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Electroanalytical applications of Prussian Blue and its analogs

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The applications of transition metal hexacyanoferrates in electroanalysis are surveyed. Prussian Blue (ferric hexacyanoferrate) is recognized as the most promising low-potential transducer for hydrogen peroxide reduction among all known systems. The advantages of Prussian Blue over platinum or peroxidase electrodes for hydrogen peroxide detection are discussed. Various types of biosensors based on transition metal hexacyanoferrates and oxidase enzymes are considered. Amperometric biosensors based on Prussian Blue-modified electrodes allow the detection of glucose and glutamate down to 10^{-7} mol L^{-1} in the flow-injection mode. The future prospects of Prussian Blue-modified electrodes in analytical chemistry for the monitoring of chemical toxic agents, in clinical diagnostics, and in food control are outlined.

Key words: Prussian Blue, hexacyanoferrates, modified electrodes, transducer, hydrogen peroxide, biosensor, oxidases, flow-injection analysis.

Introduction

Prussian Blue or iron(III) ferricyanide is one of the longest known coordination compounds, the first reports of which date back to the early 18th century.[1,2] The studies published in 1978 [3] underlay a new line of research, namely, investigation of electrochemical applications of Prussian Blue. It was shown that Prussian Blue can form electroactive films after electrochemical deposition on the surface of electrodes. During the last 20 years, vigorous fundamental studies of the synthesis and structure of films of Prussian Blue and its analogs have been carried out. Study of the electrocatalytic properties of Prussian Blue polycrystals and the design of chemical and biological sensors are the most interesting aspects.

In this review, we survey the recent advances of our research group in the development of new electrocatalysts on the basis of Prussian Blue inorganic polycrystals. The possible analytical applications of transition metal hexacyanoferrates are specially characterized. Examples are given and advantages are discussed of chemical and biological sensors based on Prussian Blue and its analogs.

Preparation and electrocatalytic properties of iron and other transition metal hexacyanides

It was shown by spectroscopy that Prussian Blue has the structure $Fe_4^{III}[Fe^{II}(CN)_6]_3$.[4] In this complex, the Fe^{III} atom is coordinated to the N atom and the Fe^{II} atom is linked to the C atom. Chemical synthesis of Prussian Blue can be accomplished by mixing Fe^{II} or Fe^{III} salts with hexacyanoferrate ions containing iron atoms in the other oxidation state, *i.e.*, in Fe^{3+} + $[Fe^{II}(CN)_6]^{4-}$ or Fe^{2+} + $[Fe^{III}(CN)_6]^{3-}$ mixtures.

Prussian Blue is deposited on electrodes from aqueous solutions containing iron (Fe^{3+}) and hexacyanoferrate ($[Fe^{III}(CN)_6]^{3-}$) ions; the process takes place either spontaneously or under the action of cathodic current. Chronopotentiometric studies of equimolar solutions of ferricyanide and iron(III) demonstrate the presence of two plateau sections, at 0.7 and 0.4 V.[5] These sections correspond to the reduction of the previously discovered $Fe^{III}[Fe^{III}(CN)_6]$ complex [6] and iron(III) ions.

The cyclic voltammogram of an electrode modified by Prussian Blue is presented in Fig. 1. Three redox states can be distinguished.

In the 0.2—0.9 V range, the inorganic polycrystal exists as brightly colored Prussian Blue. The reduction at 0.2 V results in discoloration of the coating to give

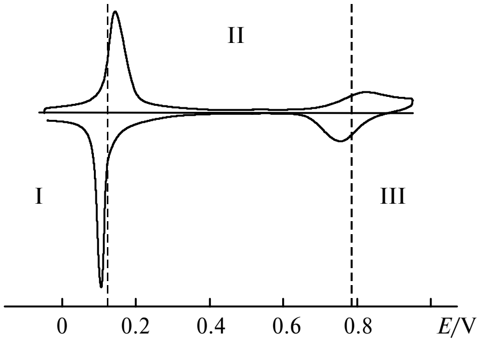

Fig. 1. Cyclic voltammogram of a glass carbon electrode modified by Prussian Blue: (I) Prussian White, (II) Prussian Blue, (III) Prussian Green; 0.1 *M* KCl, 40 mV s^{-1}, Ag/AgCl.

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the crystals of Prussian White. Conversely, when the potentials are >0.9 V, Prussian Blue is oxidized to Prussian Green. The electron transfer upon the reduction of Prussian Blue to Prussian White is counterbalanced by incorporation of the cations into the film according to the equation

$$Fe_4^{III}[Fe^{II}(CN)_6]_3 + 4e^- + 4\,K^+ \rightleftharpoons K_4Fe_4^{II}[Fe^{II}(CN)_6]_3. \tag{1}$$

The participation of the K^+ ions in the Prussian Blue—Prussian White reaction shows that the redox potential should depend on the activity of the K^+ ions. Indeed, this dependence does exist and corresponds to the Nernst equation: as the concentration of the potassium ions in the solution decreases by an order of magnitude, the potential shifts to the negative region by ~60 mV. Apart from K^+ ions, only NH_4^+, Cs^+, and Rb^+ ions can penetrate into the crystal lattice of Prussian Blue.

The quality of electrodes modified by Prussian Blue films can be judged by the sharpness of the oxidation and reduction peaks in cyclic voltammograms. To achieve a regular structure of the Prussian Blue films, two main factors should be taken into account, namely, the electrodeposition potential and pH of the initial solution of iron salts. The working electrode potential should not be lower than 0.2 V because otherwise, reduction of ferricyanide ions takes place. The pH value of the initial solution optimal for film deposition is 1.[7,8]

At high anodic potentials, Prussian Blue is converted into the completely oxidized form (Prussian Green). Upon the electron transfer, the charge of the complex is counterbalanced by addition of an anion (A):

$$Fe_4^{III}[Fe^{II}(CN)_6]_3 - 3e^- + 3\,A^- \rightleftharpoons Fe_4^{III}[Fe^{II}(CN)_6A]_3. \tag{2}$$

Application of Prussian Blue in electroanalysis

The development of chemical sensors for the analysis of electrochemically inactive ions is based on participation of cations in redox reactions of hexacyanoferrates of iron and analogous ions. Examples of sensors for determination of cesium,[9] thallium,[10] potassium,[11–13] ammonium,[14] rubidium,[15] and other cations[16] are listed in Table 1. In most sensors, potentiometric or amperometric detection of cations is used.

Prussian Blue and copper hexacyanoferrate have also been used for the adsorption of Cs^+ ions from aqueous solutions.[17–20]

Examples of compounds readily oxidizable by iron hexacyanoferrate that can be used for the development of electrochemical sensors are listed in Table 2. In addition, the Prussian Blue inorganic polycrystals have been used to design sensors for the analysis of moisture,[34] methanol and dichloroethane vapors,[35] and a sensor for pH determination with optical detection.[36,37]

Table 1. Sensors for analysis of electrochemically inactive cations

Cations determined	M*	Ref.
Cs^+	Cu	9
K^+	Cu	12
	Ag^I—$Mo(CN)_8^{4-}$	11
	Fe	13
Tl^+	Cu	10
K^+, NH_4^+	Cu	14
K^+, Rb^+, Cs^+, NH_4^+	Cu, Ni	15
Single- and double-charged cations	Cu, Ni	16

* M are metals in metal hexacyanoferrates.

Table 2. Chemical sensors for determination of readily oxidizable compounds and other types of nonconventional sensors

Substance determined	M*	Ref.
Ascorbic acid	Ni	21
Catecholamine	Fe	22
Catecholamine	Ni	23
Cisteine	Cu	24
Cytochrome C	Fe	25
Dopamine	Ni	26
Hydrazine	Fe	27
Hydrazine	Co, Cu	28
NADH**	Co, Ni	29, 30
Sulfite	Ni	31
Thiosulfate	Ni	32, 33
Moisture	Fe	34
Methanol, water, dichloroethane vapor	Fe (Prussian Blue)	35
H^+	Fe (Prussian Blue)	36, 37

* M are metals in metal hexacyanoferrates.

** NADH is the reduced form of β-nicotinamide adenine dinucleotide.

Prussian Blue as a highly efficient and selective transducer* for the analysis of hydrogen peroxide

The need for selective analysis of hydrogen peroxide is due to the following reasons. Hydrogen peroxide is found in underground waters and rainwater as a result of contamination coming from industrial plants and nuclear power stations.[38,39] This compound is widely used for disinfection, in particular, of swimming pools and food packages,[40,41] which accounts for the necessity of exact determination of its residual concentrations. In addition, hydrogen peroxide is the product of oxidase enzyme action. Detection of hydrogen peroxide on the

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* Transducer is a device converting chemical signals into electric signals.

basis of its reduction at low potentials is the most advanced method used in enzyme electrodes based on oxidases because it provides high sensitivity and selectivity of the analysis.

The possibility of selective determination of hydrogen peroxide based on its reduction in the presence of oxygen at Prussian Blue-modified electrodes was first demonstrated by our research group.[42] During the ensuing years, new publications appeared dealing with the use of Prussian Blue as a transducer for H_2O_2 analysis.[7,43—46]

We have studied in detail the properties of Prussian Blue as an electrocatalyst of H_2O_2 reduction.[7,8,47] The activity of modified electrodes in the reduction of hydrogen peroxide and oxygen depends on the structure of the inorganic polycrystal.[7,48,49] Upon optimization of the procedure of deposition of Prussian Blue films on electrodes, we succeeded in synthesizing a selective electrocatalyst for the reduction of H_2O_2 in the presence of O_2, active over a broad range of potentials.[7] At the zero potential (Ag/AgCl), which is the best for operation of enzyme electrodes, the recorded current of H_2O_2 reduction is several hundred times higher than the current of O_2 reduction.

Poor stability of the Prussian Blue-based transducer is always criticized when the possible practical applications of the transducer are considered. Prussian White (*i.e.*, the reduced form of Prussian Blue at a zero potential) is a thermodynamically unstable compound. In addition, the OH^- ions resulting from the reduction of H_2O_2 in a neutral medium[47] promote the dissolution of the inorganic polycrystal. However, detailed investigation of the electrochemical deposition of the Prussian Blue film and the factors that stabilize the crystal structure made it possible to attain high operation stability of modified electrodes even compared to other known H_2O_2 transducers. For example, in our recent works, the operation stability of electrodes was >20 h under a continuous flow of a 10^{-4} *M* solution of H_2O_2.[49,50]

The kinetics of H_2O_2 reduction catalyzed by Prussian Blue has been studied.[8,47] In neutral media, the reaction proceeds as follows:

$$H_2O_2 + 2e^- \xrightarrow{k_{cat}} 2\,OH^-. \qquad (3)$$

It was shown that the rate constant for the electrochemical reduction of H_2O_2 depends on the amount of the Prussian Blue deposited. This confirms the assumption that H_2O_2 can penetrate through the catalyst film and, hence, the inner layers of the polycrystal participate in the catalysis. For modified electrodes coated by $(4—6) \cdot 10^{-9}$ mol cm^{-2} of the polycrystal, the rate constant for the electrochemical reduction exceeds 0.01 cm s^{-1}.[8] This is the greatest value among those known for other hydrogen peroxide transducers. For example, the rate constant of H_2O_2 electrooxidation on platinum in a neutral medium is $7 \cdot 10^{-6}$ cm s^{-1},[51] and the rate constant for H_2O_2 electroreduction on platinum is even lower.

Since Prussian Blue exhibits a high activity and selectivity, *i.e.*, properties of a biocatalyst, we denote it as an "artificial peroxidase".[49]

Modern requirements of accurate and fast automated anatytical control are largely satisfied by flow-injection analysis with an enzyme electrode as the detector. The solution sample to be analyzed is injected into a continuous flow of a carrier. A so-called wall-jet electrode is efficient for hydrodynamic methods. This electrode operates under a flow of the solution ejected from a round nozzle normal to the electrode surface. A flow-injection system provides a sensitivity of hydrogen peroxide determination equal to 0.6 A mol^{-1} L cm^{-2}.[49,52] In the continuous substrate flow mode or in the case of measurements in a confined space with continuous stirring (batch mode), taking into account the dispersion coefficient,[53] the sensitivity of the analysis was 1 A mol^{-1} L cm^{-2}. A calibrating plot for the determination of hydrogen peroxide using a Prussian Blue-modified electrode in a flow-injection system is presented in Fig. 2.

To extend the scope of analytical applications of Prussian Blue, it is necessary to use industrial electrodes, for example, electrodes based on carbon paste and one-shot electrodes produced by screen printing. Chemically synthesized Prussian Blue can be used in the preparation of these electrodes. Recently, a successful chemical synthesis of the electrocatalyst has been carried out by depositing Prussian Blue in open-circuit mode on a graphite powder from a solution containing iron(III) and ferricyanide ions.[54] Carbon paste electrodes produced from this catalyst retain high operation stability in neutral and alkaline media up to pH 9; thus, they can be used to design enzymatic electrodes. However, the sensitivity of analysis for hydrogen peroxide by means of carbon paste electrodes is 20 to 200 times lower than the sensitivity of sensors based on carbon

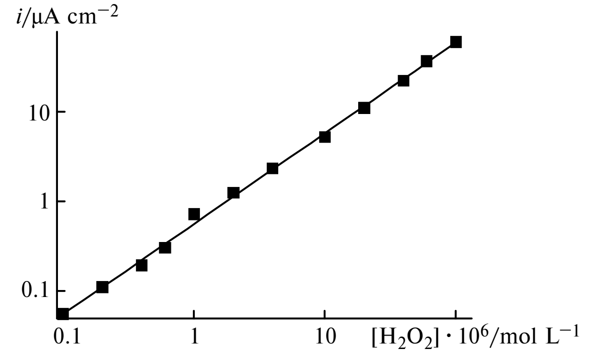


Fig. 2. Calibrating plot for determination of hydrogen peroxide with an electrode modified by Prussian Blue in a flow-injection system; a wall-jet type cell, outlet nozzle diameter 0.5 mm, H_2O_2 flow rate 0.7 mL min^{-1}, 0 mV (Ag/AgCl/0.1 *M* KCl), 0.1 *M* KCl.

glass electrodes modified by Prussian Blue by electrochemical deposition.

Several attempts have been made to use hexacyanoferrates of other metals as transducers for hydrogen peroxide. A biosensor based on copper hexacyanoferrate has been designed;[55] however, the sensitivity of analysis proved to be three orders of magnitude lower than that attained with similar electrodes on the basis of Prussian Blue. In addition, a substantial rate of hydrogen peroxide reduction at this electrode was achieved only when a high cathodic potential was established (higher than −0.7 V). No detailed kinetic study was carried out; however, these findings indicate that copper hexacyanoferrate is a much poorer electrocatalyst than Prussian Blue.

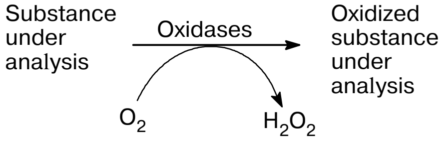
Yet another attempt has been made to produce a new hydrogen peroxide transducer by cycling titanium dioxide electrodes in a ferricyanide solution.[56] Cyclic voltammograms of the resulting electrodes indicate that Prussian Blue films are formed under these conditions. However, titanium dioxide electrodes modified by hexacyanoferrate did not exhibit high activity in the reduction of H_2O_2; the sensitivity of analysis did not exceed 0.8 mA mol^{-1} L cm^{-2}.

Recently, data on new hydrogen peroxide transducers based on cobalt[57] and chromium hexacyanoferrates[58] have been published. However, we found no experimental evidence for the synthesis of chromium and cobalt hexacyanoferrates. Apparently, in the study,[58] too, Prussian Blue rather than chromium hexacyanoferrate films were actually formed. In addition, data on the high catalytic activity of cobalt hexacyanoferrate raise doubts. Two redox potentials for the electroactivity of modified electrodes, +449 and +595 mV, were reported.[57] However, upon the addition of H_2O_2, electrcatalysis takes place in the region of 0.2 V,[58] which is typical of Prussian Blue catalysis (see Fig. 1). In addition, the film electrodeposition procedure reported in the study cited[57] includes a 6-h (!) cycling in a ferricyanide solution. In our opinion, the modified electrodes always contained some Prussian Blue because it is known to be deposited even from solutions containing only ferricyanide.[59] No experimental evidence indicating that the high catalytic activity in the reduction of hydrogen peroxide is actually due to cobalt hexacyanoferrate rather than to iron hexacyanoferrate is given in this study.

Thus, Prussian Blue is the most promising hydrogen peroxide transducer among the metal hexacyanoferrates studied.

The use of metal hexacyanoferrates for the development of biosensors

More than 90% of the commercially manufactured enzyme electrodes and analytical kits contain oxidases as the terminal enzymes responsible for the generation of the analytical signal. These enzymes catalyze oxidation of the specific substrate by molecular oxygen to give hydrogen peroxide according to the reaction



(4)

The method for hydrogen peroxide detection is considered to be the most advanced for the design of biological sensors based on oxidases because it allows analysis of low concentrations of substances.[60] However, it is important to carry out H_2O_2 analysis at low potentials in order to reduce the interfering influence of readily oxidizable compounds present in the samples.[61]

The applicability of metal hexacyanoferrates for the design of biosensors was first demonstrated by our research group.[2] It was proposed to replace platinum, used most often as a hydrogen peroxide transducer, by electrodes modified by Prussian Blue. The glucose oxidase enzyme was immobilized in a polymer (Nafion) film on the transducer surface. The resulting biosensor demonstrated high sensitivity and selectivity in glucose analysis even in the presence of reducing agents such as ascorbate or paracetamol.

Yet another method for the preparation of biosensors on the basis of Prussian Blue[44] consisted in oxidase immobilization in the films of the inorganic polycrystal during its deposition on electrodes. However, as noted above, preparation of Prussian Blue films is best performed in 0.1 *M* HCl. These deposition conditions are not favorable for most of enzymes, in particular, for glucose oxidase, and they do not ensure high enzyme activity of the modified electrodes. Therefore, the biosensor sensitivity was relatively low[62] compared to the sensitivity of the initial transducer for the detection of H_2O_2.[58] A biosensor on the basis of Prussian Blue making use of electrocatalytic oxidation of H_2O_2 has also been developed.[45,46] However, metal hexacyanoferrates are excellent electrocatalysts of oxidation of readily oxidizable substances such as ascorbic acid,[63] which can exert adverse influence on the biosensor response. The possibility of decreasing the potential of hydrogen peroxide detection based on its oxidation to 0.45 V[43,48] seems doubtful because under these conditions, Prussian Blue exists in the inactive oxidized state.

Metal hexacyanoferrates have been used to design biosensors for the analysis of glucose,[7,42–46,49,54,55,62,64–70] ethanol,[7] D-alanine,[44] oxalate anions,[71,72] glutamate anions,[49,52] and choline[54] (Table 3). In most studies, Prussian Blue was used as the transducer. As noted above, the catalytic activity of transducers based on chromium and cobalt hexacyanoferrates reported in several works[62,66,71,72] was, apparently, also due to Prussian Blue. The sensitivity displayed by copper hexacyanoferrate is several orders of magnitude lower than that of iron hexacyanoferrate.

Table 3. Transducers for detection of hydrogen peroxide and biosensors based on them

Substance determined	M*	Enzyme	Ref.
Hydrogen peroxide	Fe	—	7, 42—44, 49, 50, 54
	Co (Fe ?)	—	57
	Cr (Fe ?)	—	58
	Fe, Cu	—	77
	Ti	—	78
	Cu	—	79
Glucose	Fe	Glucose oxidase	7, 42—46, 49, 54, 64, 65
	Cr (Fe ?)	Glucose oxidase	62
	Co (Fe ?)	Glucose oxidase	66
	Cu	Glucose oxidase	55, 67—69
	Ni	Glucose oxidase	70
D-alanine	Fe	D-Amino acid oxidase	44
Ethanol	Fe	Alcohol oxidase	7
Glutamate	Fe	Glutamate oxidase	49, 52
Oxalate	Cr (Fe ?)	Oxalate oxidase	70, 71
Choline	Fe	Choline oxidase	54

* M are metals in transition metal hexacyanoferrates.

However, chemically synthesized copper hexacyanoferrate is suitable for the development of carbon paste enzymatic electrodes.[69]

In view of our conclusion that Prussian Blue prepared by electrochemical synthesis is the best currently known transducer for hydrogen peroxide, it is important to compare the properties of iron hexacyanoferrate biosensors with those of known bioanalytical systems. Let us consider, for example, the properties of biosensors for the analysis of glutamate anions. Owing to the fact that measurements are carried out at a low potential of the indicator electrode, the effect of reducing agents on the response of a biosensor based on Prussian blue[52] is insignificant, like that for biosensors containing peroxidase.[73] However, enzymatic electrodes based on Prussian Blue ensure the detection of glutamate in the flow-injection mode in concentrations ($1 \cdot 10^{-7}$ mol L^{-1}) ten times lower than those required for all other types of existing biological sensors. The sensitivity of this glutamate measurement is 0.2 A mol^{-1} L cm^{-2}, which is approximately an order of magnitude higher than the values found for the best known enzyme electrodes, moreover, in the periodic mode of testing.[73—76] The sensitivity of the Prussian Blue-based biosensor in a batch or flow mode of analysis is expected to be 1.5 times higher.

Our studies showed that the use of electrodes modified by Prussian Blue for the design of first-generation biosensors is quite promising. The replacement of a platinum electrode by an electrode modified by an inorganic polycrystal not merely decreases the cost of the biosensor. Due to the high sorption activity, catalysts based on platinum group metals can be poisoned by many low-molecular-weight compounds including thiols, sulfides, *etc.*, which are not expected to affect Prussian Blue-based electrocatalysts. Due to the multilayer structure of the coating, the current densities of hydrogen peroxide reduction at modified electrodes are higher than those for other known electrocatalytic systems. The inorganic electrocatalyst of hydrogen peroxide reduction is insensitive to oxygen, which makes it possible to decrease markedly the potential of the indicator electrode and the biosensor response does not depend on the presence of other reducing agents such as ascorbic acid.

Conclusion

Let us list the advantages and prospects of using iron hexacyanoferrates and other transition metal hexacyanoferrates in analysis. First, metal hexacyanoferrates can be used in design of potentiometric and amperometric sensors for determination of electrochemically inactive cations. Unlike so-called "smart materials," metal hexacyanoferrates exhibit sensitivity and selectivity of a thermodynamic background; electrochemically inactive cations are incorporated in films to counterbalance the charge during redox reactions. Moreover, some redox reactions in hexacyanoferrate films can also involve anions, which would enable development of sensors for the analysis of anions in the future. Second, Prussian Blue is the most promising low-potential transducer for the analysis of hydrogen peroxide not only among other hexacyanoferrates but also among all the known transducers. The catalytic activity and sensitivity of electrodes modified by Prussian Blue in H_2O_2 reduction in neutral media are characterized by an electrochemical rate constant of 0.01 cm s^{-1}. This value is three orders of magnitude (!) higher than the rate constant found for platinum electrodes, the most popular hydrogen peroxide transducers. The current of reduction of H_2O_2 at Prussian Blue-modified electrodes is 100 times as high as the current of O_2 reduction. Conversely, platinum and other noble metals are not selective catalysts for hydrogen peroxide reduction in the presence of oxygen. In addition to high sensitivity (down to 1 A mol^{-1} L cm^{-2}) and selectivity, Prussian Blue-modified electrodes, after a specially selected deposition procedure and subsequent treatment, possess also relatively high operation stability, which is comparable with or even higher than those of other transducers.

Despite the fact that iron hexacyanoferrate films are still of interest for fundamental research, electrodes modified by Prussian Blue can already be used in analytical tools both for single analysis and for continuous monitoring of chemical threat agents, food additives, and key metabolites. The use of Prussian Blue biosensors in clinical diagnostics, which requires high sensitivity and selectivity of analysis, and the possibility of designing miniature sensors are especially important for noninvasive diagnostics of blood chemistry and for neurology.

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