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Electrochemistry

PHENAZINES AND POLYPHENAZINES IN ELECTROCHEMICAL SENSORS AND BIOSENSORS

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Phenazine dyes and their polymers are reviewed with respect to their application in electrochemical sensors and biosensors, for which they are finding increasing application as redox mediators due to their unique redox and also chromatic properties, being used for the development of (bio)sensors for direct or indirect analyte determination. Electrocatalytic effects decrease the overpotential of a number of important analytes and help increase sensitivity and decrease the detection limit, in some cases, allowing the simultaneous determination of several analytes; their properties as redox mediators can be improved when combined with carbon nanotubes. Future perspectives are indicated.

Keywords: Biosensors; Electrochemical sensors; Phenazines; Polyphenazines

INTRODUCTION

A new group of phenazine-dye electroactive conducting polymers has been introduced for use in electrochemical sensors and biosensors in the mid-1990s. Phenazine dyes–aromatic azo compounds–are usually used for coloring wood, textile, as well as cell markers in microbiology. Phenazine derivatives have methyl and/or amino groups on the benzene rings and often with one N substituted by S or O in the azine ring; see the general structure in Fig. 1. Dyes discussed in this review are mainly phenazines, phenothiazines and phenoxazines, and the phenazines are either dimethylphenazines or methyl-, dimethyl-, ethyl-, diethylaminophenazines.

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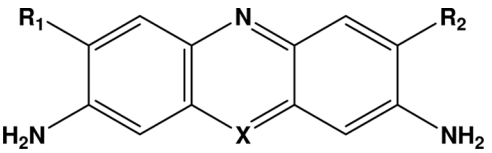

Figure 1. General structure of phenazines. X = O, N, S; R_1, R_2 = H, CH_3, C_2H_5.

Since phenazines, due to their structure, are electroactive compounds, their fundamental and applied electrochemistry have been studied, during which it has been observed that they have the ability to form polymers. The first evidence of polymerization of neutral red (NR) was observed by Nikolskii and co-authors almost 40 years ago (Nikolskii et al. 1970). Thionine (Thi) was successfully polymerized 9 years later and applied in photogalvanic cells (Albery et al. 1979). The redox kinetics of polythionine (PThi) was studied by Bruckenstein with co-authors more than 10 years afterwards (Bruckenstein, Wild, and Hillman 1990), and Tanaka's group applied this polymer for NADH determination (Tanaka et al. 1993; Oshaka, Tanaka, and Tokuda 1993). The possibility of electroactive polymer formation by other azines was reported around this time by Karyakin's group (Karyakin et al. 1993; Schlereth and Karyakin 1995; Karyakin, Karyakina, and Schmidt 1999). Several years later, polyphenazines began to be used more frequently as redox mediators in electrochemistry and bioelectrochemistry.

These polyphenazine redox polymers can be conducting polymers as well as act as redox mediators and electroactive compounds, which makes them very attractive. They can have an electrocatalytic effect, for example, on the oxidation of some carboxylic acids (Pauliukaite et al. 2007; Broncová et al. 2004) and NADH (Tanaka et al. 1993; Oshaka, Tanaka, and Tokuda 1993; Schlereth and Karyakin 1995; Karyakin, Karyakina, and Schmidt 1999). Due to these specific properties, phenazines have been widely used for the development of sensors and biosensors in recent years.

In this article some of the most-used phenazines and polyphenazines are reviewed with respect to their application in electrochemical sensors and biosensors.

STRUCTURE AND PROPERTIES OF PHENAZINES

The most used phenazines in electrochemical applications are: neutral red (NR), Nile blue (NB), methylene blue (MB), methylene green (MG), brilliant cresyl blue (BCB), safranine T (ST), phenosafranine (PS), toluidine blue (TB), thionine (Thi), azur A, B, and C (AA, AB, AC). Their chemical structures and name according to IUPAC nomenclature are given in Fig. 2, along with the color of their crystals.

All phenazine dyes discussed in this review are water soluble; however, they can be dissolved in some organic solvents. Most of the phenazines have different colors in the reduced and oxidized forms and, therefore, they can be used as redox indicators. Some of them are pH sensitive, so they can be employed as indicators, e.g., NR changes from red to yellow in the pH range from 6.8 to 8.0, and NB changes from blue to colorless in the pH range from 10.1 to 11.1 (Gundogdu et al. 2008). An optical pH indicator made from the mixed phenazine dyes, NR and Thi, was reported for use over a broad pH range from 0.5 to 11 (Hashemi and Zarjani 2008).

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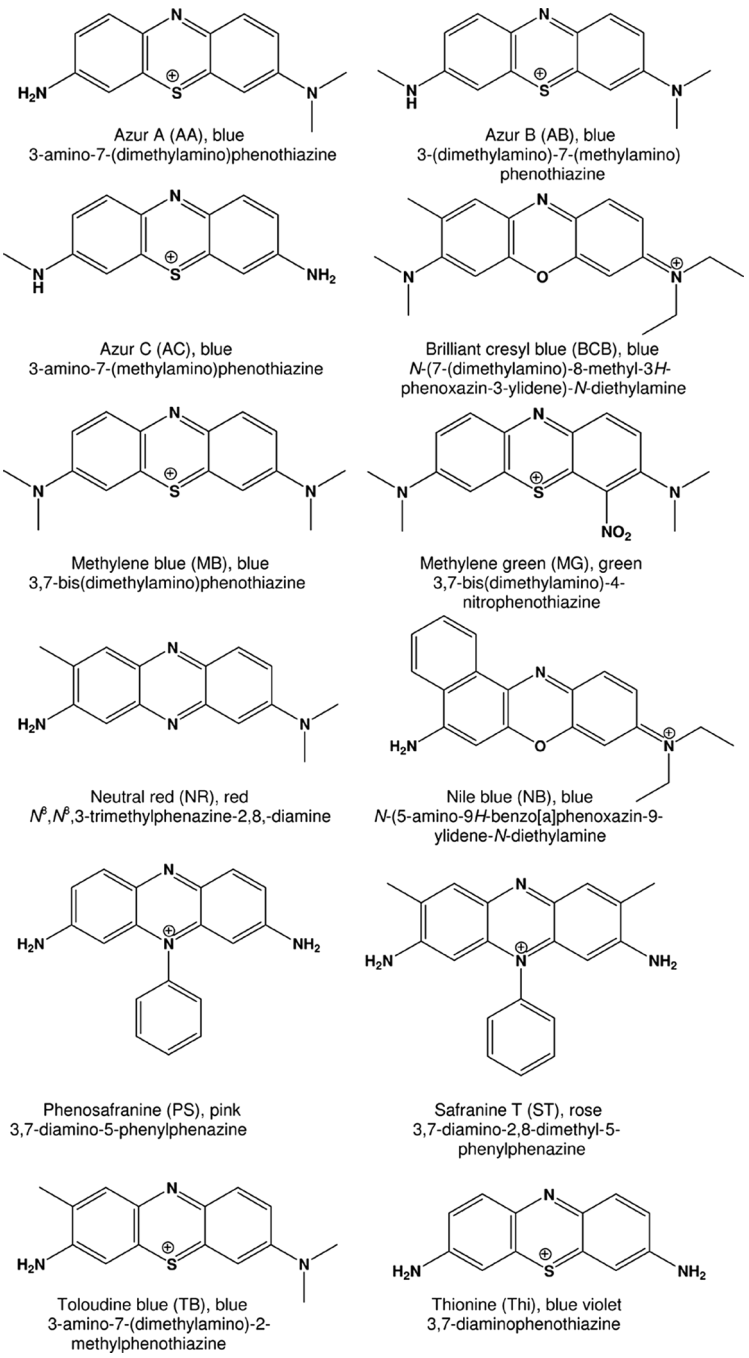

Figure 2. Chemical structures, trivial name with abbreviation, IUPAC name, and color of phenazine monomers.

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APPLICATIONS OF PHENAZINES IN ELECTROANALYSIS

Phenazines have been applied in electroanalysis, mostly for the development of sensors and biosensors. When using the monomers, it has to be remembered that the potential window available is limited in order to prevent the production of radicals and the initiation of polymerization. However, in some cases electrode modification by monomer has been reported, but due to the sufficiently wide potential window employed, polymer should be formed so that the electrode is, in fact, modified with polymer (Jeykumari and Narayanan 2007; Ensafi and Behyan 2007; Jeykumari and Narayanan 2008; Hoffmann et al. 2008).

Phenazine monomers have been applied for the electrochemical sensing of various analytes. These include the anticoagulating agent heparin using either NR in acidic Britton-Robinson buffer solution, pH 1.5, (Sun et al. 2005) or BCB in the same buffer, pH 3.0 (Sun, Ding, and Jiao 2006). H_2O_2 has been determined in neutral medium by cyclic voltammetry and amperometry using electrodes modified with carbon nanotubes (CNTs) and NR (Jeykumari and Narayanan 2007). Hemoglobin, responsible for the oxygen transport in living organisms, has been measured with either BCB in phosphate buffer saline, pH 7.0, or adsorbed on Pt electrodes using UV-VIS spectroelectrochemistry (Dong, Zhu, and Song 1989) as well as using NB cross-linked to Au electrodes by N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC) and N-hydroxysuccinimide (NHS), using cyclic voltammetry with a linear range up to 300 μM (Kuramitz et al. 1999). Nitrite can be selectively determined at Thi-CNT modified electrodes by differential pulse voltammetry and the limit of detection (LOD) and linear range of such a sensor is 1 and up to 500 μM, respectively (Zhao et al. 2007).

The phenazine MB has been used for the development of electrochemical sensors to determine low quantities of hemoglobin by cyclic voltammetry in phosphate buffer, pH 5.5, with LOD of 2 μM, and linear range up to 50 μM (Chen, Ju, and Xun 1994), adenosine by differential pulse voltammetry with a LOD of 10 nM and linear range up to 2 μM (Wang, Wang, and Dong 2009), and ascorbate by fixed potential amperometry in 1 M KCl up to 4.5 mM and with a rather low LOD of 15 μM (Hoffman et al. 2008). In this last case TiO_2 nanoparticles together with MB were used for electrode modification.

BCB-modified hanging mercury electrodes were proposed for the rapid and selective sensing of different types of DNA molecules. The best sensor was for fish sperm DNA due to the strong BCB interaction with this type of DNA (Sun et al. 2006): the linear range was 6 times longer than other methods usually used for DNA determination.

Phenazine-modified electrodes were also used in biosensor development. MG was employed in pyruvate biosensors, where MG and pyruvate oxidase were immobilized together in a carbon paste electrode (Kulys, Wang, and Daugvilaite 1992). Determination of the analyte was performed at 0.2 V vs. saturated calomel electrode (SCE) between 0.14–1.22 mM and LOD was 43 μM.

Electrodes modified with BCB and a trienzymatic system consisting of acetate kinase, pyruvate kinase, and lactate dehydrogenase immobilized into a polyethyleneglycole diglycidyl ether film were used to determine acetate by amperometry at 0.05 V vs. Ag/AgCl using FIA; the sensitivity was 30.2 nA mM^{-1} and the detection limit 130 μM (Mieliauskiene et al. 2006).

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An interesting but rather complicated approach for an amperometric glucose biosensor using NR in solution has been reported by Anik et al. (2008). The electrode was modified with a bismuth film and GOx was cross-linked with glutaraldehyde. After addition of NR to phosphate buffer saline, pH 7.0, glucose was sensed at −0.5 V vs. Ag/AgCl, leading to a LOD of 41 μM and a linear range up to 2.5 mM.

A few HRP-based biosensors for H_2O_2 determination were developed with NR or SO (Salomi and Mitra 2007), or with MB and CNT (Xu et al. 2003). The first of these operated at −0.4 V vs. SCE, in phosphate buffer, pH 7.0, and the others at −0.3 V, but the electrode with MB and CNT performed at lower pH (5.5), having the lowest LOD and the longest linear range, 1 μM and up to 2 mM, respectively.

Enzyme biosensors with phenazines can also be used for indirect analyte determination, by inhibition. A phenazine-modified HRP biosensor was used for the determination of different mercury salts such as $HgCl_2$, $Hg_2(NO_3)_2$, and its complexes by irreversible inhibition of this biosensor (Han et al. 2001); the LOD was 0.1 and 0.2 ng mL^{-1} for $HgCl_2$ and $Hg_2(NO_3)_2$, respectively.

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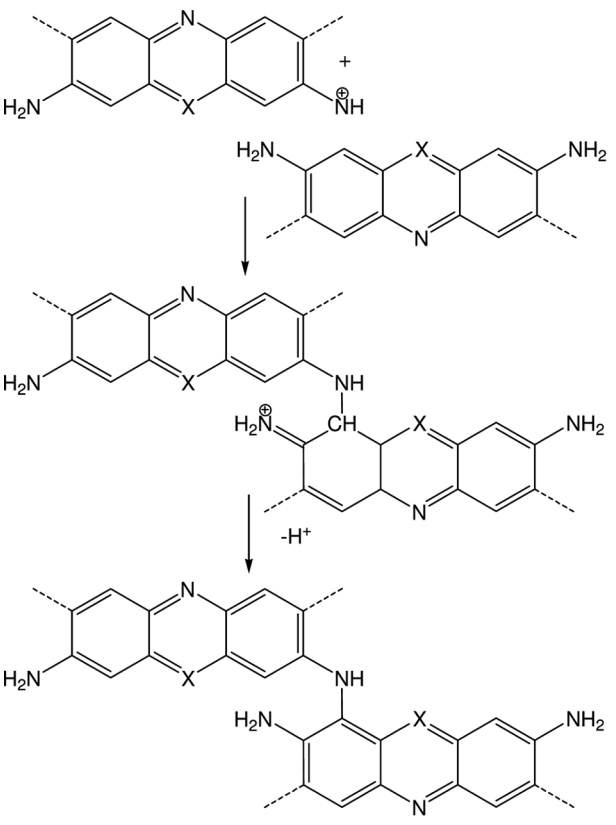
POLYMERIZATION OF AZINES

The phenazine monomers containing primary amino groups as ring substituent are able to release a proton upon oxidation, yielding a singly-charged cation-radical, which is responsible for the direct electrochemical polymerization of monomers, forming the corresponding semi-conducting polymer film (Karyakin et al. 1999).

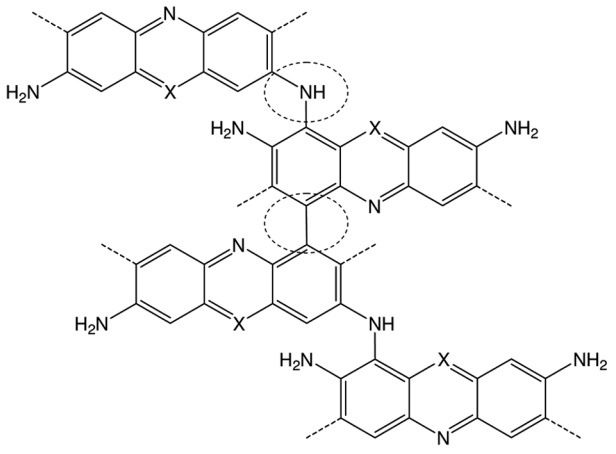
Polyphenazines are usually prepared by electrochemical polymerization of monomers (Schlereth and Karyakin 1995; Karyakin et al. 1999). The electropolymerization can be performed in the same way as any conducting polymer (Pouget et al. 1994): either by cycling the applied potential, applying a constant potential in the solution containing a phenazine monomer, or applying a constant current. The electrochemical reactions leading to polymer formation can be described as: 1) cation radicals are obtained during monomer, oxidation; 2) these radicals initiate the polymerization process and the number of radicals formed defines the structure of the polymer, which is formed via C−N coupling—the more radicals formed, the more branched the polymer obtained (Pouget et al. 1994). The electropolymerization process is controlled not only by the applied potential, which is responsible for radical formation, but also by the pH (Karyakin, Karyakina, and Schmidt 1999; Karyakin et al. 1999) as in the case of other electrochemically-formed organic polymers (Brett et al. 1993; Pouget et al. 1994).

A hypothetical structure for polyphenazines, illustrated with various phenazines, was also proposed (Karyakin et al. 1999; Pauliukaite et al. 2009; Ghica and Brett 2009). In addition to the "head-to-tail" bonding, in which amino groups bind to aromatic rings, there also exists the possibility of "ring-to-ring" coupling of aromatic amines. The possible mechanism and a tetramer of an azine are presented in generalized schemes in Fig. 3.

Typical cyclic voltammograms of NR and MB polymerization are presented in Fig. 4 (Barsan, Pinto, and Brett 2008), where both types of polymer formation are presented: the redox peaks of the monomer and of polymer overlap (Fig. 4a) or polymer and monomer oxidize at different potentials (Fig. 4b).



(a)



(b)

Figure 3. (a) Possible mechanism of azine polymerization and (b) possible tetramer structure of phenazine in general form.

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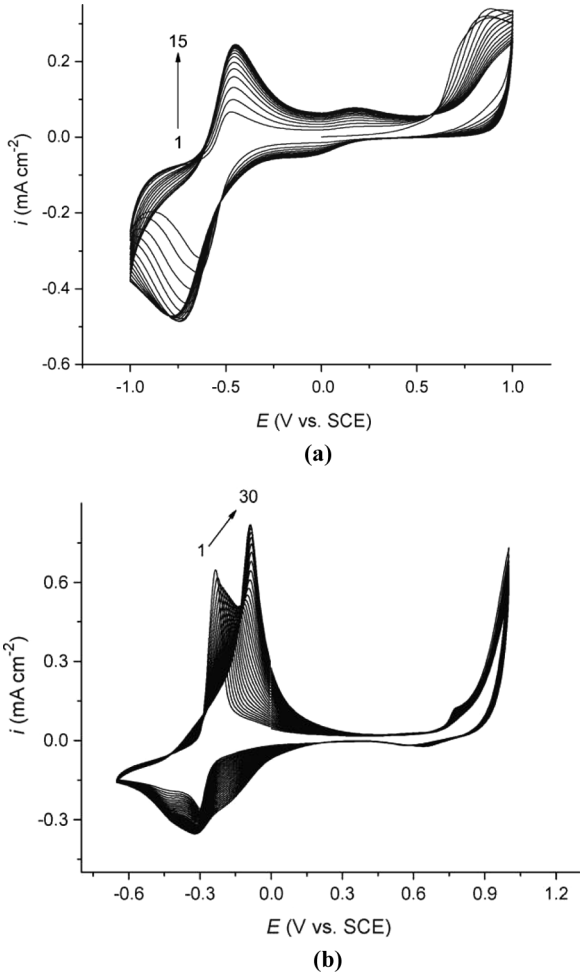

Figure 4. Electropolymerization of (a) NR from a solution containing 1 mM NR monomer in 0.025 M KPBS + 0.1 M KNO_3, pH 5.5; 15 cycles between −1.0 and +1.0 V vs. SCE at scan rate 50 mV s^{-1}, and (b) MB from a solution containing 1 mM MB monomer in 0.025 M $Na_2B_4O_7$ + 0.1 M NaOH + 0.1 M Na_2SO_4, pH 8.1; 30 cycles between −0.65 and +1.0 V vs. SCE at scan rate 50 mV s^{-1}. From Barsan, Pinto, and Brett 2008, with permission.

There are reports in the literature confirming that, besides electrochemical polymerization, it is possible to have chemical oligomerization of some monomers. There are a few reports dealing with the cases of safranine T and safranine O. The first concerns condensation of safranine with poly(methacroylchloride) in dry dimethylformamide (O'Connell et al. 1984): cross-linking due to the reaction of both amino groups in Safranine O molecules was seen as being possible. Secondly, the mode of chemical oxidative polymerization of safranine and phenosafranine using peroxydisulfate initiator (Ćirić-Marjanović et al. 2007) was determined to be coupling of the monomer molecules to give oligomeric-type structures with a molecular mass of several thousand, corresponding to about 20 monomer units. Thirdly,

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chemical synthesis of a polysafranine dye derivative poly[5-phenyl-7-(N,N-diethylamino) phenazinium] chloride occurred during decomposition of the diazonium salt of 3-amino[5-phenyl-7-(N,N-diethylamino)phenazonium] chloride (Tabakova, Petkova, and Stejskal 1998).

APPLICATIONS OF POLYPHENAZINES IN ELECTROANALYSIS

Polyphenazines used in electrochemical sensors and biosensors have been principally obtained electrochemically by potential cycling. The electrode substrate has been mainly carbon-based electrodes, such as glassy carbon, carbon film, and composites with graphite or carbon nanotubes. However, some noble metal substrates, in particular Au and Pt, have also been used (Dong and Chu 1993; Silber, Hampp, and Schuhmann 1996).

There is a variety of sensors and biosensors containing polyphenazines, which can be used in voltammetric (essentially cyclic voltammetry (CV), linear sweep voltammetry (LSV), and differential pulse voltammetry (DPV)), and fixed potential amperometric modes. A summary of polyphenazine application in sensors and biosensors, in order of the polyphenazine employed is presented in Table 1. The discussion below will compare the use of different polyphenazines for the same analytes.

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Polyphenazines in Electrochemical Sensors

Nicotinamide adenine dinucleotide (NAD^+/NADH) is a co-factor of dehydrogenases and its regeneration is extremely important for the extension of an enzyme life-time. Electrochemical regeneration of NAD^+ is one of the easiest ways but the potential is rather high (ca. -1.0 V vs. SCE) at conventional electrodes and, therefore, redox mediators are needed to decrease this potential. Polyphenazines shift the NAD^+/NADH potential significantly to less negative values; moreover, they can be used for NADH determination as NADH sensors. Polythionine (PThi) shifts the NAD^+/NADH redox potential ca. 400 mV closer to 0 V compared to a bare electrode (Tanaka et al. 1993; Oshaka, Tanaka, and Tokuda 1993); poly(neutral red) (PNR) was also applied for NADH sensing at -0.6 V (Karyakin, Bobrova, and Karyakina 1995; Chen and Lin 2001; Karyakin, Ivanova, and Karyakina 2003), as well as poly(toluidine blue) (PTB) (Dilgin, Gorton, and Nisli 2007) and polymethylene blue (PMB) (Komura et al. 2004), both between -0.5 and -0.6 V. Amperometric determination of NADH could be performed even at $+0.1$ V vs. SCE in 0.05 M potassium phosphate buffer at a poly(methylene green) (PMG) modified electrode. The sensor had a good sensitivity of 12.2 $\mu A\,mM^{-1}$ (Zhou et al. 1996).

Poly(Nile blue) (PNB), can be used for sensing of species such as nitrite in neutral medium (Chen, Wang, and Chen 2008), L-cysteine at pH 10.0 (Ensafi and Behyan 2007), and even dissolved oxygen at pH 4.8–5.5 (Ju and Shen 2001). Oxygen can be determined also at PNR in acidic medium (pH 1.5) by cyclic voltammetry (Chen and Lin 2001), whereas nitrite can be detected by amperometry at poly(brilliant cresyl blue) (PBCB) modified electrode with a sensitivity of 12.1 $nA\,\mu M^{-1}$ and LOD of 0.1 μM in 0.1 M phosphate buffer at pH 3.0 (Yang, Lu, and Hu 2006).

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Table 1. Summary of application of different polyphenazines in sensors and biosensors for different analytes

Polyphenazine film composition	Analyte	Technique	Conditions	Analytical parameters		Reference
				LOD	LR	
PAA	Hb	UV-VIS spectroelectrochemistry	–	–	–	Dong and Chu 1993
PBCB/Nafion	NO	Amperometry, 0.7 V vs. SCE	0.1 M PBS, pH 7.4	12 nM	Up to 60 μM	Chen, Wang, and Chen 2008
PBCB	Nitrite	Amperometry 1.1 V vs. SCE	0.1 M PBS, pH 3.0	0.1 μM	Up to 15 μM	Yang, Lu, and Hu 2006
PBCB-MWCNT-DHP	Epinephrine	LSV	0.1 M PB, pH 6.0	10 nM	Up to 10 mM	Yi et al. 2008
PBCB/GOx	Glucose	Amperometry, −0.3 V vs. SCE	0.1 M PBS, pH 7.0	31 μM	Up to 1.3 mM	Ghica and Brett 2009
PMalG-Nafion	NADH, dopamine, ascorbate	RRDE CV, Amperometry, FIA 0.5 V vs. Ag/AgCl	pH 4.0	–	Up to 0.3 mM	Chen, Chen, and Thangamuthu 2007
				–	Up to 0.4 mM	
				–	Up to 0.3 mM	
PMB	Hb	Amperometry-BIA, −0.55 V vs. SCE	PB, pH 8.2	0.25 g L^{-1}	–	Brett, Inzelt, and Kertesz 1999
			AcB, pH 5.0			
PMB/MWCNT	Ascorbate, epinephrine, dopamine	(CV) $E_{pa} = 81$ mV $E_{pa} = 231$ mV $E_{pa} = 272$ mV	0.1 M PB, pH 7.4	–	–	Yogeswaran and Chen 2008
PMB5/GDH	NADH, glucose	CV steady state, +0.2 V	0.1 M, PBS pH 7	–	Up to 4 mM	Silber, Hampp, and Schuhmann 1996
PMB10/GDH				–		
PMB25/GDH				–		
PMB5+PVAc/GDH				–		
PMB10+PVAc/GDH				–		
PMB25+PVAc/GDH				–		
PMB	Pyridoxine (Vit B_6)	CV ($E_{pa} = 0.57$ V)	PBS, pH 8.0	1.34 mg mL^{-1}	Up to 1.03 mg mL^{-1}	Tan, Xie, and Yao 2004
PMG	Theophylline	CV	98.9 mM $NaNO_3$ - +9.96 mM NaB_4O_7	–	Up to 0.08 mM	Ulyanova, Blackwell, and Minteer 2006

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PMG	NADH	CV, Amperometry, 0.1 V vs. SCE	0.05 M PBS	10 μM	Up to 10 mM	Zhou et al. 1996
PMG/HRP	H_2O_2	Amperometry, −0.28 V vs. SCE	0.1 M PB, pH 6.5	–	Up to 700 μM	Yang, Ruan, and Deng 1998
PNB	Hb	Amperometry, −0.41 V vs. SCE	AcB, pH 5.4	–	Up to 7.0 mg mL^{-1}	Zhou and Chen 1997
PNB-SWCNT	Ethanol	Amperometry, +0.1 V vs. SCE	0.1 M PBS, pH 8.3	50 μM	Up to 3.0 mM	Du et al. 2007
PNB	Nitrite	DPV, $E_{pa} = 0.81$ V vs. SCE	PB, pH 7.1	0.1 μM	Up to 100 μM	Chen, Wang, and Chen 2008
PNB	O_2	CV	0.1 M PBS, pH 4.8–5.5	0.78 μM	Up to 26 μM	Ju and Shen 2001
PNR	H^+, citrate	Potentiometry	0.1 M H_2SO_4 +NaOH	–	pH 2–12	Broncová et al. 2004
			pH 8.5, adjusted with NaOH	–	Up to 100 mM	
PNR	NADH	CV	NO_3^-, pH 1.0	–	–	Chen and Lin 2001
	BrO_3^-	LSV RRDE	pH 1.5; buffer, pH 10.0	–	–	
	H_2O_2	CV	pH 1.5	–	–	
	H^+	CV	–	–	–	
	NO_2^-	CV	pH 1.5	–	–	
PNR	NO	CV, $E_p = -0.6$ V vs. SCE	0.01 M PB, pH 7.0	10 μM	Up to 200 μM	Tang et al. 1999
PNR	Rutin	CV	0.1 M PBS, pH 7.0	–	–	Wang et al. 2007
PNR-CNT	Ascorbate	DPV	0.1 M PhtB, pH 4.0	–	Up to 0.2 mM	Yogeswaran and Chen; Chen, Chen, and Thangamuthu 2007
	Dopamine			–	Up to 0.2 mM	
	Ureate			–	Up to 0.2 mM	
PNR/sol-gel-GOx	Glucose	Amperometry, FIA, −0.35 V vs. Ag/AgCl	0.1 M PBS, pH 7.0	62 μM	–	Barsan et al. 2007
PNR/GA-GOx				36 μM	Up to 0.9 mM	

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Table 1. Continued

Polyphenazine film composition	Analyte	Technique	Conditions	Analytical parameters		Reference
				LOD	LR	
PNR/AlcOx	Alcohol	Amperometry, −0.3 V vs. SCE	0.1 M PBS, pH 7.5	30 μM	Up to 0.7 mM	Barsan and Brett 2008
PNR/GOx	Glucose, pyruvate	Amperometry, −0.35 V	0.025 M PB, pH 6.0	22 μM	Up to 1.8 mM	Ghica and Brett 2006a
PNR/PyrOx		−0.25 V vs. SCE	0.1 M trizma-HCl, pH 7.2	34 μM	Up to 600 μM	
PNR/GlPOx-GlK	Glycerol	Amperometry −0.35 V vs. SCE	0.1 M PB, pH 8.0	4 μM	Up to 147 μM	Ghica and Brett 2006b
PNR/GA-AldDH-NADHOx	Acetaldehyde	Amperometry, −0.5 V vs. SCE	0.1 M PBS pH 7.5	3.3 μM	Up to 100 μM	Ghica et al. 2007
PNR/sol-gel-AldDH-NADHOx		−0.4 V *vs.* SCE	pH 7.0	2.6 μM	Up to 60 μM	
PNR-CNT-GOx	Glucose	Amperometry, −0.2 V vs. SCE	PB	10 μM	Up to 10 mM	Qu et al. 2006
PNR	NAD^+, acetaldehyde	Amperometry −0.6 V vs. Ag/AgCl	0.025 M PB, pH 6.0	–	Up to 5 mM	Karyakin, Bobrova, and Karyakina 1995
PNR/AlcDH-Nafion				–	Up to 500 μM	
PNRNWs-HRP	H_2O_2	Amperometry −0.1 V vs. SCE	1/15 M PB, pH 6.98	1 μM	Up to 8 mM	Qu et al. 2007

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PNR/GOx	Cd^{2+}	Amperometry, −0.35 V vs. SCE	0.1 M PBS, pH 7.0	1 μg L^{-1}	–	Ghica and Brett 2008
	Cu^{2+}			6 μg L^{-1}	–	
	Pb^{2+}			3 μg L^{-1}	–	
	Zn^{2+}			9 μg L^{-1}	–	
PPS	AA, dopamine, serotonin	DPV $E_p = -0.05$ V, $E_p = 0.15$ V, $E_p = 0.28$ V, vs. SCE	0.1 M PBS, pH 7.1	10 nM	50 μM–1 mM	Selvaraju and Ramaraj 2003
				20 nM	50–500 μM	
				20 nM	-	
PTB	NADH	Amperometry, 0.1 V vs. SCE	0.1 M PB, pH 7.0	–	Up to 1 mM	Dilgin, Gorton, and Nisli 2007
PTB-CNT-GOx	Glucose	Amperometry −0.1 V vs. Ag/AgCl	0.1 M PB, pH 7.4	–	Up to 7 mM	Yao and Shiu 2007
PTB-Urease	Urease pH sensitive	Amperometry −0.2 V vs. SCE	McIlvine buffer, pH 7.0	20 μM	Up to 0.8 mM	Vostiar et al. 2002
PThi/HRP-Nafion	H_2O_2	Amperometry, −0.265 V vs. Ag/AgCl	0.1 M PB, pH 6.5	60 nM	Up to 1 mM	Yang et al. 1999

Abbreviations: AcB – acetate buffer; AlcDH – alcohol dehydrogenase; AlcOx – alcohol oxidase; AldDH – aldehyde dehydrogenase; BIA – batch injection analysis; CV – cyclic voltammetry; DHP – dihexadecyl phosphate; DPV – differential pulse voltammetry; FIA – flow injection analysis; GA – glutaraldehyde; GDH – glucose dehydrogenase; GlK – glycerol kinase; GlPOx – glycerol 3-phosphate oxidase; GOx – glucose oxidase; Hb – haemoglobin; HRP – horseradish peroxidase; LSV – linear sweep voltammetry; MWCNT – multi walled carbon nanotubes; NADH – nicotine adenine dinucleotide reduced form; NADHOx – NADH oxidase; PB – phosphate buffer; PBS – phosphate buffer saline; PMalG – poly(malachite green); PNRNWs – poly(neutral red) nanowires; PVAc – polyvinyl acetate; PyrOx – pyruvate oxidase; RRDE – rotating ring disc electrode; SCE – saturated calomel electrode; SWCNT – single walled carbon nanotubes; UV-VIS – ultraviolet-visible.

In the same way as its monomer, PNR is also sensitive to changes in H^+ concentration and it can, therefore, be employed as a potentiometric proton sensor in the pH range 2–12 (Broncová et al. 2004).

Hemoglobin can be determined at polyphenazines by amperometry at −0.41 V at PNB modified electrodes (Zhou and Chen 1997) by batch injection analysis using a PMB-modified electrode at −0.55 V vs. SCE (Brett, Inzelt, and Kertesz 1999) and also using UV-vis spectroelectrochemistry (cyclic voltabsorptometry) at poly(azur A) modified Pt electrodes (Dong and Chu 1993).

Polyphenazines have been found to have a catalytic effect on the reduction of different carboxylic acids, and sensors have been proposed for citric acid at PNR (Broncová et al. 2004), and uric acid at carbon nanotubes (CNT) modified with PNR (Yogeswaran and Chen 2007). Ascorbic acid can be determined simultaneously with dopamine using DPV at poly(phenylsafranine) (PPS) (Selvaraju and Ramaraj 2003) or PNR (Sun et al. 1998; Yogeswaran and Chen 2007) redox active films. The biologically-important compound serotonin, responsible for anti-aging processes, can be measured together with ascorbate and dopamine at PPS films in neutral medium using DPV, with a LOD of 20 nM (Selvaraju and Ramaraj 2003).

Hydrogen peroxide also was quantified at polyphenazine films such as PBCB (Ghica and Brett 2009) by amperometry at 0.0 V in neutral medium and at PNR by CV in acidic medium, pH 1.5 (Chen and Lin 2001). Electrocatalytic properties of PMB towards the vitamin B_6, pyridoxine, at pH 8.0 obtained by CV were reported, although the electrode was not applied as a sensor for this analyte. The pyridoxine oxidation peak was obtained at 0.57 V vs. SCE and the linear range was between 0.010 and 1.03 $mg\,mL^{-1}$, with a sensitivity of 2.5 $mA\,mg^{-1}\,mL$ and LOD of 1.34 $\mu g\,mL^{-1}$ (Tan, Xie, and Yao 2004). Rutin, another biologically important antioxidant which is also responsible for anti-aging processes as well as serotonin, was determined at a PNR film by cyclic voltammetry, an electrocatalytic effect being shown (Wang et al. 2007).

The hormone epinephrine can likewise be analyzed at polyphenazine films, in particular, PBCB with carbon nanotube modified electrode. This sensor operates in LSV mode in 0.1 M phosphate buffer solution, pH 6.0, and has an extremely wide linear range from 50 nM to 10 mM and the LOD is 10 nM (Yi et al. 2008). This hormone was successfully detected simultaneously with ascorbate and dopamine at pH 7.4 using CV at a PMB-modified electrode (Yogeswaran and Chen 2008).

Besides the analytes listed previously, polyphenazines, particularly PMG, were used for the determination of pharmaceuticals, such as theophylline, by cyclic voltammetry. The linear range obtained was up to 80 μM (Ulyanova, Blackwell, and Minteer 2006).

In summary, polyphenazine films can act as redox mediators and demonstrate electrocatalytic effects towards various analytes; therefore, they are employed increasingly often as electrochemical sensors. Moreover, some analytes can be determined simultaneously depending on the operating conditions. These films are active in different media, and reports in the literature concern their performance in acidic, in neutral, and in basic media.

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Polyphenazines in Electrochemical Biosensors

As stated previously, polyphenazines are known to decrease the overpotential of NAD^+ regeneration which is very important for the good functioning of dehydrogenase enzymes. For this reason, polyphenazines have been extensively used for the development of biosensors based on NAD^+-dependent enzymes. Karyakin, Bobrova, and Karyakina (1995) used a PNR-modified electrode with alcohol dehydrogenase (AlcDH) immobilized into a Nafion film to determine acetaldehyde at −0.6 V vs. Ag/AgCl in phosphate buffer pH 6.0 with a sensitivity about ten times higher than that at PNR modified electrode without enzyme. Following similar reasoning, by combining the ability of PNR to regenerate NAD^+ which is then used by acetaldehyde dehydrogenase (AldDH), a biosensor for acetaldehyde was described (Ghica et al. 2007) using a bienzymatic strategy. The determination of the analyte was carried out at −0.5 and −0.4 V vs. SCE at two different electrodes with enzymes immobilized by crosslinking with glutaraldehyde and encapsulation in sol-gel, with LODs of 3.3 and 2.6 μM, respectively. The biosensors were applied successfully for acetaldehyde determination in commercial wine samples.

Glucose, owing to its importance for the fermentation processes of grapes and must, has often served as an analyte for new biosensor architectures developed using polyphenazine films. Glucose dehydrogenase (GDH) has been entrapped in a PMB film or by placing an enzyme membrane made of an aqueous poly(vinylacetate) dispersion over the PMB-modified electrode. Amperometric measurements performed at +0.2 V vs. SCE using FIA showed that in the second case the biosensor had better analytical properties: a linear range from 1 to 4 mM glucose and sensitivity of $32\,\mu A\,M^{-1}$ (Silber, Hampp, and Schuhmann 1996).

Oxidase enzymes, which are not NAD^+-dependent, were also applied in biosensors. For the monitoring of glucose, several biosensors were developed with glucose oxidase (GOx) and PNR (Barsan et al., 2007): using flow injection analysis (FIA) at −0.35 V vs. Ag/AgCl, or batch amperometric determination at −0.25 V vs. SCE (Pauliukaite et al. 2006; Chiorcea-Paquim et al. 2008). Layer-by-layer films of CNT and PNR were formed (Qu et al., 2006) and glucose was determined at −0.2 V vs. SCE in the range 0.05–10.0 mM with a low detection limit of 10 μM. Beside PNR, other polyphenazines acting as redox mediators have been used in glucose biosensors. A PBCB-modified glassy carbon electrode with immobilized GOx was reported for the determination of glucose by amperometry at −0.3 V vs. SCE, with a detection limit of 31 μM and a sensitivity of $917\,nA\,mM^{-1}\,cm^{-2}$ (Ghica and Brett 2009). The PTB formed on a CNT-modified electrode also promoted electron transfer between electrode substrate and GOx active center (Yao and Shiu, 2007) at a low potential of −0.1 V vs. Ag/AgCl.

On the basis of results obtained with oxidase enzymes, mechanisms were proposed for the functioning of these biosensors. General examples illustrating the mechanisms are shown in Fig. 5, where the polyphenazine film acts as a redox mediator and can either regenerate the enzyme co-factor (in this case FAD) or reduce the product of the enzymatic reaction—hydrogen peroxide. Similar mechanisms have been proposed for PNR (Pauliukaite et al. 2007) and for PMB (Karyakin et al. 1993).

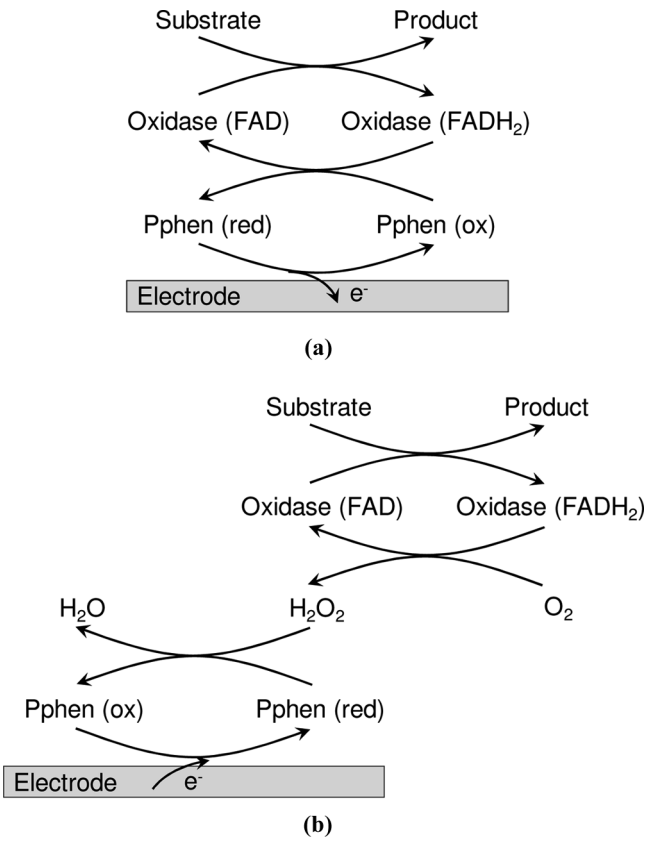

Figure 5. Proposed mechanism for polyphenazines (PPhen) acting as redox mediators in oxidase-based biosensors: (a) regeneration of FAD-cofactor and (b) reduction of H_2O_2.

For the determination of ethanol, an important analyte in food technology and clinical chemistry, a PNR-modified carbon film electrode was developed with immobilized alcohol oxidase (AlcOx), which operating at −0.3 V vs. SCE in phosphate buffer pH 7.5, was able to detect ethanol up to 0.7 mM, with a sensitivity of 171.8 nA mM^{-1} and corresponding LOD of 29.7 μM (Barsan and Brett 2007). The PNB was also used together with dehydrogenases for the determination of ethanol at electrodes modified with carbon nanotubes. In this case, the analyte was sensed between 0.1 and 3.0 mM by amperometry at +0.1 V vs. SCE with a detection limit of 50 μM and sensitivity of 6 μA mM^{-1} (Du et al. 2007).

Pyruvate is one of the most important metabolites of glucose, for which a sensitive and fast detection method is required in clinical, bioprocess, and food analysis. For this reason, pyruvate biosensors were developed using a PNR/PyrOx modified electrode (Ghica and Brett 2006a); determination of the analyte was performed at −0.25 V vs. SCE between 90–600 μM with LOD of 34 μM.

Other analytes sensed with polyphenazine modified electrodes were glycerol (Ghica and Brett 2006b) determined by amperometry at −0.35 V vs. SCE with a

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LOD of 4 μM and a sensitivity of 10 μA mM^{-1} cm^{-2} at PNR modified electrode and a bienzymatic system of glycerol kinase and glycerol-3-phosphate oxidase, urea (Vostiar et al. 2002) monitored through pH changes with at a PTB and urease modified electrode at −0.2 V vs. SCE having a detection limit of 20 μM and a sensitivity of 980 nA mM^{-1}.

The determination of hydrogen peroxide is of great relevance, ascribable both to the fact that it is the product of the reactions catalyzed by a large number of oxidase enzymes and that it is essential in food, pharmaceutical, and environmental analysis. Amperometry coupling peroxidase with mediators is one of the most sensitive procedures and such mediated H_2O_2 biosensors were developed using several polyphenazine mediators. The HRP was encapsulated in PNR nanowires grown on GC electrodes and, using amperometry at −0.1 V in phosphate buffer pH 6.98, hydrogen peroxide was determined over a wide linear range between 1 μM and 8 mM with a limit of detection of 1 μM and sensitivity of 318 μA mM^{-1} cm^{-2} (Qu et al. 2007). Another strategy involved immobilizing HRP in a PMG film (Yang, Ruan, and Deng, 1998)—determination of peroxide was achieved at −0.28 V vs. SCE in phosphate buffer pH 6.5 with a sensitivity of 0.034 μA μM^{-1}. The HRP was also immobilized into a Nafion film placed on to a PThi-modified electrode (Yang et al., 1999) and the LOD was as low as 60 nM and the sensitivity 70 μA μM^{-1} at −0.265 V vs. Ag/AgCl.

An alternative for direct analyte determination with biosensors is indirect sensing through enzyme inhibition. Biosensors based on inhibition are usually far more sensitive to the determination of the inhibitor compared to that of the enzymatic substrate. PNR also proved to be very sensitive for these applications: a glucose biosensor based on PNR/GOx electrode was used for the determination of cadmium, copper, lead, and zinc through reversible inhibition of glucose oxidase (Ghica and Brett 2008). Sensing was carried out at a fixed potential of −0.35 V vs. SCE in phosphate buffer pH 7.0 and the limits of detection obtained were 1 μg L^{-1}, 6 μg L^{-1}, 3 μg L^{-1}, and 9 μg L^{-1} for Cd, Cu, Pb, and Zn, respectively.

Thus, many biosensors have been constructed by immobilizing different enzymes, either entrapped in or on top of polyphenazines, or in layer-by-layer structures, and it is clear that the polymers can act as redox mediators that help to promote electron transfer between the electrode and the redox center of enzymes in order to allow the determination of a large variety of analytes at low working potential with low interferences.

OUTLOOK

It has been shown in many publications that phenazines and their electrochemically polymerized films can be employed as redox mediators for electrochemical sensors and/or biosensors using cyclic voltammetry, linear sweep voltammetry, differential pulse voltammetry, or fixed potential amperometry. The construction of such kinds of sensors has been generally based on carbon electrode substrates, mostly using modification by polyphenazines by electropolymerization and, in some cases, also including carbon nanotubes—the polymers can be deposited as thin film or as nanowires. Biosensor construction involves enzymes immobilized together with polyphenazine, layer-by-layer, or on top of the polymer. It is clear that

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polyphenazines have electrocatalytic effects for different analytes and enhance the sensitivity and limit of detection as well as stability of the (bio)sensors.

The main conclusion that can be drawn from this review is that polyphenazines could be exploited more often as redox mediators in electroanalysis and as electrocatalytic surfaces. Additionally, the mediation mechanism of redox mediators based on polyphenazines is still not fully understood, so that there is still work to be done in this regard. Many biologically active compounds, such as hormones, pharmaceuticals, sugars, etc., are usually determined by optical methods or chromatographically, which involve complicated sample preparation and long determination time, so that there is certainly room for further exploration into the use of polyphenazines in novel, faster, and simpler electroanalytical procedures.

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