# Electrochemical sensors based on polyconjugated conducting polymers

G. Zotti
*Istituto di Polarografia ed Elettrochimica Preparativa, Consiglio Nazionale delle Ricerche, c.o Stati Uniti 4, 35020 Padua (Italy)*

## Abstract

An overview of the applications of polyconjugated conducting polymers to electrochemical sensors is given. Gas sensors, ion sensors, and biosensors (non-enzyme and enzyme sensors) are presented and discussed. The role of the polymer as enzyme host and mediator of charge transfer is particularly emphasized in the light of recent results.

## Introduction

In the wide field of sensors, electrochemical sensors, based on current (amperometric) or potential (potentiometric) response to the analyzed species, constitute one of the main classes. The Clark electrode for oxygen detection and the pH-sensing glass electrode are well known examples of amperometric and potentiometric electrochemical sensors. Sensor applications range from simple gaseous molecules ($SO_2$, $NH_3$) to complex organic molecules such as dopamine, and even more complex species are detected with the help of the selectivity given by membranes and enzymes.

This review will consider electrochemical sensors based on polyconjugated conducting polymers (PCP). This recently developed class of materials has been proposed, among others, for sensors applications also because of a series of favourable characteristics such as: (i) direct and easy deposition on the sensor electrode by electrochemical oxidation of the monomer; (ii) control of thickness by deposition charge; (iii) redox, conductivity, and polyelectrolyte characteristics of the polymer useful for sensor applications.

These possibilities will be illustrated with polypyrrole (PP), which is the most commonly used polymer owing to water solubility, easy oxidation, low cost of the monomer, and chemical stability of the polymer. The electrochemical polymerization of pyrrole occurs by oxidative coupling. Typically, 0.1 M solutions in water, with 0.1 M sodium perchlorate or tosylate as supporting electrolytes are electrolyzed at $\sim$0.9 V versus SCE. The polymerization involves 2.25 electrons per monomer, so that 40 mC $cm^{-2}$ are required for the deposition of a 100 nm-thick film [1]. PP is reversibly oxidized in a single process with corresponding switching

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from the insulating yellow form to the conducting, deep-blue state [1]. Also, switching potentials and conductivity are dependent on the anion [2]. All these properties and their dependence on type and concentration of the analyte may be useful for the sensor ability of the polymer.

Following the classification adopted by *Analytical Chemistry*, we will consider:

(i) gas sensors;
(ii) ion sensors;
(iii) enzyme and non-enzyme biosensors.

Owing to the importance and complexity of biosensors, they will be subjected to major attention.

## Gas sensors

Operation of PCP in gas sensors is generally based on the changes in conductivity with degree of oxidation of the polymer. As the conductivity of neutral PP increases in the presence of oxidizing gases, PP has been proposed as sensor for $SO_2$, $NO_2$, $I_2$, etc. [3].

A sensor for $NH_3$, $NO_2$ and $H_2S$ has also been proposed with PP in the oxidized form [4–6], and measurements at equilibrium [6] have shown that the resistance roughly doubles for a tenfold increase in ammonia concentration over the range $10^{-5}$–$10^{-2}$ %. PP has also been proposed in a potentiometric sensor for ethanol which operates as a field-effect transistor sensing changes in the work function of the polymer [7].

Conductivity also changes with atmospheric water content in some cases, as in the case of polyaniline [8] and polyfuran [9].

## Ion sensors

PP, deposited as chloride [10] and perchlorate [11] salt, has been proposed for potentiometric sensors of chloride and perchlorate, respectively. Linear response with log of concentration in the range $10^{-1}$–$10^{-4}$ M is reported, although severe interference is caused by $I^-$, $Br^-$ and $NO_3^-$.

An amperometric detection of anions for flow injection analysis [12] applies the oxidizability of PP in the presence of anions. The sensor follows, linearly, the concentration of phosphate and carbonate in the range 10 $\mu$M–1 mM.

## Non-enzyme biosensors

The use of PCP is useful in this case, by comparison with other methods of electrode surface modification, because of the easy deposition

and the conductivity of the deposit, which allows easy charge transfer from the outer surface to the electrode by both direct and mediated electron transfer.

Thiophene/3-thiophene carboxylic acid copolymer has proved its ability for amine detection via the reduction current of the polymer [13]. This, to our knowledge, is the only reported case in which the redox properties of the polymer are used. On poly(3-methylthiophene), phenols, which usually cause fouling of platinum and glassy carbon electrodes, display stable oxidation responses [14], probably due to the polycationic character of the polymer, while preadsorption is the condition by which NADH is catalytically oxidized on the same polymer [15]. Ascorbic acid, which causes fouling of platinum and is slowly oxidized on glassy carbon, is easily oxidized on PP [16], probably because, as an anion, it binds to the polymer electrostatically; the sensor application has been subsequently reported [17].

PCP have been used for immobilizing catalytic systems and a great variety of functional groups has been introduced into the polymer matrix [18]. A significant example for possible biosensor applications is given by pyrrole *N*-substituted with viologen, which produces a polymer allowing the facile reduction of flavinmononucleotide [19].

Direct electrochemical detection of proteins though their redox centers is generally inhibited as electron transfer is hindered by the fact that the redox centres are buried in the protein. For instance, glucoxidase, (Gox) (mol wt. ~150 000) contains only two centres of $FAD/FADH_2$ coenzyme, well covered by glycoproteins. Direct redox transfer on the coenzyme is, by contrast, achieved as for the reduction of pyrrole–quinoline quinone on PP [20]. The process is reversible, and the coenzyme has also been entrapped in the polymer, keeping its electroactivity. Direct redox transfer on Gox from PP has been reported [21], but it is most likely that the transfer involves the polymer engaged with Gox as the process develops around −0.35 V, i.e., a potential typical for PP oxidation. NADH–dehydrogenase causes oxidation currents at the PP electrode in the presence of NADH at potentials at which NADH itself is not directly oxidized [22]. Thus, it has been argued that it undergoes direct redox from PP, but currents are so low ( $\sim 40\ \mu A\ cm^{-2}$) that the authors themselves suggest the responses are due to impurities. Lastly, we report the direct detection of ceruloplasmin, a copper-based redox protein, on polyaniline [23].

Access to the redox centres may be obtained with electrodes modified by chemical forms able to perform the electron transfer through the protein. Thus the easy transfer from dipyridile to cytocrome *c* is used with an electrode surface modified accordingly [24]. The general case of mediated electron transfer in proteins has been extensively considered [25, 26]. As far as PCP is concerned, we report on the oxidation of cytocrome *c* on a functionalized PP, poly(5-indole carboxylic acid) [27]. The facile electron transfer appears to be allowed by coulombic interaction of the carboxylate residue with the cationic (lysine) sites on the enzyme, since

no catalytic response is observed in the unsubstituted poly(5-indole). The catalytic process is confined to the surface and is due to a monolayer of adsorbed enzyme.

Finally, it must be mentioned that a theoretical model for charge and mass transport in PCP-based sensor electrodes has been developed [28].

## Enzyme biosensors

*Use of polyconjugated conducting polymers in enzyme biosensors*

Enzyme-based electrochemical biosensors sense non-electroactive biologic organic species by the action of one or more enzymes producing an electroactive species. Generally, in an enzyme sensor, the enzyme is fixed on the sensing surface by (i) direct adsorption on the electrode surface or in a membrane; (ii) inclusion in a gel (e.g., polyacrylamide); (iii) crosslinking with bifunctional reagents (e.g., glutaraldehyde); (iv) covalent binding to polymers (e.g., cellulose). Most often, the enzyme membrane is simply positioned close to the electrode surface as for Gox in cellulose–albumin [29] or xantine oxidase in nylon [30], and this procedure is useful for preventing fouling on the platinum electrode, but in several cases it is preferred to place it in contact with the electrode via a conducting substrate. This may be made with carbon paste or particulated platinum, and in many cases it is accompanied by incorporation of the mediator. Thus, Gox is dispersed with ferrocene in carbon paste [31, 32], or with platinum particles in Nafion [33], or in reticulated graphite [34].

The discovery that electropolymerization of heterocycles such as pyrrole easily produces polymer films of controlled thickness and of high conductivity, has suggested a new method of immobilization, and much work has been performed on this procedure, particularly with PP applied to glucose oxidation catalysed by Gox (Fig. 1). Foulds and Lowe report [35] the first enzyme biosensor based on a PCP. Pyrrole polymerization is performed in 0.2 M pyrrole solution containing $10^{-7}$ M Gox, and $H_2O_2$, produced by glucose with oxygen as mediator, is analyzed at 0.7 V. With films showing an enzyme activity up to 0.125 U $cm^{-2}$ the oxidation current follows the substrate concentration according to the Michaelis–Menten equation, with a maximum response, $i_{max} = 30\ \mu A\ cm^{-2}$ and a stability of

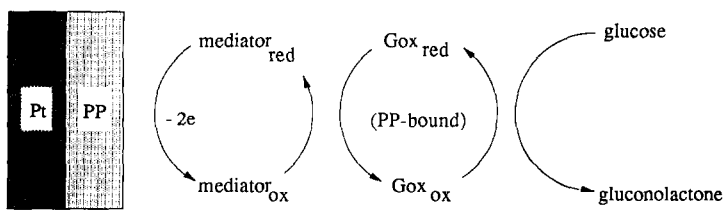


Fig. 1. Scheme for glucose oxidation on PP/Gox electrode.

at least 21 days. In order to avoid PP degradation at the high potential required by $H_2O_2$ oxidation, other mediators such as quinone have also been considered.

Subsequently, the mediator has been immobilized, both electrostatically as counterion of PP, and covalently as substituent in the pyrrole monomer. The counterion case has first been applied with ferrocene carboxylic acid [36]. This mediator is less efficient than oxygen ($K_2 = 2 \times 10^5\ M^{-1}\ s^{-1}$ versus $1.5 \times 10^6\ M^{-1}\ s^{-1}$ for oxygen), which may explain the lower maximum response ($i_{max} = 3\ \mu A\ cm^{-2}$). Also, quinonesulphonate has been electrostatically bound and the device displays $i_{max} = 5\ \mu A\ cm^{-2}$ [37]. It has been observed that the mediator and enzyme contents increase linearly with the deposition charge (thickness), while the enzyme activity tends to decrease (or level off in other cases [38]) at $50\ mC\ cm^{-2}$, corresponding to a thickness of $\sim 100$ nm, and therefore it appears that enzyme activity is surface confined. The method of covalent immobilization has been adopted using Ni–cyclam (a nickel-complexed macrocyclic polyamine) mediator for $H_2O_2$ oxidation, which has been introduced by polymerization of pyrrole *N*-substituted with a chain bearing the nickel–cyclam moiety [39], and polymer electrodes modified with Gox display electrocatalytic behaviour at low potentials (0.45 V versus SCE). Covalent binding of ferrocene to pyrrole at the nitrogen position [40] has produced a polymer displaying $i_{max} = 12\ \mu A\ cm^{-2}$, although most of the activity is lost in two days. The enzyme has also been covalently bound to PP functionalized with amino groups via formation of amide linkages [41] and, in this case too, activity is surface confined, even though the functionalization itself is on the surface.

Other polypyrrole polymers with Gox incorporation are polyindole [42] ($i_{max} = 30\ \mu A\ cm^{-2}$) and poly(*N*-methylpyrrole) [43]. Polyaniline, after Gox incorporation in acetate buffer, displays $i_{max} = 250\ \mu A\ cm^{-2}$ [44]. This system exhibits low response times (20–40 s) and a stability of greater than 60 days.

Other enzyme systems incorporated in PP are lactose dehydrogenase with NADH as mediator, used for the detection of pyruvate and lactate [45], and alcohol dehydrogenase with NADH and Meldola blue as mediators for ethanol detection [46]. In the latter case, the dye coordinates in a stable fashion to NADH without appreciably reducing the enzyme activity, and the complex is incorporated in PP. Currents are quite low ($\sim 2\ \mu A\ cm^{-2}$ under saturation conditions) but it must be recognized that this is only a first approach.

A theoretical model for PCP-based enzyme electrodes with product detection on the surface, and in the bulk on the conducting polymer, has been developed [47].

*Role of polyconjugated conducting polymers in enzyme biosensors*

The conducting polymer plays the triple role of enzyme (and possibly mediator) host, charge transducer, and permselective (or ion-exclusion)

membrane. As far as the role of host is concerned, given the polyanionic character of Gox (isoelectric point 4.2), it has been suggested that Gox binds to PP electrostatically [35]. This fact appears to be confirmed by a voltammetric analysis performed by the research group of the University of Quebec [48] in which a precycle at about $-0.4$ V versus SCE, involving about 6% of the whole reversible charge, has been evidenced in the voltammetric cycles of PP/Gox. This may be attributed to the fraction of polymer engaged with the carboxylate groups of the enzyme, as for the sulphonate groups of Nafion in the PP/Nafion composite [49]. On the basis of this hypothesis, one may estimate the enzyme concentration in the polymer in order to compare it with the experimental concentration. Thus, given the reversible charge related to Gox, and assuming that about 10% of the aminoacid residues of the enzyme (mol wt. 150 000, $\sim$1000 aminoacid residues) are constituted by anions (aspartate and glutamate), as for the average of several proteins [50], that all these units are engaged in the coordination, and that each one involves 4 pyrrole units, as is usual for PP [1], one calculates a surface concentration (for a 100 nm film) of $1.2 \times 10^{-11}$ mol cm$^{-2}$ of enzyme versus $\sim 10^{-7}$ mol cm$^{-2}$ of pyrrole units. In fact, for such a film, the experimentally found enzyme concentration is substantially higher ($\sim 2 \times 10^{-10}$ mol cm$^{-2}$) [37], which may be reasonably explained by only partial involvement of the anionic groups in Gox in the electrostatic bond. These results indicate that in terms of mass the enzyme may constitute even 3/4 of the material and, since $2.8 \times 10^{-12}$ mol cm$^{-2}$ are present in a monolayer [26], the electrode surface is covered with $\sim$100 equivalent monolayers. If one considers that the turnover of Gox (200 electrons s$^{-1}$) yields, for a monolayer, a current density of 53 $\mu$A cm$^{-2}$ [26], which has been found experimentally (e.g., 70 $\mu$A cm$^{-2}$ for Gox adsorbed on Pt in 0.1 M acetate buffer, pH = 5.5 [51]) on the PP/Gox electrode, one expects currents of the order of some mA cm$^{-2}$. In fact, experimental currents do not generally exceed $\sim$30 $\mu$A cm$^{-2}$, which means that no more than one monolayer is active.

The unexpectedly low responses may be due to (i) low permeability of the deposit to the reagents (glucose and oxygen), (ii) inactivity of incorporated Gox, or (iii) low conductivity of the PP/Gox composite. The third possibility is ruled out by the fact that PP keeps its conductivity in the composite, as demonstrated by the fact that the redox cycle of the polymer, apart from the presence of the additional cycle attributable to Gox, is unchanged with regard to pure PP. In any case, the function of the polymer as the mediator of charge transport is open to criticism, since it has been reported [48] that the system operates (at 0.4 V versus SCE) only after polarization at higher potentials (typically 0.7 V) at which PP degrades by overoxidation [52], and it therefore appears that the system only operates if non conducting.

In order to check this surprising conclusion, we performed catalytic experiments with PP/Gox under conditions such that no degradation of PP was taking place, neither from the $H_2O_2$ produced nor from the

working potential, selecting a mediator other than oxygen and operative at potentials lower than those at which PP is overoxidized. Thus, in $10^{-3}$ M quinone, which displays a reversible redox cycle over oxidized PP (Fig. 2, curve (a)), a 100 nm-thick film of PP/Gox on glassy carbon electrode displays maximum catalytic currents for glucose oxidation

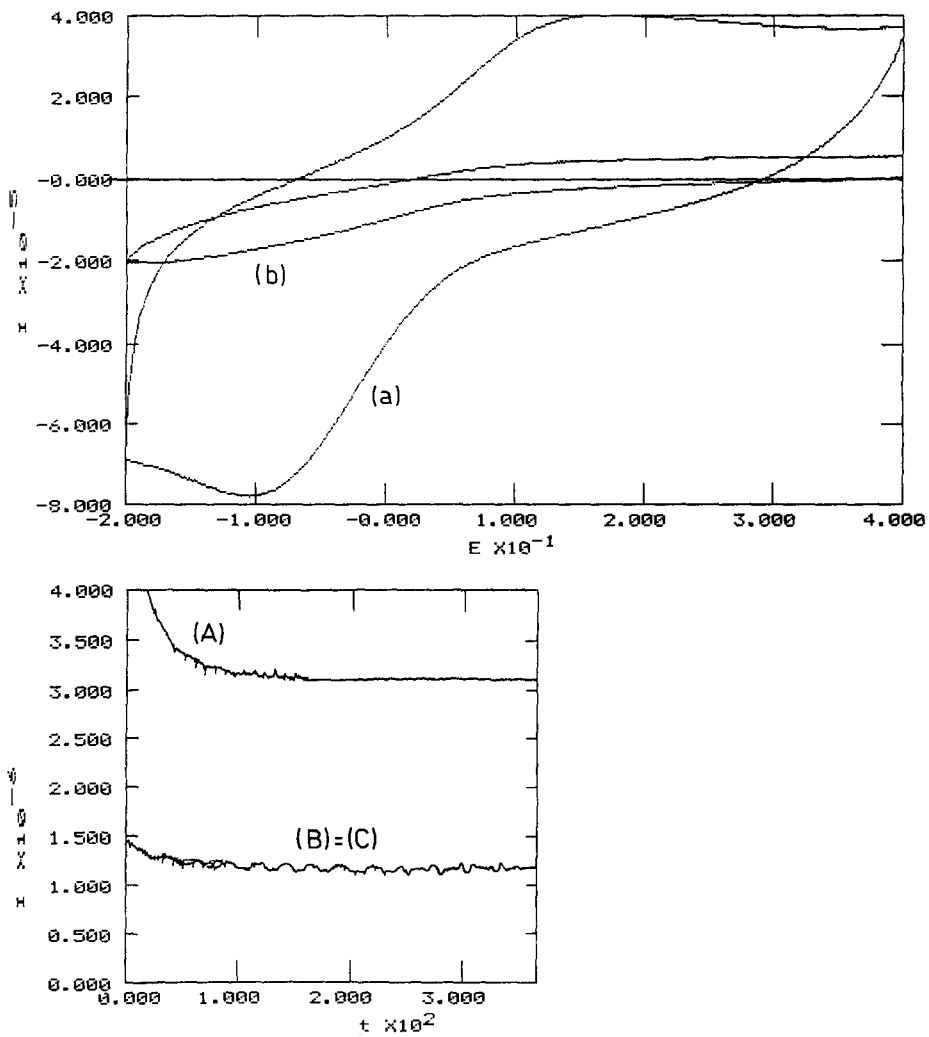


Fig. 2. Cyclic voltammogram (at 20 mV $s^{-1}$) for dissolved quinone (upper) and amperometric response to glucose (lower) on PP/Gox electrode ( ~200 nm-thick film on glassy carbon electrode) before ((a), (A)) and after ((b), (B)) overoxidation at 1.0 V vs. SCE. Conditions: 0.1 M glucose + $10^{-3}$ M quinone in $N_2$-saturated 0.1 M phosphate buffer, pH = 7. (C) response in the absence of glucose.

($\sim 10\ \mu A\ cm^{-2}$, curve (A)) analogous to those obtained with oxygen on a Pt electrode. Nevertheless, after complete overoxidation of the polymer at 1.0 V, the deposit loses, completely, both the redox response of quinone (curve (b)) following the loss of conductivity, and the catalytic activity (curve (B)); this is due to inactivation or loss of Gox, as the same treatment of the PP/Gox film on a platinum electrode does not quench the response with oxygen as mediator.

We have also repeated the experiment reported from the Canadian group and found that at 0.45 V on the as-deposited film significant catalytic currents are obtained, but it is also true that the response is greatly enhanced (by at least three times) after degradation at 0.7 V.

In order to verify whether the low responses were attributable to the diffusion limits of the reagents into the polymer films, we have also considered the catalytic responses of Gox adsorbed on Pt before, and after, PP deposition [51]. The activity of adsorbed Gox (in 0.1 M phosphate buffer, pH = 7, + 0.1 M glucose, at 0.45 V), after coverage with a 100 nm-thick PP film, is not essentially changed ($5\ \mu A\ cm^{-2}$) with regard to the uncovered, adsorbed layer ($7\ \mu A\ cm^{-2}$), indicating that diffusion is not the problem. Furthermore, similar responses have been obtained from Gox adsorbed on the outer surface of PP ($4\ \mu A\ cm^{-2}$) and on the PP/Gox composite film ($10\ \mu A\ cm^{-2}$) (Fig. 3). It is therefore evident that only Gox present at the electrode/PP and PP/solution interfaces is active. We suggest that the inactivity of polymer-entrapped Gox is due to the specific electrostatic interaction of the terminal carboxylate groups of Gox with the cationic sites of the polymer, which might cause heavy conformational changes of the enzyme. On the other hand, it is well known that enzyme immobilization may cause loss of activity of up to 90% [53]. These changes may, in our case, be reversible, and this could explain the surprising activation of the catalytic response with overoxidation. As a matter of fact, if PP degradation destroys its cation characteristics, Gox engaged in the cationic sites could be freed and regain at least part of its active conformation.

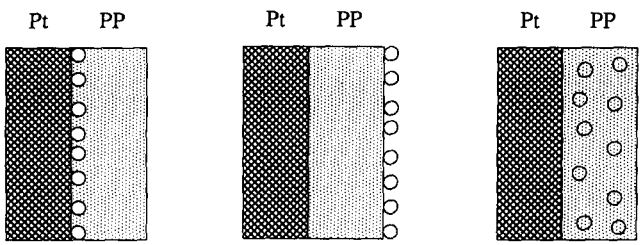


Fig. 3. Glucose response of (a) Gox adsorbed on Pt and covered with PP; (b) Gox adsorbed on PP; (c) Gox entrapped in PP. Conditions: 100 nm-thick PP film in $O_2$-saturated 0.1 M phosphate buffer, pH = 7, + 0.1 M glucose at 0.45 V vs. SCE. (a) $i = 5\ \mu A\ cm^{-2}$; (b) $i = 4\ \mu A\ cm^{-2}$; (c) $i = 10\ \mu A\ cm^{-2}$.

## Conclusions

Polyconjugated polymers have been successfully applied in the field of electrochemical sensors. Of about 50 reports that have appeared in the literature, mainly in the last 5–6 years, a majority (85%) is devoted to biosensors—most of them to enzyme biosensors—and the field is continuously expanding. Unfortunately, part of the research performed so far, particularly that concerning applications of gas sensors, has, in our opinion, a rather empiric character. We think that more fundamental research is needed to better understand the mechanisms by which the sensing action is explicated. Also enzyme-based sensors deserve greater effort in this direction in order to determine why responses are much lower than expected. Finally, we believe that for non-enzyme biosensors, improvements in selectivity, obtained both by monomer tailoring and selection of polymer morphology, will produce substantial progress in this field.

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