JIMMA UNIVERSITY

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DEPARTMENT OF CHEMISTRY

ELECTROCHEMICAL DETERMINATION OF RIBOFLAVIN (VB2) USING 1, 4-BENZAQUINONE MODIFIED CARBON PASTE ELECTRODE

A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES, JIMMA UNIVERSITY, DEPARTMENTE OF CHEMISTRY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE (MSc) IN CHEMISTRY (ANALYTICAL CHEMISTRY)

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Acronyms/Abbreviation

VB2	Riboflavin
CPE	Carbon paste electrode
CME	Chemically modified electrode
HPLC	Higher performance liquid chromatography
FMN	Flavin mononucleotide
FAD	Flavin adenine dinucleotide
CV	Cyclic voltammetry
LSV	Linear sweep voltammetry
DME	Doping mercury electrode
BR	Britton–Robinson
1, 4-BQMCPE	1,4-Benzaquinone Modified Carbon Paste Electrode
RSD	Relative standard deviation
LOD	Limit of detection

Abstract

In this work a simple and sensitive electroanalytical method for the determination of riboflavin content using 1, 4-benzoquinone modified carbon paste electrode is demonstrated. The method is based on decrease in peak current of 1, 4-benzoquinone on addition of riboflavin. Cyclic voltammetric and linear sweep voltammetric techniques were utilized for the study. The 1, 4-benzoquinone modified carbon paste electrode exhibited a well-defined peak with reproducible peak current values for repetitive measurements; and showed a decrease in peak current value with an increase in riboflavin concentration. The result showed two linear range regions between 0.1 µM - 10 µM and 20 µM - 100 µM, with detection limits of 0.087 µM and 11.51 µM, respectively. The proposed method presented higher sensitivity compared to bare carbon paste electrodes. The modified electrode showed good reproducibility, stability and recovery for the analysis of riboflavin. The developed method would used for electroanalysis of riboflavin in pharmaceutical tablet.

Keywords: Electrochemical determination, riboflavin, carbon paste electrodes, 1, 4-benzaquinone.

1. Introduction

1.1. Background of the study

Vitamins are organic compounds found in foods. They are important for usual functioning of metabolism. They are not produced by the human body and almost all vitamins require to be obtained from dietary sources[1]. Vitamins differ with respect to their structure, chemical and biological properties and solubility. The main categorization for vitamins is based on solubility, as some are soluble in fat (A, D, E and K) while others are soluble in water (B and C)[2-3].

Riboflavin (7,8-dimethyl-10-ribityl-isoalloxazine) is a water soluble vitamin found in a wide variety of foods and pharmaceutical products[4]. It is a component of two coenzymes; flavin adenine dinucleotide (FAD) and flavin mononucleotiede (FMN), and plays important role in biological oxidation and reduction[5].

Riboflavin (VB2) is a coenzyme involved in sugar, protein and fat metabolism, promoting enlargement and cell renewal. VB2 acts as an intermediary in the transport of electrons in biological redox reactions and has an important function in cell growth[6]. It can promote skin, nails, hair normal growth, and eliminate the mouth, lips, tongue inflammation, encourage vision and reduce eye failing. At the same time, riboflavin is a kind of phototropic compound, photo taxis, and photodynamic treatment photosensitive agent[7].

As with all vitamins of the B complex, VB2 is only synthesized by microorganisms and higher vegetables, but animals achieve it from their food. High concentrations of B complex vitamins present in meat and milk, these foods are the best natural sources of riboflavin in the human diet[8].

VB2 is stable during heat processing, such as pasteurization, evaporation or condensation, which have little result on the variation in VB2 present in dairy products. Even so, significant loss may occur due to photo-degradation from open of food to light. With respect to water solubility information, VB2 declination takes place such as in juices, whey and cooking waters[4]. Hence, it is interesting not only to monitor the VB2 concentration in dairy products but also to study the influence of manufacturing and storage processes on its variation[9-10]. In addition, studies have shown that alterations in some of the organoleptic features of cattle milk and other liquids, like

wine and beverages, have association with VB2 photodegradation, which induces the degradation of sulfur-containing compounds by photochemical sensitization. In these cases, the loss of VB2 can be used as a natural indicator for the degradation parameters, such as source and intensity of light, treatment forms, heat treatment and storage time. Thus the determination of riboflavin is necessary, either from the nutritional point of view or to quantify parameters in several dairy foods and beverages[10].

Analytical methods for the determination of VB2 need to be both selective and sensitive because of the presence of potential interferences and low concentration of analytes. Many analytical methods for the determination of VB2 have been reported, including spectrophotometry[11], high-performance liquid chromatography (HPLC)[12]-[13], fluorescence spectrometry[14]and gas chromatog raphy[8]. But, these techniques are not available in all laboratories, consume time and complicated instrumentation[15].

Electroanalytical methods have received a great deal of interest because of its simple, portable, low cost, fast response time ,reduce solvent and sample consumption and fast analysis[7]. For this reason, the alternative electrochemical method can be simpler. In this context, nowadays, it is well established that one of the more promising areas of analytical applications of chemically modified electrodes is that in which immobilized redox mediators are used to make possible the charge-transfer between the electrode and analyte in solution at much lower potentials than unmodified electrodes[16].

There are few electroanalytical techniques reported for the determination of VB2 using mercury electrode[17], gold electrode[18], glassy carbon electrodes[19], and manganese dioxide modified glassy carbon electrode[20]. However, the direct electrochemical detection of VB2 at common electrode materials showed important practical drawbacks, such as high over-potential, poor selectivity, high irreversibility and electrode fouling.

This research work aims to determine VB2 using electrochemical method at 1, 4-BQMCPE. The electrode process would be investigated by cyclic voltammetry method. The influence of different experimental parameters such as pH, scan rate and effect of modifier composition would be investigated to optimize the proposed method. Finally the method would be applied for

the determination of VB2 in tablets, and the results obtained will be compared with earlier reported method.

1.2. Statement of the Problems

VB2 is one of the most widely distributed B vitamins, which participates in several cellular processes. VB2 plays vital roles in biological systems such as cellular respiration and metabolism of fats, carbohydrates, and proteins. Therefore, sensitive analytical methods for the routine determination of riboflavin in samples are highly desired.

The surface modification of the electrodes gives rise to high catalytic activation, which has been used in electrochemical catalytic reactions. Thus, a description of electroanalytical properties of several modification electrodes was done for electrochemical analysis of VB2 in different samples**.** Their drawbacks are high overpotential, less selectivity, less sensitivity, less efficiency with low concentration of analytes.

Therefore this study may answer for the following questions:

- Is 1, 4-BQMCPE having higher sensitivity for determination of VB2?
- Is bare carbon paste electrode has higher sensitivity for determination of VB2?
- What will be the responsible reason for having different response of electrodes?

1.3. Objective of the Study

1.3.1. General Objective

The general objective of the study is:

- ✓ To develop a sensitive, low-cost electrochemical method for the determination of VB2.

1.3.2. Specific Objectives

The specific objectives of the study are:

- ✓ To optimize electroanalytical parameters for determination of VB2 at 1,4-BQMCPE.
- ✓ To determine VB_2 under optimized condition at 1, 4-BQMCPE.
- ✓ To compare the performance of bare CPE with 1, 4-BQMCPE.
- ✓ To optimize parameters for modification of CPE with 1,4-BQ.

- ✓ To apply the modified electrode for real sample analysis.

1.4. Significance of the study

The main significance of this work can be:

- ➢ Development of simple sensor electrode for electrochemical determination of VB2 in tablet.
- ➢ Method development for simple and fast electrochemical determination of VB2 using 1, 4-BQMCPE.

1.5. Scope of the Study

This study was conducted to determine riboflavin (VB2) by 1,4-benzaqunone modified carbon paste electrode. The proposed electrochemical method was used as simple and sensitive analysis for VB2 at 1,4-BQMCPE. The optimum conditions were selected based on response to the intensity of VB2 reduction current during fabrication of 1,4-BQMCPE. These are: effects of composition of the modifiers and effects of pH buffer solutions.

1.6. Hypothesis

The use of modification in electroanalytical chemistry has been explored by several research groups because these 1, 4-BQMCPE surface electrochemically in a simple and controlled way. Therefore, electrochemical modifications of carbon paste electrode with 1, 4-BQ minimizes the high over potential which makes the electrode sensitive to different interferences from many electro active species. As a result; the proposed electrode may able to reduce over potential by minimizing the size, having high selectivity, high sensitivity and low detection limit to VB2 study.

2. Literature Review

2.1. Nomenclature, structure and physiochemical properties of VB2

First isolated, though not purified, as yellow fluorescent compounds from whey and different biological matrices more than 100 years ago, VB2 was originally known as lactochrome or lactoflavin[21]. It was not until the 1930s that the structure and synthesis of VB2 were determined by Karrer[22]. In the same decade, the coenzyme forms of VB2, namely flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) were isolated and structurally identified[23]. In fact, these terms are misnomers because FMN and FAD are basically not mono and dinucleotide respectively[24]. However, the names are still accepted and widely used. In the scientific context, the term “riboflavin” is used to refer to the parent VB2 molecule and in many cases can be used synonymously with VB2. The official IUPAC name of VB2 is 7, 8-dimethyl-10-ribityl-isoalloxazine[5]. The name "riboflavin" originates from the sugar moiety (ribitol) which is the reduced form of the pentose sugar ribose and the isoalloxazine ring moiety (commonly referred to as flavin ring) which imparts the yellow color to the oxidized molecule[25]. The reduced form, which occurs in metabolism along with the oxidized form, is colorless. The addition of phosphate group or adenosine-5′-diphosphate at the 5′ position of the ribityl side yields FMN and FAD respectively[25]. These groups can be removed through acid hydrolysis, which is utilized in analytical procedures to liberate the free form for the quantization of the total VB2. The structure of VB2 and other related compounds are shown in Figure .1 VB2 7, 8-dimethyl-10-ribityl-isoalloxazine.

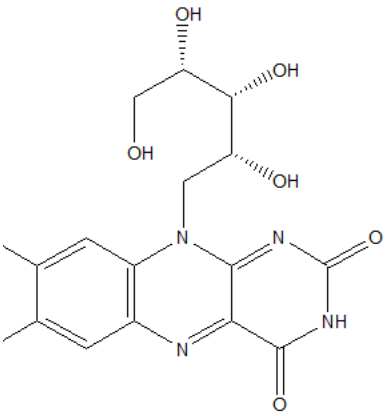


Figure 1. Chemical Structures of VB2

VB2 is an odorless orange crystalline powder with an unpleasant bitter taste and a melting point of about 280^{o}C[26]. In neutral aqueous solution, VB2 exhibits a strong yellow-green fluorescence. Though classified as a water-soluble vitamin, VB2 has a low solubility in water (10–13 mg/100 ml at 25–27.5^{o}C; 19 mg/100 ml at 40^{o}C; 230 mg/100 ml at 100^{o}C) [17]. It is carefully soluble in absolute ethanol (4.5 mg/100 ml at 27^{o}C) and not at all in acetone, diethyl ether, or chloroform. VB2 solubility can be better in dilute acid or alkali though it is not very stable in alkali. The presence of aromatic compounds is known to make VB2 more soluble in aqueous solution, which is utilized in pharmaceutical preparations. In compare, FMN and FAD are much more soluble than VB2 [5].

2.2. Stability and Degradation of VB2

In crystal form, VB2 is stable when stored in dry conditions[27]. Stability becomes a concern when VB2 occurs in solution as it is easily degraded by introduction to both UV and visible light[28]. The rate of this photodegradation process is speed up with elevated temperature and pH with the wavelength range of 350–520 nm exerting the greatest critical effects[3]. As shown in Figure 2, the nature of this degradation is the reduction of the isoalloxanzine ring by the ribityl side chain, resulting in the formation of lumichrome and lumiflavin under alkaline and acidic conditions, respectively[29]. Both of these degraded products do not exhibit VB2 activity.

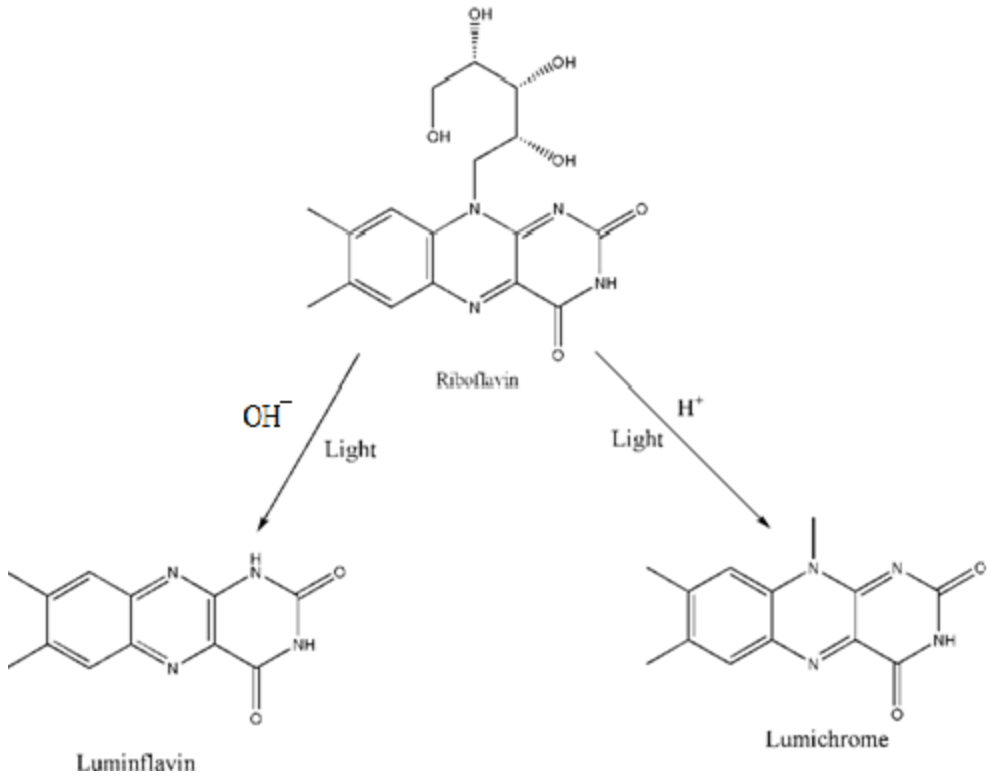


Figure 2. Photo-degradation of VB2 under basic and acidic conditions

Except for being light sensitive, VB2 is generally stable to heat and oxidation. If light is excluded, most food processing operations or normal cooking have little effect on VB2 content. Its stability increases as acidity increases with optimal stability to heat degradation being between pH 1.5 and 5.0. Low stability of flavins upon exposure to light is taken into consideration in food packaging[4]. Containers made of glass or other translucent materials are subjected to sunlight, which induces significant loss of VB2 in the food products such as milk and juices[30]. The same phenomenon even occurs in dry products such as enriched pasta over a protracted exposure to light during storage[24].

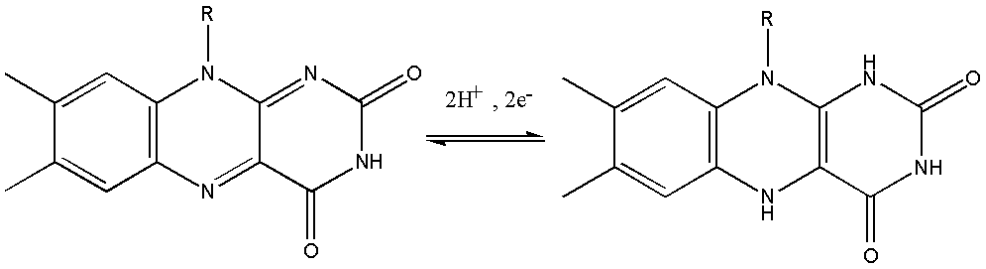
2.3. Nutritional and physiological functions of VB2

The three major biologically active forms of VB2 found in nature including VB2, riboflavin-50-phosphate (FMN) and riboflavin-5'-adenosyldiphosphate (FAD) have equal vitamin activity in human[31]. With the ability to participate in either one- or two-electron redox reactions, FMN and FAD can either act as cofactors for several flavoprotein enzymes that catalyze redox reactions in cells or serve as electron carriers in the mitochondrial electron transport system[32]. Some typical

reactions in agent metabolism that need VB2 include dehydrogenation, hydroxylations, oxidative decarboxylations, dioxygenations, and reductions of oxygen to hydrogen peroxide[31]. Performing as coenzymes of dehydrogenases, FMN and FAD are necessary to both glucose and fatty acid metabolism[27]. VB2 deficiency may also increase plasma homocysteine concentration, which is thought to be linked to an increased risk of cardiovascular disease[4]. Moreover, impaired iron absorption and even night blindness have been found to be associated with VB2 deficiency[33]. Like other water soluble vitamins, urinary riboflavin excretion occurs on a daily basis, therefore deficiency can happen when the dietary intake is low[17]. Symptoms of VB2 deficiency (a riboflavinosis) may include glossarist, angular stomatitis, angular cheilitis and dermatitis[31].

2.4. Mechanism of the Electrochemical Redox Reaction of VB2

The electrochemical determination of VB2 is possible due to its electro-reduction on the electrode surface. In general, it is believed that electro-reduction of VB2 is a reversible process involving two electrons and two protons (Figure 3)[34].



$R = C_5H_{11}O_4$

Figure 3: The electro-reduction mechanism of VB2.

2.5. Methods VB2 Detection

Many analytical methods for the determination of VB2 have been reported, including spectrophotometry[11], higher performance liquid chromatography (HPLC)[12]-[13], fluorescence spectrometry[14] and gas chromatography[8]. But, these techniques are not available in all laboratories, consume time and complicated instrumentation. Therefore, chemically modified electrodes were extensively used due to they are highly selective, sensitive, low cost of fabrication and low detection limit. To increase the sensitivity and other parameters of voltamme tric techniques with different modifiers has been cited[35]. Of the modifiers reported zeolite modif ied carbon-paste electrode with the limit of detection 0.71 μM[36], Bi-film copper electrode with

the limit of detection 100 nM[37], manganese dioxide modified glassy carbon electrode, with limit of detection 15 nM[20], [38], and hematite (α-Fe_2O_3) film modified rotating disk glassy carbon electrode[39] were employed in the detection of VB2 electrochemically.

2.6. Carbon Electrodes

Solid electrodes based on carbon are currently in widespread use in electroanalysis, primarily because of their broad potential window, low background current, low cost, chemical inertness, and suitability for various sensing detection applications[40]. In contrast electron transfer rates observed at carbon surfaces are often slower than observed at metal electrodes. A variety of electrode pretreatment procedures have been proposed to increase the electron transfer rates. The most popular carbon electrode materials are those involving glassy carbon, carbon paste, carbon fiber, screen printed carbon strips, carbon films, or other carbon composites[40].

2.6.1. Unmodified Carbon Paste Electrodes

In 1958, R.N. Adams discovered a new type of electrode by using a mixture of carbon powder with a liquid non-electroactive binder and called it as carbon paste. His original idea was to develop a dropping carbon electrode (DCE) that could be constructed similarly like the mercury electrode (DME). Although practical experiments with DCE failed, the mixture of carbon powder and a binder prepared in thicker consistency was presented as a new type of electrode material[41].

Binary Mixtures prepared from carbon powder and organic liquid of non-electrolytic character are known as bare or virgin or plain or unmodified carbon pastes[42]. As non-electrolytic binders paraffin oils are commonly used. These nonpolar pasting liquids should be chemically inert, insulating, nonvolatile, water immiscible and forming paste mixtures of fine consistency[41]. Silicone oils also represent common type of pasting liquids, especially when the problem of paraffin oils vulnerability in media with organic solvents is considered. Another group of binders is some liquid organophosphates. Their attractive property is a high ion-pairing ability[41].

The electric conductor in carbon pastes is graphite powder with micrometric particles. Carbon paste electrodes offer an easily renewable surface, low cost and very low background currents especially in the anodic region[41-43]. A disadvantage of carbon pastes is the tendency of the organic liquid binder to dissolve in solutions containing an appreciable fraction of organic

solvents. And also, the conventional paste mixtures from spectroscopic graphite powder and paraffin oil suffer from interferents when being polarized cathodically, and consequently they have been used mainly for the determination of easily oxidizable organic compounds[41-43].

2.6.2. Modified Carbon Paste Electrodes

The effort to make use of the favorable mechanical and electrochemical properties of carbon pastes for the preparation of a new design of sensors started at the beginning of the 1980s[43].

Modification of a carbon paste by impregnating the carbon particles with methanolic solution of dimethylglyoxime represents a milestone in the history of carbon paste electrodes. It was a first effort when a classic analytical reagent had served as elective modifier, thus initiating a very successful role of chemically modified carbon paste electrodes in electrochemical analysis[43].

Hand in hand with a rapid development of chemically modified carbon paste electrodes, the favorable mechanical and electrochemical properties of carbon pastes were tested for the preparation of special sensors containing enzymes allowing one to examine some enzymatically catalyzed reactions of biological substances. This way of attaching enzymes to an electrode material immediately attracted bioanalysts and carbon paste-based enzymatic biosensors had rapidly come to the front[44].

A modified carbon paste is a mixture of powdered graphite, nonelectrolytic liquid binder and a modifying agent. A modifier is usually one substance, but the carbon pastes can also be modified with two or more components. Among modifiers recently used [45].

2.6.3. 1,4-Benzaquinone Modified Carbon Paste Electrode (1, 4-BQMCPE)

Quinones are a class of compounds consisting of conjugated cyclic diketone organic systems as shown in Figure 4. Naturally, quinones function in cellular respiration, photosynthesis, and blood coagulation that occur in certain plants and animals. Their biological functions are mostly related to their electron transfer rates and redox potentials [44].

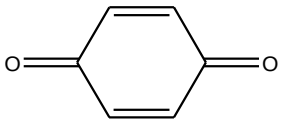


Figure 4: The structure of 1, 4-benzaquinone.

In aprotic solvents, 1,4-benzoquinone, shown in Figure 2, is reduced in two consecutive one electron steps to form hydroquinone radical anion and hydroquinonedianion[44] . BQ and hydroquinone redox couples have been widely used in electrochemical studies [44]. Moreover, BQ have been found important as modifying agents for the preparation of modified carbon paste electrodes.

The BQMCPE had shown remarkable advantages from its low noise levels. In another study, the electrochemical behavior of a tetrabromo-p-benzoquinone modified carbon paste electrode has been investigated [45]. The function of the BQ modified electrode for the electrocatalytic oxidation of ascorbic acid, dopamine and uric acid was found to be attractive. In addition, the simultaneous determination of the three components in the mixture was made possible based on differential pulse voltammetric technique by using the BQMCPE.

It was revealed that in aqueous buffer, at acidic, neutral and alkaline pH anthracyclines, anthraquinones, and other para-quinones are reduced by two electrons generating one reversible wave in cyclic voltammetry. At acidic pH the reduction is a single step two-electron two-proton process (Figure 5) and in alkaline pH the reduction does not involve proton and is only a case of two-electron reduction (Figure 6). At neutral pH, the reduction is either by one proton two electrons or only two electrons without the participation of proton [46].

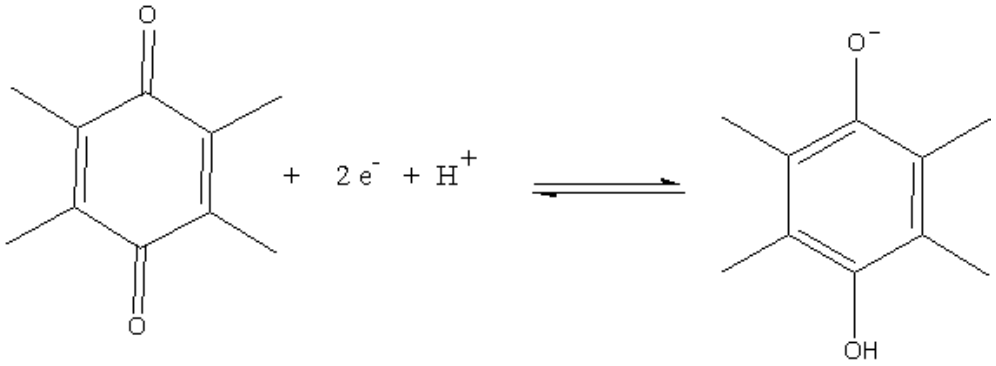


Figure 5: Two electron one-proton reduction of quinone in aqueous buffer

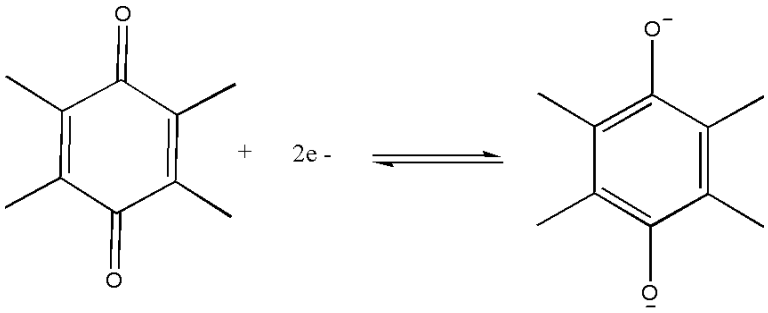


Figure 6: Two electron one-proton reduction of quinone in aqueous buffer

2.7. Voltammetric Techniques

Voltammetry is an electroanalytical technique based on the measurement of current flowing through an electrode dipped in solution containing electro active compounds while a potential is imposed upon it[46]**.** It is typically performed using a three electrode potentiostat, which accurately controls the applied potential. The redox reaction takes places at working electrode, because the working electrode is where the reaction of interest is taking place. If the working electrode is formed by drop of mercury, the analytical technique is called polarography [47]**.**

The second electrode is a reference electrode, which maintains a constant potential all through the experiments and the third electrode the counter electrode, which complete the electrical circuit. The counter electrode also known as the auxiliary electrode, is frequently much larger than working electrode to minimize current density at the electrode surface[47]**.** The common characteristic of all technique is that they occupy the application of a potential (E) to an electrode and monitoring of the resulting current (I) flowing throughout electrochemical cell[48]**.**

2.8. Cyclic Voltammetry (CV)

CV is one of the first electroanalytical techniques reported in 1938 and described theoretically in 1948 by Randles and Sevcik. It has developed extremely in popularity over the past few decades, so much so that obtaining CV is often the first experiment performed by the electrochemist, giving invaluable information as to the presence of electroactive species in solution or at the electrode surface. The effectiveness of CV results from its ability for fast observing redox behavior over the entire potential range available[49].

CV is an essential technique for initial electrochemical studies of new systems and has proven very useful in obtaining mechanistic information about fairly complicated electrode reactions.

Although CV is particularly useful in qualitative studies of electrode processes, it is less well suited for quantitative determinations. Its merits are, thus, largely in qualitative experiments. Quantitative measurements are best obtained by employing other (step or pulse) techniques[48-49]. The resulting plot of current versus potential is termed a cyclic voltammogram (Figure 7).

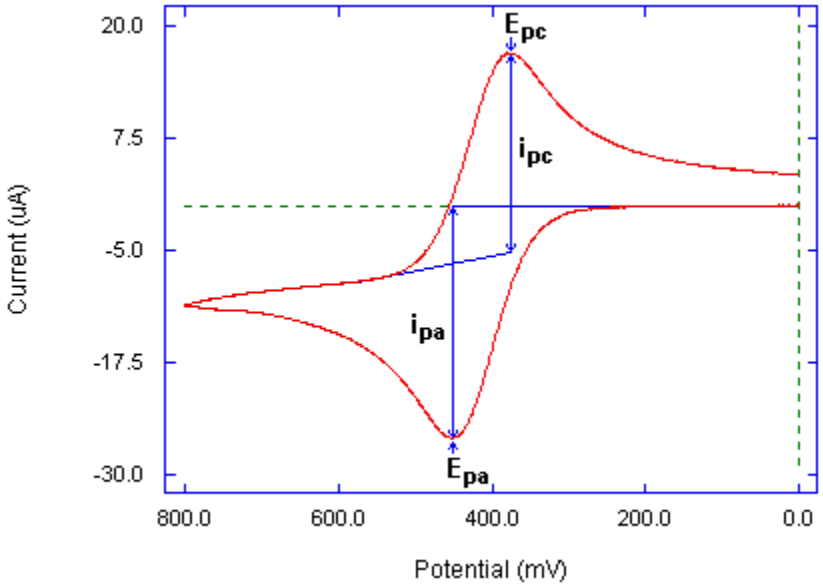


Figure 7: A typical cyclic voltammogram showing the important peak parameters

3. Materials and Methods

3.1. Chemicals

VB2 (Sigma Aldrich), 1, 4-benzaquinone (Riedel-De Haen,), paraffin oil (Fulka, Switzerland), graphite powder (BDH, UK), H_3BO_3 (Techno Pharmachem), HCl, 37% (Riedel de Ha en), NaOH (LeSOL laboratory reagent), H_3PO_4, 85% (Riedel de Haen), CH_3COOH, 100% (BDH), VB2 tablet (Ningbo Shuangwei Pharm. Co., Ltd) distilled water will be used to prepare all aqueous electrolyte solutions throughout the study.

3.2. Instruments

The electrochemical measurement was performed by using voltammetric analyzer with Epsilion EC-Ver 1.40.67 voltammetric analyzer (Bioanalytical System, USA), software, using a standard cell with three electrodes. The three electrode system consists of unmodified carbon paste electrode (UMCPE) or 1,4-bezaqunone modified carbon paste electrode (1, 4-BQMCPE) which was used as working electrode, Ag/AgCl reference electrode and a platinum wire counter electrode, all measurements were carrying out at the laboratory temperature.

3.3. Procedure for Preparation of Solution

3.3.1. Buffer solution

Britton–Robinson (B–R) buffer was prepared by dissolving 0.004 M H_3BO_3(Boric acid), 0.004 M H_3PO_4(Phosphoric acid) and 0.004 M CH_3COOH (Glacial acetic acid) in distilled water and adjusting to the required pH value with dilute hydrochloric acid solution (HCl) and sodium hydroxide (NaOH)[50].

3.3.2. Standard preparation

VB2 was prepared by dissolving 1.882 g of the component into 100 mL of distilled water. The solution was then stored in reagent bottle and covered with aluminum foil to prevent degradation on contact to light. Working solutions of vitamin standards was prepared daily by mixing and diluting the stock solutions in supporting electrolyte to preferred concentrations. Preparation steps was performed in the passive light condition using glassware covered with foil to continue vitamins from degradation[51-52].

3.4. Preparation of Electrodes

3.4.1. Preparation of Carbon Paste Electrode (CPE)

Unmodified carbon paste (0.1 g) was prepared by mixing graphite powder with paraffin oil. The composition of the paste is 70% (w/w) graphite powder and 30% (w/w) paraffin oil[16,53,54]. The mixture was homogenized with mortar and pestle for 30 minutes and allowed to rest for 24 hours. The homogenized paste is packed in to tip of Teflon tube (3 mm diameter, 7 mm deep). A copper wire was inserted from the backside of the Teflon tube to provide electrical contact. Then the surface of the electrode was smoothed against a smooth white paper until a shiny surface was emerged.

3.4.2. Preparation of 1, 4-Benzaqunone modified Carbon Paste Electrode (1, 4-BQMCPE)

The modified electrode containing the following percentage ratio of graphite powder, 1, 4-Benzaqunone (1, 4-BQ) and paraffin liquid were homogeneously mixed for 30 min. to give a uniform paste. For 5% (w/w) 1, 4-BQ, 65% (w/w) graphite powder, and 30 % (w/w) paraffin liquid utilized. In the same way, for 10% (w/w) 1, 4-BQ; 70 %(w/w) graphite powder, and 20 % (w/w) paraffin liquid used. For15 % (w/w) 1, 4-BQ; 65 % graphite powder, 20 % (w/w) paraffin liquid mixed. For 20 %(w/w) 1, 4-BQ; 60 %(w /w) graphite powders, and 20 %(w/w) paraffin liquid mixed. For 23 % (w/w) 1, 4-BQ; 57 % (w/w) graphite powders and 20 % (w/w) paraffin liquid mixed. For 25 %(w/w) 1, 4-BQ; 55 % (w/w) graphite powders, and 20 % (w/w) paraffin liquid mixed. And homogenized with mortar and pestle for 30 minutes[16,42]. The homogenized paste was packed at the tip of Teflon tube and kept at room temperature for 24 hrs. The electrode surface was smoothed against smooth white paper until a shiny surface was emerged before electrochemical measurements.

3.5. Procedure of Reproducibility, Repeatability and Stability Study of 1, 4-BQMCPE

The reproducibility of the 1, 4-BQMCPE was examined by measuring the same concentration of VB2 on three modified electrodes on three consecutive days with triplicate measurement on each day. The RSD of the measured current signal was calculated to determine reproducibility of the modification among the electrodes.

For the repeatability study, the 1, 4-BQMCPE was prepared under the optimum conditions. Triplicates successive voltammetric measurements of VB2 were made on the same day for a modified electrode. The RSD of the measurements was used to estimate the repeatability of the measurement.

For stability study, three 1, 4-BQMCPE were prepared on the same day. The LSV of VB2 was measured on different days. On each day, triplicate measurements were be made at an electrode. The average current signals of the triplicate measurements in the first day were compared to those of the last day to determine the stability of the electrode developed.

3.6. Interference Study

For the applicability of the 1, 4-BQMCPE for the determination of VB2 was evaluated by studying the selectivity of the method for the determination of VB2. Various possible interfering species such as, 0.1 mM ascorbic acid (AA), uric acid (UA), glucose (GL), Vitamin B1 (VB1) and Vitamin B6 (VB6) were added into the solution containing 1 μM of VB2. Then the percent change in the reduction current of VB2 was calculated up on addition of these interfering substances.

3.7. Procedures of Real Sample Preparation

VB2 tablets were purchased from local drug store (Ningbo Shuangwei Pharm. Co., Ltd.) and it was weighed and powdered in a mortar and pestle. 0.18 g of the powdered tablet was dissolved in to 100 mL volumetric flask and diluted with BR buffer. It was labeled that one multivitamin tablet contains 10 mg of VB2. Therefore, an amount corresponding to a stock solution of 10 mg of VB2 in the tablet in 100 mL is 0.266 mM. The diluted solution was directly analyzed by proposed method. Finally, 20 μL of tablet sample solutions was diluted in 10 mL BR buffer. Triplicate of LSV was measured and the mean values were recorded. Also three different solutions of VB2 solutions were prepared by mixing 20 μL of tablet sample solution with 1.00 μM, 3.00 μM and 6.00 μM standards of VB2 and the % recovery was calculated. The determination of VB2 in the tablet was carried out according to the linear regression equation formulated for the calibration curve.

3.8. Recovery Study

To evaluate the determination of VB2 by the developed method, 0.18 g of VB2 tablet was dissolved in 100 mL volumetric flask and filled with distilled water. And diluted with BR buffer solution of pH 1.5 and the concentration of VB2 were determined by standard addition method. And finally recovery was calculated by:

$$\%\,\mathrm{Recovery} = \frac{Amount\ of\ \ VB2\ actually\ found}{Amount\ \ VB2\ expected\ \ to\ found} \times 100$$

4. Results and Discussion

4.1. Cyclic Voltammetric Study of UMCPE and 1,4-BQMCPE in Buffer and in the Presence of VB2

The cyclic voltammogram of 1, 4-BQMCPE recorded in the buffer solution showed two peaks at potentials of 0.12 V and 0.56 V as shown in Figure 8B (a). The Figure shows that the electrochemical reaction of 1, 4-BQ was reversible. Whereas the cyclic voltammogram of UMCPE in buffer solution the two peaks are not observed (Figure 8A (a).

Cyclic voltammogram for the redox behavior of VB2 at the UMCPE and 1, 4-BQMCPE at pH 1.5 is shown in Figure 8A. As can be observed in Figure 8A (b), VB2 was undergoes no oxidation and reduction at UMCPE. The electrochemical redox behavior of 1, 4-BQMCPE in the presence of VB2 was shown in Figure 8B (b). The 1, 4- BQMCE peak current was found to decrease when VB2 was added to the buffer solution. This allows the determination of VB2 by analyzing the decrease in peak current up on addition of different concentration of VB2.

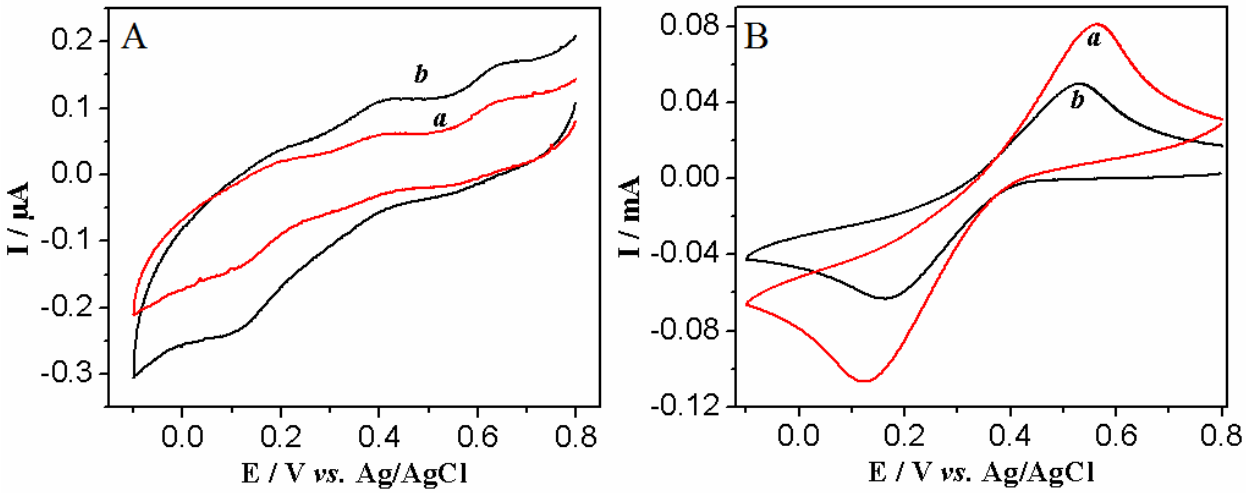


Figure 8. Cyclic voltammogram of a) BR buffer pH 1.5 b) 5 µM VB2 (pH 1.5) at UCPE (A) and 1, 4 BQMCPE (B); scan rate 50 mVs^{-1}.

The increase in the concentration of VB2 resulted in a successive decrease of 1, 4-BQ redox peak current, as observed in CV. The decrease in peak current was found to be directly correlated with the concentration of VB2.

The reason for the reduction of the peak current with increase in VB2 concentration is not well understood [16,55]. The assumptions were carefully studied for the responsible decrease of the voltammetric peak current of 1, 4- BQ in the existence of VB2 at 1, 4-BQMCPE. The assumption could be the formation of an electro-active complex at the surface of the electrode, which could result in a competitive surface adsorption as a possible reason for the decrease in peak current and it was studied by varying scan rate using 1, 4-BQMCPE. The result showed that the peak current of VB2 linearly increases with the square root of scan rate which shows diffusion controlled mechanism (Figure 11). Therefore, competitive surface adsorption is not a reason for the decrease in peak current up on successive addition of different concentrations of VB2.

4.2. Optimization of Experimental Conditions

The optimum experimental parameters have been studied to obtain optimum experimental conditions for cyclic and linear sweep volammetric determination of VB2 at 1, 4-BQMCPE.

4.2.1. Effects of pH

In order to improve the performance of 1, 4-BQMCPE, the pH of supporting electrolytes influences the response of the electrode. Therefore, the effect of pH was studied. The effect of the pH of supporting electrolyte on the electrode response was tested in the range from 1.5 to 6.0, and shown in Figure 9 below.

Optimization of pH was performed by reading the CV maximum reduction current response to 1, 4-BQCPE. So with increasing of buffer pH, the oxidation and reduction current decreased. The electrochemical reaction of 1, 4-BQ and the reduction of 1, 4-BQ becomes irreversible at higher pH[16]. Maximum peak current is observed at pH 1.5; therefore, pH of 1.5 was selected as optimum pH for further study.

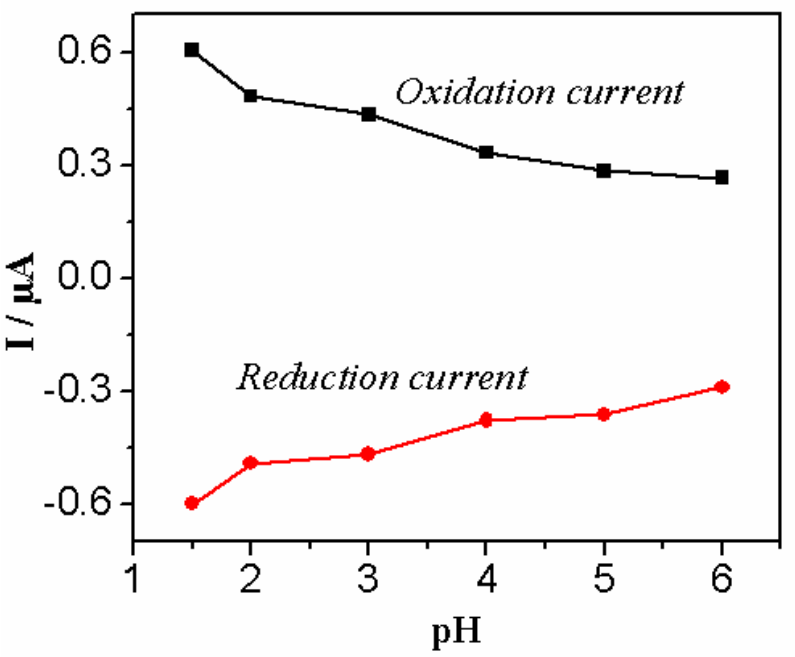


Figure 9. Plot of the response of 1, 4-BQCPE to various pH values containing 5 µM VB2.

4.2.2. Effects of Composition of Modifiers

The amount of 1, 4-BQ in the carbon paste on the voltammetric response of the modified carbon paste electrode was studied by varying the amount of 1, 4-BQ, graphite powder and paraffin oil. As shown on Figure 10, the maximum peak current was observed at 20 % (w/w) composition of 1, 4- BQ in the carbon paste. For amounts higher than 20 % (w/w) the peak currents decreased. This may be due to a decrease in the graphite content in the paste and, consequently reduction of the electric conductivity of the electrode[53]. The best composition for the electrode was found to be, 20 % (w/w) 1, 4-BQ, 60 % (w/w) graphite powder and 20 % (w/w) paraffin oil.

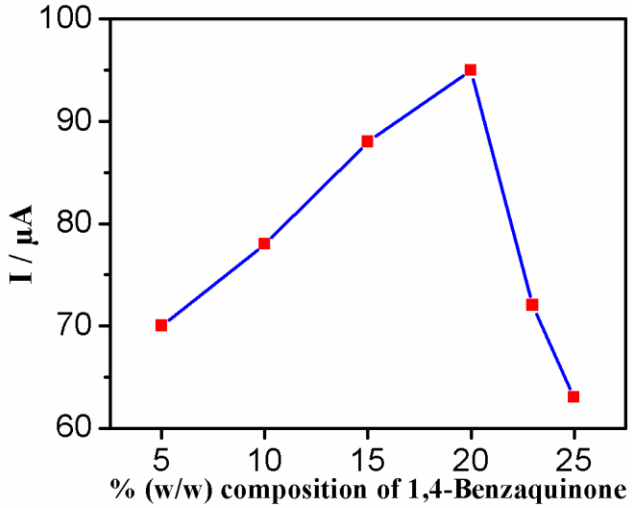


Figure 10. Effect of composition of 1, 4-Bezaquinone on the reduction peak current

4.2.3. Effects of Scan Rate

Effect of scan rate on peak currents and peak potentials was studied under optimized parameters. It was observed that peak current for oxidation and reduction of riboflavin linearly increases with the square root of scan rate, representing that both processes on the electrode surface are controlled by diffusion rather than adsorption. The linear dependence for both peaks can be expressed by the regression equations:

$y = 0.0182\,x + 0.00993$, $R^2 = 0.992$, for oxidation, and

$y = -0.0176\,x + 0.00522$, $R^2 = 0.993$ for reduction.

With increasing of scan rate, peak potentials for both processes shift to negative and positive potential which is characteristics of reversible electrochemical reaction[20], and the anodic peak and cathodic peak current increases at both oxidation and reduction. Figure 11 cyclic voltammograms were recorded in 5 µM VB2 for the modified electrodes at scan rate from 10 to 300 mVs^{-1}.

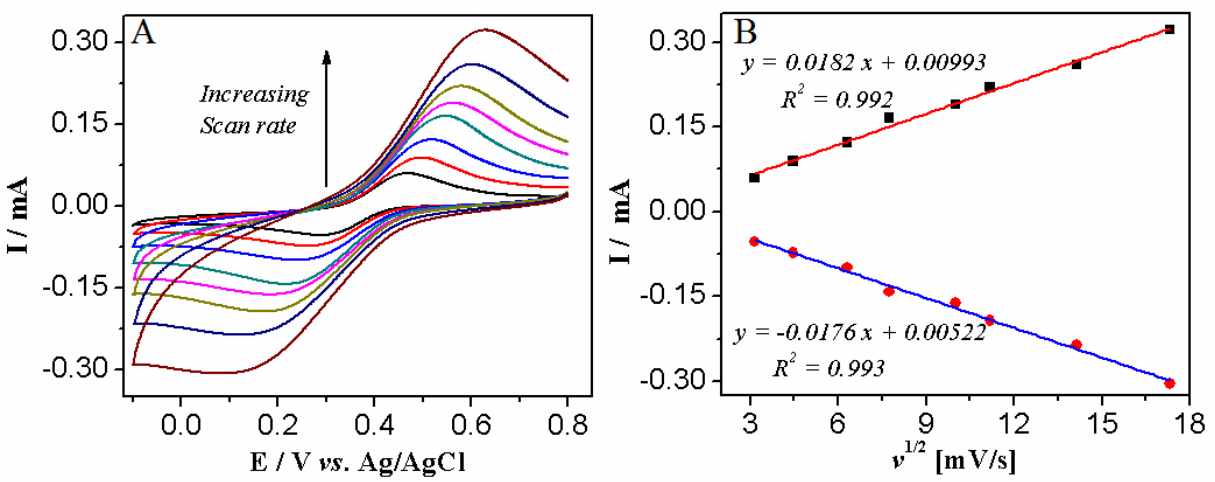


Figure 11. CV voltammograms of 5 µM VB2 at 1, 4-BQMCPEs in BRBS at pH 1.5 at different scan rates, from 10- 300 mVs^{-1}.

4.3. Calibration Plot for Riboflavin

The 1, 4-BQMCPE exhibited a well-defined peak with reproducible peak current values for repetitive measurements; and showed a decrease in peak current value with an increase in riboflavin concentration as shown in Figure 12. The result shows two linear range regions between 0.1 µM and 10 µM and 20 µM and 100 µM, with regression equations:

$y = -4.33\,x + 88.43$ (*x* concentration in µM), $R^2 = 0.995$

$y = -0.123\,x + 37.94$ (*x* concentration in µM), $R^2 = 0.992$, respectively.

A limit of detection of 0.087 µM and 11.51 µM (*3s/m*), respectively for the two linear ranges.

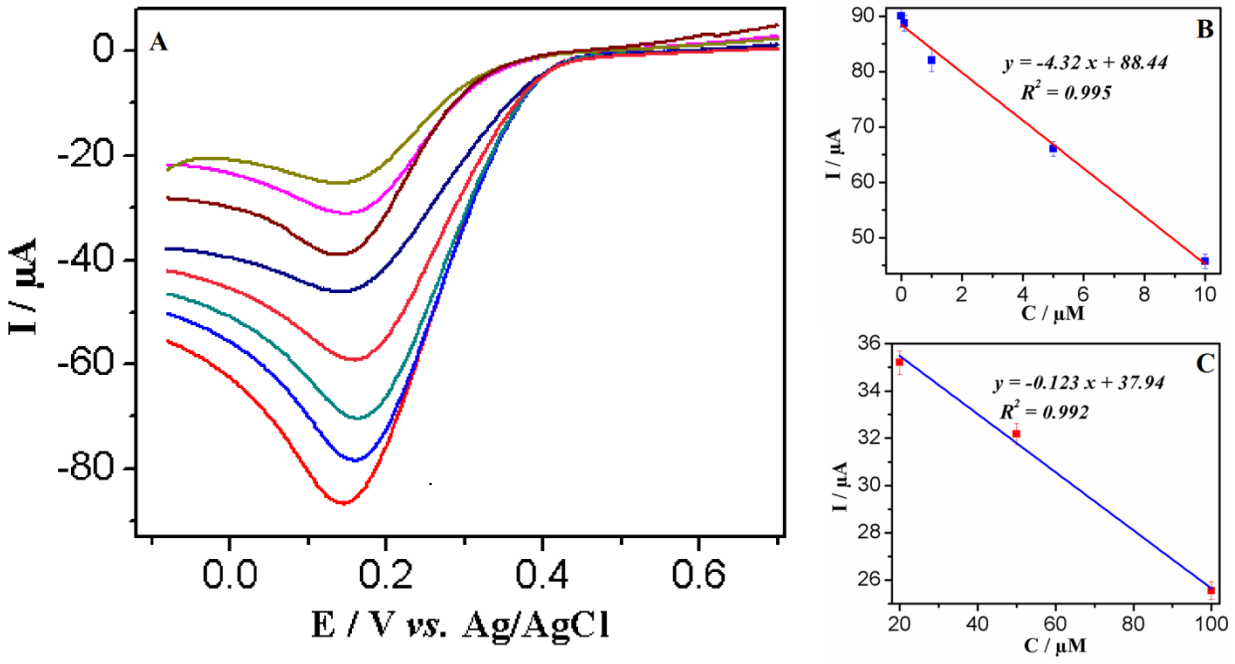


Figure 12. Linear sweep Voltammgram of 1, 4-BQCPE at different VB2 concentrations (A), the corresponding calibration curve from 0.1 – 10 µM (B) and from 20 – 100 µM (C).

The analytical performance of the proposed electrode was compared with other reported electrodes and results are summarized in Table 1. It can be seen from the Table that the proposed electrode has wider linear range with a good sensitivity and reproducibility in contrast with previously reported data this sensor acquire comparable for the quantification of VB2.

Table 1. Comparison between the Developed Method and Other Reported Method.

Electrode	**Modifier**	**Method**	**LOD(µM)**	**Linear range (µM)**	**Ref.**
Carbon paste electrode	Zeolite	CV	0.71	1.7 – 34	[36]
Copper electrode	Bi	SWAdSV	0.1 and 1.1	0.3-0.8 and 1.0-9.0	[37]
Rotating disk glassy carbon	Hematite(α-Fe_2O_3)	SWV	8.4	1.3-100	[39]
Glassy carbon electrode	MnO_2	DPV	0.015	0.02 - 9	[20]
Carbon paste electrode	1,4-BQ	LSV	0.087	0.1 - 10 and 10 – 100	This work

4.4. Repeatability, Reproducibility and Stability of 1,4-BQMCPE

The repeatability of 1, 4-BQCPE was expected through the relative standard deviation of three replicate determination of a solution of 5 μM VB2. The RSD were obtained to be 4.7%.

The reproducibility between electrodes was measured through the similar approach utilizing three different electrodes. The RSD was found 7.7% in a solution 5 μM VB2. This result confirmed that the repeatability and reproducibility of the electrode was appropriate.

The stability of electrode was assessed by inquiring of the electrode response to 5 μM of VB2 after being kept at room temperature for 14 days. It was seen that, the current response conserved almost 95.4 % of its initial value.

4.5. Interference Study

The applicability of the 1, 4-BQMCPE for the determination of VB2 was evaluated by studying the selectivity of the method for the determination of VB2. Various possible interfering species were added into the solution containing 1 μM of VB2. The result was shown in Table 2. It was observed that 0.1 mM ascorbic acid (AA), uric acid (UA), glucose (GL), and VB1 have negligible interference with the determination of 1 μM VB2. But 8.2% of the reduction current of VB2 was decreased by adding 10 μM VB6.

Table 2. Study of effect of interferences

Interferences	**Change in reduction currents (%)**
Ascorbic acid	- 0.91
Glucose	1.78
Uric acid	1.3
VB1	1.1
VB6	-8.2

4.6. Real Sample Analysis and Recovery study

The applicability of 1, 4-BQMCPE for the determination of VB2 was demonstrated by applying it to determine the VB2 content in pharmaceutical tablets. This sample was prepared as described in Section 3.7. Briefly, the tablet from Ningbo Shuangwei Pharm. Co., Ltd. was weighed and

powdered in a mortar and pestle. 0.18 g of the powdered tablet was dissolved in to 100 mL volumetric flask and diluted with BR buffer. It was labeled that one multivitamin tablet contains 10 mg of VB2. Therefore, an amount corresponding to a stock solution of 10 mg of VB2 in the tablet in 100 mL is 0.266 mM. The diluted solution was directly analyzed by proposed method. Finally, 20 µL of tablet sample solutions was diluted in 10 mL BR buffer. Triplicate of LSV was measured and the mean values were recorded. Also three different solutions of VB2 solutions were prepared by mixing 20 µL of tablet sample solution with 1.00 µM, 3.00 µM and 6.00 µM standards of VB2 and the % recovery was calculated. The determination of VB2 in the tablet was carried out according to the linear regression equation formulated for the calibration curve. The percent recovery was performed to evaluate the accuracy of the method and the results are presented in Table 3 and 4.

Table 3. Amount of VB2 detected in the multivitamin tablet using the developed method.

Expected (µM)	Detected		Labeled value (mg/tablet)	% recovery
	In µM	mg/tablet		
0.54	0.53	9.82	10.00	98.15

Table 4: Percentage recovery of VB2 from pharmaceutical tablet.

Present (µM)	Added (µM)	Expected (µM)	Found (µM)	%Recovery	%RSD
0.54	1.00	1.54	1.59	103.25	4.50
	3.00	3.54	3.61	101.98	4.91
	6.00	6.54	6.52	99.69	5.21

5. Conclusions

In summary, in this work the application of 1, 4-BQMCPE peak current reduction for the determination of VB2 is investigated .The method presented is simple, sensitive and cost effective. It can thus be concluded that the 1, 4-BQMCPE method is comparable with well established techniques for the quantitative analysis of VB2. The linear working range for the first one is very lower and the detection limit was enhanced to allow a sensitive detection of VB2. The developed method provided a low limit of detection, good repeatability and appropriate selectivity. The method is simple; fast and applicable in the regular analysis. The proposed method here can be successfully applied for determining VB2 in pharmaceutical tablets.

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