



## Electrochemical Sensor Based on Poly(Methylene Blue) with Zinc Oxide Nanoparticles on Glassy Carbon Electrodes for Quantitative Determination of Vitamin B<sub>12</sub>

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### Abstract

Vitamin B<sub>12</sub> is classified as being amongst the hydrophilic vitamins, and plays a crucial role in mortal physiology, and hemoglobin formation and function. It also promotes anti-inflammatory action and moderates risk from viral infection. This paper established an electroanalysis method to quantify vitamin B<sub>12</sub> in commercially available supplements by designing a sensor using methylene blue and zinc oxide nanoparticles on a glassy carbon electrode (PMB/ZnO NPs/GCE) via cyclic voltammetry. This offers a wide linear range with high sensitivity for vitamin B<sub>12</sub> detection via differential pulse voltammetry using the Co(II/I) redox pair. Sensor morphology and thickness were studied, as was the pH of the supporting electrolyte. To understand the influence co-species, an interference study was also conducted. Under optimized conditions, the redox peak current for Co(II/I) pair at -0.8 V vs. Ag/AgCl, with the linear relationship  $I_p = 0.0673x + 0.3449$ ,  $r = 0.9942$ , showed a linear quantification range for vitamin B<sub>12</sub> concentrations of 0.099–69.51 μM. The limit of detection was 0.0104 μM. Repeatability, sensitivity, and stability were evaluated. The PMB/ZnO NPs/GCE electrode developed was successfully applied to the determination of vitamin B<sub>12</sub> in commercially available supplements. The recoveries obtained were in the range 97.1-104% for the injection and 95.9-103.3% for the tablet. The results obtained herein compared favorably with those from the current standard quantification via UV-vis spectrometry.

**Keywords:** electrochemical sensor, glassy carbon electrode, determination of VB<sub>12</sub>, polymethylene blue, zinc oxide nanoparticles

### Introduction

Vitamin B<sub>12</sub> (VB<sub>12</sub>) or cobalamine, is grouped amongst the hydrophilic (water-soluble) vitamins and considered one of the more unique organometallic complexes, if not the most complicated [1]. The Co atom is equatorially coordinated by a corrin ligand to form a corrin ring (four reduced pyrrole rings); whilst a nucleotide loop with histidine nitrogen base is bound to the cobalt at the axial position on the lower side [2, 3]. VB<sub>12</sub> has a crucial role in mortal physiology [4] after conversion to its active form inside animal cells, and then drives catalytic reactions like metallization and isomerization [5], as well as in maintaining the nervous system, hemoglobin formation and function, and genetic material expression [6]. Further, VB<sub>12</sub> has a crucial role in refining immune functioning, as its anti-inflammatory action reduces the risk of viral infection [7]. The latest clinical studies show a

potential role of VB<sub>12</sub> supplements in significantly reducing or the severity of COVID-19 and its complications preventing them altogether [8].

Based on the above, an increased interest in prescribing VB<sub>12</sub> supplements over the last year has been noted, where VB<sub>12</sub> can be administered in the form of an injection, tablet, healthy medicinal drink, or as a nasal spray to treat VB<sub>12</sub> depletion; more recently, toothpaste has been used to avoid VB<sub>12</sub> deficiency [9,10]

This in turn implies the need for the development of simple and accurate analytical methods for the quantitative analysis of VB<sub>12</sub> in pharmaceuticals in order to ensure they are of high quality. To date, the most commonly used methods are highly involved, i.e., chromatographic [11], spectrophotometric [12,13], electrophoretic [14], chemiluminescence, radioimmunoassay, ELSA, or even microbiological tests [15]. However, there are many requirements one

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must address to apply the previous method such as the pretreatment of VB<sub>12</sub> specimens, high cost, interference with other components that are present in the sample, in addition to narrow linear range. Considerable attention is currently being given to electrochemically designed sensors, which offer ease of operation, high sensitivity, and low cost, as well as the portability of the equipment used [16,17,18]. In this regard, different electrode types have been fabricated for the electroanalysis of VB<sub>12</sub>, such as boron-doped diamond electrodes [19] carbon paste electrodes modified by trans-1,2-dibromocyclohexane [20], disposable pencil graphite electrodes with single-walled carbon nanotube-chitosan layers [21], fabricated gold electrodes with

which requires the use of poly(methylene blue) and zinc oxide nanoparticles. The proposed method can also be applied to accurately and sensitively estimate VB<sub>12</sub> without any interference or influence of other ingredients of locally available pharmaceutical preparations.

## Experiment

### Apparatus

All voltammetry assessments were carried out using a 797 VA Computrace Stand (Metrohm, Switzerland) connected to a PC and controlled via the VA Computrace 2.0 software. For voltammetry studies, an electrochemical glass cell (working volume 10 ml) containing three electrodes was used: a glassy carbon electrode (GCE) with a 1.6 mm diameter as the working electrode, an Ag/AgCl reference electrode (KCl saturated), and a 1.5 mm platinum wire as the auxiliary electrode. pH measurements were carried out via a HANNA pH meter model 211 (manufactured in Romania). A digital ultrasonic cleaner was used to sonicate the GCE. A scanning electron microscope (SEM) (JEOL, JSM-5310, Japan) was used to characterize the morphology of the different PMB films deposited on glassy carbon film electrodes. Spectroscopic measurements were carried out using a photometric spectrophotometer (Japan), and using quarter cells with a 1 cm optical path.

The buffer solution was entirely altered by deaerating using nitrogen gas. The electrochemical cells were kept fully sealed to avoid interference due to atmospheric oxygen. All electrochemical experiments were performed under anaerobic conditions to avoid any voltametric response due to oxygen reduction in the system [25].

## Reagents and solutions

self-assembled monolayers of mercaptoacetic acid [22], and bismuth film electrodes [23].

The use of redox phenazine polymers has been shown to be effective in many forms of electroanalytical detection [24], with the design of nanostructure sensors using this polymeric film still being investigated.

The present work can be considered the first study to design a sensor using methylene blue and zinc oxide nanoparticles on a glassy carbon electrode via cyclic voltammetry. This offers a wide linear range with high sensitivity for the detection of VB<sub>12</sub> via differential pulse voltammetry (DPV) that monitors such via the Co(II/I) redox pair.

In this context, this paper describes the construction of an electroanalytical sensor on a glassy carbon electrode

All reagents were of analytical grade and were used without further purification. All solutions were prepared in distilled water.

Methylene blue (MB), monobasic sodium phosphate, NaH<sub>2</sub>PO<sub>4</sub>, dibasic sodium phosphate, Na<sub>2</sub>HPO<sub>4</sub>, Vitamin B<sub>12</sub>, Vitamin B<sub>1</sub>, Vitamin B<sub>6</sub>, sodium hydroxide, sodium nitrate, zinc oxide nanoparticles, benzoyl chloride, and absolute ethanol were purchased from Sigma Aldrich, Germany. The supporting electrolyte solutions used for electropolymerization of MB consisted of 0.2 M monobasic potassium phosphate and 0.2 M dibasic potassium phosphate (PB) with different pH.

The proposed method was applied to a group of locally available pharmaceuticals, namely Neurobine<sup>TM</sup> – forte Manufactured by Acino Pharma AG, (Aesch, Switzerland) and Monovit-B12 (cyanocobalamine) manufactured by Koçak Farma (Turkey).

### Preparation of PMB-Modified Electrode

Prior to surface modification, the GC electrode was mechanically polished with 0.05 μm alumina powder on a flat pad. After polishing, in order to remove any adsorbed substance from the surface, the electrode was ultra-sonicated in ethanol and distilled water (1:1) for 5 min. Thereafter, the electrode was washed with ethanol to remove residual alumina particles that could be trapped on the surface of GC electrode, and then washed with water [26].

To provide a more effective electrode surface, the electrode was electrochemically pretreated in 0.1 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> at the beginning of each working week, applying 30 min of cyclic voltammetry treatment (-1.0 V to +1.0 V). At the beginning of each working day, the electrode was washed with ultrapure water and its surface subjected to a mild 5 min CV treatment (-1.0 V to +1.0 V) [18].

About 0.005 g ZnO nanoparticles were dissolved in 1 ml of absolute ethanol, and this solution was stirred with a magnetic stirrer at ambient temperature for 30 min to obtain a homogenous suspension of ZnO nanoparticles. About 5  $\mu\text{L}$  ZnO NPs was then transferred onto the surface of the GC electrode and allowed to dry for 30 min. Thereafter, electropolymerization was performed via cyclic voltammetry (CV) from the supporting electrolyte solution described above, containing 0.5 ml of 0.1 M monomer MB freshly prepared by dissolving 0.3198 g MB in 10 ml distilled water, at a scan rate of 0.05  $\text{Vs}^{-1}$ . The potential was cycled between -0.5 V and +1.25 V versus an Ag/AgCl reference for 20 cycles. To get a more stable response from the designed electrode the polymer-modified electrode was left aging overnight in electrolyte solution at 4°C [27].

#### Morphological and electrochemical characterization of GCE

SEM is commonly used to evaluate the morphology (grain size, surface coverage, and orientation) of synthesized films.

#### UV-visible spectrometry

To qualify the proposed sensor, the method documented in British Pharmacopeia was chosen [28]. Spectra were recorded in the wavelength range of 200 to 600 nm using a UV-1800 ultraviolet-visible spectrophotometer. A quartz cell with an optical path length of 1.0 cm was used to perform the measurements. The maximum absorbance of the samples and solutions containing different concentrations of  $\text{VB}_{12}$  was monitored at a wavelength of 362 nm.

#### Preparation of standard, stock, and $\text{VB}_{12}$ supplementation

Standard solutions for both electrochemical and spectrometry methods were prepared as follows: 0.1355 g of  $\text{VB}_{12}$  was dissolved in 10 ml distilled water to prepare a fresh stock solution of  $\text{VB}_{12}$  (0.01 M) from which further dilutions of the desired concentrations were prepared. Then, 22  $\mu\text{L}$  was taken from the stock solution (0.01M) and added to 10 ml distilled water to prepare a standard solution of  $\text{VB}_{12}$  (22  $\mu\text{M}$ ). Two samples of commercial supplements of  $\text{VB}_{12}$  were purchased: Neurobine<sup>TM</sup> – forte, manufactured by Acino Pharma AG (Aesch, Switzerland), and Monovit- $\text{B}_{12}$  (cyanocobalamine), manufactured by Koçak Farma (Turkey).

For  $\text{VB}_{12}$  supplement tablets, ten of the tablets were macerated and weighed, and a fraction was diluted in

ultrapure water, gravity filtered with filter paper (25  $\mu\text{m}$ ), and made up to 25.0 mL.

#### Electrochemical analysis studies

-Electrochemical analysis of  $\text{VB}_{12}$  on modified electrodes using the DPV technique

The electrochemical behavior of  $\text{VB}_{12}$  on the polyMB/ZnO NPs/GCE electrode was determined using DPV techniques in a PBS solution (pH7).

To prepare the  $\text{VB}_{12}$  calibration curve, different concentrations of  $\text{VB}_{12}$  (0.099 – 69.513  $\mu\text{M}$ ) were studied over a -1.1 V to +0.65 V potential range under optimized conditions. All electrochemical experiments were performed under a nitrogen atmosphere and at ambient laboratory temperature (25°C).

Analytical evaluation of modified electrodes using the DPV technique Limit of detection (LOD), and sensitivity

In this study, the data obtained from applying the electroanalytical methodologies was evaluated with respect to the linear range, limit of detection (LOD) and precision. The criteria used to determine the LOD were based on the standard deviation (SD) of the intercept and the calibration plot gradient (s) using the formula  $\text{LOD} = 3 \times (\text{SD}/s)$  [29-30]. Sensitivity was evaluated under optimized conditions by analyzing five replicates containing 29.9  $\mu\text{M}$  of  $\text{VB}_{12}$ . The sensitivity is reported in terms of relative standard deviation (RSD%).

#### Storage Stability

The DVP response recorded every three days, and over a period of 40 days. At day 40, the activity of polyMB/ZnO NPs/GCE was about 66.9%. During this time interval, the modified electrode was stored in buffer solutions at 4°C.

#### Interference Study

The effect of some of the co-components might be considered to potentially cause interference, such as benzyl alcohol and vitamins  $\text{B}_1$  and  $\text{B}_6$  in various concentrations, with the determination of  $\text{VB}_{12}$  concentrations. The concentration of  $\text{VB}_{12}$  was fixed at 29.9  $\mu\text{M}$ . The ratio of the coexisting interferences was varied in the ratios 1:1, 1:50, 1:100 and 1:200. The intensity changes obtained by excess concentrations of Vitamins  $\text{B}_1$ ,  $\text{B}_2$ , and benzyl alcohol in the same ratio do not produce any interference, even at higher concentrations, which indicates the good selectivity of the system.

## Result and discussion

Electrochemical behavior of poly-MB on the GC modified with ZnO NPs:

To enhance the electro-polymerization of MB, nitrite or hydroxide ions should be present during the growth of the polymer on the electrode surface using CV.[31-32]. Fig. 1 shows the cyclic voltammogram of the electro-polymerization of 4 mM MB at GCE/ZnO NPs, which showed one peak in PBS (pH 8.0) at 0.14V and the revised one at -1.02 V, in the first cyclic voltammogram. In successive cyclizing voltammograms, the anodic peak was shifted towards the negative potential, at 0.05 V, and the cathodic peak, at -0.6 V. In addition, a new anodic peak was observed at about 1.12 V which was attributed to the formation of the polymer[33-34]. These results indicate that polymerization of MB was successfully achieved on GCE.

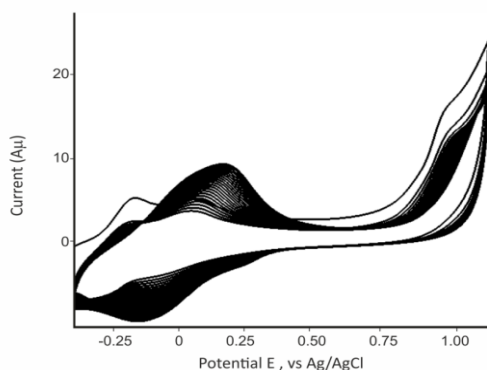


Fig. 1: Cyclic voltammograms of 4 mM MB in 0.1 M phosphate buffer and 0.1 M NaNO<sub>3</sub> at a scan rate of 50 mVs<sup>-1</sup> and scanned over 20 cycles.

Electrochemical active surface area of PMB/GE/Zn NPs:

It is an important thing to calculate the electroactive area of the electrode, which should give an idea about the effective electrochemical surface area. Here, the effective electrochemical surface area was obtained from a Randles-Sevcik equation[35], where the modified electrode was dipped into an electrochemical cell containing 1 μM K<sub>3</sub>[Fe(CN)<sub>6</sub>]. The effective surface area was estimated to be 23.3 cm<sup>2</sup>, where the geometrical surface area was 0.025 cm<sup>2</sup>, which refers to the role of the modified electrode in terms of increasing the effective

electrochemical surface area, and hence increased the current response.

Surface characterization of the fabricated electrode:

SEM was used to analyze the morphology of the modifier PMB and Zn films on the GCE surface. Fig. 2 shows the significant differences in surface morphology between bare GCE, PMB-modified GCE, and PMB/ZnO NPs-modified GCE. All images were obtained at a magnification of 100,000x. The GCE surface with ZnO NP appears to have a number of benefits as it is highly dispersed, uniformly coated. As seen in Fig. 2C, PMB covered the ZnO on the GCE surface and formed a composite film with particles of about 100 nm, which indicates that the prepared polymer has a large surface area.

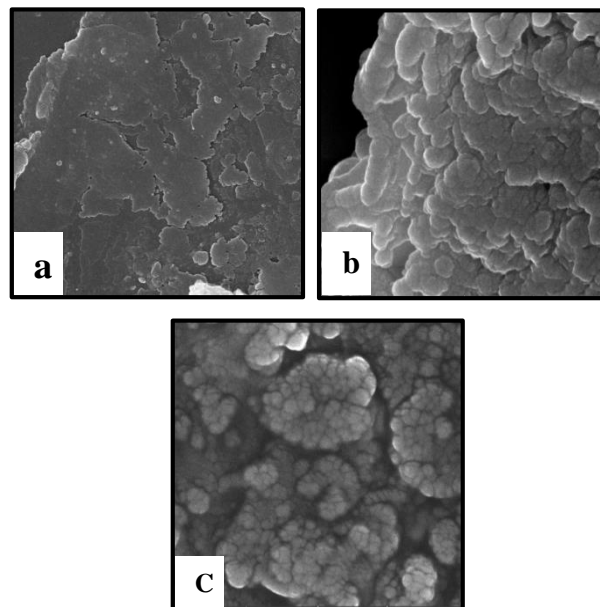


Fig. 2. Scanning electron microscopy (SEM) images of the glassy carbon electrode (a), Bare GCE (b) PMB/GCE (c) PMB/ZnO NPs/GCE

Electrochemical behavior of VB<sub>12</sub> at the PMB/ZnO NPs/GCE surface:

The electrochemical behavior of VB<sub>12</sub> on the polymeric MB with ZnO NPs at GC electrode nanocomposite, to the best of our knowledge, has not been discussed previously in other literature.

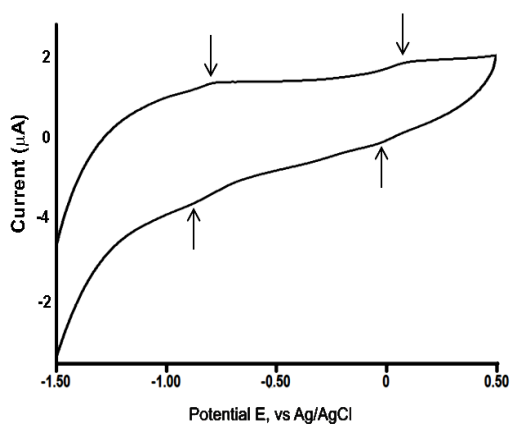
Fig 3 A shows the cyclic voltammogram of VB<sub>12</sub> with PMB/ZnO NPs/GC, which shows four peaks in PBS (pH 7.0) containing 29.9 μM VB<sub>12</sub>. There are two anodic peaks at -0.82 V and 0.06 V with peak

currents equal to 0.29 and 0.13  $\mu\text{A}$ , respectively, and two cathodic peaks at  $-0.79\text{ V}$  and  $-0.03\text{ V}$  with peak currents equal to  $-0.59\text{ }\mu\text{A}$  and  $-0.05\text{ }\mu\text{A}$ . The two anodic peaks are due to the Co(I/II) and Co(II/III) oxidation processes, respectively, and the two reduction peaks are due to the Co(III/II) and Co(II/I) reductions, respectively [36-37].

In this study, an interesting approach was followed to monitor the electroanalysis of  $\text{VB}_{12}$  via the Co(II/ I) redox reaction, which may have a faster electronic transfer via Co(III/II) redox reaction [19].

Following this, DP voltammetry was performed to detect  $\text{VB}_{12}$  using different electrodes, as per Fig 3 B For bare GC, ZnO NPs/GC, PMB/GC electrodes, and finally a PMB/ZnO NPs/GC electrode, weak peak currents were detected at 0.86 V for 29.9  $\mu\text{M}$   $\text{VB}_{12}$  of 0.05 ,0.21, and 0.33  $\mu\text{A}$ , respectively, in comparison with a suggested electrode (PMB/ZnO NPs/GCE) 0.61  $\mu\text{A}$ , whereas a superior DPV response was detected in the case of the PMb/ZnO NPs/GC electrode in comparison to the ZnO NPs/GCE and PMb/GCE. The relatively greater DPV response for  $\text{VB}_{12}$  in the material was attributed to the formation of a  $\text{VB}_{12}$ -Zn complex, wherein the PMb/ZnO NPs acted as the electrocatalyst catalyzing the reduction of  $\text{VB}_{12}$ . The polymer not only facilitated electron transfer but also increased the surface area of the electrode, which in turn helped to increase the sensitivity towards  $\text{VB}_{12}$  in aqueous solution. Surface coverage of the substrate with PMB also enhances various electrode properties such as biocompatibility, electroconductivity, and the electrochemical active surface area[24,26], which increases the response and sensitivity during measurement of  $\text{VB}_{12}$ .

**A**



**B**

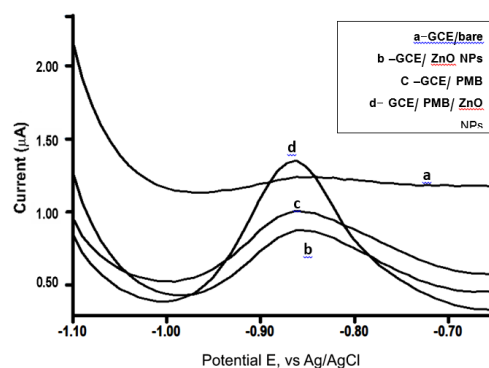


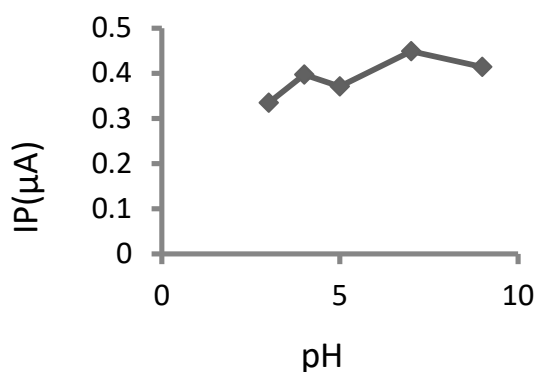
Fig 3 : (A) CV for  $\text{VB}_{12}$  (29.9  $\mu\text{M}$ ) in PB solution (pH 7) ; (B) data for  $\text{VB}_{12}$  (29.9  $\mu\text{M}$ ) for different modified GCEs in PB solution (pH 7)

Effect of pH on the electroanalytical response of  $\text{VB}_{12}$  :

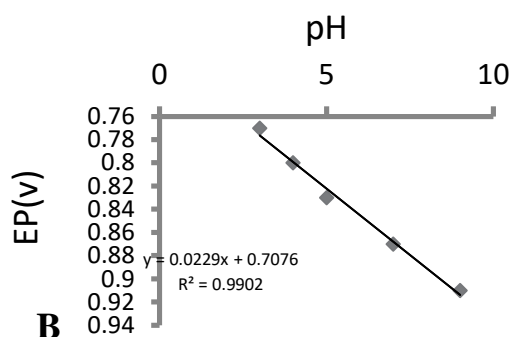
The pH of the supporting electrolyte has an important influence in the detection of  $\text{VB}_{12}$ . Fig. 4 illustrates the dependence of the electroanalytical response on pH of 29.9  $\mu\text{M}$   $\text{VB}_{12}$  on the PMb/ZnO NPs/GC electrode using DPV. When pH was varied from 3.0 to 9.0, the peak potential for  $\text{VB}_{12}$  shifted almost linearly toward the negative potential, implying that protons directly participated in the rate determining step of the reduction reaction of  $\text{B}_{12}$ [16] which also means no adsorption processes occurred on the electrode surface as observed in a previous study [17]. The linear relationship between the peak potential and pH was found to be  $E_{p}/\text{V} = 0.0229x + 0.7076$  pH ( $r = 0.9950$ ) (see Fig. 4 B). Moreover, it can be seen from Fig. 4 A that the maximum peak current appeared at pH 7.0 for  $\text{VB}_{12}$ . Therefore, considering the sensitivity, 0.1 M PBS (pH 7.0) was selected as the supporting electrolyte for subsequent electroanalysis of  $\text{VB}_{12}$  in this paper.

The establishment of a calibration plot

Under optimized conditions,  $\text{VB}_{12}$  was directly recorded using the PMb/ZnO NPs/GC electrode. From the DPV curves (Fig. 5 A) it was found that significant change occurred in the current response with change in  $\text{VB}_{12}$  concentration; the current increased with increasing concentration (Fig. 5 B). It was reasonable to assume that the electrocatalytic activity of both ZnO NPs and the polymeric layer of Mb enhanced electron transfer between  $\text{VB}_{12}$  and the electrode surface. This may attribute the electrochemical response of PMb/ZnO NPs/GC electrode to the chelating characteristics of  $\text{VB}_{12}$  with the Zn as oxide nanoparticles and polymeric MB [39-41].



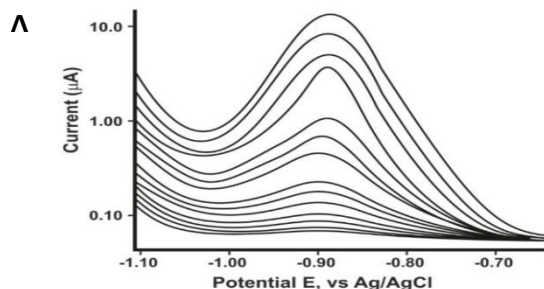
A



B

Fig. 4:(A) response the  $VB_{12}$  peak current to change in pH, (B) response of the  $VB_{12}$  peak potential to change in pH.

The sensor displayed a wide linear range over 0.099–69.51  $\mu\text{M}$ , with linear range over the DPV measurements as  $I_p = 0.0673x + 0.3449$ ,  $r = 0.9942$  (Fig. 5 B). Furthermore, the limit of detection was calculated to be 0.0104  $\mu\text{M}$ . The outcome of the study revealed that the proposed method is highly precise. The sensor fabricated here was found to be superior in terms of (low) detection limit and (linear) response over a wide concentration range.



A

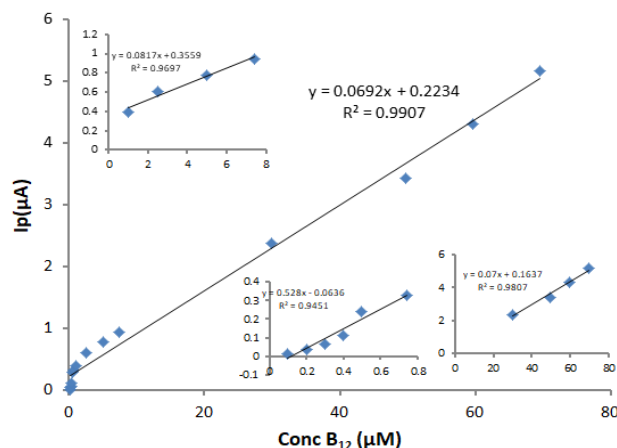


Fig 5:(A) DPV response of  $VB_{12}$  at different concentrations ( $\mu\text{M}$ ) on PMB/ZnO NPs/GCE under optimum conditions: Sta

rt potential (v)= -1.5, First vertex potential (v)= 0.5, Second vertex potential (v)= -1.5, Voltage step (v)=0.0059; Sweep rate (V/s)= 0.05; Deposition potential (v)= -0.9; Deposition time (s)= 60; Equilibration Time (s)= 5

(B) Calibration plot of the DPV current response for  $VB_{12}$  from 0.099  $\mu\text{M}$  to 69.51  $\mu\text{M}$ .

Repeatability, sensitivity and stability properties of the PMb/ZnO NPs/GC electrode:

Repeatability, sensitivity and stability of a sensor are important to evaluate the suggested methodologies. Here, repeatability was determined by repeating the measurement for the same electrode five times, the standard deviation was  $\pm 0.000241 \mu\text{M}$ , further more RSD was 0.398 % only. It was concluded that the method developed is characterized by high sensitivity, and thus that the protocol devised is suitable for  $VB_{12}$  analysis in pharmaceuticals.

The storage stability of the present Vitamin  $B_{12}$  sensor was determined using DPV. After keeping it at a temperature of  $4^\circ\text{C}$  for 40 days, the measurement had decreased by only 29.1% compared to the original response.

Pharmaceutical analysis:

Analysis of  $VB_{12}$  can be conducted by means of commercially available supplement preparations (MONOVIT  $B_{12}$  and Neurorubineforte). Selectivity is considered one of the most important properties for the sensor. Accordingly, the effect of the presence of other components on the  $VB_{12}$  peak was studied using a variety of substances, such as benzoyl alcohol,  $VB_1$  and  $VB_6$ , which are present in the product obtained, under optimum conditions. The

results show the height, shape, and position of VB<sub>12</sub> oxidation peak current during the addition of various amounts of the above, up to a 200-fold concentration, demonstrating the high selectivity of the designed

electrode. The numbered results depicted in Table (1) show the impacts of the selected species on the peak current for VB<sub>12</sub>. [38]

Table 1: investigation the effect of substances which are present in the product obtained on redox peak current of 19.96 μM VB<sub>12</sub> under its optimum conditions.

Species present in selected drugs	Conc. ratio VB <sub>12</sub>	Redox peak current of 19.96μM VB <sub>12</sub>	Recovery [%]
VB <sub>1</sub>	1:0	1.68	99.3
	1:100	1.70	101.1
	1:200	1.69	100.1
VB <sub>6</sub>	1:0	1.71	101.6
	1:50	1.68	99.3%
	1:100	1.69	100.1%
Benzyl alcohol	1:0	1.05	104.8
	1:0.5	1.03	101.8
	1:0.15	1.03	101.8
	1:1	0.993	96.4
	1:2	0.994	96.5

Pharmaceutical product electroanalysis and comparison of the voltammetric method with the UV-Visible method Based on the interference studies conducted, no significant changes were observed in the height, shape, and position of the VB<sub>12</sub> redox peak. DPV were recorded for monovitamin B<sub>12</sub> injection and the Neurorubine-forte tablet at various concentrations. From the numbered results reported in Table 2, it was observed that the recovery is in the range 97.1-104% for the injection and 95.9-103.3% for the tablet, which are satisfactory results, and which revealed that the designed voltammetric sensor holds considerable promise for the detection of VB<sub>12</sub> in different pharmaceutical products.

Moreover, a comparative study was carried out between the proposed voltammetric sensor with the British Pharmacopoeia method [28], where both VB<sub>12</sub> supplements were determined at three different concentrations, as shown in the table 2, with acceptable recovery obtained for the PMb/ZnO NPs/GC electrode. It can be concluded that there is good agreement with the results found by the British Pharmacopoeia method, where the method developed is characterized by high selectivity, and thus the devised protocol is suitable for VB<sub>12</sub> determination in real samples.

Table 2: Determination of VB<sub>12</sub> in commercial pharmaceutical products via both voltammetric and spectrometric methods.

Drug	Taken Conc. [μM]	Found Conc. [μM] Electrochemical GCE/NP <sub>s</sub> /MBP	Recovery % Electrochemical GCE/NP <sub>s</sub> /MBP	Error %	Taken Conc. [ppm]	Found Conc. [ppm] Spectro- photometer	Recovery % Spectrophot- ometer-	Error %
Monovit B <sub>12</sub> ampule	0.249	0.242	97.1	2.9	0.33	0.316	95.9	4.1
	4.975	0.911	98.7	1.3	6.74	7.03	104.3	-4.3
	9.99	10.41	104.2	-4.2	13.5	13.69	101.4	-1.4

Neurorubi	0.249	0.239	95.9	4.1	0.33	0.294	89.3	10.7
na- Fort	4.975	4.78	96.2	3.8	6.74	6.589	97.7	2.3
	9.99	10.32	103.3	-3.3	13.5	14.3	106	-6

### Conclusion

An electroanalytical method for the quantification of VB<sub>12</sub> was realized through designing a sensor using methylene blue and zinc oxide nanoparticles on a glassy carbon electrode via cyclic voltammetry. This was found to have a wide linear range with high sensitivity for the detection of VB<sub>12</sub> via differential pulse voltammetry (DPV), as dependent on the associated Co(II/I) redox pair. Under optimized conditions, the electrode was able to sense VB<sub>12</sub> at concentrations in a linear range of 0.099–69.51  $\mu$ M, with an LOD of 0.0104  $\mu$ M. In addition, the proposed method can also be applied to accurately and sensitively estimate VB<sub>12</sub> without any

### References

- [1] Vogeser, M., & Lorenzl, S. (2007). Comparison of automated assays for the determination of vitamin B12 in serum. *Clinical biochemistry*, 40(16-17), 1342-1345.
- [2] Hodgkin, D. C., Kamper, J., Mackay, M., Pickworth, J., Trueblood, K. N., & White, J. G. (1956). Structure of vitamin B12. *Nature*, 178(4524), 64-66.
- [3] Li, H. B., Chen, F., & Jiang, Y. (2000). Determination of vitamin B12 in multivitamin tablets and fermentation medium by high-performance liquid chromatography with fluorescence detection. *Journal of Chromatography A*, 891(2), 243-247.
- [4] Fedosov, S. N., Fedosova, N. U., Kräutler, B., Nexø, E., & Petersen, T. E. (2007). Mechanisms of discrimination between cobalamins and their natural analogues during their binding to the specific B12-transporting proteins. *Biochemistry*, 46(21), 6446-6458.
- [5] Banerjee, R., & Ragsdale, S. W. (2003). The many faces of vitamin B12: catalysis by cobalamin-dependent enzymes. *Annual review of biochemistry*, 72(1), 209-247.
- [6] Stabler, S. P., & Allen, R. H. (2004). Vitamin B12 deficiency as a worldwide problem. *Annu. Rev. Nutr.*, 24, 299-326.
- [7] Mikkelsen, K., Stojanovska, L., Prakash, M., & Apostolopoulos, V. (2017). The effects of vitamin B on the immune/cytokine network interference or influence due to the other constituent species in locally available pharmaceutical preparations. For the analysis of VB<sub>12</sub> in commercially available supplements, there was no need for an extra sample purification step, as shown by the absence of matrix effects, thus reducing the analysis time.

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and their involvement in depression. *Maturitas*, 96, 58-71.

- [8] Shakoor, H., Feehan, J., Mikkelsen, K., Al Dhaheri, A. S., Ali, H. I., Platat, C., ... & Apostolopoulos, V. (2021). Be well: A potential role for vitamin B in COVID-19. *Maturitas*, 144, 108-111.
- [9] Shakoor, H. (2021). Be well: A potential role for vitamin B in COVID-19 (2021) *Maturitas* 144. P108-111, Feb.
- [10] Zant A., Awwad H.M., Geisel J., Keller M. and Obeid R., Vitamin B12-fortified toothpaste improves vitamin status in elderly people: a randomized, double-blind, placebo-controlled study, *Aging. Clin. Exp. Res.*, 31, 1817–1825(2019).
- [11] Rizzo G., Laganà A.S., Rapisarda A.M.C., La Ferrera G.M.G., Buscema M., Rossetti P., A. Nigro, Muscia V., Valenti G., Sapia F., Sarpietro G., Zigarelli M., Vitale S.G., Vitamin B12 among vegetarians: status, assessment and supplementation. *Nutrients*, 8, 676 (2016).
- [12] Berton, P., Monasterio, R. P., & Wuilloud, R. G. Selective extraction and determination of vitamin B12 in urine by ionic liquid-based aqueous two-phase system prior to high-performance liquid chromatography. *Talanta*, 97, 521-526 (2012).
- [13] Samadi-Maybodi, A., & Darzi, S. H. N. Simultaneous determination of vitamin B12 and its derivatives using some of multivariate calibration 1 (MVC1)



- techniques. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 70(5), 1167-1172(2008).
- [14] Wang, M., Liu, Y., Ren, G., Wang, W., Wu, S., & Shen, J. Bioinspired carbon quantum dots for sensitive fluorescent detection of vitamin B12 in cell system. *Analytica chimica acta*, 1032, 154-162(2018).
- [15] Baker S. A. and Miller-Ihli N. J., *Spectrochim. Acta Part B*, 55, 1823–1832(2000).
- [16] Kumar S.S., Chouhan R.S., Thakur M.S. Trends in analysis of vitamin B12. *Anal. Biochem.* 398, 139–149(2010).
- [17] Bard A.J., Faulkner L.R., *Electrochemical Methods: Fundamentals and Applications*, 2nd ed. John Wiley & Sons.
- [18] ElShal, M. A. Copper Oxide Nanoparticle Modified Screen-printed Electrode for Determination of Mirtazapine. *Egyptian Journal of Chemistry*, 62(9), 1739-1748(2019).
- [19] Al-Tae, A. T., Al-Layla, A. M., & Mahmoud, A. R. Electrochemical Behavior of Aminoguanidine and Its Detection by Hybrid Polymer of Theophylline with Copper Oxide Nanoparticles. *Analytical. Bioanalytical Electrochemistry*, 13(2) 283-295(2021).
- [20] Pereira, D. F., Santana, E. R., Piovesan, J. V., & Spinelli, A. A novel electrochemical strategy for determination of vitamin B12 by Co (I/II) redox pair monitoring with boron-doped diamond electrode. *Diamond and Related Materials*, 105, 107793(2020).
- [21] Tomčík P., Banks C.E., Davies T.J. and Compton R.G., A self-catalytic carbon paste electrode for the detection of vitamin B12. *Anal. Chem.* 76 ,161–165(2004).
- [22] Kuralay F., Vural T., Bayram C., Denkbaz E.B, Abaci S. Carbon nanotube-chitosan modified disposable pencil graphite electrode for vitamin B12 analysis. *Colloids Surfaces B Biointerfaces*, 87, 18–22(2011).
- [23] Parvin, M. H., Azizi, E., Arjomandi, J., & Lee, J. Y. Highly sensitive and selective electrochemical sensor for detection of vitamin B12 using an Au/PPy/FMNP@ TD-modified electrode. *Sensors and Actuators B: Chemical*, 261, 335-344(2018).
- [24] Kreft G.L., De Braga O.C., Spinelli A. Analytical electrochemistry of vitamin B 12 on a bismuth-film electrode surface, *Electrochim. Acta*, 83, 125–132(2012).
- [25] Liu, B. Electrochemical Polymerization of Methylene Blue on Glassy Carbon Electrode. *International Journal of Electrochemical Science*, 9907-9913(2017).
- [26] Lin, K. C., Yin, C. Y., & Chen, S. M. An electrochemical biosensor for determination of hydrogen peroxide using nanocomposite of poly (methylene blue) and FAD hybrid film. *Sensors and Actuators B: Chemical*, 157(1), 202-210(2011).
- [27] Hosu, O., Bârsan, M. M., Cristea, C., Săndulescu, R., & Brett, C. M. Nanostructured electropolymerized poly (methylene blue) films from deep eutectic solvents. Optimization and characterization. *Electrochimica Acta*, 232, 285-295(2017).
- [28] Braun, W. A., Horn, B. C., Hoehne, L., Stülpe, S., ROSA, M. B., & Hilgemann, M. Poly (methylene blue)-modified electrode for indirect electrochemical sensing of OH radicals and radical scavengers. *Anais da Academia Brasileira de Ciências*, 89, 1381-1389(2017). Stationery Office (Great Britain). (2008). British pharmacopoeia 2009. London: Stationery Office.
- [29] Ozkan S.A (Ed.), *Electroanalytical Methods in Pharmaceutical Analysis and their Validation*, HNB Publishing, New York, 2012.
- [30] Ruqaya M. Hamid Al-Sultan, Ammar Abdulsalaam Al-Sultan, Mohammed A. Hayawi, Bilal J M Aldahham, Mohanad Y. Saleh, Hazim A. Mohammed. The effect of subclinical thyroid dysfunction on B- type natriuretic peptide level. *Revis Bionatura* 2022;7(2) 21. <http://dx.doi.org/10.21931/RB/2022.07.02.21>
- [31] Karyakin A.A, Karyakina E.E. and Schmidt H.-L, *Electroanalysis*, 11 ,149(1999).
- [32] Aldahham, B. J., Al-Khafaji, K., Saleh, M. Y., Abdelhakem, A. M., Alanazi, A. M., & Islam, M. A. (2020). Identification of naphthyridine and quinoline derivatives as potential Nsp16-Nsp10 inhibitors: a pharmacoinformatics study. *Journal of Biomolecular Structure and Dynamics*, 1-8.
- [33] Karyakin A.A., Strakhova A.K., Karyakina E.E., Varfolomeyev A.K., Yatsimirsky A.K. The electrochemical polymerization of methylene blue and bioelectrochemical activity of the resulting film. *Bioelectrochem. Bioenerg.* 32 ,35(1993).
- [34] Ayoob, A., Sadeek, G., Saleh, M. (2022). Synthesis and Biologically Activity of Novel 2- Chloro -3-Formyl -1,5-Naphthyridine Chalcone Derivatives. *Journal of Chemical*

- Health Risks*, 12(1), 73-79. doi: 10.22034/jchr.2022.688560
- [35] Karadas N. , Sanli S. , Akmese B. , Dogan-Topal B. , Can A., Ozkan S.A. Analytical application of polymethylene blue-multiwalled carbon nanotubes modified glassy carbon electrode on anticancer drug irinotecan and determination of its ionization constant value. *Talanta*, 115 ,911-919(2013).
- [36] Lexa D., Saveant J.M., The electrochemistry of vitamin B12. *Acc. Chem. Res.*, 16 ,235–243(1983) .
- [37] Rabeea Banoon, S., Narimani-Rad, M., Lotfi, A., Shokri, S., Abbaszadeh, S., & Özlman, S. (2021). Phytotherapy in prostatitis: A review of the most important medicinal plants affecting prostatitis in Iranian ethnobotanical documents. *Plant Biotechnology Persa*, 3(2), 68-74.
- [38] Saeed, N. H. M., & Abbas, A. M. (2020). Kinetics and mechanism of tetrahydrofuran oxidation by chloraminet in acidic media. *Periodico Tche Quimica*, 2020, 17(35), pp. 449–461
- [39] Brown, K.L. Chemistry and enzymology of vitamin B12. *Chem. Rev.*, 105, 2075–2149(2005).
- [40] Toohi, H. T. A. S., Rabeea, M. A., Abdullah, J. A., & Muslim, R. F. (2021). Synthesis and characterization activated carbon using a mix (asphalt-polypropylene waste) for novel azo dye (HNDA) adsorption. *Carbon Letters*, 31(5), 837-849.
- [41] Giedyk, M.; Goliszewska, K.; Gryko, D. Vitamin B12 catalysed reactions. *Chem. Soc. Rev.*, 44, 3391–3404(2015).