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Dependence of Catalytic Activity of Prussian Blue Particles Towards Reduction of Hydrogen Peroxide on Their Size

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A study on the catalytic reduction of Prussian blue modified electrode to hydrogen peroxide was carried out in this paper. Prussian blue was modified on glassy carbon electrode (GCE) *via* cyclic voltammetric process in solution of 5×10^{-4} mol L^{-1} $FeCl_3$ and 5×10^{-4} mol L^{-1} $K_3Fe(CN)_6$. The results indicated that the reduction of H_2O_2 on the Prussian blue modified electrode was controlled by diffusion. Moreover, Prussian blue modified electrodes with different sizes of Prussian blue particles exhibited different electrocatalytic activity to reduction of hydrogen peroxide. Lower amount and smaller size of Prussian blue particles modified on GCE gave a lower reduction signal and Prussian blue with smaller size exhibited improved catalytic activity to reduction of H_2O_2. Analytical performances of resulting Prussian blue based electrode have been studied in course of hydrogen peroxide detection by amperometry. Linear calibration range of sensor for H_2O_2 is over 1.3×10^{-7}-2.1×10^{-4} mol L^{-1} with correlation coefficient of 0.999 and sensitivity of 887 mA M^{-1} cm^{-2}. Average recovery of H_2O_2 in river water and whey was 101 and 103 %, respectively.

Key Words: Prussian blue, Catalysis, Modified electrode, Nanoparticles, Cyclic voltammetry.

INTRODUCTION

As a side product of many enzyme catalytic reactions, hydrogen peroxide exists in the reactive enzyme system[1-3]. Determination of hydrogen peroxide is of great important for monitoring bio-processes[4-6], but direct determination of the reduction signal of hydrogen peroxide is possibly interfered by coexisting oxygen. In addition, a great drawback is presented by the high over-potential needed for direct hydrogen peroxide oxidation (*ca.* 0.7V *vs.* Ag/AgCl) at which many electroactive substances (such as ascorbic acid, uric acid, *etc.*) presenting usually in real samples, could also be oxidised and interfer the determination of hydrogen peroxide[7]. In the last decade, electrochemical inorganic mediators, especially Prussian blue, which catalyzed the reduction of hydrogen peroxide, were used for the assembling of oxidase-based biosensors[8,9]. This realizes decrease of the potential applied and consequent elimination of much electrochemical interferences[10].

Recently, some works indicated that microelectrode presented novel property compared with conventional electrode. Profile of substrate diffusion around a microelectrode surface is semispherical. For such diffusion type the inversed current density has linear dependence on the electrode radius; the slope is positive and is inversely proportional to substrate concentration[11-13]. Poly(vinylpyrrolidone)-protected Prussian blue nanoparticles were used to modify glassy carbon electrode (GCE) for determination hemoglobin and the GCE modified by nano-Prussian blue exhibited obvious catalytic activity to reduction of hemoglobin. The smaller Prussian blue particles with diameter in range of 8-18 nm show the stronger catalytic effect[14]. After Prussian blue nanoclusters was used to assemble glucose biosensor[15] Prussian blue nanoparticles protected by poly (diallydimethylammonium chloride) was assembled on Au electrode *via* layer-by-layer deposition and the resulting glucose biosensor can catalyze the electroreduction of hydrogen peroxide formed in the course of enzymatic reaction at low potential and inhibit the responses of interferences, such as ascorbic acid and uric acid[16]. Surfactants Brij-56, Tween-60 and AOT (Dioctyl sulfosuccinate) were used in deposition of nano-Prussian blue on glassy carbon disk electrode and lower detection limit for H_2O_2 was obtained[17], followed by preparation of nano-Prussian blue modified glassy carbon disk electrode through direct deposition of Prussian blue for detection of H_2O_2[18]. Zhai *et al.*[19] prepared amperometric glucose biosensor based on Prussian blue nanoparticles and studied synergistic electrocatalytic effect of Prussian blue and MWNT toward the reduction of hydrogen peroxide.

In this study, nano-Prussian blue protected by poly(vinylpyrrolidone) was modified on GCE and was used to catalyze reduction of H_2O_2, however, no obvious catalytic effect was observed. It is clear that the catalytic activity of Prussian blue particles to reduction of H_2O_2 is relative to their chemical environment. As demonstrated by Rolf *et al.*[20], many physical and chemical propertied of materials are determined not only by the materials themselves, but also by their geometric dimensions. Purpose of this work is to study the relationship of electrochemistry of Prussian blue particles on the surface of electrode and their sizes and an improved method for determination of H_2O_2 by the Prussian blue modified electrode electrochemically. Furthermore, some available sensors based on Prussian blue modified electrode are potential to be established to measure substance with redox activity.

EXPERIMENTAL

H_2O_2 (purchased from Shentai Reagent Corporation, 30%) was determined first by titration of standard $KMnO_4$. $FeCl_3 \cdot 6H_2O$ (AR, Tianjin Tianda chemical engineering experiment factory). $K_3[Fe(CN)_6]$ (AR, Developing

Center of Tianjin Yongda Chemical Reagent). KCl (AR, Tianjin Beifang Tianyi Chemical Reagent Factory). HCl (38 %, AR, Tianjin Chemical Regent Fifth Factory). All other reagents were commercially available and of analytical grade. Deionzed water from a Millipore Milli Q system was used throughout this research.

All electrochemical measurements were performed with model LK-98BII electro-analytical system (Tianjin Lanlike Chemical and Electron High Technology Co., LTD). A three-electrode system was used, including a platinum wire electrode as counter electrode, a saturated calomel electrode (SCE) as reference electrode and GCE or Prussian blue modified GCE as working electrode, respectively. Scanning electron microscope (Hitachi, S-3500N) was used to characterize the morphology and size of Prussian blue. pH values were measured with Model PHS-3C pH meter (LIDA).

Titration of H_2O_2 with $KMnO_4$: 1.0 g $KMnO_4$ was dissolved in 300 mL boiling water and kept boiling for 1 h. About 0.1600 g standard $Na_2C_2O_4$ solid was weighed precisely and was used for titrating $KMnO_4$ solution. Initially, $Na_2C_2O_4$ solid was dissolved in 80-90 mL H_2O, added by 20 mL 3 mol L^{-1} H_2SO_4, then $KMnO_4$ solution was added slowly to the $Na_2C_2O_4$ solution drop by drop until final point. The concentration of $KMnO_4$ solution was counted through known amount of $Na_2C_2O_4$. 1mL H_2O_2 purchased (30 %) was diluted to 250 mL with water. 5 mL 3 mol L^{-1} H_2SO_4 solution was added to 25 mL diluted H_2O_2 solution. The H_2O_2 solution containing H_2SO_4 was titrated with $KMnO_4$ solution of known concentration and resulting concentration of initial H_2O_2 is 12.96 mol L^{-1}.

Preparation of Prussian blue modified GCE: Deposition of Prussian blue on GCE was carried out by cyclic voltammetric process with switching potentials of 0.3V and 0.8V (*vs.* SCE) at a scan rate of 0.02 Vs^{-1} in solution of 5×10^{-4} mol L^{-1} $FeCl_3$ and 5×10^{-4} mol L^{-1} $K_3Fe(CN)_6$. The supporting electrolyte is composed of 0.1 mol L^{-1} KCl and 0.1 mol L^{-1} HCl. Then in order to stabilize response of Prussian blue particles on the surface of electrode, the Prussian blue modified electrode was taken out from electrolyte, was washed with water and kept at 60 ºC for 2 h, followed by cooling it to room temperature.

Electrochemical measurement: Electrochemistry of electrode was determined by cyclic votammogram from 0.5 to -0.1 V in supporting electrolyte containing 1 mol L^{-1} KCl and 0.1 mol L^{-1} HCl. Sequential addition was used in measurement of H_2O_2 by micro-injector under electromagnetic stirring condition. The i-t curve was recorded under constant potential of 0 V for calibration curves. Practical samples were measured with the same method.

Characteristics of PB by scanning electron microscope: A GCE containing a thin glassy carbon slice was used in measurement of scanning electron microscope (SEM). The thin glassy carbon slice can be taken down

from the electrode. Same modifying method with conventional GCE as described above was used to prepare Prussian blue modified the GCE. Then the glassy carbon slice was taken out and the morphology of Prussian blue modified on carbon slice was recorded by SEM.

Pretreatment of samples: River water samples were collected locally and filtrated immediately after collection. 1 mol L^{-1} KCl and 0.1 mol L^{-1} HCl solution were prepared with the river water. Certain quantity of KCl and HCl was added in the purchased milk sample to obtain final concentration of 1mol L^{-1} KCl and 0.1 mol L^{-1} HCl. Under effection of salt, whey was separated from milk sample by vacuum filtration. pH values of these samples were measured, respectively. pH of river water was 1.0, while that of whey was 3.26. Then pH of whey was adjusted with 4.8 mol L^{-1} HCl to 1.0 for determination of H_2O_2.

RESULTS AND DISCUSSION

Modifying of Prussian blue on GCE: Electrochemical means for synthesizing Prussian blue includes generally cyclic voltammetry, constant potential and constant current. Deposition by cyclic voltammetry is scanning cyclic voltammograms at bare electrode in electrolyte containing Fe^{3+} and $K_3Fe(CN)_6$ in constant potential range. Constant potential and constant current methods are depositing Prussian blue on electrode by controlling constant potential and constant current respectively. Another method is that only $K_3Fe(CN)_6$ is initial reactant without Fe^{3+} when Prussian blue was deposited[21]. In the work, Prussian blue was deposited by scanning cyclic voltammograms between 0.3 and 0.8 at 0.02 V s^{-1} in the freshly prepared solution contains 5×10^{-4} mol L^{-1} $FeCl_3$, 5×10^{-4} mol L^{-1} $K_3Fe(CN)_6$, 0.1 mol L^{-1} KCl and 0.1 mol L^{-1} HCl. The presence of water lowered the electroactivity of the Prussian blue[22]. Therefore, Prussian blue modified electrode was kept at 60 ºC for 2 h to remove some water from Prussian blue crystalline framwork, then was activated in electrolyte consisting of 0.1 mol L^{-1} KCl and 0.1 mol L^{-1} HCl by cycling between 0.5 and -0.1 V until voltammograms became stable.

Morphology of Prussian blue deposited on GCE: Due to SEM images have a characteristic three-dimensional appearance and are useful for judging the surface structure of the sample, they were used to study morphology of Prussian blue deposited on GCE (Fig. 1). Prussian blue existed in the form of particles on surface of electrode. These Prussian blue particles are different from each other obtained by different potential cycles. Diameters of particles were distributed mostly in 30-80 nm for 1 potential cycle, 50-130 nm for 5 potential cycles and 200 nm - 4 μm for 10 potential cycles respectively. With the increase of potential cycles of deposition, sizes of Prussian blue particles on surface of GCE increased. Congregation of Prussian blue particles occurred obviously when number of potential cycles was increased to 10.

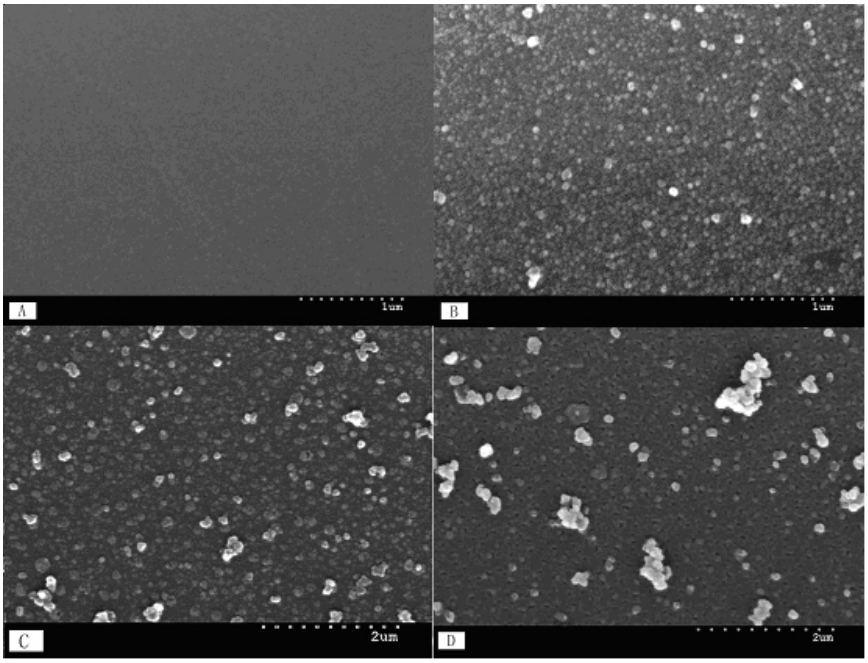

Fig. 1. Scanning electron microscope images of Prussian blue on GCE by different potential cycles: A. 0; B. 1; C. 5; D. 10, respectively

Electrocatalysis of Prussian blue modified electrodes to reduction of H_2O_2: Catalytic procedure of Prussian blue towards reduction of H_2O_2 was demonstrated by Itaya *et al.*[23]. The PB can obtain an electron from the surface of electrode and transform into the reduced form (also called Prussian white). Prussian white had a catalytic effect to the reduction of hydrogen peroxide. Prussian white changes to Prussian blue when it catalyzes reduction of hydrogen peroxide and losses electron. With the potential cycles between positive and negative value, transformation happens constantly between Prussian blue and Prussian white.

As showed in Fig. 2, cyclic voltammograms of bare GCE in the absence H_2O_2 and in the presence 1.3×10^{-3} mol L^{-1} H_2O_2 almost overlap each other and no redox of H_2O_2 happened in potential range of 0.5 – -0.1 V at bare GCE. As for Prussian blue modified GCE, cyclic voltammograms exhibited obvious redox peaks. The addition of H_2O_2 made the reductive current grow remarkably at about 0.18 V. It was obvious that Prussian blue catalyzed reduction of H_2O_2.

Effect of scan rate: The cyclic voltammograms of Prussian blue modified GCE by potential cycles in supporting electrolyte in the presence of 1.3×10^{-4} mol L^{-1} H_2O_2 were carried out. Dependence of i_{pc} on scan rate

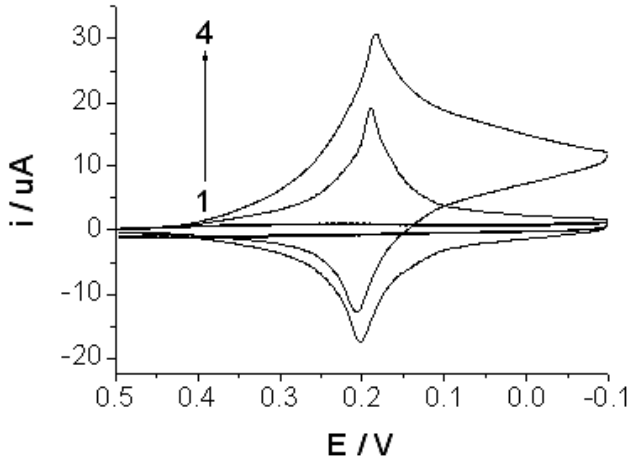

Fig. 2. Cyclic voltammograms of bare GCE (1, 2) and Prussian blue modified GCE (3, 4) in the absence of H_2O_2 (1, 3) and in the presence of 1.3×10^{-3} mol L^{-1} H_2O_2 (2, 4)

ν or square root of scan rate $\nu^{1/2}$ is shown in Fig. 3. When the reaction is controlled by diffusion dominantly, the peak current and the scan rate are also related as[24]

$$i_p = kn^{3/2}AD_0C_0\,\nu^{1/2},$$

where k, n, A, D_0, C_0 and ν represent a constant, electron transfer number, working electrode area, diffusion coefficient, concentration of reactant and scan rate, respectively.

Linear regression equations of current *vs.* $\nu^{1/2}$ were carried out. As can be seen from Table-1, reductive peak cerrent i_{pc} is linear to square root of scan rate $\nu^{1/2}$ for these modified electrodes by different potential cycles. The reaction was almost controlled by diffusion process obviously. While with increase of cycles and sizes of Prussian blue particles on GCE, R of i_{pc} versus $\nu^{1/2}$ decreased and electron transfer in Prussian blue on surface of electrode grew difficult.

TABLE-1
REGRESSION EQUATIONS OF i_{pc} *vs.* $\nu^{1/2}$

Electrode	Equation of i_{pc} *vs.* $\nu^{1/2}$	Correlation coefficient (R)
1	Y = -2.31 + 30.68X	0.998
2	Y = -7.10 + 90.70X	0.995
3	Y = -20.13 + 223.18X	0.994
4	Y = -27.22 + 321.93X	0.994

1 = deposition by 1 potential cycle; 2 = by 5 potential cycles;
3 = by 10 potential cycles; 4 = by 20 potential cycles.

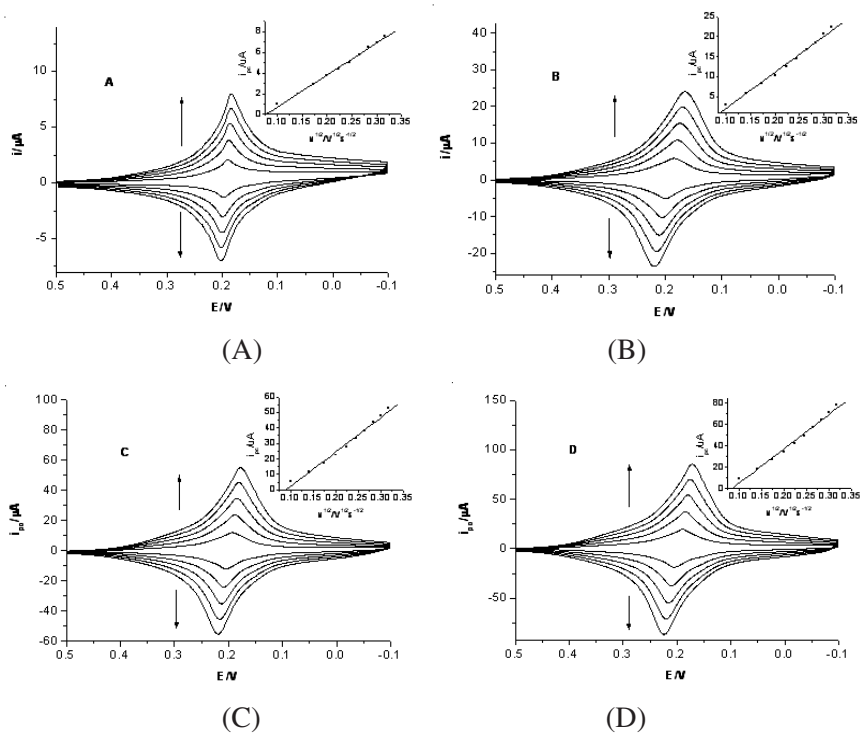

Fig. 3. Dependence of i_{pc} on square root of scan rate ($v^{1/2}$) at Prussian blue modified GCE by different number of cycles. Inserts are linear regression curves of i_{pc} *vs.* $v^{1/2}$. (A) 1 potential cycle (B) 5 potential cycles (C) 10 potential cycles (D) 20 potential cycles

Electrochemistry of Prussian blue modified electrodes deposited by different potential cycles in supporting electrolyte: GCE was modified by Prussian blue using 1, 5, 10, 20 potential cycles, respectively. Cyclic voltammograms were measured at modified GCE in supporting electrolyte composed of 1 mol L^{-1} KCl and 0.1 mol L^{-1} HCl (Fig. 4). Number of potential cycles in the process of Prussian blue deposition on GCE affected its electrochemical behaviour. When the number was low (1 and 5), redox peaks were around 0.2 V (*vs.* SCE) and no separation of peaks potential was found in the absence and presence of H_2O_2. However, with the increasing of cycles, effect of H_2O_2 on separation of redox peaks emerged gradually. Separation of peaks potential (ΔE) increased from 0.018 to 0.137 V when 1.4×10^{-6} mol L^{-1} H_2O_2 was added in the supporting electrolyte at modified GCE by 10 cycles. It increased from 0.028 to 0.288 V for 20 cycles and the separation of peaks became more obvious with the increase of cycles. As the Prussian blue particles grew larger gradually with the increase of cycles, electron transfer in Prussian blue framework became difficult and reduction of H_2O_2 was not easy.

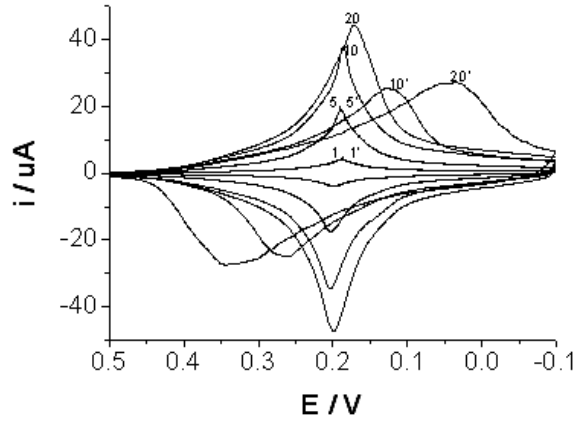

Fig. 4. Cyclic voltammograms of Prussian blue modified GCE by different potential cycles 1, 5, 10, 20 are in the absence of H_2O_2 and 1', 5', 10', 20' are in the presence of 1.4×10^{-6} mol L^{-1} H_2O_2

Analytical performance of H_2O_2 at sensor based on Prussian blue modified GCE: Sensors were prepared by 1, 5, 20, 50 potential cycles, respectively. Amperometry was used to detect concentration of H_2O_2 at 0 and 0.1 V with sequential addition by micro-injector separately. Due to addition of H_2O_2, the reductive peak shift to more negative potential, sensitivity obtained at 0 V is higher than that at 0.1 V. So applied potential was selected as 0 V. With the increase of cycles, the resulting linear range and detection limit of sensor varied. In general, with the increase of potential cycles which caused size of Prussian blue particles increase, the upper limit of linear range tend to be larger and the lower limit also increased. The sensor obtained by 5 cycles exhibited lowest value of H_2O_2 concentration in the linear range of 1.3×10^{-7}–2.1×10^{-4} mol L^{-1} among these sensors. The regression equation is i (μA) = 0.15 + 23951C (mol L^{-1}), with a correlation coefficient of 0.9998 and sensitivity of 887 mA M^{-1} cm^{-2}. It concluded that amount of Prussian blue particles obtained by less than 5 cycles is not enough to catalyze reduction of H_2O_2 and larger Prussian blue particles (when cycles is more than 10) are disadvantage to catalyze reduction of H_2O_2.

One river water sample and one milk sample were determined using the modified electrode by 5 cycles and recovery were calculated by exact addition of H_2O_2 (Tables 2 and 3). According to linear equation mentioned above, content of H_2O_2 was calculated. The results showed no H_2O_2 was found in these two samples. Average recovery of H_2O_2 in river water and whey was 101 and 103 %, respectively. The method is useful for determining H_2O_2 in practical samples.

TABLE-2
RECOVERY OF H_2O_2 ADDED IN RIVER SAMPLES OF PRUSSIAN BLUE MODIFIED GCE BY 5 POTENTIAL CYCLES

n = 3	Found (M)	Added (M)	Determined (M)	Recovery (%)	Average recovery (%)
1	N/D	1.30×10^{-5}	1.25×10^{-5}	96.2	
2	N/D	5.20×10^{-5}	5.12×10^{-5}	98.5	101
3	N/D	1.43×10^{-4}	1.55×10^{-4}	108.0	

TABLE-3
RECOVERY OF H_2O_2 ADDED IN WHEY SAMPLES OF PRUSSIAN BLUE MODIFIED GCE BY 5 POTENTIAL CYCLES

n = 3	Found (M)	Added (M)	Determined (M)	Recovery (%)	Average recovery (%)
1	N/D	1.30×10^{-5}	1.38×10^{-5}	106.0	
2	N/D	5.20×10^{-5}	5.14×10^{-5}	98.8	103
3	N/D	1.43×10^{-4}	1.48×10^{-4}	103.0	

Conclusion

The mechanism of electrocatalysis of Prussian blue towards hydrogen peroxide reduction was investigated by steady-state voltammetry at glassy carbon electrode, assisted by scan electron microscopy. Prussian blue was deposited on glassy carbon electrode by cyclic voltammetry and the electrocatalytic activity of resulting modified electrode to reductive reaction of H_2O_2 is dependant on the size and amount of Prussian blue particles existing on electrodes surface. Amount and size of Prussian blue particles on GCE affects the catalysis to reduction of H_2O_2, *i.e.*, smaller size and more amount of Prussian blue particles are favour to catalytic reduction of H_2O_2. The size of nanoparticles influences the catalytic activity, not only due to the enhanced surface area, but also due to particular electronic properties, which are different from those of bulk material[20].

Analytical performances of the resulting Prussian blue based electrode have been studied in course of hydrogen peroxide detection by stirred batch amperometry. Linear calibration range of sensor for H_2O_2 shifts to be larger with increase of potential cycles between 5 and 20 in course of Prussian blue deposition. As for the sensor obtained by 5 potential cycles, the linear range of H_2O_2 concentration is over 1.3×10^{-7}–2.1×10^{-4} mol L^{-1} with sensitivity of 887 mA mol^{-1} L cm^{-2}. Measurement of recovery values showed the sensor is capable to determinate H_2O_2 in practical samples.

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