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Electrochemical DNA sensors based on electropolymerized materials

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ABSTRACT

The use of electropolymerized materials in the DNA sensors is reviewed with particular emphasis on their functions and specific interactions with DNA and oligonucleotides. Polyaniline, pollypyrrole, polythiophenes and polymeric forms of phenazines play significant role in the immobilization and signal transduction of DNA sensors for the detection of hybridization events, DNA–protein and other specific interactions on the sensor surface. The mechanism of electropolymerization and the influence of oligonucleotides are also considered for various types of polymers. The DNA sensor performance is classified in accordance with the biological targets and composition of the surface layer.

1. Introduction

The interest to the development of DNA sensors has dramatically increased in the past decades [1,2]. This is mainly due to the great significance of DNA analysis in many areas related to human health, e.g., diagnostics of pathological microorganisms and viruses, genetic mutations, new drug discovery, especially in oncology, food technology, etc. [3,4]. Besides conventional techniques intended for the detection of specific genes based on direct sequencing or DNA hybridization, there is an urgent demand for developing compact, sensitive and inexpensive measurement devices for such purposes. They fill the gap between the sophisticated approaches to the DNA assay requiring complex specialized equipment and qualified personnel [5], and the requirements for the point-of-care diagnostics and field application [6]. Biosensor technologies are most suitable for such goals because of the broad possibilities of tuning their assembly and biorecognition elements and meeting specific requirements, e.g., simplified data readout, exclusion of the sample pre-treatment and autonomous operation [7,8].

From the early 1990-s, the DNA bioassay technologies utilizing radioactive or fluorescent labels exerted a serious influence on genomics and proteomics applications, specifically for the human genome project [9,10]. They were inspired by the appropriate protocols successfully elaborated in molecular biology starting from in 1970-s. Being very sensitive and effective in encoding primary nucleotide sequence, the appropriate devices called DNA sensor arrays have some drawbacks, e.g., the use of hazardous chemicals for their manufacture, the multi-step analysis protocol, the limitations of labeling and DNA probe stability, and the precision of oligonucleotides tagging and positioning on a solid support [11]. This complicated their routine application especially for a limited number of samples or in field conditions. The development of more simple and rather cheap DNA sensors instead of sensor arrays made it possible to extend their application area to the detection of low-molecular compounds specifically interacting with DNAs and the DNA damage caused by antitumor drugs, reactive oxygen species or some genotoxicants [12]. Such DNA sensors are certainly less productive and efficient in mass screening of a great number of samples, but they were found rather suitable for future applications in local doctor's offices or portable alarm systems directed to single use of the biosensors or a very modest number of assays per day/sensor.

The development of rather simple and inexpensive DNA sensors does not presume the application of radiolabels or lasers in readout systems. Even though the cost of appropriate detection systems has been dramatically decayed to date, they are still rather sizeable and complicated in manufacture and use. Meanwhile, electrochemical transducers have had privilege because of the possibility to use conventional measurement techniques, well elaborated backgrounds of signal transduction and user friendly design [13]. At the beginning, the intrinsic electroactivity of guanine restudies was used in electrochemical DNA sensors [14,15] but at present main attention is focused on the application of mediators and labels which alter the redox signals in hybridization events or to any other changes resulting from the interaction of the DNA probes with the analyte molecules [16]. The efficiency of such measurement protocols which are derived from the appropriate immunoassay techniques significantly depend on the way DNA is connected with the electrode. Direct electron transfer in accordance with Marcus Eq. (1) [17] requires an

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intimate contact of the reducing/oxidizing domain with the electrode surface.

$$k_{et} \sim \exp\left[-\beta(d-d_0)\right]\exp\left[-\frac{(\Delta G^0+\lambda)^2}{4RT\lambda}\right] \quad (1)$$

Here, k_{et} is the rate constant of electron transfer, d and d_0 are the Van der Waals and real distances of the electron transfer, ΔG^0 and λ are Gibbs and reorganization energies. Bulky and rather rigid DNA helixes cannot provide a high density of the contact points when adsorbed on the solid surface so that the signal of direct guanine oxidation is rather small and requires special processing to subtract the non-Faradaic current [18]. For this reason, mediators, both covalently linked to the functional groups and diffusionally free, are mostly used in the electrochemical DNA sensors. The amplification of the signal is reached due to consecutive electron exchange between the redox centers of the biomolecule and appropriate mediators.

More recently, the nanosized functional materials offering both DNA immobilization and signal transduction become popular in DNA sensor design [19]. Due to the increased specific surface, high charge density and reduced steric hindrance of access, they significantly improve the sensitivity of the DNA binding detection. Besides carbon nanotubes [20,21] and metal (metal oxides) nanoparticles [22,23], electropolymerized materials are considered as a promising solution for the DNA sensor development. They are synthesized by the electrochemical oxidation of monomers performed prior to or simultaneously with the DNA immobilization [24]. In the latter case, the biopolymers are entrapped in the growing polymer film and affect the electropolymerization as templates for the deposition of oppositely charged oligomeric products [25]. Certainly, most of the polymers obtained by electrochemical approaches have alternative routes of chemical synthesis, preferably by oxidation in organic media. However, chemical synthesis has some limitations related to the pollution of the polymeric products with the oxidant residues, limitation of the implementation of the low-soluble polymerization products into the biosensor layer and a lesser compatibility with biomolecules. The use of electrolysis provides both the formation of dense adhesive coatings directly on the transducer surface and the tool for monitoring the quality of electropolymerized products in accordance with their redox activity.

To some extent, oligonucleotides or DNA molecules can arrange the microenvironment like the molecular imprinting techniques used for establishing the specific binding of target analytes [26]. This offers additional advantages for tuning the electrochemical activity of the polymeric layer and the binding abilities of the DNA fragments. Besides immobilization, electropolymerized films offer other possibilities, e.g., DNA electric wiring [27], mediation of redox reaction involving target analytes or redox centers introduced either into the surface layer or present in solution [28]. Finally, the polymers are easily modified by introduction of appropriate functional groups in the monomer structure, co-polymerization of various units or treatment of the polymer with specific binding reagents [29]. On the same stage, some other important characteristics, e.g., swelling, hydrophobicity, stability towards active chemicals, adhesion to the transducer material, etc., can be adapted to reach maximal compatibility with other sensor materials and efficiency of the biosensor operation. Summarizing the above advantages, the use of electropolymerized materials offers broad opportunities for the further progress of DNA sensors which have only at present been discovered for application. In this review, the application of various electropolymerized materials in the assembly of DNA sensors is considered. Previously, various aspects of the application of electrosynthesized polymers in the biosensor assembly were reviewed with particular emphasis on immobilization [30] or electroconductivity properties [31].

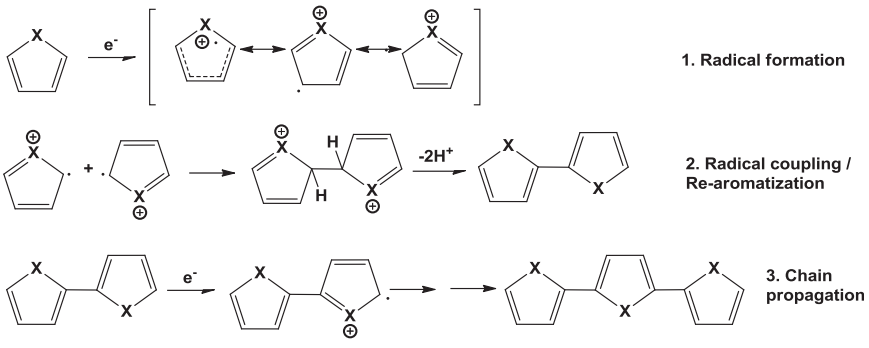
Here, the literature covering the past ten years is summarized in accordance with the polymer nature (biosensors based on polyaniline (PANI), polypyrrole (PPY), polythiophenes and polymeric forms of phenazines), and the DNA sensor purpose (detection of hybridization event, determination of specific proteins, low-molecular compounds and DNA damage). Also, brief description of electropolymerized materials and tools for their investigations is given.

2. Electropolymerized materials used in DNA sensor assembly

2.1. General characteristics of electropolymerization techniques

Many characteristics of the DNA sensors depend on the way the receptor layer is assembled. For this reason, it seems useful first to consider the electropolymerization of the polymers used in the biosensors as the DNA carriers and signal transduction systems.

Electropolymerization is initiated by the anodic oxidation of unsaturated monomer units followed by coupling cation radicals usually with the removal of the hydrogen ion. Mostly in these reactions aromatic systems with substituents containing heteroatoms are used. If the intermediate products are stable enough, the propagation stage can continue to form oligomeric and polymeric products with no additional electrochemical stimuli similarly to the ion-radical polymerization initiated by chemical oxidants. But, most frequently, the electrochemical stage is necessary within the whole polymerization period because of the low efficiency of coupling of the neutral monomer molecule. The propagation stage involves the formation of the cation radicals of intermediate dimers/ oligomeric products of the previous stages in which the electrochemical activity does not differ dramatically from that of the monomer. Termination stage can include a deeper oxidation of the intermediates, the formation of stable radicals by homogeneous electron exchange and the reactions with molecular oxygen or nucleophilic substances present in solution. An example of electropolymerization process for five membered heterocycles is given in Eq. (2) as an example (X=S, NH) [32].



(2)

If the products of polymerization are electrochemically inactive and insoluble they precipitate on the electrode surface and hence suppress the electron transfer due to the electric insulation of the interface. This limits the polymerization similarly to the phenomenon of electrode fouling well known for corrosion [33] or electrochemical sensors exploited in biological liquids [34].

In general, three types of electropolymerization products are used in the DNA sensor assembly:

- electroconductive polymers that exhibit electronic–ionic conductivity comparable to that of semi-conductors and participate in the effective electron transduction between the mediators or redox centers of biomolecules and electrodes [35,36]. Three polymers are mostly investigated as DNA supports and components of the signal transduction systems of biosensors, i.e., PANI, PPY and polythiophene. Among them, some structurally relative products exert similar features, i.e. polythionine for PANI and poly(3,4-ehtylenedioxythiophene) (PEDOT) for polythiophene;
- electrochemically active but non-conductive polymers which can mediate electron exchange [37,38]. They exert electroconductivity properties only in a very narrow potential window near their standard redox potential but are quite effective as mediators especially if their formation has been conducted to reach intimate contact of redox centers with the biomolecules. Polycyclic heteroaromatic systems obtained from phenazines, phenoxazines and phenothiazines belong to this group of polymers. Many of them are known under trivial names as dyes or redox indicators (Neutral Red [39], Methylene Blue [40], etc.). The structures of frequently used monomers of this group are presented on Fig. 1;
- non-conductive polymers that do not support electron transduction. Their use in the DNA sensor assembly is aimed at the mechanical protection of the biorecognition layer or the mechanical support for the immobilization of the DNA probes or mediators. The thickness of such polymers is naturally limited to their permeability for small charge carriers, i.e., ferricyanide ions or other mediators required for wiring redox centers of the components attached on the interface [41,42]. Such coatings are obtained by the oxidation of substituted phenols and some aromatic amines [43–45]. Besides, non-conductive films are obtained by overoxidation of electroconductive materials like PPY or PANI [46,47].

There are two main strategies for the deposition of the electropolymerized film followed by the biomolecule immobilization. The first one is performed in a polar organic solvent preferably in the potentiostatic regime. Then the electrode is covered with the layer of a DNA probe or an aptamer and then transferred into the aqueous media to record voltammograms and measure target analytes specifically bonded to DNA onto the electrode surface. The second approach involves electropolymerization in the aqueous solution of a monomer, in some cases containing the DNA oligonucleotides so that the immobilization can be performed simultaneously with the polymerization. In other case, assembly of a biorecognition layer can be performed after polymerization from an aqueous solution with no dramatic difference from that based on electropolymerization in an organic solvent.

The first strategy allows using monomers insoluble in water and results in formation of more dense and compact films with lesser permeability for ionic substances in comparison with the products of electropolymerization in aqueous media. Lack of the hydrolysis and/or oxidation product and a lower significance of dissolved oxygen is another advantage of electropolymerization in an organic solvent. For some systems, e.g., substituted polymers, this is the only way of providing reproducible characteristics of the films.

Aqueous polymerization is more preferable for biosensor development due to its simpler realization and a better compatibility of the product with biopolymers bearing a sufficient amount of water and metal ions stabilizing the steric structure of the molecules and counterbalancing the negative charge of the phosphate skeleton of DNA and oligonucleotides. The low solubility of some monomers mentioned earlier can be partially compensated for by addition of surfactants or emulsifying agents [48,49]. Though this significantly decreases the regularity of the polymer structure, especially taken in thin films, and the reproducibility of their electrochemical characteristics.

The dynamic regime, i.e. multiple scanning the potential in monomer solution is often used besides potentiostatic electrolysis for electropolymerization of redox active species. This procedure presumes the reversible reduction/oxidation of the oligomers on the electrode so that only a small portion of the charge is spent on the target process, i.e. monomer oxidation. Being less effective in the sense of the current yield, such a regime has some other advantages particularly attractive for biosensor applications. First, the shape of the peaks on cyclic voltammograms provides information on the success of the electrolysis, i.e. amount, redox activity and reversibility of

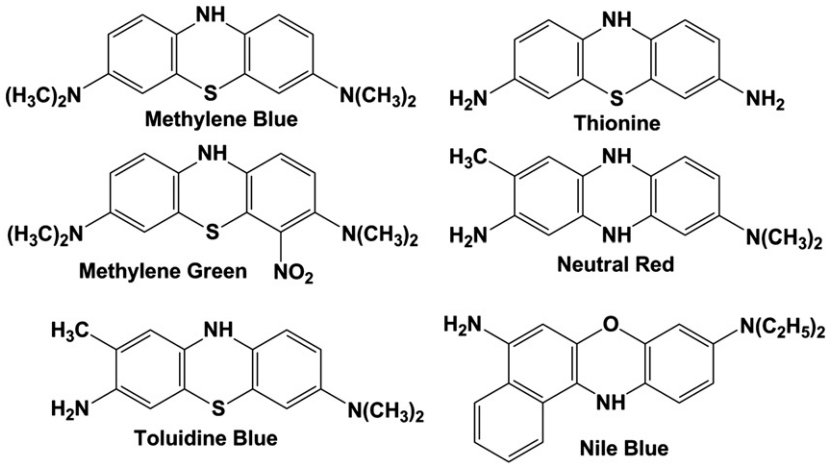


Fig. 1. Monomers used for the synthesis of redox-active polymers and application in DNA sensor assembly.

the electron exchange within the growing polymer film. Second, this makes it possible to control the possible formation of by-products, commonly related to the oxidative degradation of the functional groups in the side chains of the polymer. Third, the cycling of the potential offers criteria of the full coverage of the electrode surface necessary for the optimization of the surface layer assembly. As regards the low efficiency of electrolysis, the amounts of the electrolysis products required for biosensor development are so small (several micrograms) that the low efficiency of the process does not affect the general immobilization protocol.

In most cases, the number of cycles required for electropolymerization is rather small. The changes of the redox activity of the electrode interface indicating the film deposition began from 3–5 cycles of the potential. Indeed, this is insufficient to obtain a high-molecular product and actually oligomeric species can also be precipitated on the electrode. There have been only a few attempts to estimate the real mass distribution of the electrolysis products as well as real structure of the polymers especially in dynamic electrolysis. PANI and PPY are the only examples of rather intensive investigations including the morphology of the particles deposited on different supports. However, most of the research with these polymers was carried out with chemically oxidized analogs that showed the redox features similar to those of the electrolysis products but obviously differing in their steric structure and mass.

Regarding electrosynthesis, growing attention has been paid in the past decade to the combination of electropolymerized layers with other mediator systems which are pre-deposited onto the surface or implemented into the polymer structure by physical entrapment, simultaneous synthesis or covalent linking. The use of mediators is commonly aimed to the improvement of the electron transfer conditions especially for redox-active but not electroconductive coating. The use of mediators also increases the efficiency of electropolymerization and to some extent alters the morphology of the films. Thus, an increase in the roughness or formation of nanosized domains with regular structure and positioning on the transducer is preferable for the following DNA immobilization. Metal nanoparticles (Au [50,51] and Pt [52,53]) and carbon nanotubes [54,55] are mostly used for such an electrode pre-treatment although some other common mediator systems has been described [56].

2.2. Measurement techniques for electropolymerization products

Besides voltammetry, there are two common tools providing information on the characteristics of the electropolymerized layers on the electrode surface, i.e. electronic impedance spectroscopy (EIS) [57] and electronic microscopy. Measurements of electrochemical impedance estimate the charge transfer processed in order to interpret the charge distribution, permeability of the surface layer for small particles and ions as well as electroconductivity of the film. Comprehensive consideration takes into account the non-uniform structure of the polymeric film including the existence of the pores with charged walls and cracks providing the direct access of the charge carriers to the electrode [58,59]. On the other hand, the deposition of DNA mostly suppresses such phenomena and allows simplified approaches based on the Randles equivalent circuit assuming homogeneous structure of the film with a thickness near zero.

Electronic spectroscopy as well as atom force microscopy (AFM) make it possible to visualize the insoluble products on the electrode surface which can form a great variety of the shapes, e.g., rods, beads, star like particles, dendritic structures, nanofibers, etc. [60]. The form and size distribution mainly depends on the polymerization conditions and is characterized better for chemically synthesized polymers. Meanwhile, there are remarkable examples of morphology study that consider the influence of DNA and DNA involving biochemical reactions on the surface morphology [61,62]. Unfortunately, mostly results present only a qualitative description of the surface morphology. Even though the distribution of the particles can be quantified by appropriate techniques, there are two principal limitations. First, the AFM or electron microscopy covers a very small part of the surface, which is several micrometers square in size, whereas the whole working surface of a common electrode used in a voltammetric DNA sensor is much bigger and often contains inclusions unpredictably affecting the features of the biosensor. Second, the supports used in such investigations are not the same as those used in biosensor assembly. Highly ordered pyrographite and even gold, conventional supports in such investigations, have specific features affecting the electropolymerization process. Certainly, the coverage of the electrode with initially formed polymer layers suppressed the influence of the support, but the holes and cracks in the film cast some doubts on this statement.

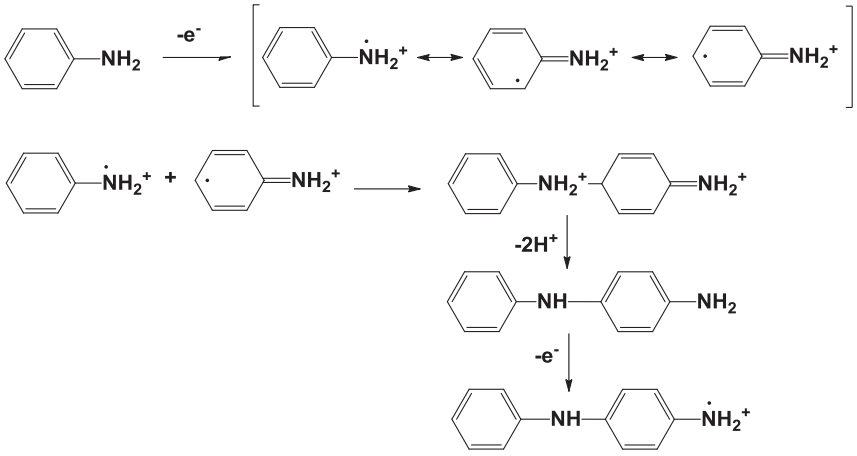
Spectroscopic studies (Fourier transformation-infra red (FTIR), UV–vis, surface plasmon resonance, (SPR)) have found increasing attention because of the electrochromic features of PANI [63] and some other polymers which can be obtained in electrolysis. ITO electrodes and thin transparent films of Au on a glass provide the opportunity for simultaneous monitoring of redox and optical features of the polymeric layers. Thus, for PANI – ClO_4^- films electrochemically deposited onto the ITO electrode the peaks at 793 and 1055 cm^{-1} in FTIR spectra were assigned to stretching and bending vibrations associated with the C–N linkage, and the strongest band at 1469 cm^{-1} was assigned to the C–C stretching. The 1545 and 3014 cm^{-1} peaks were ascribed to N–H bending and C–H group, respectively [64]. Similarly, the carbodiimide binding of the DNA probe to the polymeric chain can be proved by observation of appropriate bands assigned to the $>C{=}N-$ bonds in the products. Comprehensive consideration of spectroelectrochemistry approaches is beyond the scope of this review but it should be mentioned that the use of these approaches offers unique opportunities for flow-through systems, including microfluidics and thin layer cells [65].

Among AFM, the direct observation of the polymer growth is achieved by quartz crystal microbalance (QCM) especially in the mode providing simultaneous recording frequency changes and voltammograms (EQCM) [66]. Although the experiments in liquids do not provide exact mass measurements, there are some approaches to obtain their estimates against the current or electricity applied for polymerization [67]. The same techniques make it possible to detect the absolute amounts of biomolecules attached to the DNA binding sites onto the surface of piezosensors. Although the sensitivity of the detection is sufficient only for high-molecular analytes, QCM and EQCM give direct evidence of the recognition event and assist in specifying other measurement protocols based on indirect phenomena like changes in the conductivity, charge transfer or redox activity of appropriate labels.

2.3. General characteristic of electrosynthesized polymers used in the assembly of DNA sensors

PANI is a linear polymer which is commonly obtained in the media of strong mineral acids which stabilize the primary cation radical required for polymerization initiation (3). This step is performed at rather high anodic potentials close to the oxidative degradation of the reactants.

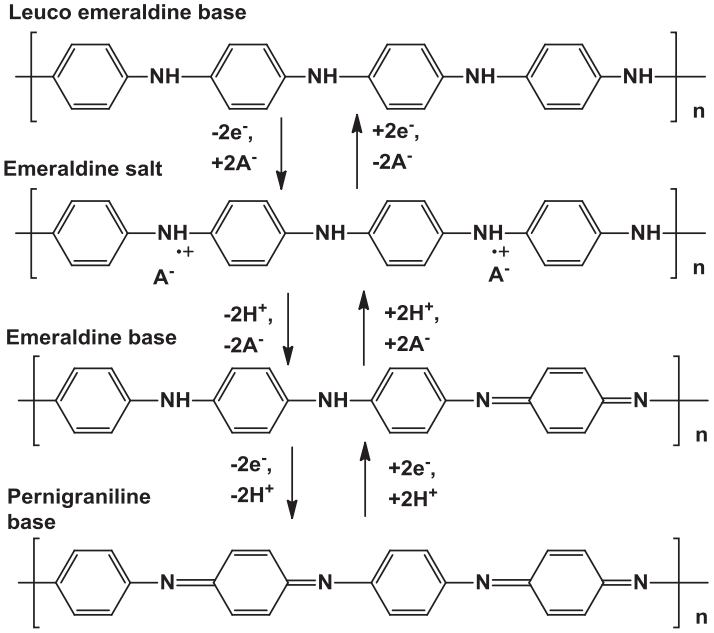
This limits the electrolysis conditions and often results in the formation of quinoid by-products which participate in the reversible electron exchange and worsen the electroconductivity of the polymer. Meanwhile such processes do not seriously affect the electrocatalytic features of PANI although they decrease the stability of the products especially in basic media.



(3)

PANI is a "ladder" polymer that polymerizes head-to-tail. The nitrogen atom directly participates in the polymerization and redox conversion of PANI forms. The process is self-catalyzing, i.e. the rate of the polymer precipitation increases with amount of the reaction product [68]. Many characteristics of the PANI depend on the redox equilibria of various reduced and oxidized forms (4) which take place in accordance with the redox potential and the pH of the media [69,70].

Only a half-oxidized form, the emeraldine salt, exerts electroconductivity. The conductivity window as well as the average molecular weight, adhesion of the film to the electrode and some other characteristics depend on the electrode nature, the electrolysis regime and temperature and the counter ion used. The latter determines also the swelling of the polymeric film in pH switching processes.



(4)

The transfer of anions with the hydrate sphere can change the specific volume of the polymer by 30%. This is one of the reasons to use the potential scanning which eliminates water and low molecular components from the growing polymer film. Meanwhile the decrease of the temperature down to -30 to -70 °C in chemical oxidation of aniline for improving structure and increasing the molecular weight is less appropriate in electrosynthesis due to obvious technical problems.

PANI is insoluble in most organic solvents and requires some special treatment like grinding with doping agents (camphorsulfonic acid [71] and some other strong organic acids insoluble in water) and plasticizer (phenol). The possibility to cover the electrode with thin regular PANI films is one of the most important advantages of electrosynthesis for sensor and biosensor development.

The electropolymerization of aniline in strong acids is easily monitored by appropriate changes in the redox peaks corresponding to the reversible conversion of leuco-emeraldine and emeraldine salt at low potential and the emeraldine salt and perngraniline at a high potential. In-between two peak pairs, reversible peaks corresponding to the quinoid products of the aniline degradation are often exist. The peaks grow with the number of potential cycling and became broader. If the acidity of the solution is insufficient, the peaks can amalgamate with the formation of one very broad peak in the middle area of the potentials.

Many *o*- or *m*-substituted anilines are able to polymerize similarly to the unsubstituted monomer. Aniline-like polymerization is proposed for thionine [72] and some other aminated heterocyclic compounds [37]. Besides, the use of sulfonic and carboxylic derivatives is of special interest because of the self-doping effect [73,74]. The anchoring counter ion covalently attached to the polymeric chain

stabilizes the reaction product and the electrochemical properties of modified electrodes. Insoluble organic acids [75] and sulfonated or carboxylated MWCNTs have a similar effect [76,77]. For the same reason, PANI are easily combined with anionic polymers and polyelectrolytes to form layer-by-layer constructions [53,78–80]. The formation of multilayered complexes of PANI with negatively charged components is also aimed at the enhancement of the redox activity of the polymer which is usually inert in the middle of pH scale, which is most appropriate for biomolecule functioning. The doping of cationic oxidized forms with large polycharged counterparts makes it possible to detect the electrochemical signals at pH=4.0–6.5.

As other polyanionic species (nafion, polystyrene sulfonate, polyacrylic acid), DNA interacts with positively charged oligomers of aniline during polymerization and hence affects the characteristics of the coating. Thus, the DNA–PANI complex was obtained on the Au electrode preliminary treated with 2-aminoethanethiol [25]. The electrode was first immersed into the DNA solution to obtain a monolayer by electrostatic accumulation. After that, electrolysis was performed in the acetate buffer solution, pH=4.2, by potential cycling between 0 and 0.7 V vs. Ag/AgCl. The formation of the polymer was confirmed by UV–vis and NIR spectroscopy and SPR measurements. The modification of the Au with the DNA monolayer significantly decreased the polymerization pH to the region acceptable for most other biomolecules. This makes the approach promising for the development of biosensors.

Another approach is described in [81]. The poly(dA) and poly(dT) oligonucleotides were electrostatically adsorbed on a thin Au film deposited onto a borosilicate substrate and covered with a monolayer of 4-aminothiophenol. After that, the electropolymerization of aniline was performed from the acetate buffer solution (pH=4.2). The efficiency of PANI deposition was controlled by EIS and AFM. The thickness of the polymer layer was estimated as 1.4 nm.

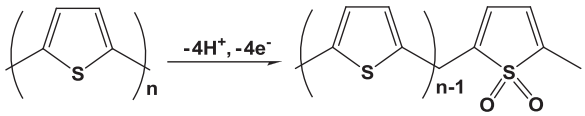
The electrocatalytic polymerization of aniline pre-adsorbed onto the DNA helix was described in [27]. The Au electrode was first covered with a self-assembled monolayer consisting of thiolated DNA primer and thiohexanol. Then, the double stranded (ds-) DNA was formed by hybridizing the primer with a complementary DNA sequence. After that, the product was saturated with an intercalator daunorubicin and aniline electrostatically accumulated onto the DNA molecule. Later, the electrode was transferred into the solution of horseradish peroxidase and cathodically polarized at -0.65 V vs. SCE. The oxygen was electrocatalytically converted into the H_2O_2 which oxidized aniline to PANI. Based on the coulometric experiment, the density of the coating was estimated as 0.066 nanomol cm^{-2}. The resulting PANI–DNA layer showed redox-activity in neutral media contrary to PANI which revealed redox functions only in acidic solutions.

PPY is formed in a cation radical process similarly to PANI (compare Eqs. (2) and (3)). However, chain propagation results in the formation of C–C bonds and does not involve nitrogen atoms which could be easily modified either in the monomer or polymer to reach specific features (solubility, hydrophilicity) or covalently attach the DNA probes. The electropolymerization reaction is limited by the formation of cation radicals. Other stages, i.e. radical coupling, deprotonation and doping with counter anions, are fast enough. The precipitation of the insoluble product of polymerization onto the electrode presumes the neutralization of the polymer chain charge. For this reason, the final product contains rather high (up to 50 mass%) amount of counter ions located between the planes formed by pyrrole heterocycles. The solubility of PPY in organic solvents is even lower than that of PANI. Together with moderate adhesion to electrode materials this makes the chemical synthesis less effective in comparison with electrochemical approaches.

The structure and morphology of the PPY particles strongly depend on the electrolysis regime, the supporting electrolyte and the electrode used. It should be mentioned that the redox equilibria are much less affected by the pH of the microenvironment than those of PANI. This results in a lesser pH dependence of the electrochemical characteristics of the polymer and much weaker counter ions flow through the interface in the redox conversion. Also, PPY provides only one redox transfer and one pair of peaks on cyclic voltammograms as well as a wider conductivity window against that of PANI. Irrespective of that, the PPY films are commonly used as inert supports for the DNA probe immobilization which can be achieved by covalent binding based on carbodiimide linking, avidin-biotin interactions or electrostatic accumulation. In the latter case, over-oxidized PPY [82,83] is used. Overoxidation is performed by the anodic polarization of the electrode modified with PPY in aqueous solution or in the presence of dissolved oxygen. This is a partially destructive irreversible process with a loss of electroconductivity and conjugation in the primary polymer chain [84,85] due to the formation of carboxylic, hydroxy and carbonyl groups in the rings. The film becomes permeable for cations and can also eject a small amount of counter anions while polarized. Overoxidation is applied for the molecular imprinting of the PPY as well to provide selectivity toward biomacromolecules [86].

PPY was the first electropolymerized material applied in the DNA sensor assembly [87,88]. The investigation of the DNA adsorption kinetics made it possible to conclude that single- and double stranded DNA molecules are adsorbed onto the PPY film with a significant alteration of their structure and the kinetics was moderately affected by the ionic strength and pH in the area pH=5–9. In the second report [88], pyrrole was co-polymerized with oligonucleotides bearing terminal pyrrole groups to manufacture a 50×50 μm chip for hybridization detection. A similar approach assuming the formation of mixed polymeric products by electrolysis in the presence of pyrrole and other monomers has been successfully applied for the introduction of other fragments providing either electrocatalytic activity or the binding sites for DNA probe immobilization. Thus, the copolymers of PPY and PANI [89,90], PPY and its derivatives [91,92] have been synthesized from the mixture of appropriate monomers. Their use is due to the necessity of combining the electroconductivity of the polymer with the ability to bind the DNA probes by involvement in self-assembled monolayers or Langmuir–Blodgett films or by the covalent attachment to carboxylic groups via carbodiimide binding. The use of substituted pyrroles only increases the density of the DNA binding sites and decreases the permselectivity of the surface coating for charge carriers.

Polythiophenes are synthesized similarly to PPY in a general reaction sequence (2) including the formation of cation radicals and their coupling either in solution or on the electrode surface. The oligomeric products are easily oxidized to sulfones (5).

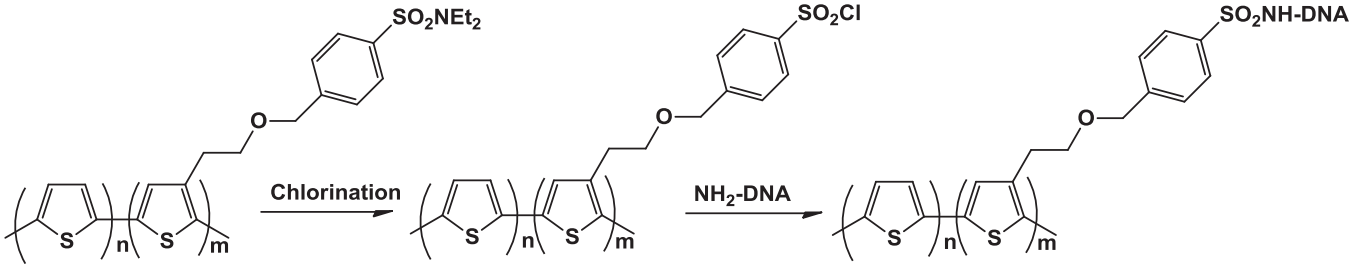


(5)

This results in a significant worsening of electrocatalytic and electroconductive abilities in the usual process. The product of electropolymerization consists of a mixture of oxidized and overoxidized polythiophene. The working potential of polymerization can be decreased by the addition of dimers and tetramers to the monomer solution [93]. At present, substituted polythiophenes are more

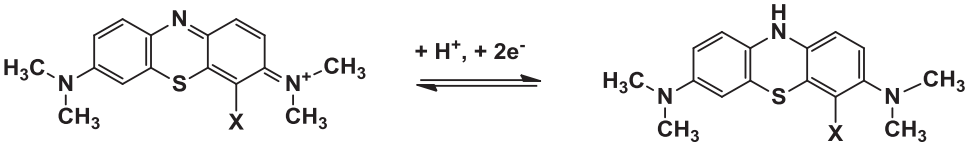
frequently used in the biosensor assembly due to the higher solubility of appropriate monomers (PEDOT) or to self-assembling onto the solid support (3-alkyl- or alkoxysubstuted polythiophenes). PEDOT was introduced in the chemical sensors development in the early 1990-s [94]. The electrolysis is performed by cyclic potential scanning in aqueous or organic media in a broad potential range, from about −0.8 to 1.0 V. The initiation step of the cation radical formation yields an irreversible peak near 0.8–1.0 V which becomes lower starting from the second cycle till about its full disappearance by the tenth cycle. Meanwhile, an ill-defined pair of reversible peaks related to redox conversion of PEDOT appears at about −0.5 to −0.3 V. All the peaks are less pronounced in comparison with PANI or PPY signals. The substitution of appropriate monomer at the ethylene bridge does not significantly alter the electrochemistry of the polymer. PEDOT in oxidized doped form is much more stable than PPY and PANI and shows high adhesion to the solid support because of the 3,4-dioxyethylene cycle which provides favorable geometry of the polymeric chain and leaves no sites free for carbonyl formation [95]. The electron transduction via the PEDOT film is a complex process which probably involves several overlapping redox steps [96] and demonstrates a high rate of the transfer of charge carriers and electroconductivity of the final products. This stimulates application of PEDOT in various chemical sensors and biosensors. 3-Alkyl or alkoxy substituted thiophenes are produced preferably in organic solvents, i.e. acetonitrile [97]. They form dense films with a regular orthogonal position of hydrophobic substituents towards the electrode interface. Such polymers are compatible with other self-assembled monolayers on gold or lipid films. The substituents can also be used for derivatizing the polymer chain, among others, for the covalent immobilization of the DNA probes [98] (see (6) as an example). Steric access of the bulky reactant can be improved by the co-polymerization of substituted and un-substituted thiophenes on the same support.

Polyphenoxazines. Contrary to other polymers considered here, polyphenoxazines are mostly obtained in neutral and basic aqueous media. Moreover, the efficiency of the polymerization and the mass of deposited product increase with the pH value. The structures of the monomers most frequently used in the DNA sensor assembly is presented in Fig. 1.



(6)

In the repeated cycling of the potential the polymerization results in a continuing decrease of the irreversible anodic peak at high potential and increase of a pair of the peaks at much lower potentials which formally correspond to the redox conversion of the monomeric form of the phenazine (see (7) for Methylene Blue (X=H) and Methylene Green (X=NO_2) as an example).



(7)

Probably, the characteristics of the oligomeric forms deposited on the first steps of polymerization do not differ significantly from those of the monomers. But, then novel peaks appear at more positive potentials, which are les resolved and significantly broadened in comparison with those of the monomers. They are commonly attributed to the polymeric forms of the phenoxazines. On Fig. 2, the examples of voltammograms are presented for Methylene Blue and Neutral Red on glassy carbon electrode. If the electrodes are transferred into the solution with no monomeric phenoxazines, the peaks remain but their relative height and position on voltammograms can shift in favor of polymeric forms in multiple cycling the potential [99]. This procedure is also recommended for the stabilization of the redox characteristics of the electrode prior to modification with biomolecules.

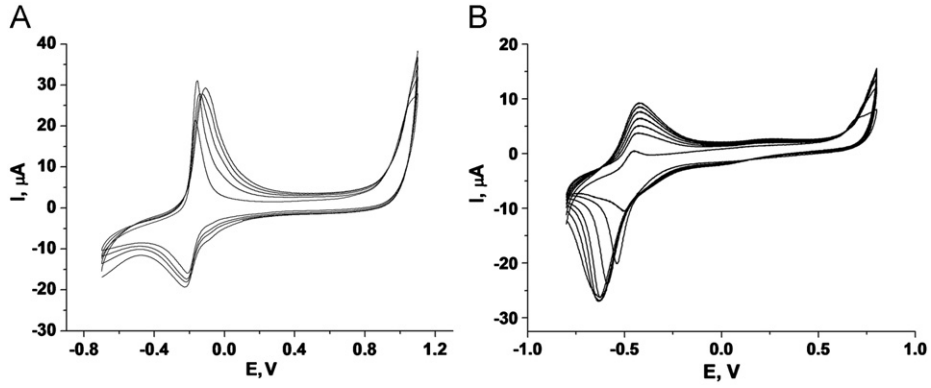
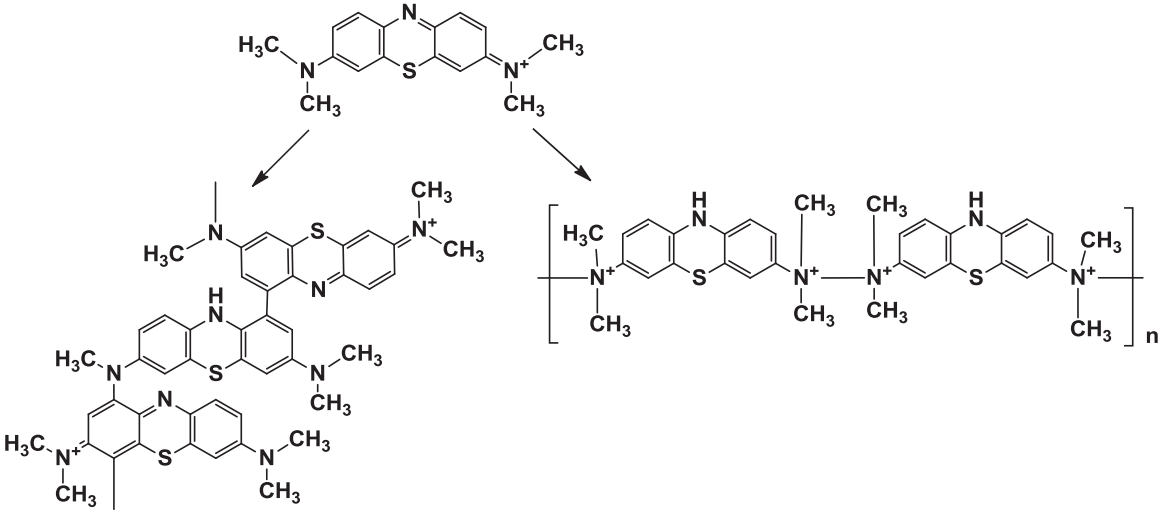


Fig. 2. Voltammograms of 2 mM Methylene Blue recorded on glassy carbon electrode in TRIS buffer, pH=7.6 (A) and 0.4 mM Neutral Red in HEPES buffer, pH=7.0 (B). Scan rate 100 mV/s.

The products of polymerization are mostly undiscovered and their structure is supposed on the basis of cyclic voltammetry and spectroelectrochemistry. Thus, the polymerization of Methylene Blue results in the preferable N-demethylation of the initial molecule (N-to-ring coupling [100]). Another mechanism presumes ring-to-ring coupling which might involve the following benzidine rearrangement [101], (8).

The possible structures of oligomers were confirmed by on-line electrochemistry - electro spray mass spectrometry [102]. The electrochemical and SPR study of poly(Methylene Blue) deposited onto a gold electrode showed that the use of a high enough concentration of the monomer yielded the formation of the leuco-form of the polymer with reduced electroactivity [103].

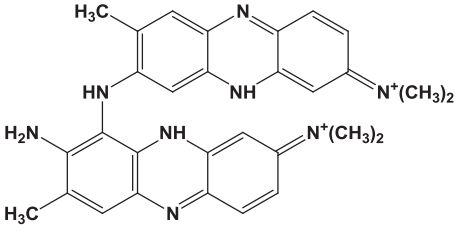
Methylene Green shows rather small changes in repeated cycling on a glassy carbon electrode. The peaks related to the reversible redox reaction (8) shift to anodic potentials and a minor increase of the current in the area corresponds to potential polymerization products [104]. This made it even possible to conclude that the redox characteristics of the electrodes after such a modification correspond rather to chemosorbed monomers that participate in charge directed desorption and re-sorption in potential cycling [105].



(8)

Neutral Red is preferably oxidized in neutral media to form linear polymers. The cycling potential increases the primary pair of peaks at −700 to −500 mV vs. Ag/AgCl with insignificant changes of the rest of the voltammogram [38]. Changes in the peak currents coincide well enough with the mass of the surface film estimated with QCM techniques [106].

In some reports, smaller reversible peaks at about 0 to 200 mV are mentioned which increase with the number of cycles but their relation to the primary polymerization product has not been discussed. The N-to-ring polymerization products are proposed for the Neutral Red (see (9) for the proposed dimer structure [107]).



(9)

3. DNA sensors based on electrosynthesized PANI

3.1. Hybridization detection

The determination of the primary nucleotide sequence specific for target analytes is achieved by involvement of the oligonucleotides in the hybridization reaction with complementary oligonucleotides called also DNA probes attached to the electrode surface. Such DNA sensors are mainly on demand in the medical diagnostics of pathological microorganisms and viruses but also find growing application in food chemistry (detection of transgenic tissues in the foodstuffs) and environmental monitoring (bacterial contamination).

Three strategies are mainly applied for the detection of a hybridization event:

- determination of the changes in the redox signal related to the electrochemically active labels incorporated in the DNA probe or to the redox indicators specifically interacting with the hybridization product (see a principal scheme in Fig. 3a as an example);
- monitoring of the changes in the electrochemical characteristics of the polymeric support resulting from its interaction with the DNA probe and/or target oligonucleotide. In this case, the equilibria (4) are altered due to changes in the increased negative charge of the hybridization product;
- Recording changes in the electrode characteristics due to different permeability of the surface layer caused by hybridization (Fig. 3b).

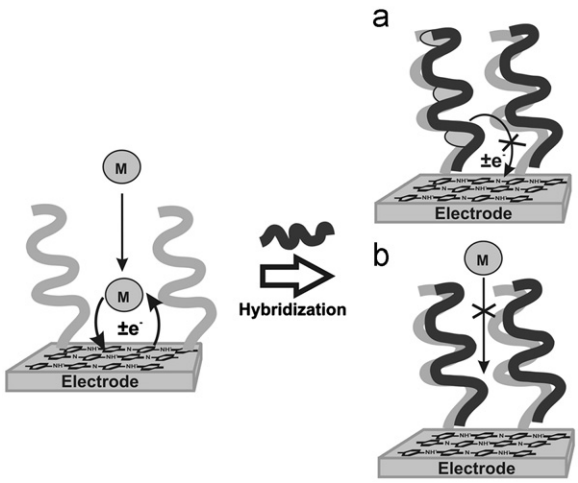


Fig. 3. Schematic representation of the hybridization detection based on the use of diffusionally free mediator M: (a) – Mediator intercalates the DNA duplex; (b) – Mediator access is limited by decreased permeability of the surface layer.

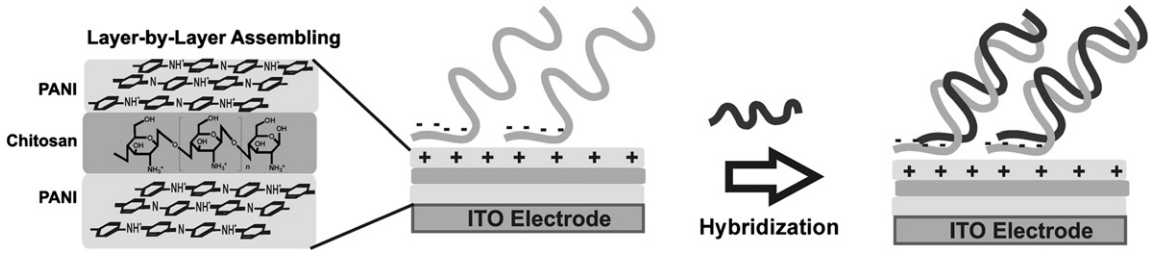


Fig. 4. Layer-by-Layer assembling of the PANI–chitosan multilayer and electrostatic accumulatin of the DNA probe for the hybridization detection.

Not all of them are realized in appropriate DNA sensors involving PANI. The main attention was paid to the determination of the intrinsic PANI activity related to the reversible conversion reduced and oxidized forms of a polymer. Meanwhile, the mechanism of the signal generation is commonly suggested but not proved by appropriate techniques.

An adapted procedure of electrosynthesis [60] was employed to obtain aligned PANI nanofibers onto the glassy carbon electrode modified with oxidized graphene [108]. The electrosynthesis was performed in three steps which involve galvanostatic initiation at high and then reduced (two steps) current density in the aniline–HCl solution. The immobilization of the 24-mer DNA probe was performed by carbodiimide binding. The hybridization of the complementary DNA target resulted in a significant increase of the peak current measured by differential pulse voltammetry (DPV) and referred to as the oxidized graphene redox conversion. The PANI exerts synergetic effect on the graphene redox activity. The DNA sensor makes it possible to distinguish complementary and three-mismatch oligonucleotides and quantify the content of the target DNA sequence between 2.12×10^{-6} and 2.12×10^{-12} M with the limit of detection (LOD) 3.25×10^{-13} M. A similar mutual enhancement of the redox activity was also observed for similar composite electrodes based on multi-walled carbon nanotubes (MWCNTs) and PANI nanofibers obtained by physically mixing the components with their following deposition on the glassy carbon in a chitosan matrix [109].

The ITO coated glass electrodes were modified with PANI obtained in the potentiostatic regime at 0.9 V vs. Ag/AgCl from 0.5 HCl containing 1% chitosan [110]. After that the 42-mer DNA probe was immobilized onto the polyelectrolyte layer by electrostatic accumulation (Fig. 4). The content of the surface layer as well as the hybridization were investigated using EIS with $[Fe(CN)_6]^{3-/4-}$. As was shown, the formation of double-stranded DNA onto the electrode decreased the ferricyanide peak current in the range from 0.1 to 25 fM (LOD 0.05 fM). This makes the DNA sensor attractive for the early diagnostics of the breast cancer susceptibility.

Few examples of the use of diffusionally free indicating system have been reported. Thus, the highly sensitive detection of the pathogen of *Mycobacterium tuberculosis* based on Methylene Blue reduction signal has been described [111]. PANI obtained by potentiostatic electrolysis on Au electrode was first treated with glutaraldehyde and then incubated in the solution of aminated DNA probe complementary to the target DNA sequence. The polymerization was controlled by EIS, FTIR and SEM. The signal of Methylene Blue was recorded in the direct current mode. The cathodic peak current decreased in the presence of target oligonucleotide due to the involvement of the indicator in the hybridization product on the electrode surface. The DNA sensor made it possible to determine from 2.5×10^{-18} to 15×10^{-18} M of target oligonucleotide. The hybridization time did not exceed 5 min and up to 15 measurements were performed with each DNA sensor. The sensitivity toward the biological target was then increased by the displacement of DNA probe with the appropriate protein nucleic acid (PNA, the lowest quantification level of 0.125×10^{-18} M).

Nanowires of PANI were synthesized by a three step potentiostatic electrolysis as described in [60] to form the tubes of 80–100 nm in diameter which showed high conductivity and permeability for ferricyanide ions [112]. The characteristics of the coating were optimized with EIS and SEM data. The aminated oligonucleotides were immobilized onto the PANI by carbodiimide binding. For signal measurement, the electrode was consecutively incubated in the solution of the target DNA sequence and Methylene Blue and then transferred into the TRIS buffer solution which did not contain the indicator. The signal was recorded at $+0.3$ V in the DPV mode.

The decrease of the peak current against the value obtained prior to hybridization depended on the biological target content in the range from 0.225 nM to 2.25 pM (LOD 1.0 pM).

The ITO electrodes were used for deposition of PANI film in a potentiostatic regime for 600 s with intermediate washing steps [113]. As was shown by SEM, the additional treatment of the polymer resulted in a decreased chain length and an increased number of amino groups suitable for the following DNA probe immobilization. The PANI coating was then treated with avidin and biotinylated oligonucleotides related to the gene of *Neisseria gonorrhoeae*. The intrinsic redox activity of PANI–DNA adduct was characterized by the current plateau measured at 200–400 mV by DPV. In addition, the guanine oxidation peak at 0.85 V was observed. Both currents decreased with the hybridization progress. Meanwhile, the oxidation of Methylene Blue was found more appropriate for the target DNA quantification. The range of the concentrations determined was found to be 1×10^{-6}–1×10^{-16} M (logarithmic scale) and LOD 0.5×10^{-16} M.

The same target oligonucleotides were detected by ITO electrodes covered with the composite consisting of MWCNTs (20–80 nm in diameter), Fe_3O_4 nanoparticles (20 nm) and PANI electropolymerized in a glavanostatic mode at 50 mA for 900 s with two intermediate washing steps [114]. The principal scheme of the biolayer preparation and signal measurement is given on Fig. 5. The immobilization of DNA probes was performed by biotin–avidin binding followed by the detection of the hybridization by the current of Methylene Blue accumulated in the DNA helix. The use of additional nanosized mediator systems decreased the lowest detectable concentration down to 1×10^{-19} M. The use of glutaraldehyde for covalent attachment of the DNA probe, vice versa, has worsened this value to 1×10^{-17} M [115]. For both DNA sensors [113–115] the results of the assay were validated with the samples of the biological fluids taken from patients and healthy donors and on the positive and negative amplicons of PCR.

3.2. Aptasensors based on PANI

The aptasensors involve aptamers, i.e. synthetic oligonucleotides which are synthesized by combinatorial chemistry approach and can specifically bind various biomolecules [116]. Being sensitive as conventional antibodies, aptamers are more stable and easier to modify for implementation in the biosensor assembly. Aptamers find increasing application in the detection of biopolymers and low-molecular compounds capable to selective DNA binding [117].

PANI provides unique tools for the detection of such interactions in a micro scale offering new ways for microfluidics and microelectronic devices for point-of-care diagnostics. Thus, a single wire of PANI has been grown in the microchannel obtained by e-beam lithography between two Au electrodes separated by a 5 μm gap [118]. The interaction of DNA aptamers with immunoglobulin IgE resulted in remarkable increase of the PANI conductivity due to the changes in the charge and redox status caused by aptamer–protein interaction. The aptasensor makes it possible to detect 1.0 pg/mL – 22.0 ng/mL (LOD 0.56 pg/mL, or 2.8 fM).

3.3. Other specific interactions

DNA sensors are also employed for the determination of proteins specifically bonded to the biopolymers within a surface layer. The anti-DNA antibodies specific against native ds-DNA are released in the blood in some autoimmune diseases and pathologies like autoimmune thyroiditis and systemic lupus erythematosus [119]. Their interaction with native DNA alters both the permeability of the surface layer for hydrogen ions and the charge distribution in the PANI–DNA system. These changes are monitored by EIS (increase in the charge transfer resistance and capacity of the surface layer) and potentiometry [120]. In the latter case, the PANI electropolymerized from 0.1 M H_2SO_4 was covered with physically adsorbed DNA and then transferred into acidic media (pH = 3.0). The shift of the potential recorded in open circuit mode decreased in the presence of specific anti DNA antibodies probably due to the influence of the protein on the doping ability of PANI.

An unusual influence of insecticides, i.e. cipermethrin and thrichlorfon, onto the electrochemical activity of PANI–DNA adduct was observed [64]. The recognition layer was obtained by galvanostatic electrolysis in the $LiClO_4$ solution followed by the DNA covalent binding with carbodiimide. The analytes suppressed the guanine oxidation peak recorded by square-wave voltammetry at 0.85 V. The maximal effect was achieved with about 2 ppm solution of the insecticides whereas the minimal detectable concentration was 0.005 and 0.01 ppm for cypermethrin and trichlorfon, respectively (incubation period 30–60 s). Although the authors presented some evidence of the interaction between DNA and insecticides in solution, the mechanism of the biosensor response remained undiscovered.

3-Aminophenylboronic acid was electropolymerized onto a gold electrode with physically adsorbed single-walled carbon nanotubes [121]. To promote the adsorption of the negatively charged polymers, the self-assembled monolayer of 2-aminoethanothiol was obtained prior to the deposition of a recognition layer. Contrary to similar experiments with aniline, aminophenylboronic acid did not concentrate onto the DNA surface as was shown by AFM. Instead, in similar conditions nanowires along the surface about 2 nm high were obtained. They were twice higher than those of PANI in similar conditions. The increased efficiency of polymerization was related

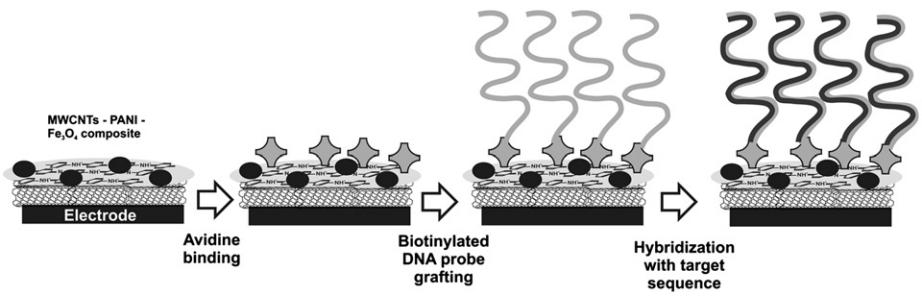
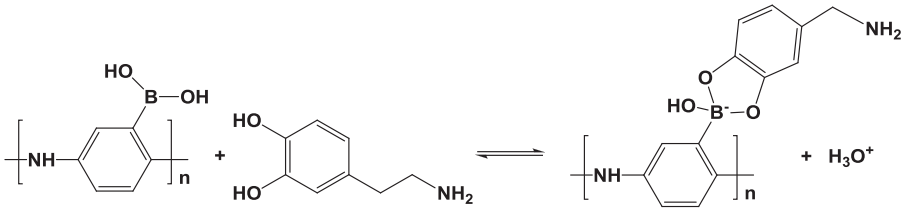


Fig. 5. General scheme of the DNA sensor preparation based on PANI and avidin–biotin binding of DNA probe.

to the emulsifying ability of the DNA–MWCNTs composite and the electrostatic repulsion of anionic species near the electrode surface. Besides, the redox peaks of PANI conversion were shifted by 150–200 mV to a less anodic potential indicating the promotion of the redox activity. The modified electrode was successfully applied for determining dopamine with the LOD of about 1 nM. The addition of the analyte resulted in the suppression of the redox activity because of the chemical binding of dopamine with a boronic acid residue and the formation of an anionic cycle (10). This affects the redox activity of the primary PANI chain probably due to steric hindrance of the protonation reaction required for the redox conversion of the polymer.



(10)

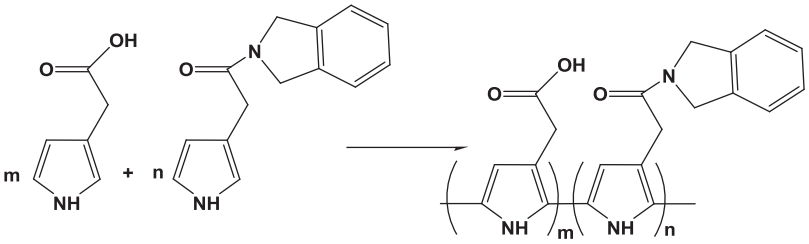
The addition of polyelectrolytes sufficiently decreases the pH required for polymerization and extends the electroconductivity window of the polymer [122]. Aniline, poly(vinyl sulfonate) and ds-DNA were polymerized in the 0.1 M $LiClO_4$ solution in a galvanostatic regime at 0.6 mA/cm^2 on the ITO electrode. Spectroelectrochemical and voltammetric investigations confirmed the electroconductivity of the film deposited and the entrapment of DNA resulting in the formation of irreversible peak at 700 mV on SWV referred to a guanine oxidation. The addition of organophosphates, i.e. 0.5–100 ppb of chloropyrifos or 0.01–10 ppm of malathion suppresses the peak current due to the suggested DNA damage caused by the phosphorylation activity of the pesticides.

4. DNA sensors based on electrosynthesized PPY

4.1. Substituted PPY as a platform for covalent DNA immobilization

The application of substituted PPY was first related to the approaches towards the directed positioning of DNA oligonucleotide probes on the solid support required for the DNA chip development. This was an alternative to the use of covalent modification onto activated nylon beds [123] which offers better opportunities for the automation of the process by passing electricity through the appropriate electrodes fabricated by lithography or other related techniques on a dielectric substrate. For targeted immobilization, various substituents were introduced in the 3-position of pyrrole ring. To get access of bulky DNA oligonucleotides to the functional groups of the substituents, it was suggested to perform the electrolysis in the mixture of variously substituted monomers different in their ability to bind biomolecules.

As an example, pyrroles bearing carboxylic groups and phtalimide fragments (11) were co-polymerized from an aqueous solution at −0.2 V vs. SCE [124]. The film about 200 nm thick exerted reversible behavior at about −200 mV indicating the favorable influence of the carboxylic group at 3-position. The reaction with aminated 14-mer oligonucleotide resulted in grafting the DNA probe. This was confirmed by IR spectroscopy when vibration frequencies at 1650 cm^{-1} associated to the pyrrolidinone moiety of the leaving group disappeared and new bands at 695 cm^{-1} and about 790–820 cm^{-1} related to the phosphorylamide and oligonucleotide groups, respectively, appeared. The hybridization of the DNA probe with the complementary DNA sequence suppressed the redox peak of PPY probably due to the charge influence exerted on the electron transfer in the polymer film.



(11)

The terminal covalent immobilization of DNA probes can be also achieved by N-substituted pyrrole monomers bearing terminal carboxylic groups. Thus, 4-(3-pyrrolyl)butanoic acid was first treated with N-hydroxysuccinimide and then electropolymerized in acetonitrile by scanning the potential between 0 and 0.95 V vs. Ag/AgCl [125]. The polymerization product exhibited nearly reversible behavior with standard potential of 0.58 V and the surface coverage of 3.5×10^{-7} mol cm^{-2}. Aminated 21-mer oligonucleotides specific for West Nile virus were added and the biosensor washed to remove unlinked biomolecules. The hybridization event was monitored using competitive assay mode. For this reason, after the incubation in the sample the biosensor was additionally treated with biotinylated complementary DNA sequence and then glucose oxidase was attached via biotin–avidin–biotin linking. The concentration of an enzyme label was determined after the substrate injection at 0.6 V vs. Ag/AgCl which corresponded to H_2O_2 oxidation. The principal scheme of the signal measurement is presented on Fig. 6. The total measurement period was about 2 h. and the range of the DNA target sequence from 10^{-10} to 10^{-15} g/mL.

The co-polymer of 4-(3-pyrrolyl)butanoic acid and unsubstituted pyrrole was synthesized by potentiostatic electrolysis of the monomer mixture at 0.75 V vs. Ag/AgCl and then modified with the DNA probe specific for *Salmonella* virulence *invA* gene by carbodiimide binding [126]. The hybridization resulted in a remarkable change of the charge transfer resistance or suppression of PPY redox peak on cyclic voltammgrams. The measurements were performed in the nanomolar range of the target DNA concentration.

A similar immobilization scheme was used in [127]. However, instead of the carbodiimide binding, the DNA probe specific for the West Nile virus was attached to the PPY film by intercalation. The acridone derivative that anchored the DNA sequence was covalently immobilized onto PPY bearing succinimide groups. Labeling the hybridization product with glucose oxidase made it possible to reach a 1 pg/mL detection limit.

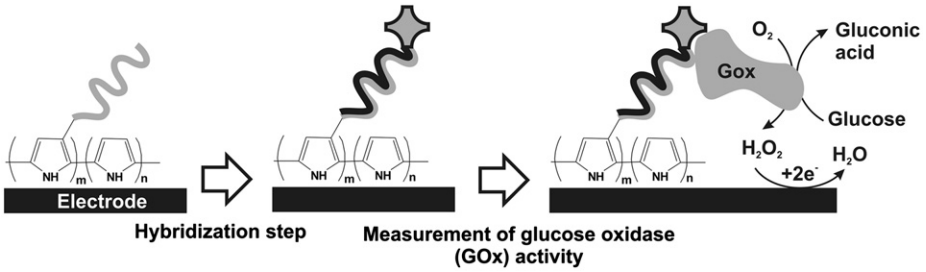


Fig. 6. Competitive scheme of the hybridization detection with glucose oxidase (GOx) as a label linked to DNA helix via avidin–biotin bridge.

Impedimetric DNA sensor with PPY–carbon nanotube composite and aminated DNA oligonucleotide covalently attached to the terminal carboxylic groups of the carrier was used for the detection of 1.0×10^{-11}–1.0×10^{-7} M of target DNA complementary to 24-mer DNA probe (LOD 5.0×10^{-12} M) [128]. Contrary to many other reports, the authors noticed decrease of the charge transfer resistance due to hybridization which is explained by own electric conductivity of the DNA helix even though increased negative charge of the surface should repel anionic redox probe $[Fe(CN)_6]^{3-/4-}$.

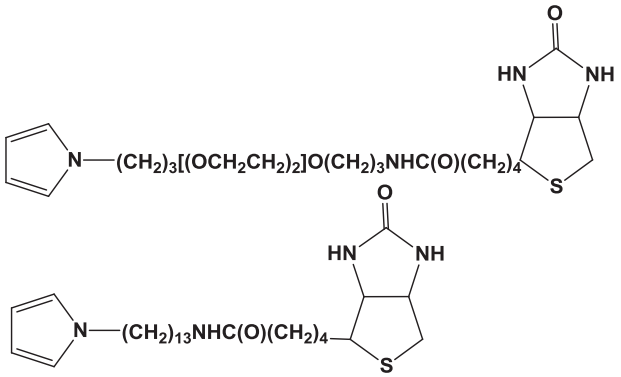
Covalent immobilization via carbodiimide binding was compared with affine immobilization by avidin–biotin linking in [129] where the PPY – polyvinylsulfonate film was deposited onto the Pt electrode in galvanostatic regime similarly to that of PANI-polyvinylsulfonate-DNA composite [122]. The $(dA)_{25}$ was used as a model DNA probe in the reaction with poly(dG) as a complementary target and poly(dA) is non-complementary sequence. In both immobilization protocols, the PPY reversible redox peak was recorded at 0.6 V together with the irreversible peak of the guanine oxidation at higher potentials (0.82–0.86 V). The carbodiimide binding showed a broader range of concentrations and a lower LOD against the avidin-biotin binding. The minimal LOD for the complementary target was found to be 0.01 fmol (hybridization period 30 s).

4.2. Hybridization detection based on non-covalent PPY–DNA composites

Avidin–biotin binding is one of the most effective immobilization ways for targeted binding the DNA oligonucleotides via terminal groups [130–132]. Some of the monomers used for this purpose are shown below (12). They are commonly electropolymerized together with unsubstituted pyrrole, for example, in the aqueous $LiClO_4$ solution by potential cycling between −0.25 and 0.85 V vs. SCE. The DNA grafting can be performed prior to electrolysis as well. The products of electropolymerization containing DNA probes are suitable not only for voltammetric but also for photovoltaic [133], SPR or fluorescence signal measurements as well [134].

Avidin–biotin binding provides renewal of the biorecognition surface either by solubilization of the avidin or denaturation of the DNA duplex. In both cases, new biotin/avidin/DNA construct can be built onto the PPY film.

The avidin can be excluded from the surface layer by using chelate complex of Cu(II) obtained at poly(pyrrole-triacetic acid) film [135]. The latter one was obtained by potentiostatic electrolysis in acetonitrile at 0.95 V vs. Ag/AgCl and then soaked in $CuCl_2$ aqueous solution. Biotinylated 48-mer DNA target specific for HIV was immobilized by incubation of the electrode for 30 min in oligonucleotide solution. Besides DNA sensor, glucose oxidase and polyphenol oxidase were immobilized in a similar manner via biotin–chelate complex bridge to proof the immobilization concept. The hybridization was indicated by increase in the resistance of the charge transfer measured in the presence of neutral redox probe, hydroquinone, at 0.4 V. Although the concept of avidin free immobilization of biomolecules seems attractive, the shift of the resistance reported (about 28% of initial value) seems rather small for quantification of the target content.



(12)

Entrapment in the growing polymer film provides a rather simple and one-step manufacture protocol for the preparation of DNA sensors for hybridization detection [136]. For better adhesion, Pt electrodes can be additionally covered with dispersed platinum by cathodic polarization from the H_2PtCl_6 solution. After that, the 0.05 M pyrrole solution on 0.1 M KCl was mixed with 0.5 mg/mL of equal amount of 23-mer oligonucleotide and electrolysis was performed by application of 30 potential pulses at 950 mV vs. Ag/AgCl. The hybridization of complementary DNA sequence could be monitored using EIS or pulse amperometry. The hybridization decreased the current shift in comparison with a blank PPY film due to the influence of the negative charge of the ds-DNA onto the redox equilibrium of PPY.

Carbon paste electrode with MWCNTs added to the electrode material was used for the electropolymerization of pyrrole from a phosphate buffer solution containing 32-mer DNA probe [137]. The hybridization with target DNA was monitored at 0.56 V vs. Ag/AgCl by the oxidation current of ethidium bromide, an intercalator often used for the fluorescence detection of DNA helixes. The oxidation current on the DPV voltammogram increased with the target DNA sequence in the range from 1.0×10^{-10} to 1.0×10^{-8} M

(LOD 8.5×10^{-11} M). A five-point mismatch sequence can be distinguished from a fully complementary oligonucleotide at the lowest level of quantification. The role of carbon nanotubes in the biosensor assembly remains unobvious and is probably related to the simplification of polymerization due to the negative charge of MWCNTs partially oxidized prior to their application.

The composite of the MWCNTs and poly(*trans*-3-(3-pyridyl)acrylic acid) was used for sensitive detection of biological target with 21-mer DNA probe bearing terminal thiol group on long chain methylene linker [138]. The immobilization of DNA probe was achieved by its anchoring to silver nanoparticles deposited onto the PPY composite (Fig. 7). After hybridization, the DNA sensor was treated with Adriamycin, a DNA intercalator, and then the current of its cathodic reduction was recorded in DPV mode. Under the optimal conditions, the signal linearly depended on the logarithm of the concentration of the complementary oligonucleotides from 9.0×10^{-12} to 9.0×10^{-9} M (LOD 3.2×10^{-12} M).

Among acrylic derivative, structurally relative monomers with unsaturated substituents bearing terminal carboxylic groups have been successfully employed in the DNA sensor assembly [139]. The influence of the number of carboxylic groups and linker length was evaluated using EIS measurement. The 5-(3-pyrrolyl)-2,4-pentadienoic acid showed better results in comparison with 3-pyrrolylacrylic and 3-pyrrolyl-pentanoic acids. The LOD of 0.5 nM was achieved for hybridization detection. Similar investigations performed with poly[pyrrole-co-4-(3-pyrrolyl) butanoic acid] [140] showed lower sensitivity toward target sequence in comparison with acrylic acid derivative and its analogs probably to a lesser regularity of the DNA probe positioning in the surface layer and hence lower changes of the charge transfer resistance.

Bilayer DNA microsensor was developed by combination of PPY and polymer of 2,5-bis(2-thienyl)–N-(3-phosphorylpropyl)pyrrole which were formed in two separate steps of electrolysis onto the Pt microelectrode [141]. The electrolysis was performed from acetonitrile in potentiostatic regime with control of the charge passed to reach reproducible conductive films. The 27- or 18-mer synthetic oligonucleotide probe was linked to the conducting polymer by forming a bidentate complex between Mg^{2+} and an alkyl phosphonic acid group of the polymer. The hybridization increases electrostatic barrier onto the layer which prevents chloride anion exchange. As a results, the charge passed in potential cycling decreased with the hybridization step. The DNA sensor can be chemically regenerated by incubation in HCL to restore the chloride content in the DNA layer. In double logarithmic scale, the peak current related to the redox conversion of PPY was dependent on the complementary sequence concentration in the range from 10^{-16} to 10^{-6} M. This protocol of the DNA sensor development was successfully used for sensitive determination of HIV DNA sequences (244-mer) resulting from the reverse transcriptase-linked PCR amplification of the original viral RNA (LOD 1.82×10^{-21} M [142]).

DNA planar sensor based on interdigitated Au electrode covered with PPY–DNA film was developed for fast and simple detection of DNA sequence of herpes simplex virus [143]. The immobilization was performed by entrapment of the DNA probe in the PPY layer by cycling the potential of the electrode between -0.7 and 0.6 V vs. Ag/AgCl. The PPY redox peak was suppressed by hybridization product within 4–22 nM of target DNA (LOD 2 nM).

Ferrocene functionalized PPY was synthesized onto gold macro- and microelectrodes [144]. The mixture of 1-(phthalimidylbutanoate)-1′-(N-(3-butylpyrrole)butanamide)ferrocene and pyrrole was potentiostatically polymerized in acetonitrile at 0.7 V vs. SCE. Then 25-mer DNA oligonucleotides with terminal amino group were covalently grafted by substitution of phtalimide fragment. For hybridization detection, the redox signal of the ferrocenyl group (Fc) which decreased with the concentration of target DNA (13).

(13)

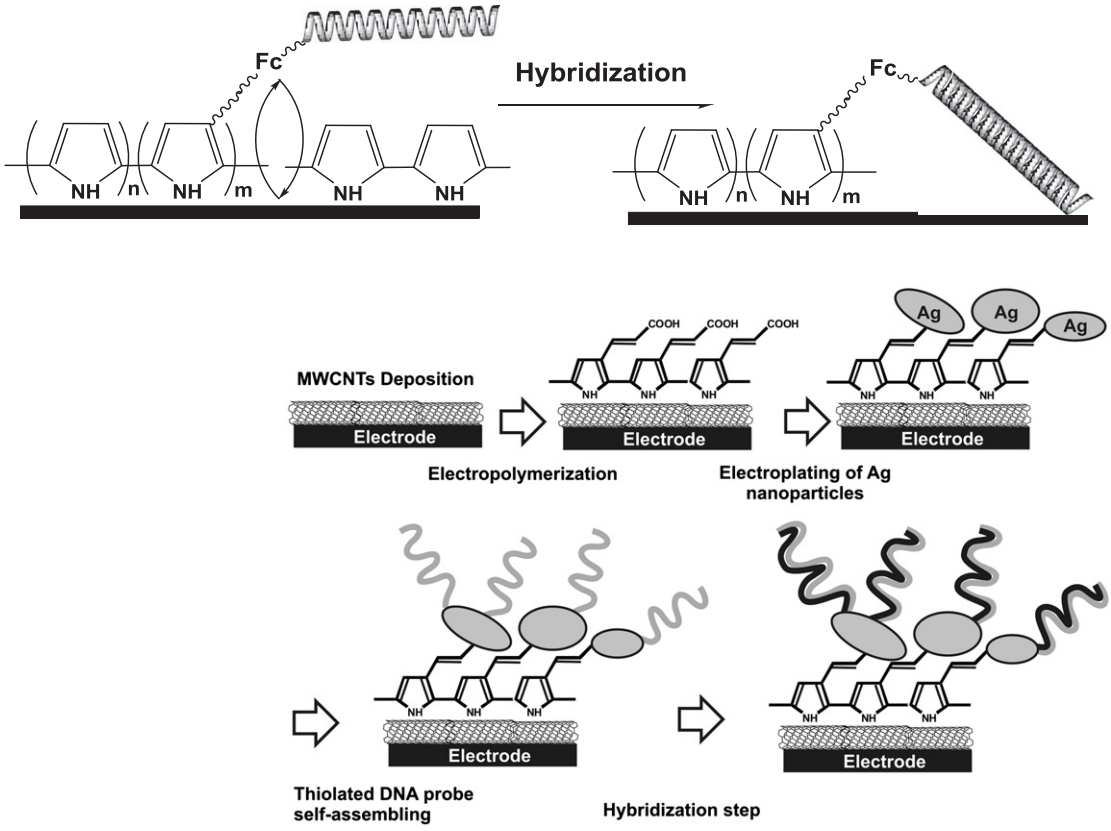


Fig. 7. The immobilization of thiolated DNA probes onto Ag nanoparticles electrodeposited onto multi-walled carbon nanotubes covered with electropolymerized poly(*trans*-3-(3-pyridyl)acrylic acid).

The results of SEM showed that reducing the size of the electrodes allowed controlling the nucleation and the growth rate of the polymer film during the polymerization. This enabled an increase in the sensitivity and selectivity of DNA hybridization in the chip format. It should be mentioned that the range of the peak current changes (about 30% of initial value) is small enough and does not coincide well with the mechanism assuming steric hindrance of the electron exchange in the surface layer caused by electrostatic interaction of the DNA helix with positively charged PPY and ferrocene units. Indeed, such interactions would change the ratio of anodic and cathodic peak currents related to ferrocene redox conversion. Meanwhile the cyclic voltammograms indicates the shift of fully reversible pair of the peaks to anodic potentials but not changes in their shape or symmetry.

Other examples of the use of PPY in the multi-sensor chip format different in geometry and number of individual sensors are given in [145,146]. They are mainly intended on signal on-off registration based on the intrinsic PPY activity and to a less extent on the quantification of the target analyte. Although high sensitivity of the response is always reported, in should be noticed that the metrological characteristics of the chips presented are mainly related to the single measurement and rarely consider the reproducibility of the absolute response on a set of the chips. In all such applications, main attention is paid to the synthesis of well-structured PPY surface to promote the changes in the charge distribution and increase the specific concentration of the binding sites.

Among diseases and pathologies, DNA hybridization can be used for the detection of tissues from genetically modified organisms which are under special attention in food industry by European legislation. The exogenous DNA integrated into a crop consists of a promoter, structural gene ('encoding region') and transcription terminator. In most available transgenic products, subunit 35S of ribosomal RNA of Cauliflower mosaic virus is used as promoter and NOS gene from *Agrobacterium tumefaciens* as transcription terminator. Thus their indication testifies the presence of such tissues in foodstuffs. For this purpose, DNA sensor has been proposed on the base of PPY doped with MWCNTs [147]. Herbicide-resistance soybean (Monsanto Inc.) was chosen as a model. The DNA probes specific for 35S promoter and NOS gene were added to the 0.3 M pyrrole in phosphate buffer, pH = 7.4. Pre-oxidized MWCNTs were placed onto the Au planar interdigitated microelectrode and then the electrolysis was performed at constant potential of 0.7 V vs. Ag/AgCl (30 min) followed by multiple potential scanning between 0 and 0.9 V. The hybridization of a target was monitored by QCM and EIS. The increase of the charge transfer resistance made it possible to detect 25–292 pM of 35S sequence.

The same protocol of PPY–DNA immobilization but on a glassy carbon was used for impedimetric detection of 24-mer oligonucleotide [148]. After hybridization, the metallation of the DNA helix with Zn^{2+} ions was performed. Specific changes of the charge transfer resistance on various steps of the measurement allowed detecting 0.05 nM of target sequence and distinguish one- and two-point mismatches.

The 2,6-pyridinedicarboxylic acid was first deposited together with single-walled carbon nanotubes on glassy carbon electrode from the DMF solution [149]. Then the electrode was dried and the electropolymerization performed by potential cycling in aqueous 0.1 M KCl from −0.6 to 2.0 V vs. SCE. The immobilization of DNA probe was performed by electrostatic accumulation onto the layer of poly(diallyldimethylammonium chloride). The detection of a target was performed by monitoring changes in the reduction signal of Methylene Blue in direct current voltammetry. The hybridization decreased the signal due to involvement of the label in the DNA helix. The DNA sensor was applied for the detection of two sequences specific for transgenic organisms, i.e. phosphinothricin acetyltransferase (PAT) and NOS gene were detected from model solutions and PCR products. Thus, the PAT gene sequence was detected from 1.0×10^{-11} to 1.0×10^{-6} M (LOD 2.6×10^{-12} M). In comparison with similar detection techniques based on self-assembled layers on the Au electrode [150], significant enhancement of the signal and extension of the concentration range was reported due to the higher surface square and improved electron transduction conditions. Also, the same electropolymerized layer was obtained on zirconia nanoparticles [151]. The biosensor was used for determination of 1.0×10^{-11}–1.0×10^{-6} M PAT gene specific sequence (LOD 1.38×10^{-12} M).

4.3. PPY–DNA for voltammetric determination of analytes based on non-specific interactions

As in the case of PANI, PPY–DNA composites can be used for the specific accumulation of the substances which do not interact specifically with DNA. This might be due to extended electrocatalytic characteristics of the polymer with DNA as a template or due to the electrostatic pre-concentration of the analyte followed by their direct or mediated oxidation onto the electrode.

Thus, overoxidized PPY was synthesized onto calf thymus DNA as a template by scanning the potential between −0.25 and 0.85 V vs. Ag/AgCl [152]. Carbon fiber was used as a working electrode. Prior to electropolymerization the electrode was dipped into the DNA solution containing cetylpyridinium chloride. The modified electrode was then polarized at 1.8 V to obtain an overoxidized PPY layer for 30 min. The electrode obtained showed a remarkable signal of dopamine and serotonin oxidation at about 0.25–0.40 V. The linear response was obtained in the range from 1.0×10^{-8} to 1.0×10^{-6} M (LOD 7.0×10^{-9} M) for serotonin and from 3.0×10^{-7} to 1.0×10^{-5} M (LOD 5.0×10^{-8} M) for dopamine. The microsensor was used for the determination of biogenic amines in human blood serum.

Pulse electrolysis techniques were also used for electrosynthesis of PPY nanofibers on the Pt electrode and electrostatic accumulation of double-stranded DNA [153]. The electrode was used for the sensitive detection of salicylic and acetylsalicylic acids which affect the guanine oxidation peak measured by DPV. The increase of the current depended on the content of the analytes in the range 0.1–2 μM and 0.05–1 mM for salicylic and acetylsalicylic acids, respectively.

Nanofibers of PPY were synthesized by potentiostatic pulse electrolysis carried on by stepwise increase of the working potential from 0 to at 0.80 V vs. Ag/AgCl with intermediate equalization at 0 V. The Pt disk was used as a working electrode and double-stranded DNA from calf thymus electrostatically adsorbed on the PPY fiber surface [154]. The interaction of DNA with PPY was confirmed by FTIR and EIS and the morphology of the fibers on the electrode surface by SEM. The DNA sensor was used for the detection of spermine, an indicator of cell apoptosis and a model of polyamine compounds which play significant role in diagnostics of many diseases such as cancer, infections, insulin-dependent diabetes mellitus and Alzheimer's disease [155]. The electrostatic interaction of DNA with protonated amino groups of spermine suppressed the anodic oxidation of guanine due to shielding oxidizable groups. The reaction is time dependent and can be ascribed by logistic curve in the range from 0.05 to 1.0 μM (LOD 0.02 μM).

In the above examples, DNA acts a template and support for the PPY layers which exert electron mediation functions. Due to high density of the negative charge, ds-DNA can accumulate positively charged or protonated analyte molecules providing amplification of the signal of its oxidation. The use of guanine oxidation peak seems interesting from the point of view of the mechanism of electrode

reactions. However, as was mentioned before, the signal is complicated by rather high overpotential of guanine oxidation which exerts moderate effect of mediation via PPY chain. For this reason, the working potential of such sensors is high enough to compete with mediated sensors based on conventional redox systems.

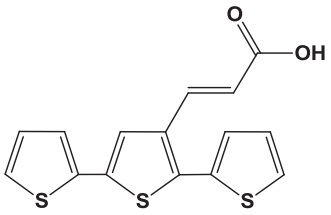
5. DNA sensors based on polythiophene and polyphenazines

5.1. Polymers of thiophene and its derivatives in the DNA sensor assembly

Many concepts of polythiophene based DNA sensors do not differ from those already described in previous section. Polythiophene moiety exhibits like PPY one broaden reversible peak on voltammogram which changes with the DNA specific interactions, namely, hybridization. All the changes observed can be related to either the electron exchange suppression or the counter ion transfer. The use of the substituted polythiophenes enhances the possibilities of the DNA sensor development due to the increased capacity of the polymer film toward the anionic biomolecules or improved procedure of polymerization. The steric limitations of the DNA hybridization initiated the application of co-polymers with the unsubstituted thiophenes or rather small substituents. In polythiophenes synthesis only, the di- and trimeric structures with long or bulky substituents were chemically synthesized and then used for electrochemical polymerization to avoid steric hindrance and improve regularity of the polymer structure against common polymerization products based on appropriate monomers.

Thus, cyclopentadithiophene was electropolymerized with 4-cyclopenta[1,2-b;3,4-b']dithiophene-4-ylidenemethyl-N,N-diethylbenzene sulfonamide from acetonitrile solution by potential cycling between −0.75 and 0.40 V vs. SCE [156]. After that, the 42-mer DNA oligonucleotide was covalently attached to the terminal groups of a co-polymer as was described previously [98]. The 675-mer target was hybridized via 3′-terminal sequence to the DNA probe. Changes in the polythiophene redox peaks, permeability of the surface film for ferricyanide ions as well as mass shift of the surface film were monitored using direct current voltammetry, EIS and QCM, respectively.

The terthiophene structure functionalized with the alkylenecarboxylic fragment (14) was synthesized and used for the DNA hybridization detection [157]. The polymerization was performed on the Pt electrode by potential cycling between 0 and 1.2 V vs. Ag/AgCl from methylene chloride.



(14)

After polymerization, the 18-mer DNA oligonucleotide bearing amino group was grafted to the surface layer by carbodiimide binding. The electropolymerization and formation of regular surface films of the polymer were confirmed by the FTIR and Raman spectroscopy and SEM. The hybridization with the complementary target resulted in regular increase of the charge transfer resistance measured in the presence of $[Fe(CN)_6]^{3-/4-}$.

The DNA chip on glass 2 × 3 cm with thin Pt electrodes was prepared by the consecutive deposition of the polymeric film from 3-carboxylic derivative of thiophene and aminated DNA probe [158]. The carboxylic group of the substituents was protected by benzyl derivatives removed after polymerization. All the reactions including the hybridization detection were performed in acetonitrile. The formation of ds-DNA onto the electrode surface resulted in suppression of the polythiophene redox activity. Besides, the role of protecting group on the polymerization step and reversibility of redox reaction was considered. It should be mentioned that all the articles devoted to the polythiophene application in the electrochemical DNA sensors do not report the quantitative characteristics of the target determination.

Hybrid coating of poly(3-methylthiophene) and Pt nanoparticles was obtained in two-step protocol including potentiostatic polymerization of 3-methylthiophene from acetonitrile solution at 1.42 V vs. Ag/AgCl and incubation of the film obtained in the dispersion of Pt nanoparticles obtained by chemical reduction of H_2PtCl_6 by $NaBH_4$ in the presence of citrate ions as stabilizers [159]. The attachment of Pt nanoparticles can be monitored by their electrocatalytic effect on cathodic reduction of dissolved oxygen. Thiolated 15-mer oligonucleotides were covalently attached to the metal surface and then involved in hybridization with 38-mer target sequence. Both processes resulted in specific changes of the mass of the coating recorded with QCM. The immobilization protocol provides the specific concentration of the DNA probes (4.7×10^{-11} mol/cm^2) comparable with that on Au electrode.

Contrary to other polythiophenes, PEDOT films can be obtained from aqueous solution. This simplifies the modification protocol. Thus, PEDOT-ds-DNA film was obtained by cycling the potential in the monomer solution between −0.5 and 1.1 V vs. Ag/AgCl and physical adsorption of DNA followed by drying the surface [160]. After that, the modified electrode was immersed in the solution of Nile Blue for 10 min. Phenazine dye intercalated the DNA helix by preserved its electrochemical and electrocatalytic activity. This was shown on the example of H_2O_2 detection. The reversible peak of Nile Blue at −0.37 V regularly changed with the analyte concentration between 6 μM and 0.2 mM (LOD 0.1 μM).

5.2. DNA sensors based on polyphenazines

As was mentioned before [37], thionine is electropolymerized similarly to aniline with formation of the product that exerts the pH dependent redox activity and rather high electron mediation rate. However, contrary to PANI the poly(thionine) retains its redox activity in neutral media. This offers good opportunities for the application of this material in the DNA sensor assembly.

The electropolymerization of thionine can be performed in the pH range from 6 to 8 [161]. The position of redox peaks corresponding to the polymer conversion shift with the pH value to higher cathodic potentials. The 40-mer DNA probe was covalently attached to the polymer via terminal phosphate group. The hybridization was monitoring by intrinsic redox activity of poly(thionine) measured by DPV. A clear decrease in the reversible peak current was observed probably due to suppression of the rearrangement of the polymer chains required for electron transfer. The LOD of 1.0×10^{-10} M was reported for complementary target. It should be mentioned that maximal shift of the current did not exceed 35% of initial value. This seems insufficient for quantification of the target sequence content but can be used for qualitative estimation of its presence.

The redox activity of poly(thionine) can be applied for the detection of DNA by recording the guanine and adenine oxidation [162]. For this purpose, glassy carbon electrode was first covered with physical mixture of MWCNTs and Au nanoparticles from their dispersion in DMF and then thionine was electropolymerized from aqueous solution by the potential cycling between −0.4 and 0.4 V. Is should be mention that the use of MWCNTs/Au composite decreased the polymerization potential which was reported much higher for blank electrodes [161]. Nevertheless the resolution of thionine redox peaks in-between −0.2 and −0.3 V was rather modest and did not differ dramatically from that obtained in the absence of MWCNTs. The oxidation of nucleotides can be performed in DPV mode either individually or simultaneously by their oxidation peaks recorded at high anodic potentials (0.6 and 0.9 V). The analytical applications of the method involve the detection of individual nucleotides (LOD about 1 nM) and estimation of their ratio for ds-DNA samples after their digestion with concentrated HCl.

The ss-DNA covalently attached to the surface of carbon paste electrode via carbodiimide binding was used as a template for thionine electropolymerization. For this purpose, the electrode was first soaked in the monomer solution and then its potential was cycled between −0.6 and 0.4 V vs. SCE for 60–200 s. The modified electrode showed electrocatalytic response on 0.99–2.86 mM H_2O_2 at −0.2 V (LOD 0.16 mM). Although the concentrations detected are rather moderate, the immobilization protocol makes it possible to manufacture miniaturized sensors and is quite inexpensive.

Among phenothiazines, Methylene Blue has found great attention in the DNA sensor development due to high electrochemical activity and intercalator ability. Monomeric forms of the dye are often used as covalently attached labels [163,164] or diffusion free indicators [111,113–115,165]. Meanwhile, the polymerization of Methylene Blue retains its ability to specific interactions with DNA due to electrostatic attraction and geometric concordance with the helix surface.

Electropolymerized phenothiazine dyes Methylene Blue and Methylene Green were used for the detection of specific anti-DNA antibodies [166]. Contrary to similar system based on PANI [120], the antibody binding was not complicated by the pH sensitivity of the redox activity typical for PANI. This made it possible to simplify the measurement procedure and increase the sensitivity toward the analyte molecules. The same protocol was used for the detection of thrombin with aptamer introduced in the biosensor assembly instead of ds-DNA. In both cases, the electorpolymerization was performed by potential cycling and the stationary potential or charge transfer resistance were measured prior to and after the contact of the DNA sensor with an analyte solution. For thrombin, the LOD of 1 nM was obtained. It should be mention that the aptasensors based on polymeric phenothiazine dyes show high sensitivity and are less affected by serum proteins than other electrochemical DNA sensors especially based on gold electrodes. This was confirmed by comparative investigation of the response toward serum albumin and direct measurements in spiked serum samples.

The impedimetric aptasensors have been developed for the detection of thrombin. The aptamer was immobilized onto the poly(Methylene Blue) layer by avidin–biotin binding [167]. Electropolymerization was performed by the potential cycling between −0.7 and 1.1 V vs. Ag/AgCl from TRIS buffer solution, pH=7.6 on the glassy carbon electrode covered with MWCNTs. The interaction of the aptamer with thrombin resulted in an increase of the charge transfer resistance and decrease of the capacity of the surface layer. Aptasensors make it possible to detect thrombin in the concentration range 1 nM − 1 mM with the LOD of 0.7 nM (monitoring resistance changes) and 0.5 nM (capacitance changes), respectively.

Poly(Neutral Red) was employed as a matric for immobilization of Au nanoparticles with the thiolated 26-mer DNA probes attached. The hybridization event was recorded by the changes in the reduction peak at −0.078 V which decreased with the concentration of complementary sequence. The LOD of 4×10^{-12} M was obtained. It should be noted that the peak used for hybridization indication is not referred to polymeric dye in accordance with other works [38,168]. Probably, some other products of oligomerization and/or oxidative destruction of the monomer can be responsible for such a response.

6. Conclusion

Summarizing the results obtained in the review area, three approaches to the use of electropolymerized materials in the DNA sensors can be specified:

- electropolymerization as a tool for the DNA immobilization;
- electrosynthesis of polymeric mediators for the DNA wiring;
- electropolymerized materials as new source of the generation of the signal about biorecognition event.

Electrosynthesis provides simple and cost effective route to the direct control of the polymer growth and hence to the variation of the amount of biomolecules onto the electrode surface. This seems not so much important when macro electrodes are taken as signal transducers. The affinity immobilization via avidin–biotin binding or self-assembling of thiolated probes onto Au successfully competes with electropolymerization protocols. However, the impact of electropolymerizations increases remarkably for miniaturized systems and planar electrodes with complex geometry of the surface. For this reason, the use of PPY for the DNA chip development was described even earlier than that for macro systems. Contrary to related techniques of immobilization in the polymer film (swelling, solvent evaporation or displacement), the electropolymerization provides instruments for tuning the characteristics of the films obtained. Thus the use of pulse techniques or combination of chemical and electrochemical stages makes it possible to obtain 3D nets which not only adsorb the macromolecules but also offer enough space for the access of rather bulky biological targets like oligonucleotide sequences and proteins specifically interacting with DNA. The electropolymerization in the presence of DNA molecules requires milder conditions

and can often be performed in neutral media compatible with other biomolecules and biological fluids. This enhances the capabilities of such techniques in developing other biosensors including immunoregents or enzymes. In the future, the electrosynthesis can find adequate solution in manufacture of implantable biomedical devices including DNA sensors for antitumor drugs or gene therapy control.

The application of electrosynthesized polymers as mediators is mostly based on the experience of successful use of the structurally relative compounds in the DNA sensor assembly. The use of phenothiazine dyes, especially Methylene Blue, is one of the most evident examples. This dye has found broad application as one of the best electrochemically active intercalators and meanwhile it showed specificity in the interaction with DNA in polymeric form, too. PPY and polythiophene derivatives often include ferrocene units in the substituents.

The composites of electrosynthesized materials with metal nanoparticles and carbon nanotubes belong to the group of the polymer applications, too. However, it is rather difficult to distinguish the real function of nanoparticles which can either promote the electron transduction or act as DNA probe support with increased density of the binding site against unmodified analogs. The statements about electrocatalytic activity of carbon nanotubes or enhanced electron transduction in the presence of noble metals do not often find adequate arguments or are not supported by appropriate experimental data. It should be noted on the contradiction between the EIS data indicating increase in the charge transfer resistance and cyclic voltammetry showing increased currents for such systems. This calls for the further efforts in clarifying the role of specific components in the composite films obtained by electropolymerization. These investigations can result in improved characteristics of the DNA sensors already existed or development of new systems with unique performance.

Some of the polymers, especially PANI and PPY, offer absolutely new opportunities for the detection of DNA related biorecognition events. In many cases, the hybridization affects the intrinsic redox reactions in the polymer film which are commonly discussed in the terms of doping–undoping equilibrium or the stabilization of the charged–uncharged form of the polymer. Indeed, in most cases the DNA hybridization results in suppression of redox activity either due to shielding redox active layer from the solution or prevention of the anion transfer on the interface of the surface layer. When such changes are discussed it should be taken into account that most of the EIS experiments are performed in the presence of anionic species (redox buffer $[Fe(CN)_6]^{3-/4-}$) which are attracted to the positively charged polymer layer but are repulsed by negatively charged DNA. Such a twofaced behavior complicates the consideration of EIS data together with voltammetry or QCM results. It would be interesting to see in the future investigations similar works with positively charged redox systems.

The following progress in the development of the electropolymerized materials for DNA sensors can be related to the molecular imprinting techniques which improve the performance of electrochemical sensors by mimicking multi-point interactions on the electrode surface. There are few examples of such approach that show the increased sensitivity of the target detection even though the DNA interactions are specific enough without these extra-efforts [169,170]. The molecular imprinting can be used to achieve special positioning of oligonucleotides against redox centers of polymeric supports. Finally, molecular imprinting is considered as one of the promising directions of direct coupling of biological components with microelectronic parts of microsensors which can find application in development of ''smart'' bioelectrically driven prostheses or biocomputers.

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