A New Potentiomertic Sensor for a Preventing Alzheimer's Disease Drug Based on Nano-Silver Modified Carbon Paste Electrode

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ABSTRACT
Due to the importance of vitamin B6 (VB6) drug for human health, especially in the treatment of Alzheimer's disease, it is useful to develop new, simple, cheap and rapid methods for the determination of this compound. A high linear range and a sensitive potentiometric sensor for VB6 based on carbon paste electrode (CPE) modified with synthesized silver nanoparticles by co-precipitation method were prepared. The sensor fully characterized in terms of distinctive nanoparticles quality, electrode composition, and usable pH range. Under optimal conditions, a linear calibration curve with range 1×10^{-6} to 1×10^{-2}mol L^{-1} and a detection limit of 4.84×10^{-7}molL^{-1} was obtained for VB6 determinations. The research introduces the design and construction of the nanocomposite modified CPE for simple and low-cost determination of VB6 in pharmaceutical formulation. Validation of the method indicates the suitability of the sensor for application in quantity control analysis of VB6 drug in pharmaceutical formulation preparations.

Keywords: Potentiometric Sensor, Modified Carbon Paste Electrode, Vitamin B6, Silver NPs

INTRODUCTION
Vitamin B6 (VB6) or Pyridoxine drug (Fig. 1) plays a vital role in many enzyme activities. It is essential for the breakdown of proteins, carbohydrates and fats from food, and also for the release of stored carbohydrates to energy yield. It is involved in the production of red blood cells and antibodies and the maintenance of skin and digestion health. It is also necessary for the normal function of the nervous system and several hormones [1, 2]. Scientists showed that high-dose B-vitamin treatment slowed shrinkage of the whole brain volume over 2 years and preventing Alzheimer's disease by as much as seven-fold [3, 4]. Developing a sensitive and simple analytical method for VB6 sensing is very important in the food and pharmaceutical industries [1, 2]. Special attention has been paid to VB6 in the clinical and pharmaceutical sciences because of its extensive and essential applications in bio-metabolisms. Researchers believe that deficiencies in the VB6 result in reducing dopamine in kidneys, which leads to an increase in sodium excretion. As result symptoms in the body like swelling and pain in the chest as well as stomach, are caused. VB6 decreases the symptoms and prevents acne [5, 6]. Increased level of Prolactin at the end of the luteal phase is one of the reasons for chest pain and swelling, and probably physical exercise among female athletes reduces levels of this hormone and decreases the symptoms [5, 7, 8].

Vitamins B6 and D are among those vitamins, whose performance relationship and interference with magnesium is approved [9]. Likewise, VB6 is regarded as a non-toxic food supplement. However, long-term prescription and arbitrary use of VB6 leads to peripheral neuropathy (among patients using 2 to 6 g daily for 2 to 40 months) [10].

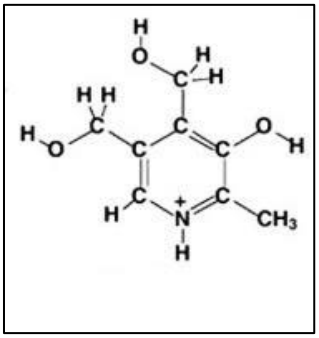

Fig. 1: Chemical structures of Pyridoxine (VB6)

Various analytical methods have been developed for the determination of VB6, such as flow injections [11, 12], high-performance thin-layer chromatography [13], and electrochemical detection [14, 15]. Spectrophotometric determination in presence of other vitamins has also been described by multi calibration techniques [9, 16]. Algar *et al*. [17] have determined Pyridoxine in pharmaceutical preparations by native fluorescence method. Kaynar in 2013 indicated that quantitative determination of VB6 in the foods can be achieved by the use of the microbiological assay

method [18]. Potentiometric sensors have found the most widespread practical applicability due to their simplicity, fast responsibility and cost. Potentiometric carbon paste electrodes with nano-sized materials have increased industrial and clinical interest [19-22]. Pires *et al.* [19] introduced a potentiometric sensor for vitamin B1 and VB6 in order to multivitaminic control. The construction and application of ion-selective electrodes, as well as modified CPEs, applied for the determination of pharmaceutical compounds, such as acetylsalicylic acid and VB6, has been described [23, 24].

The advantages of modified carbon paste electrodes (MCPE), such as chemical inertness, robustness, low cost, renewability, very low background current, stable response, low ohmic resistance and no need for internal solution over conventional polymeric membrane electrodes has attracted wide attention of researchers in recent years [25-28].

The aim of this study is VB6 measuring by synthesised silver nanoparticles (Ag NPs) modified CPE. To the best of our knowledge, there is no report of VB6 detection based on Ag NPs modified electrode. We checked the useful ability of the proposed nanoparticles as an accelerator for electron transferor in carbon paste electrode for VB6 sensing. As compared to the reported results, the improvement in sensitivity, linear range and response time was achieved successfully. Also, the sensor is successfully used in the estimation of low concentrations of VB6 in pharmaceutical real samples.

MATERIALS AND METHODS

Materials and equipment

VB6 drug, silver nitrate ($AgNO_3$), sodium boron hydrate ($NaBH_4$), dry methanol, graphite powder, silicon oil, phosphoric acid, all purchased from Merck. All potentials were measured with a potentiometer (model PTR 79), which is made of a calomel electrode as a reference and a MCPE as an identifier. SEM images were provided by Zeiss, Dsm-960A instrument. A pH meter (Model EIL 744 Metrohm) was used to measure pH solutions. All solutions prepared with double distilled water.

Synthesis of silver nanoparticles

The silver nanoparticles have been synthesized simply upon routine methods by reducing silver nitrate similar to the previous procedures [29]. In a typical experiment, 0.025 g $AgNO_3$ was dissolved in 15 mL anhydrous methanol under stirring at 25 ºC. Next, 0.0945 g $NaBH_4$ as a mild reducing agent was dissolved in 7.5 mL anhydrous methanol and added to stirred solution drop by drop. Vigorous stirring of the solution was continued for 2 hours. Then, the solution was centrifuged and the resulting precipitate was decanted.

Preparation of modified carbon paste

The modified carbon paste electrode was prepared similar to our previous report [30], by mixing mineral oil, graphite powder and silver nanoparticles with various weight ratios. Typically, 0.058 g graphite powder and 0.012 g silver nanoparticle were mixed till a homogeneous mixture was obtained. Then, 0.006 g silicon oil was added to the mixture and the paste was used for the construction of the MCPE. The pastes were packed into a Teflon microtip (diameter 0.5 mm) and a copper wire inserted into the paste established an electrical contact. A new surface was regenerated by pressing out an excess of paste out of the tip and manually smoothed by polishing it on clean paper. The MCPE was immersed in the supporting electrolyte placed in the cell and several sweeps were applied to obtain a low background current.

RESULTS AND DISCUSSION

Here we check the ability of the silver nanoparticles as an accelerator for electron transferor in carbon paste electrode for VB6 sensing. Owing to their small dimensional size, good conductivity and excellent catalytic activities, noble nanoparticles have potential applications in the construction of electrochemical sensors and biosensors [26, 27]. They function as electron antennae efficiently channelling electrons between the electrode and the electroactive species that promoting better electron transfer between the electrode surface and the electrolyte [20]. Based on the obvious results, we have utilized silver nanoparticles to modify the CPEs and use them as a sensor for potentiometric determination of VB6. The obtained results from the experimental works revealed that the proposed electrode exhibits excellent performance for VB6 sensing and has two additional advantages of simplicity of operations and low-cost instrument as well.

Characterization of the Nanomodifier

In order to get the best response upon optimal synthesized nanomodifier, optimization of the effective parameters in the synthesis of the silver nanoparticles was investigated by one variable at a time method. The amount of silver nitrate ($AgNO_3$), $NaBH_4$ and dry methanol were examined to obtain the best potentiometric response utilizing the MCPE with synthesized nanoparticles. They compared results of the synthesis processes are shown in Table 1. It is clear that $NaBH_4$ as a reductant has an important role in nanoparticles synthesis. An increase in the amount of $NaBH_4$ leads to an increase in the reaction rate, which makes the synthesis process out of the normal state and eventually leads nanoparticles to lose their high quality. On the other hand decrease in the concentration result in decreasing the synthesis yield

and potentiometric response. That's because the low Ag NPs level leads to a decrease in the electrode conductivity due to its ability of electron transfer. Also, the concentration of $AgNO_3$ in the synthesis directly influences the size, quality and characteristics of the NPs. Finally, the NPs with 0.09 g of $NaBH_4$ and 0.025 g of $AgNO_3$ with 15 ml of anhydrous methanol as optimized values showed the best electrode responses for VB6 determination (Table 1).

Table 1: The optimization of effective parameters in silver nanoparticles synthesis

Electrode	Anhydrous Methanol (ml)	$NaBH_4$ (g)	$AgNO_3$ (g)	Slope mv/Decade	R^2
1	15.0	0.05	0.025	36.2	0.7448
2	15.0	0.07	0.025	40.5	0.8679
3	15.0	0.09	0.025	59.8	0.9923
4	15.0	0.11	0.025	70.3	0.6735
5	15.0	0.09	0.005	40.7	0.6519
6	15.0	0.09	0.015	54.5	0.9905
7	15.0	0.09	0.035	44.8	0.6178

Optimized synthesized silver nanoparticles were characterized by scanning electron microscopy (SEM), before utilizing the carbon paste electrode (Fig. 2). SEM images of the particles, as shown in Fig. 2, revealed the average diameter of synthesized nanoparticles below 100 nm.

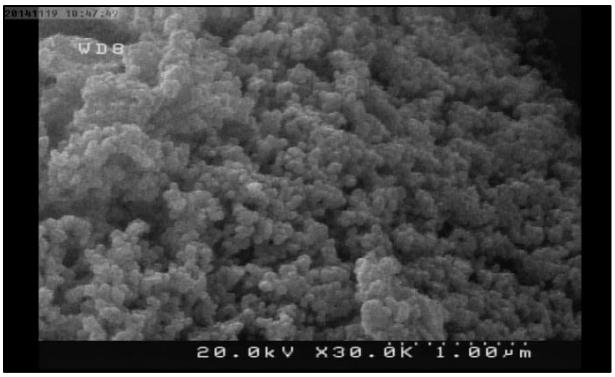

Fig.2: SEM image of synthesized silver nanoparticles

Electrode composition and modification

Here, a modified paste was prepared similarly with unmodified CPE consist of silicon oil and graphite powder, except that the graphite powder was replaced with a desired weight of nanomodifier, to get a different electrode mixture as given in Table 2. The optimized amount for all factors in the carbon paste, which result in better performance and potential response, was determined in solutions of 1×10^{-6} mol L^{-1} VB6 at pH= 6. It was concluded that at least 0.006 g silicone oil and 0.012 g amounts of nano-silver with 0.06 g graphite powder in carbon paste is favourable for obtaining the electrode with proper physical characteristics. This nano-composition was selected for further examinations.

The most optimized amount for silicone oil factor, which was determined by running a comparison analysis with electrodes by various amounts of silicone oil in a mixture of carbon paste, shows that at too high amounts, preparing carbon paste layer on the electrode surface is impossible (adjacent surface of the electrode to aqueous solutions adsorbs water and collapses). Likewise, fewer amounts of silicone oil have a negative effect on electrode performance because paste mixture is not favourable due to weak adhesion between silver nanomodifier and graphite in the mixture (leading to an increase in hydrophobicity at electrode surface). Also, it is clear that increasing in Ag NPs amount in the electrode, resulted in increasing the electrode capability due to the high ability of their electron transfer, until the diminution of catalytic properties of electron transfer and as a result the conductivity at too high percentages [23] (Table 2).

Table 2: Optimization of the MCPE mixture, (pH=6) 1×10^{-1} mol L^{-1} PBS and 1×10^{-6} mol L^{-1} of VB6

Electrode	Silicone Oil (g)	Graphite Powder (g)	Silver Nano-particles (g)	Slope mv/Decade	R^2
1	0.006	0.06	0.009	58.23	0.974
2	0.006	0.06	0.012	60.03	0.996
3	0.006	0.06	0.014	61.17	0.965
4	0.006	0.06	0.016	61.94	0.948
5	0.002	0.06	0.012	62.94	0.872
6	0.004	0.06	0.012	62.75	0.924
7	0.008	0.06	0.012	58.18	0.645
8	0.006	0.04	0.012	46.42	0.937
9	0.006	0.08	0.012	57.28	0.981
10	0.006	0.11	0.012	42.35	0.925

Optimization of pH

pH of the VB6 drug solution has a great impact on the electrode response. To examine the effect of pH, electrode potential was measured in different PBS buffer solutions with pHs from 5.0 to 8.0 at the concentrations of 1×10^{-6} mol L^{-1} VB6 (Table 3). The

mixture composition of the electrode was kept constant during all experiments. Here pH=6 is regarded as an optimized value due to its best slope and correlation coefficient. The results showed that the potential of the electrode is nearly constant between pH 5.5 to 7.5. Thus, the electrode works satisfactorily in this range of pH. The decrease in potential at high pH values might be justified by the competition between the VB6 drug and hydroxyl groups in the solution. Also, the decrease in potential at too acidic media could be attributed to a change in the VB6 drug structure and also the dissolution of the nanoparticles.

Analytical characteristics

The analytical characteristics of the electrode were investigated. The precision of the proposed sensor was evaluated by caring out an analysis using standard working solutions with the same electrodes for various VB6 concentrations in different conditions, to determine the stability of the constructed electrode. The R.S.D results for the measurements are shown in Table 4. Limit of detection was obtained according to $3S_b/m$, where S_b is the standard deviation of the blank (by 20 times determination in PBS buffer solution, pH=6) and m is the slope of a calibration curve, as 4.8×10^{-7} mol L^{-1}. Also, the proposed electrode demonstrated potential responses across the range of 1×10^{-6} -1×10^{-2} mol L^{-1} obtained from the calibration curve.

Table 3: Optimization of pH in a range from 5.0 to 8.0 in VB6 solution (1×10^{-2} mol L^{-1})

pH	Slope mv/decade	R^2
5.0	61.3	0.8157
5.5	63.4	0.9847
6.0	64.7	0.9954
6.5	62.5	0.9923
7.0	60.9	0.9721
7.5	59.1	0.9632
8.0	57.7	0.7962

Table 4: The precision results of the modified CPE at optimal conditions

Concentration (mol L^{-1})	E_1 (v)	E_2 (v)	E_3 (v)	E_{mean} (v)	RSD%
1×10^{-2}	295	297	299	297	0.673
1×10^{-3}	257	255	257	256.3	0.448
1×10^{-4}	198	199	199	198.6	0.293
1×10^{-5}	123	124	126	124.3	1.22
1×10^{-6}	55	56	59	56.6	3.68
1×10^{-7}	20	21	22	21	5.90

The effect of interfering ions

One of the most important characteristics of the ion-selective electrode is its selection ability of the target ion. Here, the common interferences of VB6 were checked as potential interfering species in the analysis. The results indicate that modified carbon paste electrode and silver nano-particles do not exhibit a considerable response to interfering blood electrolyte cations such as Cd^{2+}, Co^{2+},Cu^{2+}, Fe^{2+} and K^{2+}. It was found that the common existing cations in real samples did not cause interference even up to 100-fold excess over the analyte. As it is evident, the interfering ions don't have any effect on the response of electrodes. The electrode provided an appropriate response to the VB6 drug, showing favourable selectivity of MCPE for the drug compared to other ions.

Real sample analysis

For the overall purpose of this study, VB6 was measured with the developed potentiometric sensor in samples of injectable ampoules of the vitamin. The resulted data by the proposed method are shown in Table 5 which are obtained without any treatment. Therefore development and validation of the method were investigated by the use of the sensor for real sample analysis by simply measuring the potentials by the use of a potentiometer with construct electrodes as a sensor in ampoules of vitamin with pH adjusted by PBS buffer solutions. As can be seen from Table 5, the amounts of VB6 determined by our MCPE electrode in real samples were in good agreements with the expected values. Therefore, the sensor is very suitable for the potentiometric determination of VB6 in real samples.

Table 5: Determination of VB6 in real ampule samples

Concentration (mol L^{-1})	Added (mol L^{-1})	Obtained (mol L^{-1})	Recovery%
1.0×10^{-5}	0.0	1.9897×10^{-5}	98.97
1.0×10^{-5}	1.0×10^{-6}	2.1998×10^{-5}	99.98
1.0×10^{-5}	1.4×10^{-6}	2.2798×10^{-5}	99.98
1.0×10^{-5}	2.0×10^{-6}	2.3998×10^{-5}	99.98

CONCLUSION

VB6 deficiency has been shown to be a potentially modifiable risk factor for Alzheimer's disease. Here, a new potentiometric sensor is constructed for the determination of VB6 concentration in pharmaceutical samples. To the best of our knowledge, there is no previous report for the determination of VB6 based on

silver nanoparticles modified CPE. Also, VB6 electrochemical status by use of the potentiometric technique was surveyed for the first time. The ability of silver nanoparticles as an accelerator for electron transferor in carbon paste electrode for VB6 sensing is investigated. Since the electrode used in the present research, is based on silver nanoparticles, the conditions to achieve the ideal nano-silver particles and ultimately highest conductivity, initially optimized by the proper selection of reagents in nanosilver synthesis. In the next step, the best electrode performance in the case of MCPE was obtained by paste composition optimization. The offered sensor demonstrated potential responses across the range of 1×10^{-6} -1×10^{-2} mol L^{-1} with a detection limit of 4.8×10^{-7} mol L^{-1}. The electrode is stable, selective and illustrated highly reproducibility and repeatability which show a Nernstian response over a relatively wide concentration range. The proposed electrode also exhibits excellent potentiometric performance for simple and low-cost determination of the drug in real samples.

ETHICAL ISSUES
There are no ethical issues in this study.

CONFLICT OF INTEREST
There is no conflict of interest to be reported by the authors

AUTHORS' CONTRIBUTIONS
This work is the result of the full cooperation of all mentioned authors.

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