Polymer Modified Carbon Paste Electrode as a Sensitive Sensor for the Electrochemical Determination of Riboflavin and Its Application in Pharmaceutical and Biological Samples

Edwin S D' Souza[a,b], J.G. Manjunatha[a,*], C. Raril[a], Girish Tigari[a] and P.A. Pushpanjali[a]

[a]*Department of chemistry, FMKMC College, Madikeri, Constituent College of Mangalore University, Karnataka, India*
[b]*Department of Chemistry, St. Philomena College, Puttur, Karnataka, India*
(Received 7 February 2020 Accepted 12 May 2020)

Poly (threonine) modified carbon paste electrode (PTMCPE) can be used as a responsive and selective electrocatalytic sensor for the estimation of riboflavin (RF) by means of cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The proposed electrode creates a catalytic enhancement in redox behavior of RF when equated with bare carbon paste electrode (BCPE). Under optimized conditions, RF quantification at modified electrode has shown a detection limit of 1.78×10^{-7} M in the linear range of 0.2-1 μM and 1-5 μM. Simultaneous analysis of RF with folic acid (FA) and ascorbic acid (AA) shows well-separated peaks leading to a quick and selective analysis of RF. The fabricated sensor can be successfully applied to RF analysis in human blood serum and B- complex tablet with acceptable results.

INTRODUCTION

Organic compound Riboflavin (RF, vitamin B_2) is essential for the natural metabolism in the human body [1]. The water-soluble RF, a component of flavoenzymes, plays a major role in biochemical reactions [2]. It has some pharmaceutical values and functions as an antioxidant [3], anti-inflammatory [4], and analgesic [5] compound. It is not produced in the body and hence it has to be supplemented through diet. The two components present in RF are flavin adenine dinucleotide and flavin mononucleotide. These components are essential for a number of flavoprotein enzyme reactions including the activation of other vitamins. RF is found in green vegetables, enriched flour, meat, nuts and eggs [6]. Insufficient ingestion of RF can have an adverse effect on health, resulting in numerous grave diseases such as microcytic hypo-chronic anaemia, night blindness (beriberi), pellagra or sometimes even death [7].

Obviously, it is of much significance to initiate a straightforward, simple, consistent and successful process for RF determination.

Several procedures have been applied for the estimation of RF, including fluorescence [8-11], chemiluminescence [12,13] and capillary electrophoresis [14]. These approaches can be utilized for the quantification of RF, but they are tedious, expensive and time consuming [15]. Electrochemical processes have gained importance in the quantitative estimation of RF because of their simple, sensitive, cheap and quick response [16-18]. The chemically modified electrodes afford a device to advance the performance of electrodes and enormous functional materials as modifiers for vitamin sensors [19-20]. The nanoparticles of metallic or semiconducting materials with conducting polymer find massive applications in catalysis, electronics, and sensors [21-23]. Based on the constituent polymer and the metal introduced, physical and chemical properties of the electrode can be studied. Accordingly, setting a polymer with nanoscale material, nano-electronic

---

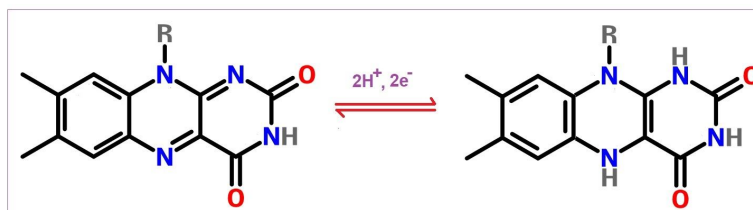*Corresponding author. E-mail: manju1853@gmail.com

Fig. 1. Electron exchange mechanism of RF.

sensors with higher performance can be designed [24-26]. Modified electrodes are established to improve the sensitivity of the electrodes to get a new sensor of better quality with desired properties [27-33]. Electropolymerization is among the diverse methods for the preparation of such modified electrodes, having a versatile approach. Modification involves flexibility due to high surface coverage of thick polymer coatings. So, the natural compounds compatible with carbon paste electrode are used to devise the electrodes of superior performance.

In the current effort, the poly (threonine) modified carbon paste electrode (PTMCPE) is prepared and applied for the detection and quantification of RF. Along with an individual study, simultaneous study of RF with FA and AA is also carried out. A susceptible speedy technique is developed using DPV for the determination of RF. The developed electrode is effectively applied for the analysis of real samples such as human blood serum and B- complex tablet. The electron transfer mechanism of RF is shown in Fig. 1.

EXPERIMENTAL

Apparatus

Voltammetry experiments are performed using CHI 6038 E (USA) connected to a personal computer for control and storing of the data. A standard three-electrode assembly is used to carry out all the electrochemical experiments. While, a bare or poly (threonine) modified carbon paste electrode is utilized as working electrode, the counter electrode used is a platinum wire and the reference electrode is a saturated calomel electrode (SCE). Field emission scanning electron microscopy (FESEM) result is obtained using an instrument operating at 5.0 kV (DST-PURSE Laboratory, Mangalore University).

Reagent and Chemicals

Riboflavin, threonine, and folic Acid are purchased from Molychem Chemicals, India, and used as received without any Purification. All other chemicals used are of analytical standards. Phosphate buffer solutions of different pH values are prepared by mixing standard solutions of 0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4. Working solutions of RF are freshly prepared using suitable solvent on the day of experiment. All other required stock solutions are prepared in distilled water.

Preparation of Carbon Paste Electrode

Carbon paste electrode is obtained by carefully hand mixing of silicon oil with graphite powder in a mortar with pestle in the ratio of 30:70 (w/w) until a consistent paste is prepared. A portion of the paste is then filled tightly in a Teflon tube of 3 mm internal diameter. The electrical contact is established by connecting a copper wire down the tube and into the backside of the mixture. The operational electrode surface is properly made by polishing with tissue paper.

RESULTS AND DISCUSSION

Characterization

The surface morphologies of BCPE and PTMCPE are characterized by FESEM as shown in Fig. 2. The surface of BCPE exhibits inconsistent structure with sub divisions as indicated in Fig. 2a. However, PTMCPE has a smooth surface with a uniform arrangement, which is advantageous for the expansion of volume of the electrode, Fig. 2b. This shows that the exterior of BCPE is sheltered by poly (threonine) layer.
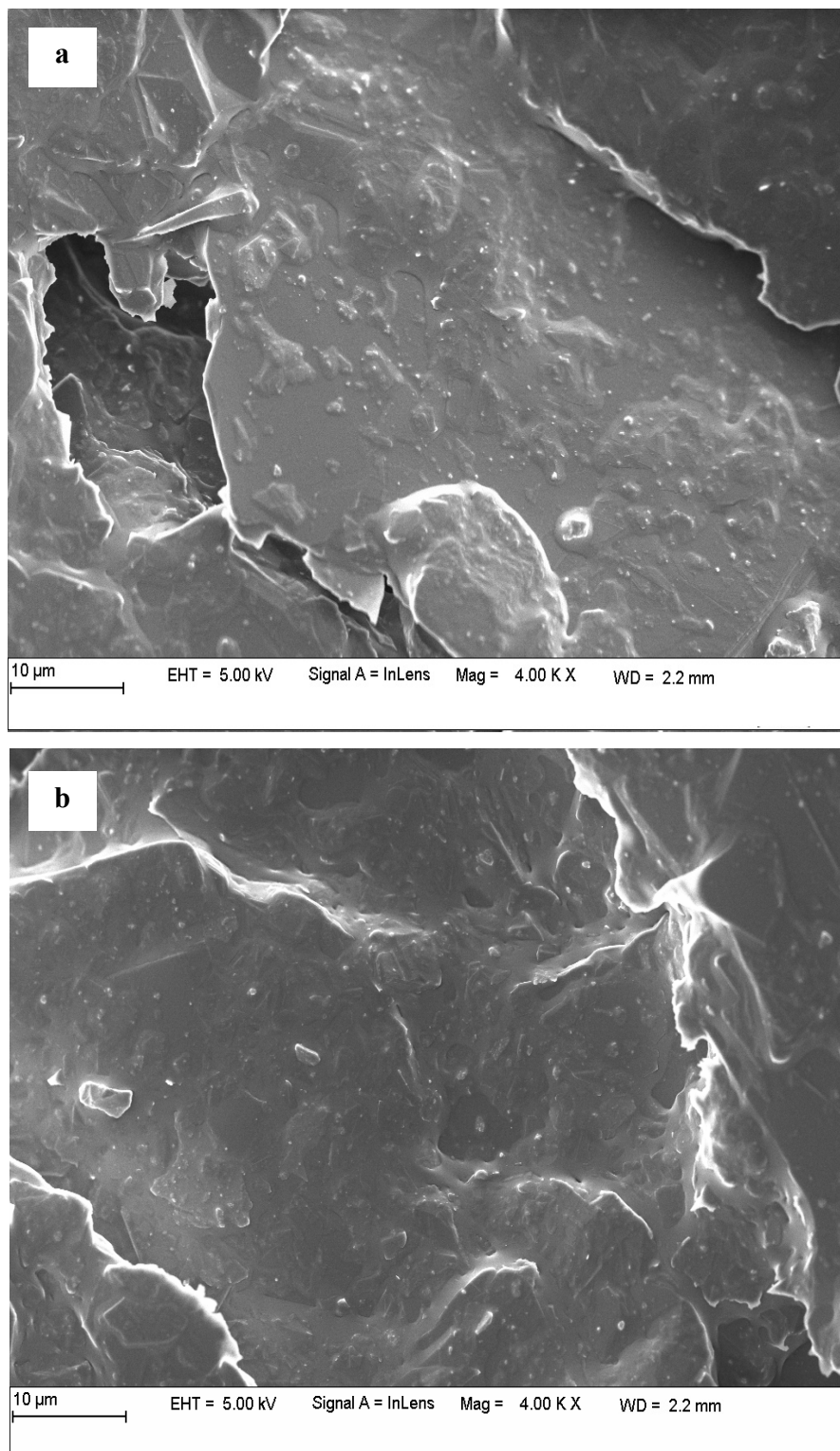
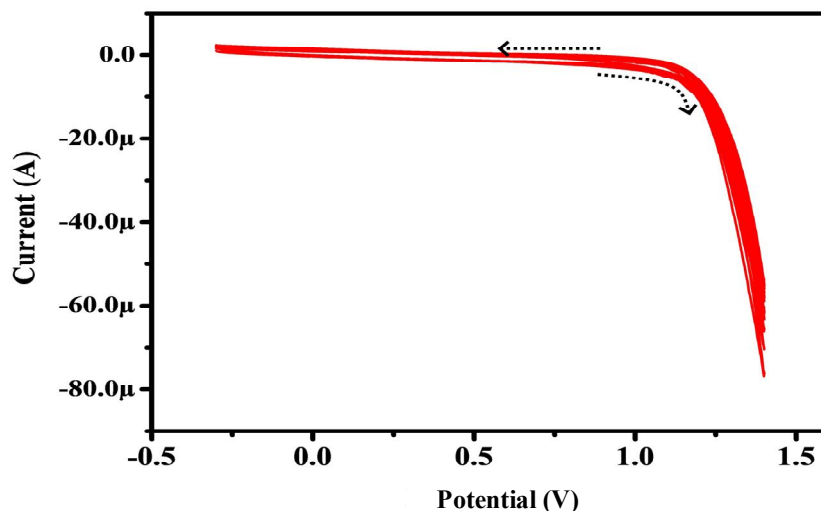**Fig. 2.** FESEM images of (a) BCPE and (b) PTMCPE.

Fig. 3. The cyclic voltammogram of poly (threonine) film coated on CPE with 1 mM threonine in 0.1 M PBS, 7.0 pH, for 10 cycles at the sweep rate of 0.1 V s^{-1}.

Preparation of Poly (Threonine) Modified Carbon Paste Electrode (PTMCPE)

A solution of 1 mM threonine in 0.1 PBS, pH 7.0, is placed in an electrochemical cell. The PTMCPE is obtained by ten times scanning of threonine solution in a potential range of 0.3-1.4 V at a scan rate of 0.1 V s^{-1}. The untreated threonine is detached by rinsing the prepared electrode with distilled water. Figure 3 indicates the cyclic voltammogram for the electropolymerization of threonine.

Study the Electrochemical Behavior of RF on PTMCPE Using LSV, CV and DPV

Linear sweep voltammetry (LSV) is a technique to determine the current at the working electrode through which the potential between the fabricated electrode and a reference electrode is swept linearly in time. Oxidation or reduction of the analyte is registered as a peak or trough in the current signal at which the analyte begins to oxidize or reduce. LSV of RF is investigated and Fig. 4a represents the linear sweep voltammogram of RF at bare (dotted line) and PTMCPE (solid line) in 0.1 M PBS, 7.0 pH, at the sweep rate of 0.1 V s^{-1}. The CV study of RF shows both the oxidation and reduction peaks in Fig. 4b. The BCPE (dotted line) in the diagram is differentiated by the PTMCPE (solid line). Figure 4c represents the DPV of carbon paste

electrodes before modification (dotted line) and after modification (solid line) with threonine. All the three techniques distinguish the peaks of BCPE from PTMCPE. The current response obtained for the modified electrode in all the above techniques is superior to that which is obtained from bare paste electrode. This study reveals that the polymer modified electrode considerably increases the electrochemical performance of RF; the developed electrode exhibits excellent electro catalytic activity towards RF oxidation.

Effect of pH on RF

In the determination of compounds, the pH of the medium is an important factor affecting both the rate of the electrode process along with the mechanism. The influence of pH on the peak current density of Riboflavin is studied by CV. The effect of buffer pH for the oxidation/reduction of RF are studied across the pH range 5.5-7.5 (Fig. 5a). The peak potential is linearly dependent on the pH with the regression equation of E_{pc} (V) = -0.2406-0.0504 pH (Fig. 5b) with R = 0.99. The obtained slope value -0.0504 V/pH, which is very near to the theoretical value of -0.059 V/pH, suggests that the ratio of electrons and hydrogen ions involved in RF oxidation is 1:1. The most symmetrical and the highest peak current of RF are found at pH 7.0 (Fig. 5c).
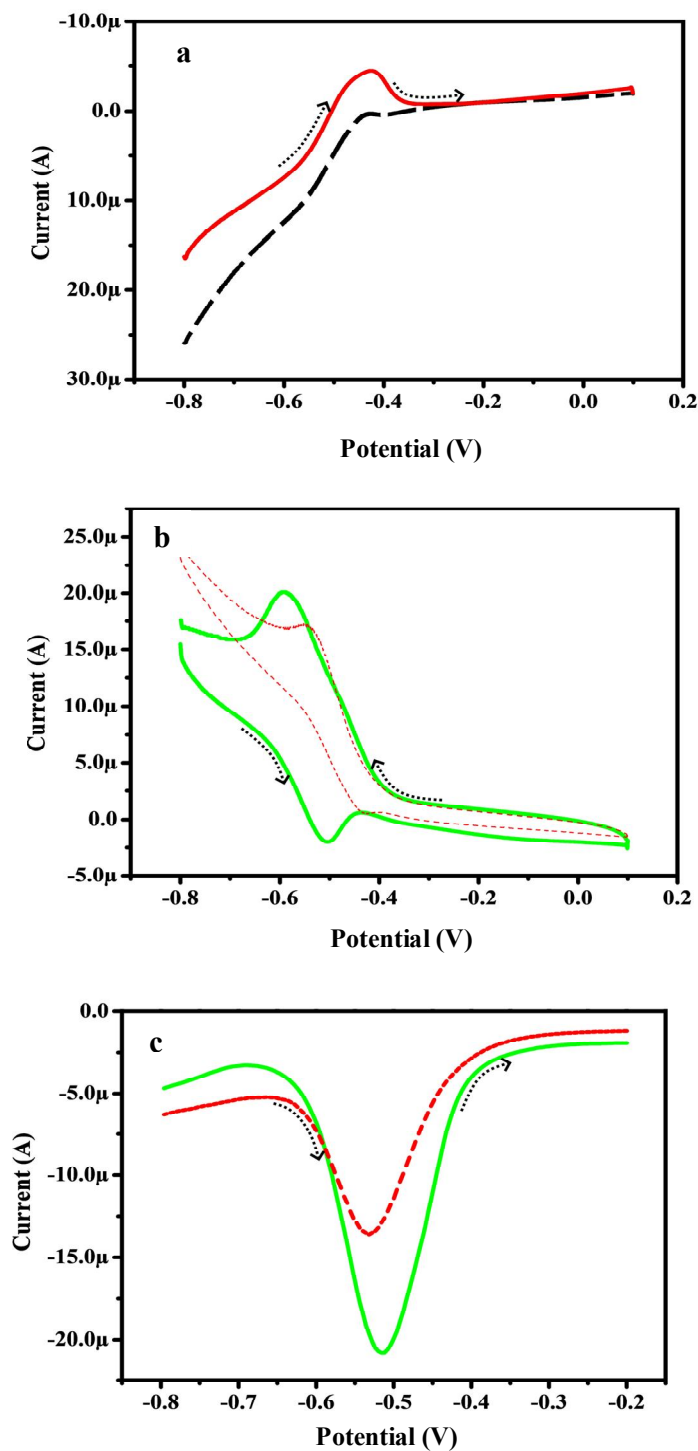
Fig. 4. (a) Linear sweep voltammogram of RF at bare (dotted line) and PTMCPE (solid line) in 0.1 M PBS, 7.0 pH, at the sweep rate of 0.1 V s^{-1}. (b) Cyclic Voltammogram of RF at bare (dotted line) and PTMCPE (solid line) in 0.1 M PBS, 7.0 pH, at the sweep rate of 0.1 V s^{-1}. (c) Differential pulse voltammogram of RF at bare (dotted line) and PTMCPE (solid line) in 0.1 M PBS, 7.0 pH, at the sweep rate of 0.1 V s^{-1}.
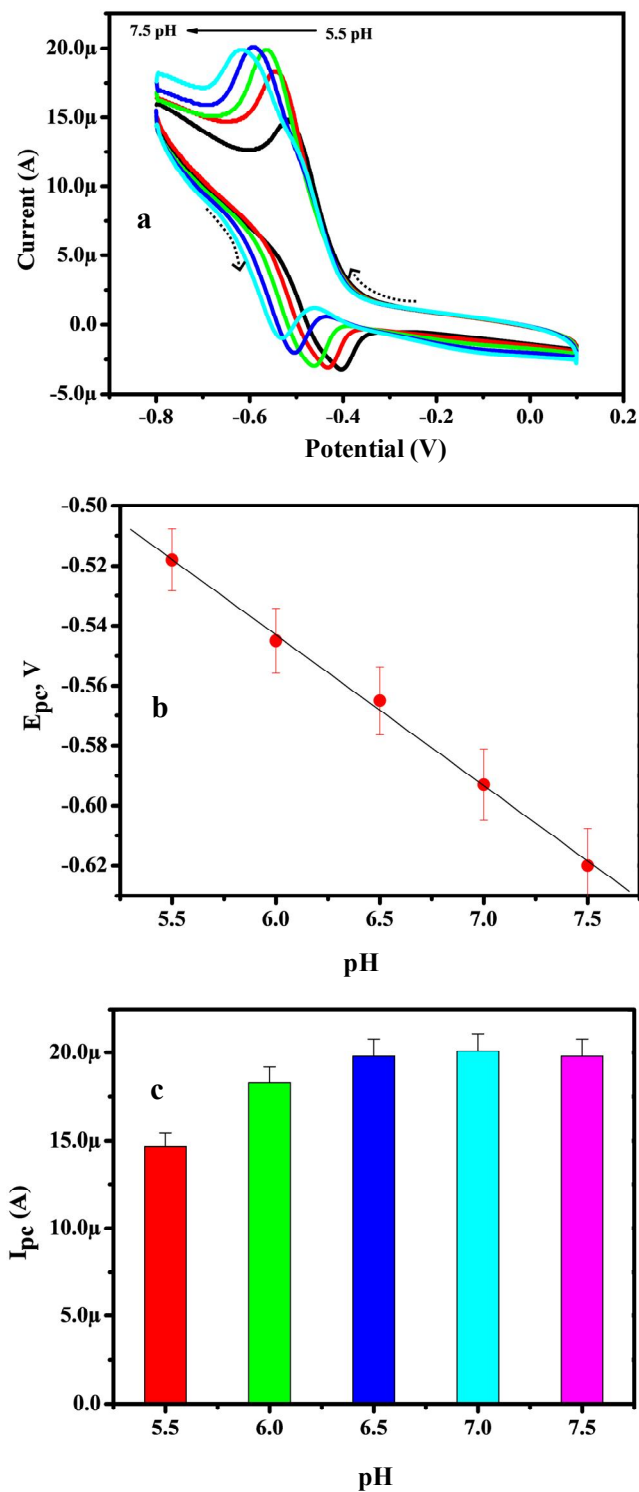
Fig. 5. (a) Cyclic voltammogram of RF (1×10^{-5} M) at PTMCPE in 0.1 M PBS at different pH values, 5.5, 6.0, 6.5, 7.0, 7.5. (b) E_{pc} *vs*. pH. (c) I_{pc} *vs*. pH.
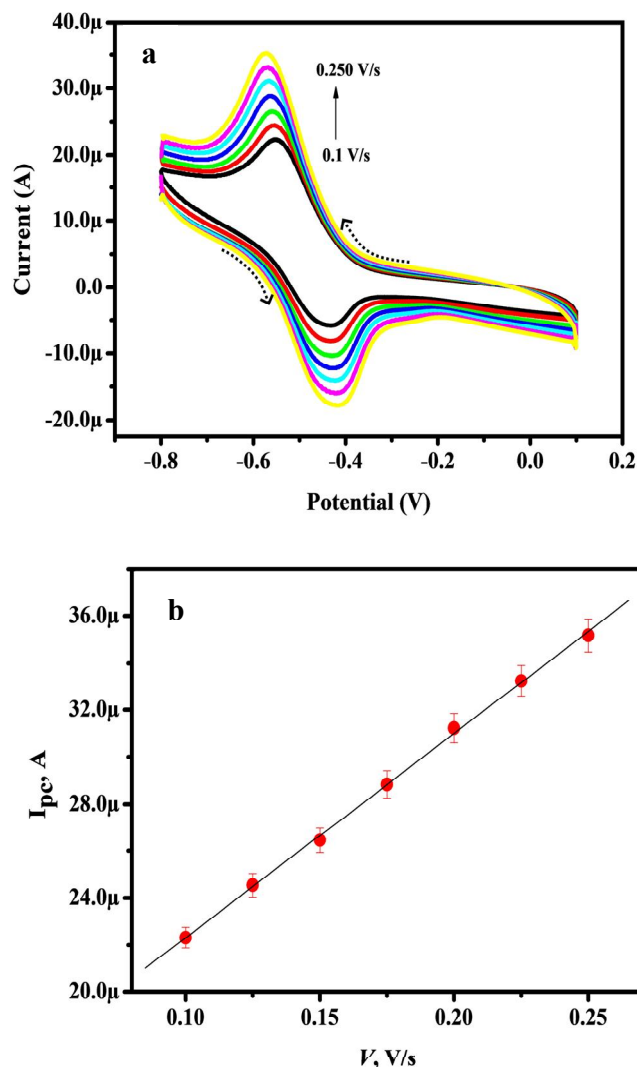
Fig. 6. (a) Cyclic Voltammogram of RF (1×10^{-5} M) at PTMCPE in 0.1 M PBS, pH 7.0, at different scan rates (0.1, 0.125, 0.150, 0.175, 0.2, 0.225, 0.250). (b) Cathodic peak current (I_{pc}) *vs.* scan rate (*v*).

Therefore, the value of pH 7.0 is chosen as the most favorable pH for better sensitivity and selectivity of RF determination.

Impact of Sweep Rate

Figure 6a shows the voltammogram of RF (1×10^{-5} M) at PTMCPE in 0.2 M PBS of pH 7.0 at different scan rates (0.1, 0.125, 0.150, 0.175, 0.2, 0.225, 0.250). It is observed that the peak current is amplified steadily with the increase in sweep rate. The peak current increases linearly with the

scan rate in a range from 0.1-0.250 V s^{-1}. It is evident from Fig. 6a that there is a linear relationship between *v* and the peak currents with the regression equation of I_{pc} (µA) = 13.63 + 86.28 *v* (Fig. 6b), and correlation coefficient 0.99. This performance shows that RF oxidation is controlled by adsorption.

Calibration Curve and Detection Limit

Differential pulse voltammetry is used to inspect the relationship between the concentration of RF and the peak
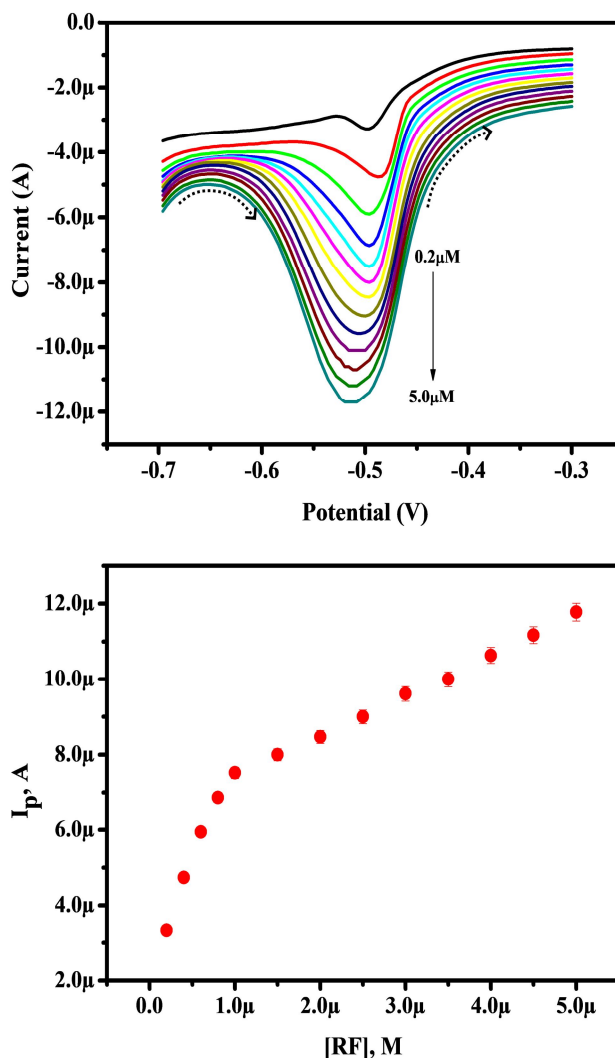
Fig. 7. (a) DPV curve for RF at the modified electrode with different concentrations in 0.1 M PBS (pH 7.0) at the scan rate of 0.05 V s^{-1}. (b) Plot of I$_p$ *vs*. RF concentration.

current. As seen in Fig. 7a, under the most favorable conditions, the peak current is reliant on the RF concentration over the range of 2×10^{-7} M and 5×10^{-6} M ; actually two linear curves are obtained from 0.2-1 μM and 1-5 μM. However, we have considered a fine linear form of 1-5 μM with a linear regression equation I$_p$ (A) = 6.35 + 1.07 C_{RF} (M), and a correlation coefficient of 0.99 (Fig. 7b). The limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3σ/m and 10σ/m [34]. Here, σ- is determined as the standard deviation of 5 DPV measurements in blank solution and m- is the slope of the

calibration curve. The LOD and LOQ were evaluated for RF estimation as 1.78×10^{-7} M and 5.95×10^{-7} M, which are close to many of the modified electrodes (Table 1) [35-39]. The developed electrochemical method in this work has shown a lot of advantages, such as high sensitivity, easy preparation, inexpensive and high steadiness.

Simultaneous Determination of FA, AA and RF at PTMCPE by DPV

A very sensitive electrochemical method named differential pulse voltammetry is used to study the

Table 1. Comparison of Linear Range and Detection Limit Obtained Using PTMCPE
for RF Determination with other Methods

Electrodes	Linear range (μM)	Detection limit (μM)	Ref.
CPE/Zeolite	1.7-34	0.71	[35]
Rotating disc/hematite	1.3-100	8.4	[36]
Glassy carbon/Cr doped SnO$_2$ nanoparticle	0.2-100	0.1	[37]
Pencil graphite electrode/DNA	1-186	0.9	[38]
Glassy carbon/Fe$_3$O$_4$/ rGO Flowers-like	0.300-1&1-100	0.089	[39]
PT/CPE	0.2-1&1-5	0.178	This work
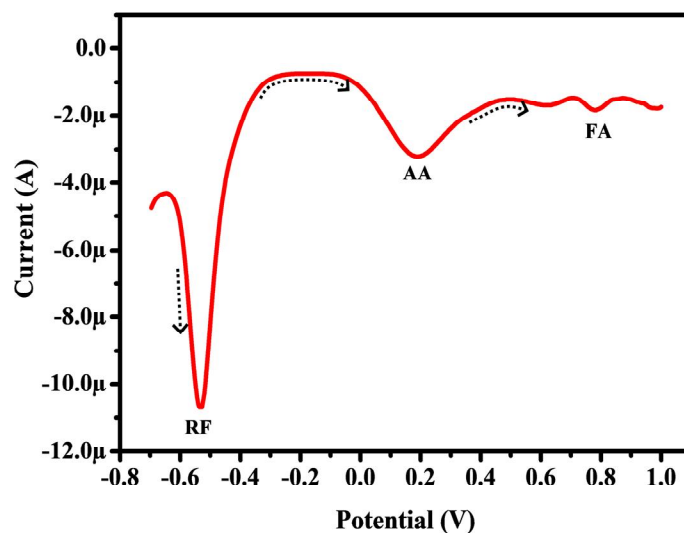
Fig. 8. DPVs of the solution containing RF (1×10^{-5} M), AA (1×10^{-3} M) and FA (1×10^{-4} M) in 0.1 M PBS (pH 7.0) at the PTMCPE.

simultaneous determination of FA, AA and RF using PTMCPE. The studies of DPV for RF, AA and FA in the mixture are represented in Fig. 8. As shown, three well separated peaks, at the potentials of -0.532, 0.188 and 0.781 V, are respectively obtained for RF, AA and FA, indicating that PTMCPE has successfully separated AA and FA in the presence of RF.
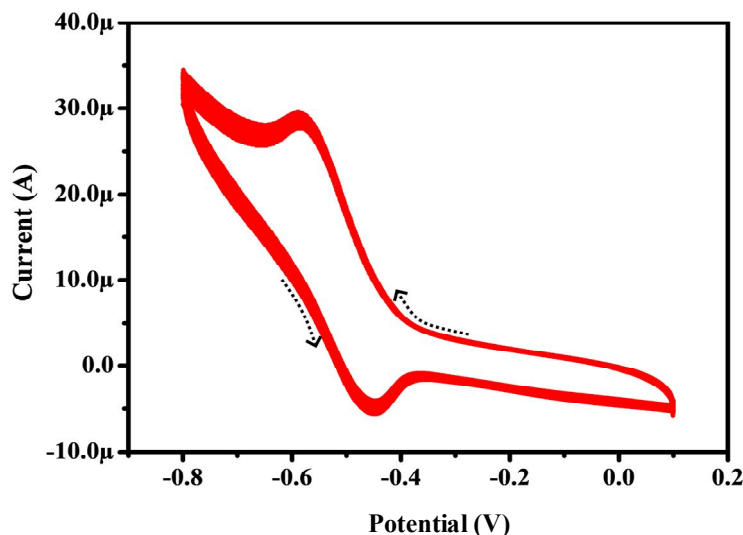
Fig. 9. Cyclic voltammogram for the stability study of RF (1×10^{-5} M) at PTMCPE in 0.1 M PBS (pH 7.0) at the scan rate of 0.1 V s^{-1}.

Stability, Repeatability and Reproducibility

The main parameters for the assessment of the feasibility of a sensor are stability, reproducibility and repeatability of the sensor. The developed electrode has retained 96.7% of its initial peak current response for a concentration of 10 µM of RF for continuous 20 cycles (Fig. 9). This exhibits the steadiness of the tailored electrode for an extended period in the determination of RF. Repetitive voltammograms (n = 3) have shown that the standard deviation is 2.6%. It shows the excellent repeatability for RF determination at the tailored electrode. Reproducibility of a constructed sensor is evaluated by the calculation of RF concentration with the three independent electrodes. The RSD for 10 µM RF determination on these three electrodes is found to be 2.8%. This indicates that the modified electrode has a good reproducibility. The outcome of work shows that the PTMCPE has an excellent reproducibility, and repeatability with an extended stability in voltammetric determinations.

Real Sample Analysis

Analysis of RF in real sample could be done by means of B-complex tablet and blood serum. The B-complex tablet was obtained from a nearby medical store and the experiment was carried out by standard addition method. It is observed that the recovery is in the range of 98.75-101.5%. Figure 10a shows the differential pulse voltammogram for the determination of RF in B-complex tablet sample. The blood sample was collected from the nearest district hospital and experiments were carried out by standard addition method. The recovery ranges from 92.11-96.48%. Figure 10b shows the differential pulse voltammogram for the determination of RF blood serum. The outcome has revealed that the equipped voltammetric sensor is extremely successful for the investigation of RF in B-complex capsule and biological samples of blood serum with satisfactory results.

Interference Study

The selectivity is one of the most important properties of a novel sensor. The effect of a variety of substances which are potentially able to interfere with the determination of RF was studied under the optimum conditions in the presence of 50 fold concentration of some compounds such as folic acid, ascorbic acid, arginine, glycine, indigo carmine, alizarin red and fast sulfone blue. It was observed that a 50 fold concentration of above compounds does not have any significant influence on oxidation potential of RF; indicating the high selectivity of the proposed electrode.
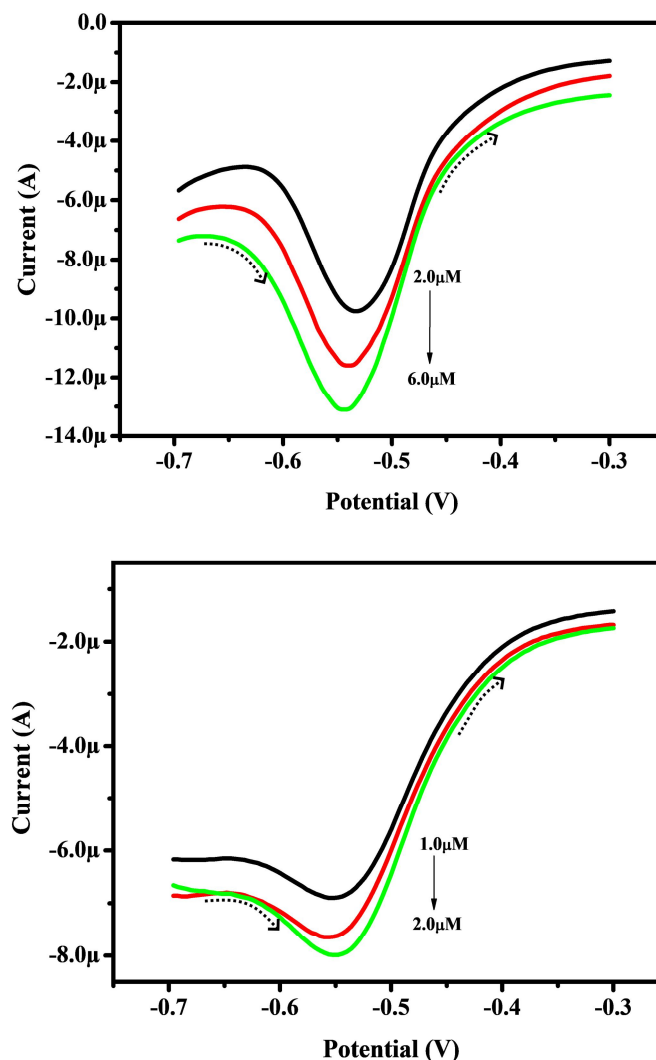
Fig. 10. (a) DPV curves for the quantification of RF in B-complex tablet in 0.1 M PBS (pH 7.0) at the scan rate of 0.05 V s^{-1}, (b) DPV curves for the quantification of RF in blood serum in 0.1 M PBS (pH 7.0) at the scan rate of 0.05 V s^{-1}.

CONCLUSIONS

Carbon paste electrode adopted with threonine can be employed for the quantification of RF by DPV. The utmost benefit of every electrode is trouble-free cleaning and revival of their surface before the sequence of measurements. It is observed that the introduction of threonine in carbon paste electrode shows affinity towards the determination of RF with good reproducibility and a lower detection limit. The electrode preparation is very simple, has high precision and better sensitivity, is inexpensive and provides rapid response in the determination of the concentration of RF in solutions. The results have also shown that the instantaneous determination of RF with elevated accurateness in the presence of some other vitamins is possible.

ACKNOWLEDGMENTS

REFERENCES

[1] J.Y. Lee, M.Y. Jung, Food Chem. 220 (2017) 289.

[2] S.E. Sa, S.P. Silva, L.C. Jost, A. Spinelli, Sens. Actuators B. 209 (2015) 423.

[3] A. Maseka, E. Chrzescijanska, M. Zaborski, M. Maciejewska, C.R Chim. 15 (2012) 524.

[4] S.C. Sanches, L.N.Z. Ramalho, M. Mendes-Braz, V.A. Terra, R. Cecchini, M.J. Augusto, F.S. Ramalho, Food Chem. Toxicol. 67 (2014) 65.

[5] M. Roushani, F. Shahdost-fard, Sens. Actuators B. 207 (2014) 764.

[6] H.J. Zhang, Y.T. Gao, H.B. Xiong, Food Anal. Methods. 10 (2017) 399.

[7] M. McGuire, K.A. Beerman, Nutritional Sciences: from fundamentals to food, 2013 Wadsworth, America.

[8] T.G. Seiler, T. Ehmke, I. Fischinger, D. Zapp, O. Stachs, T. Seiler, A. Heisterkamp, Invest. Ophthalmol. Vis. Sci. 56 (2015) 6740.

[9] Y. Wang, P.H. Zhu, T. Tian, A. Tang, L. Wang, X.Y. Hu, J. Agric. Food Chem. 59 (2011) 12629.

[10] A. Bonamore, M. Gargano, L. Calistil, A. Francioso, L. Mosca, A. Boffi, R. Feaerico, Food Anal. Methods, 9 (2016) 840.

[11] M.L. Privitera, V.A. Lozano, Michrochem J. 133 (2017) 60.

[12] Y. Meng, X. Hun, Y. Zhang, X.L. Luo, Microchim. Acta 183 (2016) 2965.

[13] Q. Honglan, C. Zongze, H. Lina, Spectrochim. Acta A. 78 (2011) 211.

[14] S. Hustad, P.M. Ueland, J. Schneede, Clin. Chem. 45 (1999) 862.

[15] D.M. Stankovic, K. Kalcher, Electrochim. Acta 168 (2015) 76.

[16] L. Svorc, D.M. Stankovic, E. Mehmeti, K. Kalcher, Anal. Methods 6 (2014) 4853.

[17] K. Schachi, H. Alemu, K. Kalcher, J. Jezkova, I. Svancara, K. Vytras, Analyst 122 (1997) 985.

[18] Z. Taba, J. Wang, Electroanalysis 3 (1991) 215.

[19] T. Nie, J.K. Xu, L.M. Lu, K.X. Zhang, L. Bai, Y.P. Wen, Biosens Bioelectron. 50 (2013) 244.

[20] W. Qijin, Y. Nianjun, Z. Haili, Z. Xinpin, X. Bin, Talanta 55 (2001) 459.

[21] J.G. Manjunatha, Sens. Bio-Sensing Res. 16 (2017) 79.

[22] P.R. Roy, T. Okajima, T. Ohsaka, Bioelectrochem. 59 (2003) 11.

[23] J.G. Manjunatha, M. Deraman, N.H. Basri, I.A. Talib, Arab. J. Chem. 11 (2018) 149.

[24] W. Chen, X. Lin, I. Huang, H. Luo, Microchim. Acta 151 (2005) 101.

[25] D. Lakshmi, P. Sharma, B. Prasad, Biosens. Bioelectron. 22 (2007) 3302.

[26] A. Riccarda, L. Gorton, Biosens. Bioelectron. 22 (2007) 2611.

[27] J.G. Manjunatha, M. Deraman, N.H. Basri, N.S. Mohd Nor, I.A. Talib, N. Ataollahi, C.R. Chim. 17 (2014) 465.

[28] J.G. Manjunatha, J. Electrochem. Sci. Eng. 7 (2017) 39.

[29] H. Beitollahi, S.G. Ivari, M. Torkzadeh-Mahani, Mater. Sci. Eng. C. 69 (2016) 128.

[30] S. Jahani, H. Beitollahi, Anal. Bioanal. Electrochem. 8 (2016) 158.

[31] F. Karimian, G.H. Rounaghi, M. Mohadeszadeh , J. Anal. Chem. 71 (2016) 1057.

[32] E. Mehmeti, D.M. Stankovic, S. Chaiyo, L. Svorc, K. Kalcher, Mikrochim. Acta 183 (2016) 1619.

[33] Safavi, Afsaneh, Maleki, Norouz , Ershadifar, Hamid , Tajabadi, Fariba, Anal. Chim. Acta 674 (2010) 176.

[34] G. Tigari, J.G. Manjunatha, C. Raril, N. Hareesha, Chemistryselect 4 (2019) 2168.

[35] A.N. Ejheiehm, P. Pouladsaz, J. Ind. Eng. Chem. 2 (2013) 2146.

[36] L.C. Gribat, J.T. Babauta, H. Beyenal, N.A. Wall, J. Electroanal. Chem. 798 (2017) 42.

[37] N. Lavanya, S. Radhakrishnan, C. Sekar, M. Navaneethan, Y. Hayakawa, Analyst 138 (2013) 2061.

[38] A.A. Ensafi, E. Heydari-Bafrooei, M. Amini, Biosens. Bioelectron. 3 (2012) 376.

[39] R. Madhuvilakku, S. Alagar, R. Mariyappan, S. Piraman, Sens. Actuators, B. 253 (2017) 879.