Organic Conducting Polymers as Active Materials in Electrochemical Chemo-Sensors and Biosensors

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The electroanalytical applications of organic conducting polymers produced by electropolymerisation are reviewed based on 101 references. They find applications for detection of numerous gaseous analytes, for design of amperometric, voltammetric and potentiometric sensors operating in solutions. There is also increasing number of their applications in development of biosensors for immobilization of enzymes or antibodies, with simultaneous size-exclusion or ion-exchange discrimination of various interferences.

For many years organic conducting polymers have been the subject of investigation in various fields of science and technology [1–3]. They have gained wide interest as electrode materials in electrochemistry and electroanalysis since development of electrolytic procedures of their deposition on the electrode surface at the end of the seventies, initially for polypyrrole and polyaniline, and then for numerous other heterocyclic and substituted aromatic compounds [4]. Investigations carried out with
various physical and chemical methods have shown many of their unique properties. They result not only from the nature of electronic conductivity of these materials, similar to the conductivity of metals, but also from the variety of their chemical interactions. The most important feature of conducting polymers in electrochemical applications is the possibility of modification of their physical and chemical properties by appropriate polarization and doping with counterions. This allows the modification of their hydrophobic interactions, or hydrogen bonding and ionic interactions. Their properties utilized in electrochemistry and electroanalysis depend on chemical and physical conditions applied in the electropolymerisation process. The possibility of molecular switching or changes of conductivity by appropriate polarization is of great interest for electronics. Good adhesion to metallic surfaces and ability to storage the electric charge creates the possibility of their use as anticorrosion coatings and in battery production.

Many different applications of conducting polymers have also been found in electroanalysis. They have widened the possibility of modification of surface of conventional electrodes providing new, interesting properties. They were applied in electrocatalysis, membrane separations and chromatography. They also create new technological possibilities in design of chemical and biochemical sensors [5-7].

The literature concerning the electropolymerisation of conducting polymers is already very extensive. Especially much attention was devoted to polypyrrole and its derivatives, either to its electropolymerisation e.g. [8-12], as to its properties e.g. [13,14]. The potentiostatic or galvanostatic electropolymerisation is a simple and efficient method of producing conducting polymers from solutions of monomer, in which the rate and extent of the process can be carefully controlled by electrochemical conditions. Galvanostatic deposition at constant current provides better control of the film thickness and is more reproducible than the potentiostatic method, although the latter requiring simpler instrumentation is very often used. The electodeposition is carried out either in organic or aqueous solutions, however, the choice of solvent affects the morphology, conductivity and activity of obtained polymer. In the most common process, the conductive polymer is formed by mild oxidation of the monomer and it involves incorporation of counterions into the polymer matrix during electrodeposition. Application of too positive potentials results in irreversible oxidation of the polymer and a loss of its conductivity and activity. As it is often in case of polymers it is difficult to determine exact structure of the electropolymerized polymer. A common opinion is that polymers formed from heterocyclic monomers form a linear chains. It was found, however, that at least under certain polymerization conditions e.g. polypyrrole with a macrocyclic structure is formed [15]. In model considerations an observation was made about the possibility of a fullerene-like molecule constructed from pyrrole [16]. Polyaniline evolution can go towards a two-dimensional polymer with phenazine rings, which can be formed by a cross-linking reaction or by insertion of nitrenium cations of aniline [17]. During the electropolymerisation process also mediators, complexing ligands, enzymes and antibodies can be incorporated into the conducting polymer. The biocompatibility essential for clinically oriented biosensors can be achieved by incorporating polysaccharides or
heparin into the polymer during electrodeposition [18]. The combination in the same material of the chemical interaction or molecular recognition centers with electronic properties provides a convenient basis for electronic signal generation for the design of chemical or biochemical sensors both for gaseous species and analytes in solutions. Amperometry, potentiometry and conductivity are most commonly employed as detection methods with these sensors.

Response to gaseous species

Chemiresistors with conducting polymer layers respond to a variety of gases including many organic vapors [19–21] and inorganic species such as ammonia [20,22,23], hydrazine vapor [22,24], hydrogen cyanide [25], NO$_2$ [26], PCl$_3$ and SO$_2$ [27]. The adsorbed organic molecules affect the electronic charge-transfer process and have a solvent-type action on the polymer causing physical swelling of the polymer structure. The arrays of different conducting polymer sensors can be successfully employed for olfactory sensing with various types of advanced multivariate processing of measured signals [28,29]. Illustration of signals obtained for several different polymers to beer headspaces is shown in Fig. 1 [28].

![Figure 1](image.png)

Figure 1. Typical responses of conducting polymers to beer headspaces [28]: 8a,b,c – polypyrrole doped with p-toluenesulfonate; 9 – polypyrrole doped with tetraethylammonium toluenesulfonate; 11a,b – polyaniline doped with hydrogensulfate

The interaction with electrophilic gases attracts electrons out of the polymer phase causing an increase in conductivity, whereas the interaction with nucleophilic gases increase the resistance of the polymer. The irreversible increase in resistance of the polypyrrole film in the presence of ammonia was attributed to nucleophilic attack by ammonia on the polymer leading to loss of conjugation and ring opening [30]. Reversible response to ammonia gas was observed in piezoelectric measurements with polypyrrole sensor interpreted as adsorption by the polymer coating of the quartz crystal with simultaneous conductivity changes [31]. D.c. conductivity measurements with polypyrrole films initially doped with Fe(CN)$_6^{4-}$ shown that
ammonia acts as a reversible dedopant, but a poisoning effect of NO$_2$ was observed [32]. Sensitivity of polypyrrole to ammonia was utilized in design of air-gap enzyme microelectrode for urea [33]. Thin films of the electrically conductive polymer poly(3-hexylthiophene) were found as ultrasensitive chemical sensors for hydrazine and monomethylhydrazine vapor in the presence of NH$_3$, amines, and ambient water [24]. The sensitivity is based on irreversible increase in the electrical resistance based on annihilation of delocalized charge carriers on the polymer backbone. Composite membranes from anion-exchange membrane and polypyrrole were employed as potentiometric humidity sensors [34], whereas polyaniline with metal clusters incorporated into the bulk of the polymer served as a sensing layer for gaseous hydrogen cyanide [25]. In the latter system the interaction of the gas with this layer causes a reversible and reproducible change of its work function measured with a Kelvin probe. The polyaniline film was conditioned in aqueous mercuric chloride solution prior to exposure to gaseous HCN. When nitrotoluenes are incorporated electrochemically into the bulk of the polypyrrole it becomes sensitive to some aromatic and hydrogen bonding compounds that interact with the film by hydrogen bonding or through aromatic $\pi$ systems [35]. The copolymerisation of polypyrrole with nitrotoluenes involving the nitrotolyl radicals produced a substantially different material with much higher electron affinity.

**Amperometric and voltammetric chemical sensors**

The voltammetric response of electrodes modified with conducting polymers essentially depends on the kind of counter ion incorporated into the polymer layer during the electropolymerisation process [36]. The detection with such electrodes is exceptionally advantageous for ions electrochemically inactive which, however, influence the oxidation–reduction equilibrium of the polymer. Determination of electroinactive anions is based on the incorporation of anions during the oxidation of the polymer as they neutralize the positive sites generated during oxidation. The application of the reduction potential causes the disappearance of the positive charges from the polymer, which is associated with ejection of anions from the polymer matrix. This mechanism was confirmed by piezoelectric measurements [37]. The electrodes modified with polypyrrole and polyaniline were applied for amperometric detection of inorganic and organic anions [38–42]. Such a detection was utilized also in high-performance ion-chromatography of anions [41,42]. A significant improvement of detection limit in amperometric determination of anions was observed by using platinum microelectrodes (10 $\mu$m diameter) [43]. Then using larger electrodes, it was shown that enhanced sensitivity for detection of electroinactive ions was obtained when a pulsed potential waveform was used [44]. The same pulsed potential waveform was applied in work with polypyrrole coated microelectrodes for flow-injection detection of nitrate, chloride, carbonate, phosphate, acetate and dodecylsulfate with detection limits from 50 nmol l$^{-1}$ to 5 $\mu$mol l$^{-1}$ [43].

Large surfactant anions such as dodecylsulfate or toluenesulfonate used as dopants in conducting polymers are captured irreversibly by the polymer and during
reduction process they are not ejected and rather cations are incorporated, which can be utilized for amperometric detection of electroinactive cations [45]. Paratoluene-sulfonate doped polypyrrole electrode was also used in normal pulse voltammetric determination of proteins [46]. For a polyaniline and polypyrrole film formed in the presence of a cation exchanger Nafion it was observed that immobilized sulfonate groups of Nafion serve as a charge compensator in the course of anodic polymerization of monomers [47,48]. The immobilization of anionic sites causes that cations are diffusing species accompanying the redox processes and the polyaniline–Nafion composite film electrode can be used for sensitive amperometric detection of alkali and alkaline earth metal ions in ion-chromatography [49] (Fig. 2).

The interaction of ammonia with some conducting polymers utilized for ammonia detection in gaseous phase can be also exploited for amperometric detection of ammonia in solution. This can be carried out with polypyrrole [50,51] and polyaniline [52]. Polypyrrole based sensor shown satisfactory dynamic characteristics of detection that permits its application to the determination of ammonia in a flow injection system up to 100 μmol l⁻¹ of analyte with detection limit of 0.6 μmol l⁻¹ [51]. The ammonia sensitivity of polypyrrole is also maintained after covering the polymer layer with bilayer lipid membrane [53], which can be used for design of biosensors. More selective and stable amperometric response to ammonia is obtained for poly-
aniline film produced by cyclic voltammetric electropolymerisation from solutions containing organic anions [52]. The example of recorded flow-injection response for such a sensor is shown in Fig. 3.

![Flow-injection amperometric response](image)

**Figure 3.** Flow-injection amperometric response recorded for polyaniline doped with p-toluenesulfonate to ammonia. Measurements carried out at +1.0 V vs. Ag/AgCl. Injected sample volume 20 μl

Detection of some organic and biological molecules was carried out by voltammetry at conducting poly(3-methylthiophene) electrodes [54,55]. The polymer modified surface catalyzes the oxidation of catechol, ascorbic acid, hydroquinone, dopamine, epinephrine, acetaminophen, p-aminophenol and NADH. Differential pulse and square wave modes were used for the analysis of binary mixtures of ascorbic acid with catechol, NADH, dopamine and p-aminophenol. Differential pulse voltammetry was also successfully used for a ternary mixtures of ascorbic acid, p-aminophenol and catechol [52]. Poly(3-methylthiophene)/polypyrrole bilayer-coated carbon fiber electrodes were shown to exhibit enhanced electrochemical reversibility for ascorbic acid oxidation and with differential pulse voltammetry simultaneous determination of ascorbic acid and dopamine was carried out [55].
In amperometric measurements it was also found that fine molecular cutoffs can be obtained by varying the electropolymerisation time and monomer concentration [56]. The exclusion of large electroactive species offers substantial improvements in the selectivity of amperometric detection in flowing streams and prevention of electrode deactivation due to protein adsorption.

### Voltammetry at ligand loaded conducting polymer films

Preconcentration on solid sorbents with immobilized complexing agents is at present the most commonly used method of preconcentration in trace metal analysis. The electrodes modified with conducting polymer with incorporated complexing centers allow the combination of the chemical preconcentration process with voltammetric detection. Preconcentration of copper was carried out using a poly(pyrrole-\(N\)-carbodithioate) coated electrode, which was obtained by chemical derivatisation of polypyrrole film [57]. The subsequent voltammetry of the complex on the electrode surface allows copper determination at the ppm level. Similar procedures were used for mercury determination [58]. It was observed that the coated electrode removed more mercury ions from solution and more rapidly than a bare platinum electrode. As a result the stripping signals with the polypyrrole electrode were much larger in magnitude. Another procedure is based on the incorporation of complexing ligand into the polymer layer by entrapment during electropolymerisation. This was employed in the determination of silver [59] and copper(I) and copper(II) [60], however, detection limits at the ppm level obtained in these determinations are substantially poorer, than in most conventional electroanalytical methods, especially in stripping methods. Determination of oxyanions of Cr(VI) can be based on trapping the analyte via ion exchange in poly(3-methylthiophene) [61] or on preconcentration in overoxidised polypyrrole upon application of an anodic potential [62], followed by linear sweep or differential pulse voltammetry.

### Potentiometric chemical sensors

Conducting polymers have found an increasing number of applications in potentiometric chemical sensors. Polypyrrole films doped with chloride, perchlorate, tetrafluoroborate or nitrate produce stable potentiometric responses to a range of anions [30–32], however, selectivity of obtained sensors is poor. In case of polypyrrole based nitrate electrode it was found that while anions more lipophilic than nitrate can be sterically hindered from entering the nitrate-doped polypyrrole films if their radii are larger than that of nitrate (e.g. perchlorate or iodide), more lipophilic anions of equal or smaller radii, still cause an interference problem [66]. It was shown theoretically that the potentiometric signal results from the transfer of the electrons (redox sensitivity) and doping ions [68]. The sensitivity of anionic potentiometric response depends substantially on kind of doping ion, on polymerization potential and the pH of monomer solution during the electropolymerisation [67]. A potentiometric iodide sensor was produced by the entrapment of iodide/iode/triiodide into various conducting polymers [69]. Superior behavior of iodine-doped poly(3-methyl-
thiophene) electrode towards iodine over that of polyaniline or poly-(N-methyl-pyrrole) (Fig. 4) was explained in terms of the iodine-induced structural changes in the poly(3-methylthiophene). A non selective cationic response to several alkali and alkaline earth metal cations was observed also for undoped poly(3-octylthiophene) [70].

![Figure 4. Potentiometric response of iodide electrodes obtained by entrapment of iodide/iodine/triiodide in poly(3-methylthiophene) (a) and polyaniline (b) 1 day (●), 7 days (○), 30 days (■), 120 days (□) (Reprinted from [69] with kind permission of Elsevier Science – NL, Sara Burgerhartstraat 25, 1055 KV Amsterdam, The Netherlands.)](image)

Another application of conducting polymers in potentiometric sensors is incorporation of polymers, which are soluble in common organic solvents, into plasticised PVC based membranes containing an ionophore [71]. This procedure enhances the signal transduction in the case of ion-selective electrodes with PVC membranes and solid electronically conducting substrates. It was shown that doped polyaniline in the membrane improves the stability of the standard potential of the electrode. A more stable potentiometric response of membrane ion-selective electrodes can be also obtained by contacting the ionophore containing membrane to the solid substrate via an intermediate conducting polymer layer having mixed ionic and electronic conductivity [72,73].

**Conducting polymer based enzyme biosensors**

There is now wide interest in development of biochemical methods of chemical analysis. The largest number of electroanalytical applications of conducting polymers is devoted to design of enzymatic biosensors (Tables 1 and 2). Performing the electropolymerisation process in the presence of soluble enzyme enables its entrapment in the polymer layer at the electrode surface without significant loss of its
biocatalytic activity. Since first works published by Aizawa and Yabuki [74] and Foulds and Lowe [75] these procedures were employed for numerous commonly employed enzymes in different polymer layers [5–7]. The alternate way can be a covalent binding of the enzyme to polymer layer via amides or secondary amines [76], or copolymerisation of monomer with a monomer-modified enzyme [77]. Recently some examples of coimmobilisation of several enzymes in the conducting polymer films were also reported. In lactate biosensor coimmobilisation of lactate dehydrogenase and lactate oxidase in poly(phenylenediamine) film yields a highly sensitive detection due to amplification by substrate recycling [78]. The creatinine electrode was made by coimmobilisation of creatininase, creatinase and sarcosine oxidase in a PPy matrix [79]. The enzyme immobilization can be also carried out by the electropolymerisation of amphiphilic pyrrolyl–alkylammonium ion mixtures with enzymes. Using tyrosinase a biosensor for the determination of cyanide, chlorophenols, atrazine, dithiocarbamate and carbamate pesticides was developed [80]. The detection was performed via inhibiting action of analytes on the tyrosinase sensor. The advantages of the procedure of enzyme immobilization in the conducting polymer were shown by sensor preparation in situ i.e. in a flow injection analysis system by injecting a plug of a solution containing the monomer and the enzyme [81].

Table 1. Developed glucose biosensors employing conducting polymers

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Polymer</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose oxidase</td>
<td>polypyrrole</td>
<td>amperometry</td>
</tr>
<tr>
<td></td>
<td>poly(N-methylpyrrole)</td>
<td>potentiometry</td>
</tr>
<tr>
<td></td>
<td>polyaniline</td>
<td>amperometry</td>
</tr>
<tr>
<td></td>
<td>poly(o-phenylenediamine)</td>
<td>amperometry</td>
</tr>
<tr>
<td></td>
<td>polyindole</td>
<td>amperometry</td>
</tr>
<tr>
<td>Glucose oxidase and peroxidase</td>
<td>polypyrrole</td>
<td>amperometry</td>
</tr>
<tr>
<td>Glucose oxidase and catalase</td>
<td>polypyrrole</td>
<td>amperometry</td>
</tr>
<tr>
<td>Glucose dehydrogenase</td>
<td>polypyrrole</td>
<td>amperometry</td>
</tr>
</tbody>
</table>

The structure of the polymer affects the sensitivity and the detection limit of the biosensor obtained, as well as the ion-exchange and size-exclusion properties, leading to improvement of the detection selectivity. Conducting polymers are highly effective in removal of interferences when used for entrapment of redox enzymes [82] and in protection against electrode fouling caused by non-specific adsorption of high-molecular compounds from natural samples [83].

The most important factor affecting the amperometric biosensor is the electron transfer between the biocatalytic molecule, usually oxidase or dehydrogenase, and the electrode surface, most often involving a mediator. The role of conducting polymers used for immobilization of enzyme in this process is discussed by numerous
authors. In one of the first approaches the reagentless glucose electrodes have been prepared by the synthesis of \(N\)-substituted pyrrole containing redox-active ferrocene side chains designed to accept electrons from the reduced form of the enzyme [84]. Ferrocene-pyrrole conjugates were efficient oxidants of reduced glucose oxidase, however, sensitivity of such a system to oxygen contrasts with other ferrocene-mediated biosensors.

Table 2. Biosensors employing conducting polymers developed for various substrates (except glucose, see Table 1)

<table>
<thead>
<tr>
<th>Substrates or species to be determined</th>
<th>Enzyme</th>
<th>Polymer</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Alanine</td>
<td>d-amino acid oxidase</td>
<td>polypyrrole</td>
<td>amperometry</td>
</tr>
<tr>
<td>Atrazine</td>
<td>tyrosinase</td>
<td>polypyrrole</td>
<td>amperometry</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>cholesterol oxidase</td>
<td>PPD</td>
<td>amperometry</td>
</tr>
<tr>
<td></td>
<td>cholesterol oxidase and cholesterol esterase</td>
<td>polypyrrole</td>
<td>amperometry</td>
</tr>
<tr>
<td>Choline</td>
<td>choline oxidase</td>
<td>substituted</td>
<td>amperometry</td>
</tr>
<tr>
<td>Dopamine; catecholamines</td>
<td>tyrosinase</td>
<td>polypyrrole</td>
<td>amperometry</td>
</tr>
<tr>
<td>Galactose</td>
<td>galactose oxidase</td>
<td>polyaniline</td>
<td>amperometry</td>
</tr>
<tr>
<td>Glutamate</td>
<td>glutamate dehydrogenase</td>
<td>polypyrrole</td>
<td>amperometry</td>
</tr>
<tr>
<td>Fructose</td>
<td>fructose dehydrogenase</td>
<td>polypyrrole</td>
<td>amperometry</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>pepsin</td>
<td>polyaniline</td>
<td>conductometry</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>peroxidase</td>
<td>polypyrrole</td>
<td>amperometry</td>
</tr>
<tr>
<td></td>
<td>polyaniline</td>
<td>amperometry</td>
<td></td>
</tr>
<tr>
<td>L-Lactate</td>
<td>lactate oxidase</td>
<td>PPD</td>
<td>amperometry</td>
</tr>
<tr>
<td>Lipids</td>
<td>lipase</td>
<td>polyaniline</td>
<td>conductometry</td>
</tr>
<tr>
<td>NADH</td>
<td>NADH dehydrogenase</td>
<td>polypyrrole</td>
<td>amperometry</td>
</tr>
<tr>
<td>Phenols</td>
<td>tyrosinase</td>
<td>substituted</td>
<td>amperometry</td>
</tr>
<tr>
<td></td>
<td>polypyrrole</td>
<td>amperometry</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>urease</td>
<td>polypyrrole</td>
<td>amperometry</td>
</tr>
<tr>
<td></td>
<td>potentiometry</td>
<td>amperometry</td>
<td></td>
</tr>
<tr>
<td></td>
<td>conductometry</td>
<td>amperometry</td>
<td></td>
</tr>
<tr>
<td></td>
<td>capacitance measurement</td>
<td>amperometry</td>
<td></td>
</tr>
<tr>
<td></td>
<td>admittance measurement</td>
<td>amperometry</td>
<td></td>
</tr>
<tr>
<td></td>
<td>conductometry</td>
<td>amperometry</td>
<td></td>
</tr>
<tr>
<td>Uric acid</td>
<td>uricase</td>
<td>polyaniline</td>
<td>amperometry</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>lipase</td>
<td>polyaniline</td>
<td>conductometry</td>
</tr>
</tbody>
</table>

PPD – poly(o-phenylenediamine).
Another observed drawback was complete loss of sensitivity after two days of use. Numerous observations indicating a participation of the polymer in direct electron transfer were made in the systems without mediators. The same oxidation and reduction peaks observed for flavin adenine dinucleotide (FAD) in poly(N-methylpyrrole) film and for glucose oxidase in the same polymer indicate that electron transfer occurs between FAD centers in glucose oxidase and the electrode [85]. Mediation by the conducting polymer was found to be very effective and no significant electron transfer to oxygen was observed for biosensors with glucose oxidase adsorbed irreversibly on conducting microtubules of polypyrrole [86,87]. The positive response to glucose was observed in oxygen-free solutions at low measuring potential (100 mV vs. Ag/AgCl), where no response to hydrogen peroxide was observed. Biosensors with conducting polymers are considered as third-generation biosensor, where reoxidation of the reduced enzyme is the rate-limiting process at low potentials [87]. Similar observations were reported for biosensor with glucose oxidase immobilized in polyaniline [88]. A similar reduction and oxidation behavior was observed for electrodes with polyaniline film loaded with glucose oxidase and isoalloxazine derivative, having the same active redox radical as the active center of FAD of glucose oxidase. The direct electron transfer between electrode modified with polypyrrole and peroxidases was also observed [89,90]. It is postulated that an oxidation product(s) of pyrrole, most probably dimeric pyrrole can mediate the enzyme-polypyrrole electron transfer, though a direct electron transfer may also be possible [90]. For fructose biosensor with fructose dehydrogenase immobilized in polypyrrole it was found that the enzyme in the conductive thin membrane exhibits a sharp increase in catalytic activity, which led also to conclusion that polymer matrix serves as an electron-shuttling medium between the enzyme and the electrode [91].

There are also more skeptic reports about the active role of conducting polymer matrix in electron transfer in biosensors. For polypyrrole based electrode with immobilized glucose oxidase the evidence was presented that electron transport between the enzyme and the electrode surface is due to the electrocatalysed oxidation of hydrogen peroxide [92]. For glucose sensor based on a pyrrole-tubule-impregnated membrane it was found that the device operates by direct electrochemical oxidation of glucose at the platinum film [93].

Potentiometric biosensors with conducting polymers can be produced using pH sensitivity of polymers [94]. An advantage of replacement of amperometric detection with potentiometric measurement has been found to be a greater independence of the sensitivity of detection on aging of the biosensor. Polypyrrole sensitivity to ammonia was used to produce amperometric biosensors [33,51]. Conductivity biosensors based on conducting polymers were developed for penicillin [95], and also for glucose, urea, lipids and hemoglobin [96]. Conducting polypyrrole molecular interfaces have been also implemented to modulate biological functions of enzymes and living cells at the electrode surface by adjustment of electrode potential [97]. Very often additionally obtained discrimination of electroactive interferences by using conducting polymers as matrices for enzymes allows the use of such biosensors for
analysis of natural samples e.g. flow-injection determination of lactate in whole blood (Fig. 5).

![Figure 5.](image)

**Figure 5.** Recorded flow-injection response for standard lactate solutions (a) and correlation plot for lactate determination in whole blood (b) with amperometric lactate biosensor with lactate oxidase immobilized in poly(o-phenylenediamine) and internal bilayer of electrodeposited polypyrrole/polyphenol for elimination of electroactive interferences

Besides the advantage of the well defined formation of the polymer layer, this methodolgy yields also some difficulties, associated with a significant chemical and electrochemical activity of the polymer matrix due to the sensitivity of these materials towards ion-exchange processes and redox equilibria. The role of the polymer conductivity in electron transfer in a biocatalytic process requires further studies, as well as the mechanism of the frequently observed, quite fast degradation of the enzyme activity. One of the reasons can be deterioration of conductivity of conducting polymers by hydrogen peroxide observed, for instance, for polypyrrole [98].

**Immunosensors based on conducting polymers**

Similarly to enzymes, antibodies can also be incorporated into the conducting polymer layer during the electropolymerisation process. Polypyrrole immunosensors have been developed for human serum albumin [99], thaumatin (artificial sweetener) [100] and for p-cresol and other phenolics [101]. Both in determination of human serum albumin and thaumatin it was shown that sensitive, reversible and rapid responses can be obtained when pulsed amperometric detection with flow-injection sample processing is employed. This led to conclusion that if the control of detection is in the millisecond time domain then the interaction of antigen with antibody immobilized in polypyrrole can be reversible [100].
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