_6]^{4-}$ species.

The surface area of BCPE and PAMCPE can be evaluated by applying the Randles-Sevcik formula [36]:

$$Ip = 2.69 \cdot 10^5 \, n^{3/2} \, A \, D^{1/2} \, C_0 v^{1/2},$$

where *Ip* is peak current, C_o is the concentration of electroactive molecule (mol cm^{-3}), n is the number of electrons exchanged, *D* is the coefficient of diffusion (cm^2/ s), *v* is the sweep rate (V/s), *A* is the surface area (cm^2). For PAMCPE the electrochemically active surface area is found to be extreme (0.0469 cm^2) as compared to BCPE (0.038 cm^2).

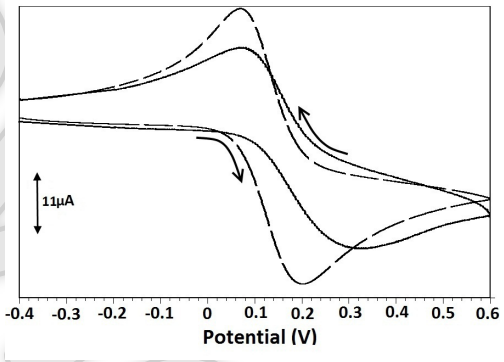


Fig. 2 Cyclic Voltammograms for Electrochemical activity of 1 mM $K_4[Fe(CN)_6]$ at PAMCPE (dashed line) and BCPE (solid line) in 0.1 M KCl at sweep rate 0.1 V/s.

FESEM surface characterization of BCPE and PAMCPE. Fig. 3 Shows the FESEM profile of BCPE (Fig. 3a) and PAMCPE (Fig. 3b). The FESEM Characterization of BCPE displays irregular shapes of graphite layers on the surface of the electrode. While FESEM Characterization of PAMCPE shows a homogeneous surface morphology. This validates the surface of BCPE is covered by thin polymer layers.

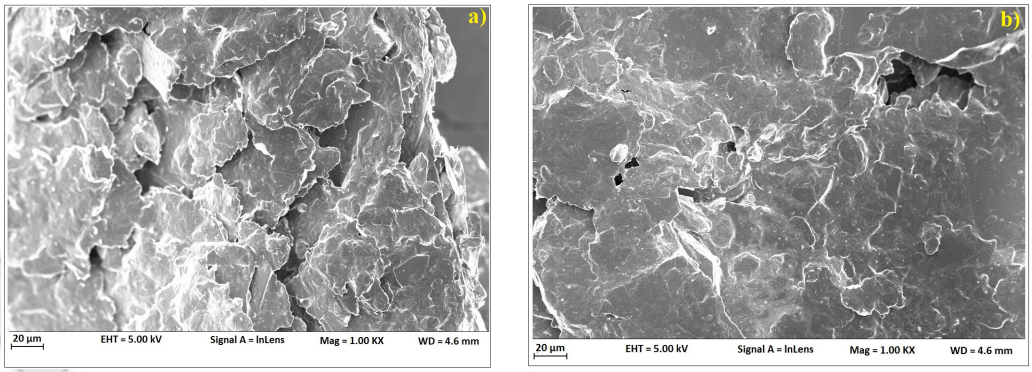


Fig. 3 FESEM magnified images of (a) BCPE (b) PAMCPE.

Electrochemistry of RF. As revealed in Fig. 4a in absence of RF (solid line) was characterized by CV in potential window - 0.80 to - 0.20 V at sweep rate 0.1 V/s in PBS of pH 6.0, no peak was received at PAMCPE. But under a parallel condition in presence RF (0.1 mM) the enhanced anodic and cathodic peaks were observed at -0.403 V and -0.512 V with an excellent current response.

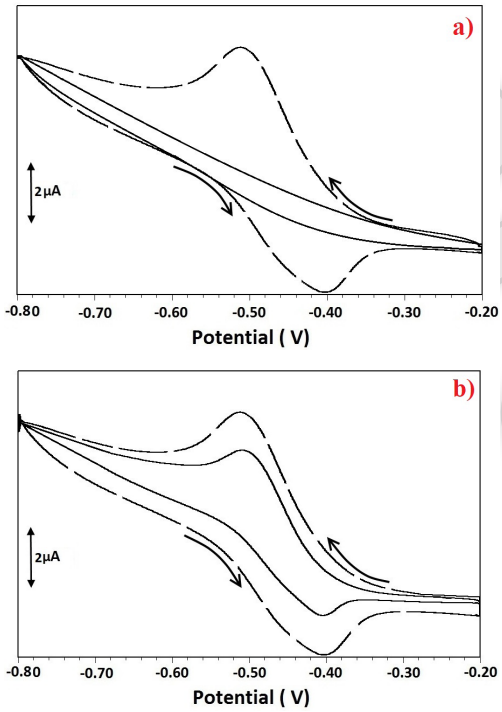


Fig. 4 (a) Cyclic voltammograms (v = 0.1 V/s) of RF (0.1 mM) in 0.1 M PBS of pH 6.0 at PAMCPE (dashed line) and without RF (solid line); (b) Cyclic voltammograms (v = 0.1 V/s) of RF (0.1 mM) in 0.1 M PBS of pH 6.0 at BCPE (solid line) and PAMCPE (dashed line).

The electrocatalytic behaviour of RF (0.1 mM) at PAMCPE and BCPE is represented in Fig. 4b At BCPE the cyclic voltammogram for RF (0.1 mM) disclose deprived oxidation and reduction responses at -0.405 V and -0.510 V (solid line) with quasi reversible behaviour at sweep rate 0.1 V/s in 0.1 M PBS of pH 6.0. However, the electrocatalytic action of RF is enlarged at PAMCPE in 0.1 M PBS of pH 6.0 at sweep rate 0.1 V/s with quasi-reversible behaviour. The anodic and cathodic potential for RF at PAMCPE were sensed at - 0.403 V, - 0.512 V vs. SCE with ΔE_p 0.105 V and upper current responses which are 3 times higher to the current obtained at BCPE.

Impact of potential scan rate. The effect of sweep rate on the anodic oxidation of RF was measured to identify the electrode process, by varying the sweep rate from 0.1 - 0.3 V/s in 0.1 M PBS of pH 6.0, the voltammograms obtained are shown in Fig. 5a. And a graphical plot of I_{pa} vs. $v^{1/2}$ (Fig. 5b) gives a straight line and it is expressed by linear regression equation $I_{pa}(A) = 45.85+13.212\ v^{1/2}\ (V/s)^{1/2}$ with correlation coefficient 0.99. This shows the PAMCPE process is under diffusion-controlled, so it is the ideal case for the quantitative measurement.

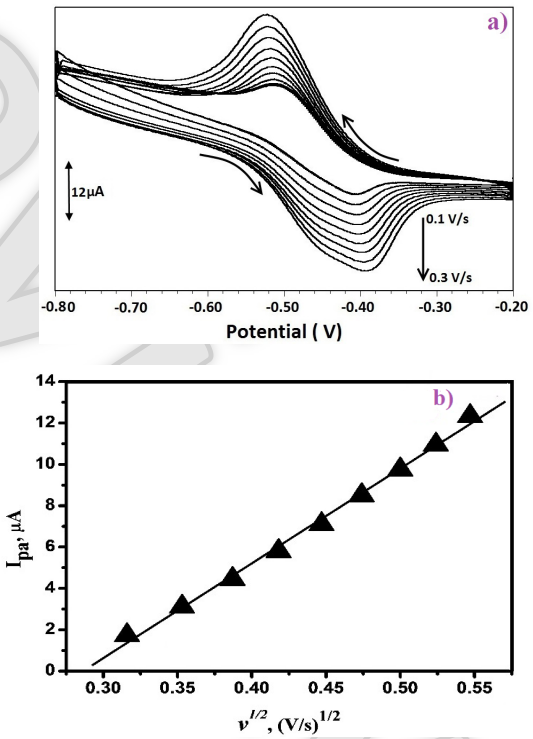


Fig. 5 (a) Cyclic voltammogram of RF (0.1 mM) in 0.1 M PBS of pH 6.0 at PAMCPE at various scan rates from 0.1 V to 0.3 V; (b) anodic peak current (I_{pa}) against square root of sweep rate ($v^{1/2}$).

Effect of pH. Its known that oxidation and reduction mechanism of organic molecules depends on pH. So its essential to know the impact of pH in electroanalysis of organic molecules. The Fig. 6a displays cyclic voltammograms of RF at various pH ranging from 5.5-7.5 of 0.1 M PBS at a potential sweep rate of 0.1 V/s. A graphical plot I_{pa} vs. pH (Fig. 6b) displays that the oxidation peak current was sensitive for slight acidic pH 6.0. So pH 6.0 is thus an optimized pH and hence, it was maintained throughout the experiment. Since at this pH, the fast electron transfer reaction will occur. The higher basic media is not appropriate for RF determination that may produce unstable lumiflavin with irradiation of light.

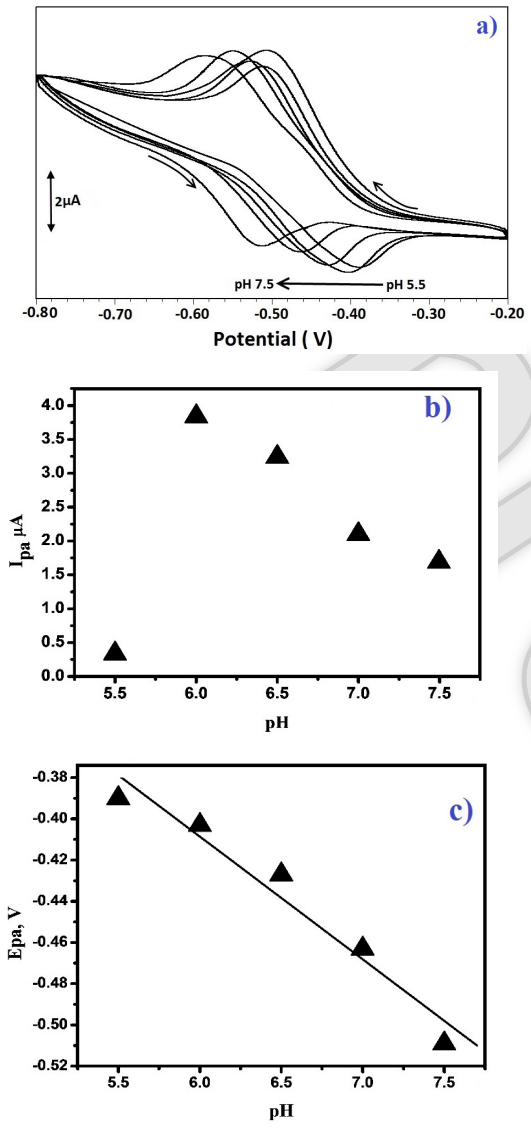
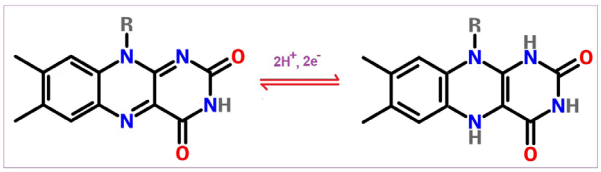


Fig. 6 (a) Cyclic voltammogram (v = 0.1 V/s) of RF (0.1 mM) in 0.1 M PBS of pH 6.0 at PAMCPE at various pH values ranging from 5.5 to 7.5; (b) plot of anodic peak current (I_{pa}) vs. pH; (c) plot of anodic peak potential (E_{pa}) against pH.

A plot of E_{pa} vs. pH (Fig. 6c) demonstrates the influence of pH on the anodic peak potential of RF is studied by CV over the range of 5.5-7.5. The graph indicates the anodic peak is shifted towards negative potential direction with a rise in pH by validating the linear equation E_{pa} (V) = -0.0596 pH - 0.05. The slope 0.0596 V/pH unit is almost equivalent to the accepted value 0.059 which elucidates the number of electrons and protons involved in the electrocatalytic reaction is in the proportion 1:1. Therefore the pathway of RF reduction/oxidation contains two electrons and two protons as shown in scheme.1.



Scheme 1. The Electron transfer process of RF.

Repeatability and stability studies. Repeatability for the determination of RF was assessed using CV in 0.1 M PBS (pH 6.0) at sweep rate 0.1 V/s. The PAMCPE electrode exhibits admirable repeatability for 5 individual measurements with relative standard deviation (RSD) 2.25 %. The stability of the fabricated sensor for the detection of 0.1 mM RF was analyzed by running 30 successive cycles and has observed that 91 % of primary outputs are retained even after 30 cycles, so the developed sensor has good stability.

The Sensing of RF at DPV. The DPV voltammogram for sensing RF (0.1 mM) in 0.1 M PBS (pH 6.0) with sweep rate 0.05 V/s at BCPE and PAMCPE in the potential scale -0.80 V to -0.20 V are depicted in Fig. 7 The peak potential for RF (0.1 mM) at PAMCPE was received at -0.492 V with current signal was 2 times higher to that current attained at BCPE. At BCPE the RF (0.1 mM) peak was sensed at -0.488 V with a minor current response. This means the electrooxidation of RF (0.1 mM) is elevated at PAMCPE.

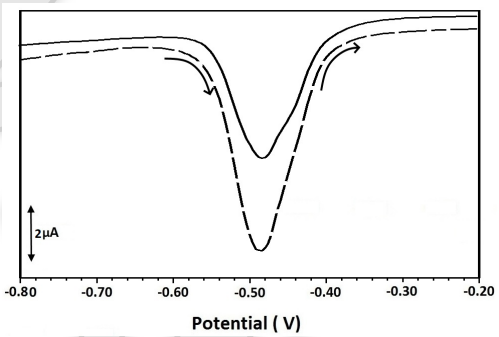


Fig. 7 DPV (*v* = 0.05 V/s) of RF (0.1 mM) in 0.1 M PBS (pH 6.0) at BCPE (Solid line) and PAMCPE (dashed line).

Calibration graph and detection limit. Fig. 8a and Fig. 8b shows DP voltammograms and calibration graph for the oxidation peak current values of RF enhanced linearly with raise in RF concentration from 2.0-50 μM and is expressed as I_{pa} (A) = 5.41×10^{-6} + 0.101 C (M) with R = 0.99.

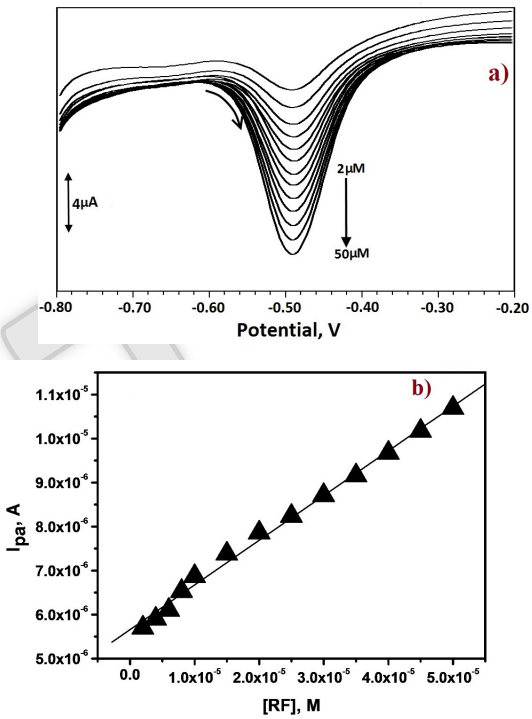


Fig. 8 (a) DPV curves for diverse concentrations of RF from 2.0 to 50 μM in 0.1 M PBS, pH 6.0 at sweep rate 0.05 V/s; (b) DPV Calibration plot (v = 0.05 V/s) for the determination of RF from 2.0 to 50 μM at PAMCPE in 0.1 M PBS (pH 6.0).

The detection limit (LOD) and the quantization limit (LOQ) were calculated by using the formulae LOD = 3. S_d/m and LOQ = 10. S_d/m, [37] where m is the slope of the standard calibration graph and S_d is the standard deviation of blank current values (five repeats). The LOD and LOQ for assessment of RF at PAMCPE were calculated as 9.3·10^{-8} M and 3.1·10^{-7} M. Table 1. [38-43] display an assessment of the PAMCPE with other electrodes described in the literature for the determination of RF. It can be seen in the table, and this sensor provides a low detection limit as compared DNA modified Pencil graphite [43] Cr doped SnO_2 nanoparticles/glassy carbon [42] and near to the Fe_3O_4/rGO/Glassy carbon electrode [41] and higher to complicated procedures like MB-SO_3H-MSM/Glassy carbon [38], α-Fe_2O_3/MWCNT/AuNPS/Glassy carbon [39], GCE/AuNPs@PDA-RGO/Glassy carbon[40]. The main advantages of our work are the simplicity of preparation, low cost, disposable, and high applicability.

Quantification of RF in vitamin B-complex capsule and Human Blood Serum. To know the applicability of the fabricated sensor, the experiments were carried out to estimate the amount of RF in B-complex capsule and blood serum. Two pieces of RF containing B-complex pill were powdered in an agate mortar. A suitable quantity of the B-complex powder to a standard solution of concentration of 1.0·10^{-4} M was prepared using distilled water and analysis was done with standard addition method in phosphate buffer solution at optimal conditions.

Blood serum samples were collected from healthy volunteers then centrifuged at 3000 rpm for 30 min. Obtained serum was diluted five times with phosphate buffer solution. These serum samples were analysed immediately or they were stored at cold condition until analysis. Further, DPV technique has been carried for the analysis of spiked RF in human blood serum samples by using standard addition method. The obtained results were shown in Table 2. As seen in table the sensor provides good recovery in B-complex tablet and complicated human blood serum, this shows the high applicability of PAMCPE.

Table 1. Comparison of some biosensors for riboflavin estimation with proposed sensor.

Electrode	Modifier	Concentration range (μM)	LOD (μM)	Technique	Ref
Glassy carbon	MB-SO_3H-MSM	0.01–15 & 15-50	0.005	DPV	[38]
Glassy carbon	α- Fe_2O_3/MWCNT/AuNPS	0.3-0.6	0.006	SWV	[39]
Glassy carbon	GCE/AuNPs@PDA-RGO	0.02−60.0	0.0096	DPV	[40]
Glassy carbon	Fe_3O_4/rGO, Flowers-like	0.300–1 & 1-100	0.089	DPV	[41]
Glassy carbon	Cr doped SnO_2 NPs	0.2-100	0.100	LSV	[42]
Pencil graphite electrode	DNA	1–186	0.900	DPV	[43]
Carbon paste electrode	Poly(arginine)	2.0-50	0.093	DPV	present work

MB-SO_3H-MSM- Methylene blue incorporated mesoporous silica microsphere, DPV-Differential pulse voltammetry, GCE/AuNPs@PDA-RGO- gold nanoparticles on glassy carbon electrode modified by a hybrid of polydopamine and reduced graphene, MWCNT/AuNPS- multi walled carbon nanotubes / gold nanoparticles, SWV- Square wave voltammetry, rGO-Reduced graphene oxide, LSV-linear sweep voltammetry, DNA - Deoxyribonucleic acid

Table 2. Estimation and recovery appraisal for RF in B-complex capsule and Human blood serum using DPV.

Sample	Added (μM)	Found (μM)	Recovery (%)	RSD* (%)
B-complex Capsule	2.0	1.91	95.5	1.28
	4.0	3.9	97.0	
	6.0	6.1	101.6	
Blood serum	4.0	3.72	93.0	3.25
	6.0	5.70	95.0	
	8.0	7.68	96.0	

Note. *relative standard deviation

RF concentration variation (0.1 mM - 0.120 mM) in the presence of AA (1 mM). The simultaneous determination of RF (0.1-0.120 mM) and AA (1 mM) in a mixture was performed at PAMCPE using DPV in 0.1 M PBS, pH 6.0 at sweep rate 0.1 V/s as depicted Fig. 9. The concentration of RF is increased from 0.1 mM to 0.120 mM while the AA (1 mM) concentration kept constant. The peak current of RF is increased linearly to each addition. There are no significant differences in the peak current and potential values occurred for AA, so the simultaneous determination of RF with AA is feasible.

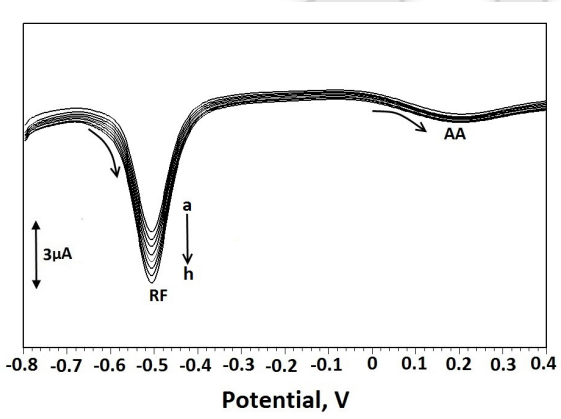


Fig. 9 Determination of RF from (a) 0.1 mM to (h) 0.120 mM in presence of AA (1mM) under optimal conditions.

RF concentration variation (0.1 mM – 0.115 mM) in the presence of FA (1mM). The Concurrent determination of RF (0.1 mM - 0.115 mM) and FA (1 mM) in a mixture was performed at PAMCPE using DPV in 0.1 M PBS, pH 6.0 at sweep rate 0.1 V/s as revealed Fig. 10. The concentration of RF is raised from 0.1 mM to 0.115 mM while the FA (1 mM) concentration kept unchanged. The peak current of RF is increased linearly to each successive addition. There are no major changes in the peak current and potential values of FA, so the simultaneous electrochemical determination of RF with FA is feasible.

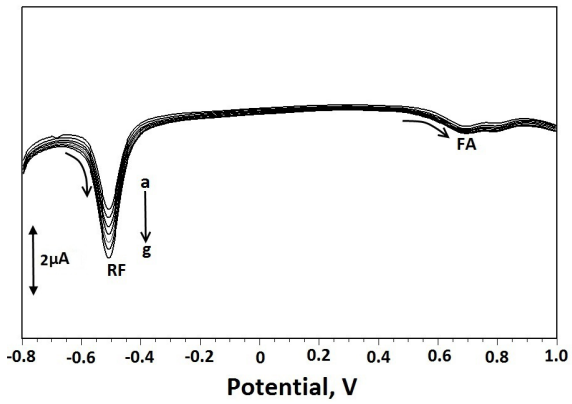


Fig. 10 Estimation of RF from (a) 0.1 mM to (g) 0.115 mM in presence of FA (1 mM) using DPV in 0.1 M PBS, pH 6.0, at sweep rate 0.05 V/s.

Simultaneous determinations of RF, AA, and FA using DPV. DPV is a highly responsive method of voltammetry as compared to CV. So it was employed for the simultaneous determination of RF, AA and FA in 0.1 M PBS of pH 6.0 at sweep rate 0.05 V/s in the potential range -0.8 to 1.0 V at PAMCPE as shown in Fig. 11. Clear separations of peaks were observed for RF, AA, and FA. The peak potentials of RF (0.1 mM), AA (1 mM), and FA (1 mM) observed at -0.552, 0.217, 0.656 V respectively with enhanced current response.

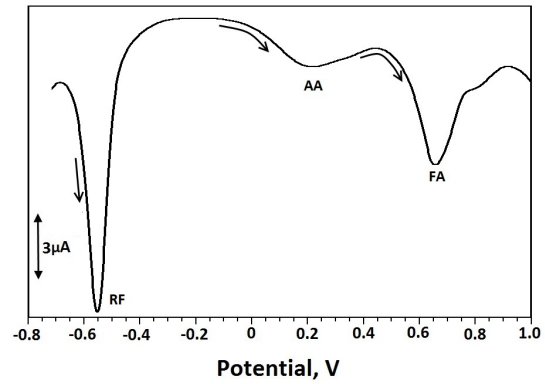


Fig. 11 Simultaneous determinations of RF (0.1 mM), AA (1 mM), and FA (1 mM) using DPV under calibrated conditions.

Simultaneous Screening of RF, AA, and FA in a B-complex capsule. To prove the practicability of devised PAMCPE, the simultaneous analysis was performed in B-complex capsule solution (1 ml) in 0.1 M PBS (pH 6.0) at sweep rate 0.05 V/s using highly sensitive technique DPV. The obtained DPV curve is shown in Fig. 12 the RF, AA, FA separated into three distinct peaks at - 0.5 V, 0.184 V and 0.812 V with excellent current responses. It can be seen in the curve the acceptable slight shift in potentials of analytes is observed due to other ingredients present in the capsule. The current responses for RF, AA is more as compared to FA because the B-complex

capsule solution contains a higher concentration of RF and AA as compared to FA.

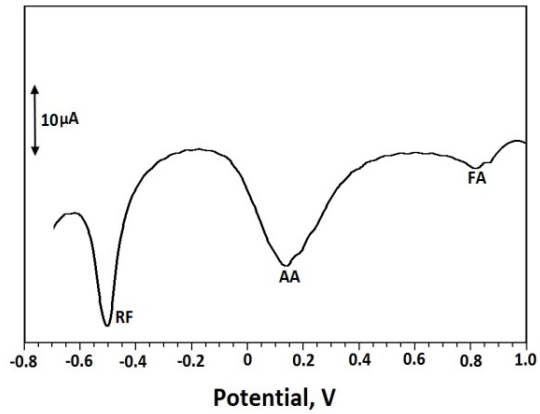


Fig. 12 DPV screening of RF, AA, and FA in a B-complex capsule in 0.1 M PBS pH 6.0 at sweep rate 0.05 V/s.

Conclusion

We developed a simple, highly effective and selective electrochemical method for voltammetric determination of riboflavin in the presence of ascorbic, and folic acid. The established sensor is characterized by CV and FESEM. The developed sensor is effectively used to the quantization of riboflavin in B-complex capsule and complicated blood serum. The results showed well - defined responses and the proposed sensor offers good sensitivity, selectivity, stability, low detection limit with good linear range. The established sensor is low cost and effective for Vitamin analysis.

Acknowledgement

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Conflict of Interest

The authors declare no conflict of interest.

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