Review

Prussian Blue and Its Analogues: Electrochemistry and Analytical Applications

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Abstract
This article reviews fundamental aspects of deposition, structure and electrochemistry of Prussian Blue and its analogues. Special attention is given to the metal hexacyanoferrates with potential analytical applications. Prussian Blue and its analogues as advanced sensing materials for nonelectroactive ions are discussed. In contrast to common 'smart materials', the sensitivity and selectivity of metal hexacyanoferrates to such ions is provided by thermodynamic background. Prussian Blue itself is recognized as the most advantageous low-potential transducer for hydrogen peroxide over all known systems. Both high sensitivity (ca. $1\,A\,M^{-1}\,cm^{-2}$) and selectivity in relation to oxygen reduction are more than three orders of magnitude higher, than for platinum electrodes. Biosensors based on different transducing principles containing enzymes oxidases are compared, and the devices operated due to hydrogen peroxide detection with the Prussian Blue based transducer are shown to be the most advantageous ones. The future prospects of chemical and biological sensors based on metal hexacyanoferrates are outlined.

Keywords: Prussian Blue, Hexacyanoferrate, Sensor, Nonelectroactive cations, Hydrogen peroxide, Transducer, Biosensor, Glucose, Glutamate

1. Introduction

Prussian Blue, or ferric hexacyanoferrate is definitely one of the most ancient coordination materials known. The earliest announcements found are from the very beginning of the eighteenth century [1, 2]. However, a quite recent investigation by Neff [3], that Prussian Blue forms electroactive layers after electrochemical or chemical deposition onto the electrode surface, has opened a new area in fundamental investigation of this unique inorganic polycrystal.

This article reviews the fundamental aspects on deposition, structure and electrochemistry of Prussian Blue and its analogues. Special attention is given to the metal hexacyanoferrates for which analytical applications are reported. The attractive performance characteristics of chemical and biological sensors based on Prussian Blue and its analogues are summarized.

2. Fundamental Aspects: Structure and Electrochemistry

2.1. Chemical and Crystalline Structure of Prussian Blue

The fact that Prussian Blue is indeed ferric ferrocyanide ($Fe_4^{III}[Fe^{II}(CN)_6]_3$) with iron(III) atom coordinated to nitrogen and iron(II) atom coordinated to carbon has been definitely established by spectroscopic investigations [4]. Prussian Blue can be synthesized chemically by mixing of ferric (ferrous) and hexacyanoferrate ions with different oxidation state of iron atoms: either $Fe^{3+} + [Fe^{II}(CN)_6]^{4-}$ or $Fe^{2+} + [Fe^{III}(CN)_6]^{3-}$. After mixing, an immediate formation of a dark blue colloid is observed. On the contrary, the mixed solutions of ferric (ferrous) and hexacyanoferrate ions with the same oxidation state of iron atoms are apparently stable.

Crystalline structure of Prussian Blue was first discussed by Keggin and Miles on the basis of powder diffraction patterns [5] and then has been determined more precisely by Ludi and co-workers from single crystals by electron and neutron diffraction measurements [6]. Prussian Blue has a basic cubic structure consisting of alternating iron(II) and iron(III) located on a face centered cubic lattice (Fig. 1) in such a way, that the iron(III) ions are surrounded octahedrally by nitrogen atoms, and iron(II) ions are surrounded by carbon atoms. The cubic unit cell dimensions are 10.2 Å.

2.2. Deposition of Prussian Blue and Its Electroactivity

The deposition of Prussian Blue on various conductive surfaces is usually carried out from aqueous solutions containing a mixture of ferric (Fe^{3+}) and ferricyanide ($[Fe^{III}(CN)_6]^{3-}$) ions, either spontaneously in an open-circuit regime or by applying a reductive electrochemical driving force. Chronopotentiometric investigations in equimolar ferric-ferricyanide mixtures has shown two basic plateaus: at 0.7 V and at 0.4 V (SCE) [7]. These plateaus have been attributed to reduction of the one-to-one complex of $Fe^{III}[Fe^{III}(CN)_6]$, discovered earlier [8], and of Fe^{3+} ions, respectively. At 0.7 V, Prussian Blue is deposited according to reduction of ferric-ferricyanide complex ($Fe^{III}[Fe^{III}(CN)_6]$). Around 0.4 V the bulk precipitation of Prussian Blue occurs due to reduction of Fe^{3+} to Fe^{2+}, the latter reacting with ($[Fe^{III}(CN)_6]^{3-}$). The open-circuit deposition is highly dependent on the electrode support. Its mechanism is probably the oxidation of the conductive material with the ($Fe^{III}[Fe^{III}(CN)_6]$) complex, which forms Prussian Blue after one-electron reduction. Posing the electrodes at potentials lower, than 0.2 V is, according to our experience, not plausible for deposition of Prussian Blue, because both Fe^{3+} and $[Fe^{III}(CN)_6]^{3-}$ ions are reduced, and the structure of the resulting polycrystal is less regular.

Cyclic voltammogram of Prussian Blue modified electrode is shown in Figure 2. In between the observed two sets of peaks the oxidation state corresponding to Prussian Blue itself occurs. Its reduction is accompanied with loss of the color, and the reduced form of the polycrystal is denoted as Prussian White. The transfer
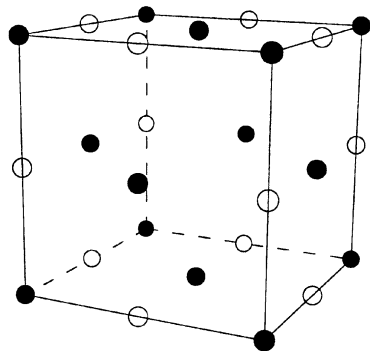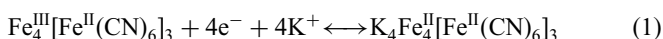
Fig. 1. Prussian Blue unit cell according to Keggin and Miles, plotted using data from measurements [6]; (●) Fe^{3+}, (○) Fe^{2+}.

of electrons is compensated by the entrapment of cations in the film according to the equation:

$$Fe_4^{III}[Fe^{II}(CN)_6]_3 + 4e^- + 4K^+ \longleftrightarrow K_4Fe_4^{II}[Fe^{II}(CN)_6]_3 \qquad (1)$$

In literature the term 'soluble Prussian Blue' introduced by Keggin and Miles [5] to determine the $KFeFe(CN)_6$ compound is still widely used. However, it is important to note, that the term 'soluble' refers to the ease with which the potassium ion can be peptized rather than to the real solubility of Prussian Blue. Indeed, it can be easily shown by means of cyclic voltammetry, that the stability of Prussian Blue films on electrode supports is nearly independent of their saturation by potassium cations. Moreover, Itaya and co-workers [9] have not found any appreciable amount of potassium ions in Prussian Blue, which makes doubtful the structures like $KFeFe(CN)_6$. Thus, the above equation fully describes the Prussian Blue/Prussian White redox reaction.

The Prussian Blue/Prussian White redox activity with potassium as the counter cation is observed in cyclic voltammograms as a set of sharp peaks with separation of 15–30 mV. These peaks, in particular the cathodic one, are like the peaks from anodic demetallization. Such set of sharp peaks in cyclic voltammograms correspond to the regular structure of Prussian Blue with homogeneous distribution of charge and ion transfer rates throughout the film. This obvious conclusion from electrochemical investigations was confirmed by means of spectroelectrochemistry [10].
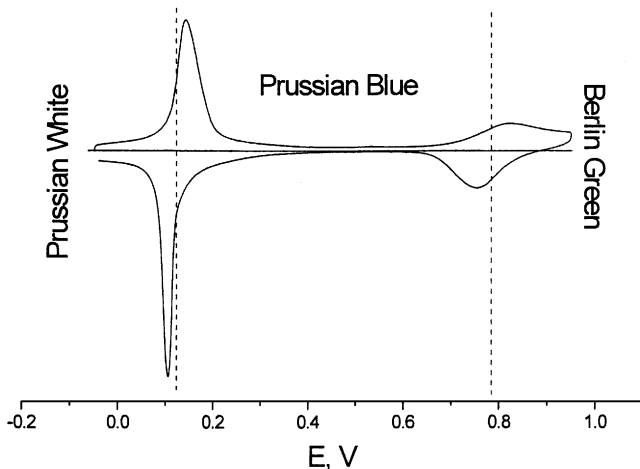

Fig. 2. Typical cyclic voltammogram of Prussian Blue modified smooth (mirrored glassy carbon) electrode; 0.1 M KCl, 40 mV s^{-1}.

The sharpness of Prussian Blue/Prussian White redox peaks in cyclic voltammograms can be used as an indicator of the quality of Prussian Blue layers. To achieve a regular structure of Prussian Blue, two main factors have to be considered: the deposition potentials and the pH of the initial growing solution. As mentioned, the potential of the working electrode should not be lower then 0.2 V, where ferricyanide ions are intensively reduced. The solution pH is a critical point, because ferric ions are known to be hydrolyzed easily, and the hydroxyl ions (OH^-) cannot be substituted in their coordination sphere in course of Prussian Blue crystallization. According to our findings initial solution with pH 1 is optimal for deposition [11, 12].

It is important to note, that not all cations promote Prussian Blue/Prussian White electroactivity. Except for potassium, only ammonium (NH_4^+), cesium (Cs^+) and rubidium (Rb^+) were found able to penetrate the Prussian Blue lattice. Other mono- and divalent cations are considered as blocking ones.

At high anodic potentials Prussian Blue converts to its fully oxidized form as is clearly seen in cyclic voltammograms due to the presence of the corresponding set of peaks (Fig. 2). The fully oxidized redox state is denoted as Berlin Green or in some cases as 'Prussian Yellow'. Since the presence of alkali metal ions is doubtful in the Prussian Blue redox state, the only possible mechanism for charge compensation in Berlin Green/Prussian Blue redox activity is the entrapment of anions in course of oxidative reaction. The complete equation is:

$$Fe_4^{III}[Fe^{II}(CN)_6]_3 - 3e^- + 3A^- \longleftrightarrow Fe_4^{III}[Fe^{III}(CN)_6A]_3 \qquad (2)$$
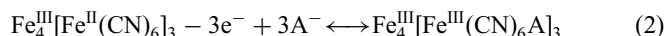
Except for deposition of Prussian Blue from the mixture of ferric and ferricyanide ions, its electrosynthesis from the single ferricyanide solution is reported [13]. Ferricyanide ions are not extremely stable even in aqueous solution, which is noticed in the change of color after a few days of storage. Thus, the coordination sphere can be destroyed also in course of electrochemical reactions. The mentioned processes may lead to formation of ferric-ferricyanide complex or 'free' ferric ions. The reduction of the resulting mixture leads to formation of Prussian Blue.

2.3. Deposition and Electroactivity of the Other Metal Hexacyanoferrates

A few years after the discovery of the deposition and electroactivity of Prussian Blue, other metal hexacyanoferrates were deposited on various electrode surfaces. However, except for ruthenium and osmium, the electroplating of the metal or its anodizing were required for deposition of nickel [14], copper [15, 16], and silver [9] hexacyanoferrates. Later studies have shown the possibilities of synthesis of nickel, cobalt, indium hexacyanoferrates similarly to deposition of Prussian Blue [17–19]. It seems that deposition of cupric hexacyanoferrate still required either open-circuit regime [17] or electroplating.

Recently, the electrodeposition of chromium hexacyanoferrate has been reported [20], raising, however, some doubt. Keeping in mind, that the difference in redox potentials of $Fe^{3+/2+}$ and $Cr^{3+/2+}$ couples is approximately 1.2 V (0.771 V for $Fe^{3+/2+}$ and −0.41 V for $Cr^{3+/2+}$, NHE [21]), it is surprising to observe, that the cyclic voltammograms of chromium hexacyanoferrate [20, 22] are quite similar to those observed for Prussian Blue (Fig. 2). There is no other metal hexacyanoferrate with Prussian Blue-type electroactivity. Our attempt to synthesize chromium hexacyanoferrate chemically failed because Cr^{3+} ions do not give any recognizable precipitate either with ferricyanide ($[Fe^{III}(CN)_6]^{3-}$)

or with ferrocyanide ($[Fe^{II}(CN)_6]^{4-}$) ions. Since during the reported electrochemical synthesis the electrode has not been poised to potentials lower than -0.2 V [20, 23], Cr^{3+} cannot be reduced even to Cr^{2+} because $Cr^{3+/2+}$ redox potential is -0.6 V (SCE). Then, what kind of film did Lin and co-workers observe [20, 23]? Since the deposition has been carried out during 0.5–1 h, the Prussian Blue itself can be crystallized even in the presence of ferricyanide alone [13]. Thus, most probably the authors [20, 23] deposited Prussian Blue instead of chromium hexacyanoferrate. In another report [22] chromium could be pre-plated during the potential cycling down to -1.5 V. However, further deposition of chromium hexacyanoferrate was carried out with the cathodic switching potential of -0.2 V, and the resulting film also showed Prussian Blue-type redox activity. Thus, there is no strong evidence for electrosynthesis of chromium hexacyanoferrate.

As mentioned, the potassium ion promotes the redox activity of Prussian Blue, where as sodium ion blocks it. However, indium, cobalt and nickel hexacyanoferrates were successfully grown and then cycled in the presence of sodium as the counter cation [18]. There are two possible explanations of redox activity in the presence of sodium. All these hexacyanoferrates give a single set of peaks in their cyclic voltammograms. This set of peaks may be attributed to the redox reaction with the charge compensation due to entrapment of anions rather than cations, similarly to the Berlin Green/Prussian Blue redox reaction. Alternatively, sodium ions may penetrate the lattice of these hexacyanoferrates.

3. Analytical Applications

3.1. Sensors for Nonelectroactive Cations

The participation of cations in redox reactions of metal hexacyanoferrates provides a unique opportunity for development of chemical sensors for nonelectroactive ions. The development of sensors for thallium (Tl^+) [15], cesium (Cs^+) [24] and potassium (K^+) [25, 26] pioneered the analytical applications of metal hexacyanoferrates (Table 1). Later the number of cationic analytes was enlarged, and included ammonium (NH_4^+) [27], rubidium (Rb^+) [28] and even other mono- and divalent cations [29]. In most cases the electrochemical techniques used were potentiometry and amperometry either under constant potential or in cyclic voltammetric regime. The apparently complete list of such sensors is presented in Table 1.

However, some monovalent ions similarly promote the electroactivity of metal hexacyanoferrates, which affects the selectivity of the corresponding sensors. In particular, it is rather hard to distinguish between the alkali metal ions and ammonium ion. Indeed, the same metal hexacyanoferrates were used in some cases as potassium, cesium or rubidium sensors, and in other cases as ammonium sensors (Table 1).

Particular cases are potassium selective potentiometric sensors based on cobalt [30] and nickel [28, 31] hexacyanoferrates. As mentioned, these hexacyanoferrates possess quite satisfactory redox activity with sodium as counter cation [18]. According to the two possible mechanisms of such redox activity (either sodium ions penetrate the lattice or charge compensation occurs due to entrapment of anions) there is no thermodynamic background for selectivity of these sensors. In these cases electro active films seem to operate as 'smart materials' similarly to conductive polymers in electronic noses.

Table 1. Sensors for nonelectroactive cations.

Analyte	Me-hexacyanoferrate, Me:	References
Cs^+	Cu	[24, 77]
K^+	Cu	[26, 78]
	Ag^+-$[Mo(CN)_8]^{4-}$	[25]
	Fe	[79]
	Co	[30]
	Ni	[31]
NH_4^+	Cu	[80]
Tl^+	Cu	[15]
	Fe	[81]
K^+, Cs^+	Fe, Cu, Ag, Ni, Cd	[82]
K^+, NH_4^+	Cu	[27]
K^+, Rb^+, Cs^+, NH_4^+	Cu, Ni	[28]
K^+, Rb^+, Cs^+, NH_4^+	Fe	[83, 84]
Mono- and divalent cations	Cu, Ni	[29]
Separation of Cs^+	Fe	[32, 33]
	Cu	[34, 35]

Except for sensors applications, the intercalation of alkali metal ions in metal hexacyanoferrates was used for adsorption and separation of cesium ions from different aqueous solutions with Prussian Blue [32, 33] and cupric hexacyanoferrate [34, 35].

3.2. Sensors for Easily Oxidizable Compounds and Other Nontraditional Sensors

The ability of metal hexacyanoferrates to oxidize some organic and inorganic compounds has been used in the 1990s, for analytical applications. A list of the analytes is given in Table 2. Despite some of the compounds being tested in real objects, a cross-selectivity of such sensors must be low.

Some nontraditional sensors based on Prussian Blue are also listed in Table 2. The Prussian Blue films grown through inter-digitated arrays are used as a humidity sensor [36] as well as a sensor for vapors of methanol and dichloroethane [37]. A non-conventional optical pH sensor based on Prussian Blue is also reported [38, 39].

3.3. Prussian Blue as an Advanced Transducer for Hydrogen Peroxide

The requirements for selective detection of hydrogen peroxide rise from the following main reasons. Hydrogen peroxide itself is a chemical threat agent present in rain and ground waters as waste product of industry and atomic power stations [40, 41]. In addition, hydrogen peroxide is used for disinfection of water pools, food and beverage packages [42, 43], which makes it important to measure its residual concentration. On the other hand, hydrogen peroxide is a side product of oxidases, the enzymes, which are included as terminal ones in more than 90 % of the existing enzyme-based biosensors and analytical kits. The low-potential detection of hydrogen peroxide was found to be the most progressive procedure for operation of the oxidase-based biosensors providing both high sensitivity and high selectivity in the presence of easily oxidizable compounds.

Table 2. Sensors for the easily oxidizable compounds and other nontraditional sensors.

Analyte	Me-hexacyanoferrate, Me:	References
Ascorbic acid	Ni	[64]
Catecholamine	Fe	[85]
	Ni	[86]
Cysteine	Cu	[87]
Cytochrome C	Fe	[88]
Dopamine	Ni	[19]
Hydrazine	Fe	[89]
	Co, Cu	[90]
NADH	Co, Ni	[91, 92]
Persulfate anion	Fe	[93]
Sulfhydryl	Cu	[94]
	In	[95]
Sulfite	Ni	[96]
Sulfur dioxide	Cu	[97]
Thiosulfate	Ni	[98, 99]
Humidity	Fe	[36]
Vapors of methanol, H_2O, dichloroethane	Fe (Prussian Blue)	[37]
pH, optical	Fe (Prussian Blue)	[38, 39]

The possibility for selective detection of hydrogen peroxide by its reduction in the presence of oxygen on Prussian Blue modified electrodes was first demonstrated by our group in 1994 [44]. Thereafter several articles on application of Prussian Blue as hydrogen peroxide transducer appeared [11, 45–48]. The approach of a British school, however, was the oxidation of H_2O_2 at high anodic potentials rather than its reduction [47, 48].

Electrocatalyic reduction of hydrogen peroxide by Prussian Blue has been thoroughly investigated [11, 12, 49]. The comparative activities of the inorganic polycrystal in oxygen and hydrogen peroxide reduction are highly dependent on the Prussian Blue structure [11, 50]. Optimizing the deposition procedure for Prussian Blue, a selective electrocatalyst for H_2O_2 reduction in the presence of oxygen able to operate in a wide potential range has been synthesized [11]. At optimal potential for sensor and biosensor applications (0.0 V, Ag/AgCl) the current of H_2O_2 reduction was several hundred times higher than of oxygen reduction.

Stability of the Prussian Blue based hydrogen peroxide transducer is a crucial point commonly raised by referees as an objection against its practical applications. Indeed Prussian White (the redox state of Prussian Blue at 0.0 V) is thermodynamically unstable on electrode surfaces. In addition, hydroxyl ions being the products of hydrogen peroxide reduction in neutral media [49] are able to solubilize the inorganic polycrystal. However, due to continuous efforts in improving the crystalline structure of the deposited Prussian Blue and its additional post treatment excellent operational stability, which even exceeds the stability of the known H_2O_2 transducers, has been achieved [51, 52].

The kinetics of hydrogen peroxide reduction catalyzed by Prussian Blue has been investigated [12, 49]. In neutral media the reaction scheme of H_2O_2 reduction has been found to be the following:

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

The electrochemical rate constants for hydrogen peroxide reduction have been found to be dependent on the amount of Prussian Blue deposited confirming that the H_2O_2 penetrates the films, and inner layers of the polycrystal take part in catalysis. For 4–6 nmol cm^{-2} of Prussian Blue the electrochemical rate constant exceeds 0.01 cm s^{-1} [12], which is higher than for all the known H_2O_2 transducers. For comparison, the electrochemical rate constant for H_2O_2 oxidation on platinum in neutral media is less than 7×10^{-6} cm s^{-1} [53]. The activity of Pt in hydrogen peroxide reduction is even lower.

Due to both its high activity and selectivity, which are commonly the properties of biocatalysis the Prussian Blue was denoted as an 'artificial peroxidase' [51]. Using Prussian Blue as transducer for hydrogen peroxide it was possible to achieve the sensitivity in flow-injection mode of 0.6 A M^{-1} cm^{-2} [51, 54], which takes into account the dispersion coefficient [55] corresponding to the sensitivity of 1 A M^{-1} cm^{-2}, in either batch regime or under continuous flow.

Of particular importance is the chemical synthesis of a Prussian Blue based hydrogen peroxide transducer, which can be used both in screen-printed and carbon paste electrodes. Despite both the commercially available and commonly precipitated Prussian Blue they show only a minor electrocatalytic activity in hydrogen peroxide reduction [56]. A successful chemical synthesis of the electrocatalyst has recently been carried out by open-circuit deposition onto graphite powder from a ferric-ferricyanide solution [57]. The resulting carbon paste-Prussian Blue transducer for H_2O_2 exhibits high operational stability in neutral and weakly basic media up to pH 9, which provides successful application in the corresponding oxidase-based biosensors. The sensitivity of the Prussian Blue based carbon paste sensors for hydrogen peroxide is rather satisfactory, being, however, from 20 to 200 times lower than the sensitivity of the Prussian Blue modified smooth (glassy carbon) electrodes.

There were several attempts to use other metal hexacyanoferrates as hydrogen peroxide transducers. Cupric hexacyanoferrate was integrated in a biosensor, but the resulting sensitivity was three orders of magnitude lower than that of a similar electrode based on Prussian Blue [58]. Moreover, cupric hexacyanoferrate has to be poised to high cathodic overvoltages (> -0.7 V vs. its redox potential) to achieve a considerable rate of hydrogen peroxide reduction. Despite the lack of a kinetic study, these observations indicate that cupric hexacyanoferrate is a poorer electrocatalyst compared to Prussian Blue.

Another transition metal based transducer for hydrogen peroxide was made by cycling of a titanium dioxide electrode in ferricyanide solution [59]. Despite the authors did not claim synthesis of a new transition metal hexacyanoferrate, the cyclic voltammograms were similar to Prussian Blue films. Unfortunately, titanium dioxide electrode modified with hexacyanoferrate did not show high activity to H_2O_2 reduction: the sensitivity calculated from the data in paper was of 0.8 mA M^{-1} cm^{-2}.

Two metal hexacyanoferrates with extremely high sensitivity to hydrogen peroxide (>1 A M^{-1} cm^{-2}) were recently reported: Co-hexacyanoferrate [60] and Cr-hexacyanoferrate [20]. However, as was mentioned above, there is no experimental evidence for synthesis of chromium hexacyanoferrate: instead [20] the Prussian Blue layers were most probably synthesized. There is also doubt concerning high catalytic activity of Co-hexacyanoferrate. Lin and co-workers reported two redox potentials for electroactivity of cobalt hexacyanoferrate: at

+449 mV and +595 mV [60]. However, after addition of hydrogen peroxide electrocatalysis is observed at a shoulder with potential of about 0.2 V [60], which has never been reported for Co-hexacyanoferrate, but is similar to one attributed to Prussian Blue (Fig. 2) with the precision of the reference electrode used. It should be noted, that the deposition of Co-hexacyanoferrate in [60] included 6 (!) hours cycling in solution containing ferricyanide, and thus, the resulting film obviously contain some amount of Prussian Blue being deposited even from a single ferricyanide solution [13]. So, there is no experimental evidence that the high catalytic activity in hydrogen peroxide reduction is peculiar to Co-hexacyanoferrate rather than to Prussian Blue. The publications under discussion [20, 60] most probably confirm the unique catalytic properties of Prussian Blue.

As a conclusion from this section, Prussian Blue has to be considered as the most advantageous hydrogen peroxide transducer over all the existing systems.

3.4. Application of Transition Metal Hexacyanoferrates for Biosensors

More than 90% of commercially available enzyme based biosensors and analytical kits contain oxidases as terminal enzymes responsible for generation of the analytical signal. These enzymes catalyze oxidation of specific analyte with molecular oxygen producing hydrogen peroxide according to the reaction:

$$Analyte \xrightarrow{\text{Oxidase}} Oxidized\ Analyte$$
$$O_2 \rightarrow H_2O_2$$

$$(4)$$
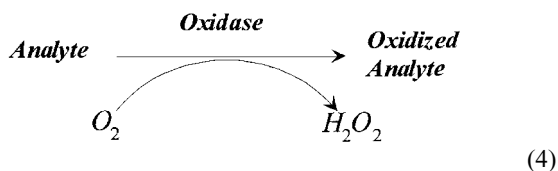
Among different approaches providing operation of the oxidase-based biosensors, the detection of hydrogen peroxide production was found to be the most progressive one, allowing detection of low levels of analytes [61]. However, the detection of H_2O_2 has to be carried out at low potentials in order to reduce the interference of easily oxidizable compounds [62].

Application of metal hexacyanoferrates for development of biosensors was first announced by our group in 1994 [44]. The goal was to substitute platinum as most commonly used hydrogen peroxide transducer for the Prussian Blue modified electrode. The enzyme glucose oxidase was immobilized on the top of the transducer in the polymer (Nafion) membrane. The resulting biosensor showed advantageous characteristics of both sensitivity and selectivity in the presence of commonly tested reductants, such as ascorbate and paracetamol.

Another approach for development of Prussian Blue based biosensors was published in 1995 and involved enzyme immobilization by entrapment into Prussian Blue films during its deposition [46]. However, as mentioned, the best media for deposition of Prussian Blue is 0.1 M HCl, is not tolerable of enzymes, in particular for glucose oxidase [63]. Moreover, the entrapment of the enzyme in metal hexacyanoferrates during their deposition does not provide enough enzyme activity of the resulting film, which resulted in a rather low sensitivity of the

Table 3. The transducers for hydrogen peroxide and the related biosensors.

Analyte	Me-hexacyano-ferrate, Me:	Enzyme	References
Hydrogen peroxide	Fe	–	[11, 44–46, 51, 52, 57]
	Co (Fe ?)	–	[60]
	Cr (Fe ?)	–	[20]
	Fe, Cu	–	[100]
	Ti	–	[59]
	Cu	–	[101]
Glucose	Fe	Glucose oxidase	[11, 44–48, 51, 57, 65, 66]
	Cr (Fe ?)	Glucose oxidase	[23]
	Co (Fe ?)	Glucose oxidase	[67]
	Cu	Glucose oxidase	[58, 68–70]
	Ni	Glucose oxidase	[71]
D-Alanine	Fe	D-Amino acid oxidase	[46]
Ethanol	Fe	Alcohol oxidase	[11]
Glutamate	Fe	Glutamate oxidase	[51, 54]
Oxalate	Cr (Fe ?)	Oxalate oxidase	[22, 72]
Choline	Fe	Choline oxidase	[57]

resulting biosensor [23] compared to the sensitivity of the corresponding H_2O_2 transducer [20].

Except for a low-potential hydrogen peroxide transducer, Prussian Blue was integrated in biosensors as electrocatalyst for H_2O_2 oxidation [47, 48]. However, metal hexacyanoferrates are ideal electrocatalysts for oxidation of easily oxidizable compounds like ascorbate [64], which would definitely interfere in the biosensor response. The detection of hydrogen peroxide at 0.45 V by its oxidation on Prussian Blue modified electrodes [48] seems to be doubtful, because the Prussian Blue oxidation state has not been found active either in oxidation or in reduction of H_2O_2 [45, 50].

Metal hexacyanoferrates based biosensors were developed for analysis of glucose [11, 23, 44–48, 51, 57, 58, 65–71], ethanol [11], D-alanine [46], oxalate [22, 72], glutamate [51, 54] and choline [57] (Table 3). Among transducers Prussian Blue undoubtedly dominates especially if one take into account that instead of both chromium and cobalt hexacyanoferrates the activity of the transducers in publications [22, 23, 67, 72] was most probably provided by Prussian Blue (see above). As was also mentioned, the sensitivity of cupric hexacyanoferrate is several orders of magnitude lower compared to Prussian Blue. However, chemically synthesized cupric hexacyanoferrate is useful for carbon paste biosensors [70].

According to the above conclusions that electrochemically synthesized Prussian Blue is the best transducer for hydrogen peroxide, it is important to compare the properties of the related biosensors with bioanalytical systems based on different detection principles. Let us consider for example biosensors for glutamate. Due to low potential of the indicator electrode, the influence of interferents on Prussian Blue based biosensor [54] was similarly low as in the case of a biosensor based on peroxidase wired in osmium hydrogel [73]. However, Prussian Blue based electrodes allows one to detect 1×10^{-7} M glutamate in the flow-injection mode, which is one order of magnitude lower, than for known biosensors. The sensitivity of a Prussian Blue based biosensor for glutamate is $0.2\ A\,M^{-1}\,cm^{-2}$ in flow-

injection mode. This is approximately one order of magnitude higher than the value observed for one of the best glutamate biosensors in the batch mode [73–76]. In our FIA experiments [51, 54] the dispersion coefficients exceeded the value of 1.5, thus in batch or flow-through conditions the sensitivity of Prussian Blue based glutamate biosensor would be at least 1.5 times higher.

Thus, Prussian Blue based biosensors are the most advantageous among the known bioanalytical devices which involve hydrogen peroxide producing oxidases.

4. Conclusions and Future Prospects

In conclusion, the unique properties of Prussian Blue and other transition metal hexacyanoferrates, which are advantageous over existing materials concerning their analytical applications, should be mentioned. First, metal hexacyanoferrates provide the possibility to develop potentiometric and even amperometric sensors for nonelectroactive cations. In contrast to common 'smart materials', the sensitivity and selectivity of metal hexacyanoferrates to such ions is provided by thermodynamic background: nonelectroactive cations are entrapped in the films for charge compensation upon redox reactions. Moreover, according to the current state of knowledge, some of the redox reactions occurring in metal hexacyanoferrates involve entrapment of anions rather than cations. Thus, in future studies the sensors for anions may also be developed.

Second, Prussian Blue is considered as the most advantageous low-potential transducer for hydrogen peroxide not only among hexacyanoferrates, but over all known systems. The specific activity and, thus, the sensitivity of Prussian Blue modified electrodes to H_2O_2 reduction in neutral media are characterized by the electrochemical rate constant of $0.01 \, cm \, s^{-1}$, which is three orders of magnitude (!) higher than in case of platinum being the most widely used hydrogen peroxide transducer. Current of H_2O_2 reduction on Prussian Blue modified electrodes is two orders of magnitude higher than of O_2 reduction, where as platinum and other noble metals are not selective to hydrogen peroxide reduction in the presence of oxygen. Except for greatly improved sensitivity (up to $1 \, A \, M^{-1} \, cm^{-2}$) and selectivity, the specially deposited and post treated Prussian Blue modified electrodes possess quite satisfactory long-term operational stability comparative with or even exceeding the known transducers. Ferric ferrocyanide films on electrodes are still an open area for 'pure' scientific investigations while Prussian Blue modified electrodes are ready-to-use in analytical devices for either sampling or continuous monitoring of chemical threat agents, important food additives and key metabolites of life pathways. Particular importance of application of Prussian Blue based biosensors is expected in certain areas of clinical diagnostics, where high sensitivity and selectivity as well as the possibility of miniaturization are required, e.g., brain research and noninvasive monitoring of blood chemistry.

5. Acknowledgements

6. References

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