Available Online at

http://www.ijcpa.in

April-June 2017

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726 ; pISSN : 2395-2466

DOI : http://dx.doi.org/10.21276/ijcpa

Research Article | Volume-4 | Issue-3 | Article ID: 1335

DEVELOPMENT OF A LOW-COST PORTABLE PAPER-BASED MICROFLUIDIC DEVICE FOR THE DETECTION AND QUANTIFICATION OF VITAMIN B12

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Received: 9 June 2017 / Revised: 15 June 2017 / Accepted: 25 June 2017 / Available online : 29 June 2017

ABSTRACT

This paper reports the development of a simple, sensitive and low-cost microfluidic paper-based electrochemical sensing device developed for the detection of vitamin B12 in foods and pharmaceutical products. This hydrophobic barrier device was constructed on paper using commercially available varnish paint. The working electrode was constructed using a mixture of varnish and graphite powder. Silver conductive ink and a 6B pencil were used to fabricate the reference and counter electrodes, respectively. Cyclic voltammograms of standard vitamin B12 and real samples were collected from -2 V to +2 V at a scan rate of 200 mV/s. Currents of cyclic voltammograms at +2 V for the concentrations of vitamin B12 from 5 – 25 mM produced a linear relationship (r^2 of 0.98). The calculated limit of detection of this paper-based device is 3.7 mM.

Keywords – *Vitamin B12; Low-cost paper-based device; Electrochemical detection; Portable*

1. INTRODUCTION

Vitamin B12 (Cobalamin) is a complex structure containing a central cobalt ion complexed with organic ligands. A *Corrin* ring binds with four binding sites of the cobalt ion and the fifth site is occupied by the dimethylbenzimidazole group to form the basic skeleton of vitamin B12.[1] The sixth site of the cobalt ion is occupied by any group of cyano, hydroxyl, methyl, and 5'-deoxyadenosyl groups. Cobalamin is required to maintain many biological functions in human body. This vitamin cannot be synthesized by the human body and it must be obtained from foods or supplements. Insufficient intake of vitamin B12 may lead to many medical complications such as pernicious anemia.[2] Quantification of vitamin B12 is a useful approach to determine the quality of foods and pharmaceutical supplementary products such as tablets, capsules, and injections. Quantification of vitamin B12 can be done using microbiological, radioisotopic dilution, electrochemical, chromatographic and spectroscopic methods.[3,4] Few drawbacks of these methods are that these methods require advanced instruments and trained technicians, expensive and cannot be performed on site. Most electrochemical methods are based on the redox activity of the cobalt metal ion attached to the *corrin* ring. Change in the oxidation state of the Co(III) ion to Co(II) or Co(I) releases the group attached to sixth site in vitamin B12.[1,3] As reported previously, reduction of Co(III) to Co(II) or Co(II) to Co(I) can be used for the electrochemical quantification of vitamin B12. [1,3] In addition to Co(III) reduction, the oxidation of the *corrin* ring to form cationic radical can also be used for the quantification of this vitamin.[1,3] This paper describes a low

cost electrochemical paper-based microfluidic device (µPAD) to quantify vitamin B12 in food samples. A paper-based tool for the quantification of vitamin B12 has not been reported previously. The test zone of the electrochemical tool described in this paper is developed on a Whatman No 1 filter paper using varnish paint. Silver conductive ink is used to develop reference electrode and the working electrode is developed using a varnish graphite powder mixture. Paper-based devices are well known for their use as portable devices that can be used onsite to collect information.[5] The proposed device can be used as a portable low-cost device for the detection of vitamin B12.

2. MATERIALS AND METHODS

2.1 Chemicals

Vitamin B12, ethylenediaminetetraacetic acid disodium salt (EDTA), and sodium hydroxide (NaOH) were used as they were received. All reagents were of analytical grade and purchased from Sigma-Aldrich. All chemicals were used as they were received. Double distilled water was used to prepare all solutions. Polyurethane varnish, 6B pencils, and silver conductive ink were purchased from the local market.

2.2 Development of the electrochemical paper-based microfluidic device

The Whatman No 1 filter paper was used as the paper substrate to develop the sensor device. The hydrophobic barriers were created manually using polyurethane varnish. The carbon paste for the fabrication of working electrode was prepared by mixing 0.2 g of varnish with 0.4 g of graphite powder. The reference electrode was fabricated using a silver conductive ink pen. A graphite pencil (6B) was used to draw the counter electrode.

2.3 Preparation of standard vitamin B12 solutions

A series of vitamin B12 standard solutions with the concentration of 5, 10, 15, and 20 mM was prepared in 0.1 M EDTA buffered at pH = 9.6.

2.4 Analysis of vitamin B12

Pine wave driver 20 potentiostat was used to perform all electrochemical experiments. A volume of 100 µL of each vitamin B12 standard solution was introduced to the device and the cyclic voltammetric analysis was carried out from -2.0 to +2.0 V at a scan rate of 200 mV/s. The Current produced at +2.0 V for each standard solution was used to develop the calibration plot.

2.5 Analysis of real samples

Egg white and milk samples were prepared by mixing 0.1 M (pH = 9.6) EDTA (pH = 9.6). These prepared samples were analyzed using the same method used for the analysis of standard vitamin B12.

3. RESULTS AND DISCUSSION

Varnish was used to fabricate the hydrophilic channels on the Whatman No 1 filter paper to develop the device platform. This approach is more convenient and economical than the previously reported wax printing techniques. The working electrode was developed using the graphite-varnish mixture. This method can be used to develop working electrodes with any modifier by mixing with the graphite-varnish mixture. The reference and the auxiliary electrodes were fabricated as reported previously using Ag conductive ink and a 6HB pencil, respectively.[5] Since the device is developed based on low-cost materials and a simple fabrication method, it can be considered as a cost-effective alternative method to determine vitamin B12. Also, this is a one time use only device and that eliminates the possible cross contamination due to the use of the same device for multiple sample analysis.[6] The proposed device is shown in Figure 01, and the red color developed at the test zone is due to the typical red color of vitamin B12.

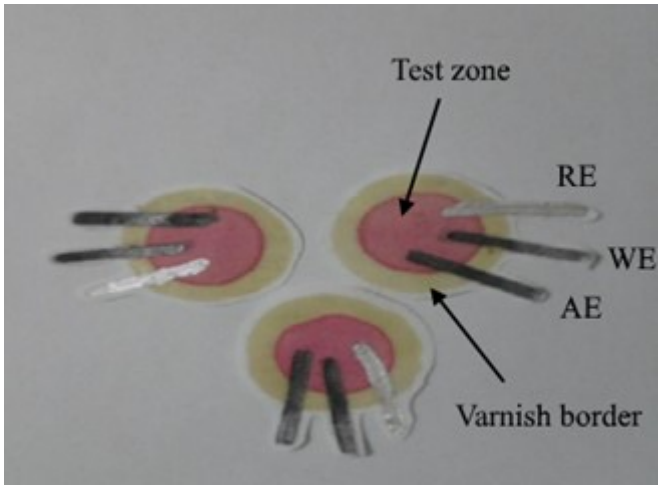

Figure 1. The device fabricated with varnish hydrophobic barrier. The working (WE), reference (RE) and auxiliary (AE) electrodes are shown on the diagram. The diameter of the device is approximately 1.5 cm.

Most electroanalytical methods used for quantification of vitamin B12 are based on the reduction process of Co(III) metal ion located at the center. However, the oxidation of the *corrin* ring also can be used for the detection and quantification of vitamin B12. Electrochemical behavior of Co(III) ion and the *corrin* ring were previously reported by many research groups. These studies were performed using glassy carbon and graphite screen printed electrodes. The oxidation process of the *corrin* ring is shown in Figure 02. Four nitrogen atoms represent the *corrin* ring of vitamin B12, and bonding at the sixth site is shown using the arrow connected to the Co ion from the top. Oxidation of vitamin B12 *corrin* ring is shown in Figure 02-(b) to Figure 02-(c). The oxidation of the *corrin* ring occurs at 1.2 V versus Ag/AgCl reference electrode.

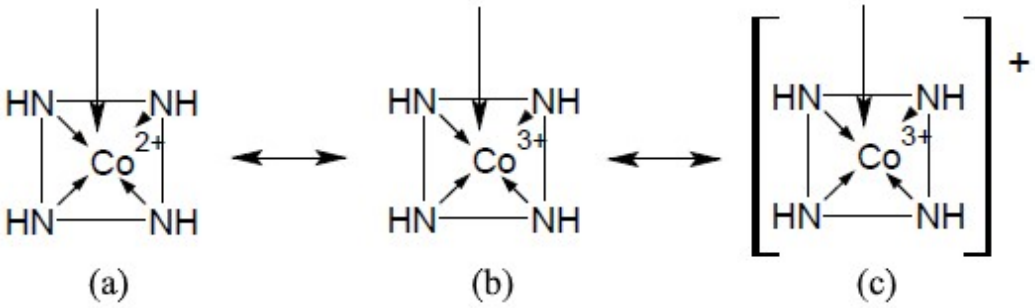

Figure 2: Reduced (a), normal (b) and oxidized (c) forms of vitamin B12.

Even though reduction currents of Co(III) ion to Co(II) or Co(I) are reported by many research groups, dissolved oxygen can act as an interfering agent in the detection process. However, dissolved oxygen does not interfere with the oxidation current of the *corrin* ring. EDTA is used to eliminate other possible interferences and pH = 9.6 and a 100 mV/s scan rate were used as optimum conditions. The cyclic voltammogram of vitamin B12 produced using the proposed low-cost paper-based device showed a rapid increase in the current beyond +1 V. Cyclic voltammograms collected with standard vitamin B12 solutions are shown in Figure 03.

The response on the paper-based device for various selected standard vitamin B12 solutions were recorded. The best linear relationship was observed between the current at +2 V and the vitamin B12 concentration range of 5 mM to 25 mM with an r^2 of 0.98. The resultant calibration plot is shown in Figure 04. The corresponding linear equation for this calibration curve was y = 3E-05*x* + 6E-05. The limit of detection was calculated using the relationship of LOD = 3.3(Sy/S), where Sy and S are the standard deviation of the slope

and the slope of the calibration curve, respectively.[7] The calculated LOD for this proposed paper-based device is 3.7 mM. The proposed device was tested by analyzing two laboratory prepared food samples.

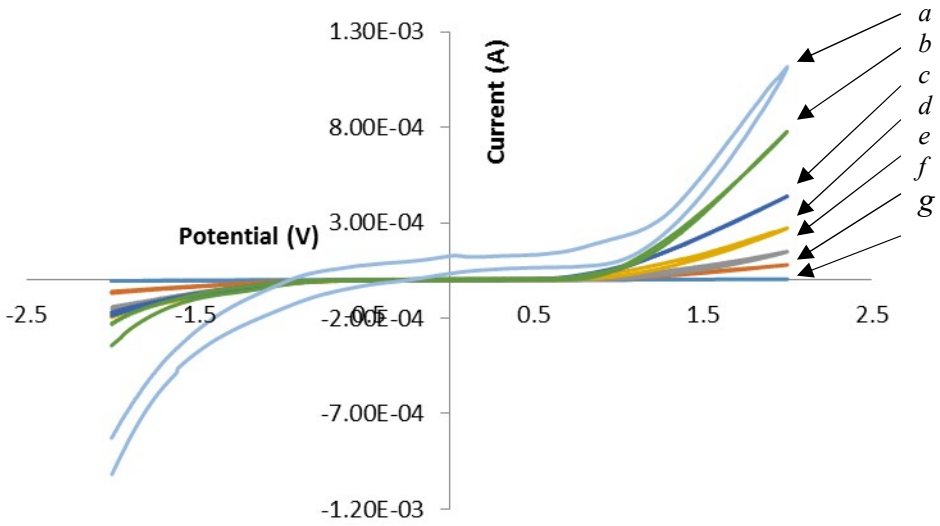

Figure 3. Cyclic voltammograms collected using the paper-based device for standard vitamin B12 solutions, *a* =25 mM, *b* = 20 mM, *c* = 15 mM, *d* = 10 mM, *e* = 5 mM, *f* = 1 mM, and *g* = blank solution.

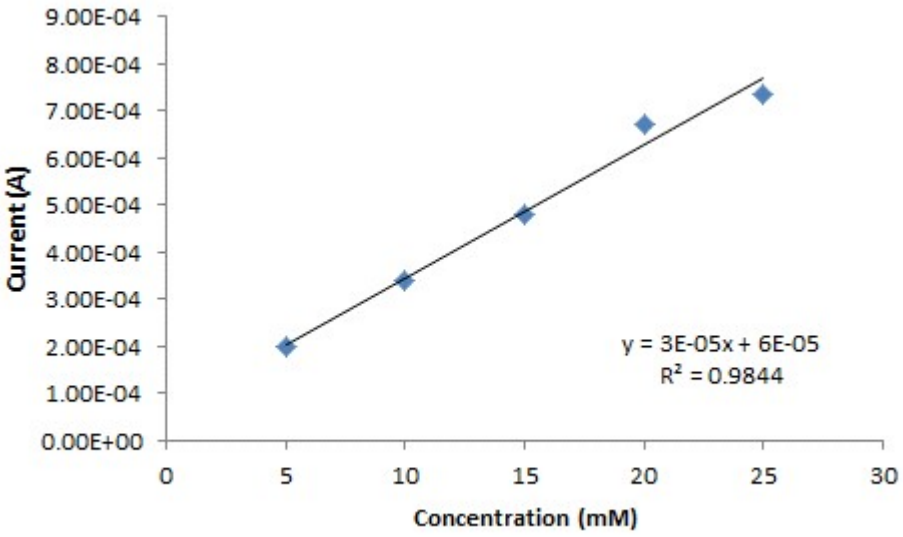

Figure 4. The calibration curve obtained between the vitamin B12 standard solutions and the current produced at +2 V for the cyclic voltammograms collected using the paper-based device.

4. CONCLUSIONS

The proposed low-cost portable device is capable of detecting vitamin B12 successfully in solutions with a concentration of 3.7 mM. This device developed a linear relationship between the concentration of vitamin B12 and current at +2 V over the concentration range of 5 mM to 25 mM. This device can be used to detect the level of vitamin B12 in pharmaceutical supplements and food products fortified with vitamin B12.

5. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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