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Review

Biosensing and drug delivery by polypyrrole

S. Geetha, Chepuri R.K. Rao, M. Vijayan, D.C. Trivedi *

Centre for Conducting Polymers, Electrochemical Materials Science Division, Central Electrochemical Research Institute, Karaikudi 630006, India

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Abstract

Conducting polypyrrole is a biological compatible polymer matrix wherein number of drugs and enzymes can be incorporated by way of doping. The polypyrrole, which is obtained as freestanding film by electrochemical polymerization, has gained tremendous recognition as sophisticated electronic measuring device in the field of sensors and drug delivery. In drug delivery the reversing of the potential 100% of the drug can be released and is highly efficient as a biosensor in presence of an enzyme. In this review we discuss the applications of conducting polypyrrole as biosensor for some biomolecules and drug delivery systems.

Keywords: Polypyrrole; Biosensor; Drug delivery; Immobilization

Contents

1. Introduction

Design, fabrication and applications of amperometric chemical and biochemical sensors have gained considerable importance in recent years. The subject of chemically modified electrodes has also remained an important area of research activity. With respect to biosensor considerable emphasis has been placed on the fabrication and characterization of electroactive polymer modified electrodes. General survey on the electroactive polymers has been provided by Hillman [1], Lyons [2] and Evans [3]. Review by Wring and Hart provides information about the application of polymer modified electrodes in analytical chemistry [4]. Enzymes can be immobilized in electronically conducting polymer films to form novel amperometric biosensor devices [5,6]. The immobilization of enzymes in electropolymerized films allows the easy electrochemical control of various parameters such as the thickness of the polymeric layer, the enzyme loading or the enzyme location. Consequently, theoretical modeling of biosensors was often coupled with the electrochemical entrapment of enzyme for the evaluation of the role of these parameters on biosensor functioning. The use of CPs in the area of bioanalytical sciences is of great interest since their biocompatibility opens up the possibility of using them as: (a) in vivo biosensor applications [7], (b) for continuous monitoring of drugs or metabolities in biological fluids [8] and (c) means of opening the field to a variety of new analytes [9]. Biosensors based on the conducting polymers are well suited to the requirements of modern biological analysis, multiparametric assays, high information density and miniaturization [10,11]. In the

* Corresponding author. Tel.: +91 4565 227775; fax: +91 4565 227713/227779.
E-mail addresses: trivedi_dc@cecri.res.in, trivedi_dc@rediffmail.com (D.C. Trivedi).

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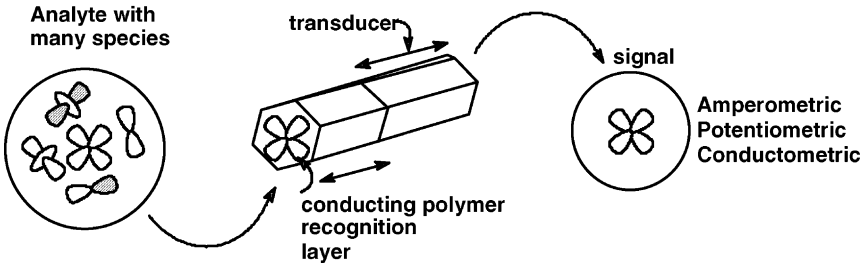

Scheme 1. Basic components and steps involved in a sensor.

present review, recent progress made on polypyrrole based conducting polymer as biosensors is discussed. Conducting polymer based biosensors work as transducers. A transducer is a device which converts energy from one form to another. Here conducting polypyrrole acts as an amperometric biosensor. The transducing processes in general are:

- electrochemical transducers,
- optical transducers,
- thermal transducers,
- piezo-electric transducers,
- cantiliver biosensing.

The electrochemical transducers are of two types, amperometric in which a potential is applied between working and reference electrode and the output current is measured continuously and second is conductometric based on measurement of conductance/resistance changes due to the charges produced during enzymatic conversion. The photometric effects like UV–vis absorption, fluorescence, bioluminescence, chemiluminescence, internal reflection and light scattering methods are exploited in optical transducers. Making/breaking of chemical bonds during enzymatic reactions results in enthalpy changes which is used in thermal transducers. The piezo-electric transducer works on the variation of frequency of piezo-electric crystal which depends on change in the mass adsorbed on its surface. Schematic representation of steps and components in a typical electrochemical sensor is shown in Scheme 1.

2. Immobilization of biomolecules on conducting polymer surface: the use of conducting polypyrrole films

The preparation of polypyrrole (PPy) is simple and is easily obtained as a thin film on an anode surface from aqueous neutral solutions around 0.8 V versus saturated calomel electrode (SCE) on any conducting substrate. Polypyrrole is biologically compatible (Fig. 1) therefore, has been widely studied for the immobilization of enzymes, antibodies and nucleic acids [12,13]. The polyaniline is an excellent conducting polymer in presence of protons which limits its application as biosensor under neutral condition [14] whereas polythiophene requires higher potentials for formation and is also water insoluble. The formation of conducting and highly stable PPy films on a Pt electrode by anodic oxidation of pyrrole (Py) in acetonitrile was first reported in 1979 by Diaz et al. [15]. At the same time Kanazawa et al. polymerized pyrrole in aqueous sulphuric acid medium onto a

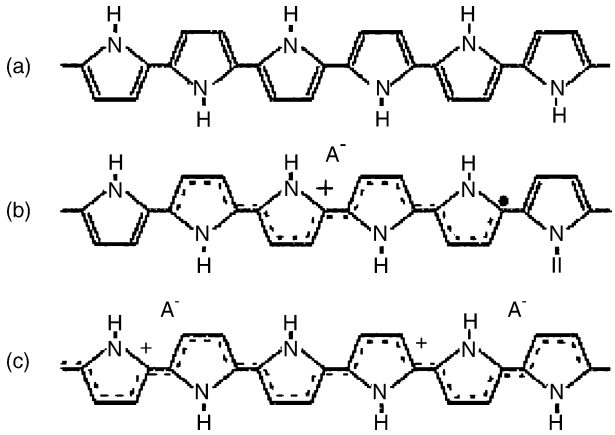

Fig. 1. Structures of polypyrrole in: (a) neutral, (b) partially oxidized (low doping) and (c) highly oxidized (highly doped) states.

Pt electrode and obtained films known as “Pyrrole black” which were insoluble in water and organic solvents with conductivities of around 10^2 S/cm. These films were air-stable [16].

Immobilization can be carried out with different procedures which are compatible with the biological activities of enzymes. The adsorption process takes place in the polymer–solution interface due to electrostatic interactions between the polycationic matrix of the conductive polymer and the total negative enzyme charge, provided the pH is higher than the isoelectric point of the enzyme. However, the amount of enzyme incorporated is very small, and is limited to single monolayer. This procedure has been used for preparation of glucose biosensors [17,18], cholesterol biosensors [19] and pyruvate sensors [20]. Enzyme adsorption onto the electrode surface and subsequent electropolymerization is also a possibility. But this process suffers by desorption of the enzyme or denaturation during formation of the polymer layer [21]. Immobilization by electrochemical doping is based on the redox switching processes of the conducting polymers between the oxidized and the reduced states, the mobility of the dopant ions and the ion exchange properties of the polymer. Covalent linking of enzymes in conducting polymer films can be performed in two ways:

- Covalent linking of enzymes to previously functionalized conducting polymers—a two-step procedure.
- Electrochemical synthesis of polymers using enzyme-functionalized monomers—a one-step procedure.

In the first method, immobilization takes place only on counter surface of the polymer layer while enzyme molecules get incorporated in the polymer matrix in the latter method.

The pioneering works by Umana and Waller [22] and Bartlett et al. [23,24], entrapment of biomolecules in electropolymerized films is the most popular electrochemical approach for fabrication of biosensor. This simple one-step method involves application of an appropriate potential to the working electrode placed in aqueous solution containing both biomolecule and electropolymerizable monomer. Biomolecules present in the immediate vicinity of the electrode surface are thus physically incorporated into the growing polymer layers. It should be noted that such immobilization occurs under mild conditions, without chemical reactions that could alter the activity of the biomolecules. This, easy electrochemical approach is applicable to a wide variety of biomolecules. It should be noted that several drawbacks may counterbalance this advantageous electrochemical entrapment. For example, the electrostatic interaction with in polymer matrix due to its disordered orientation and chemical environment and modification of permselectivity behaviour due to polymer ageing could contribute to reduce the performance of these biosensors [25,26]. To obtain a moderate thick film by electropolymerization technique, moderate concentrations of enzymes are required and hence small-volume electrochemical cells were designed to save significantly the amount of enzyme used [27].

The intrinsic electronic conductivity of polypyrrole and its derivatives, due to their conjugated structure, is crucial for the propagation step during the electrodeposition of polymer coatings. The thickness of the polymer can be thus easily tailored and successive polymeric layers can be generated. The latter may lead to spatially controlled biomolecule location in the polymeric structure or to a multienzyme configuration exhibiting heterogeneous enzyme location [28,29]. For instance, the enzyme horseradish peroxidase (HRP) was immobilized into a conducting polypyrrole film electrogenerated over an underlying regular polypyrrole [30]. This was designed to avoid the enzyme folding onto the Pt surface and to reinforce the adhesion of the biopolymer on the electrode surface. Dale et al. reported the sequential electrochemical immobilization of three enzymes (xanthine oxidase, purine nucleoside phosphorylase, and adenosine deaminase) in polypyrrole films generated on the microelectrode [31]. The controlled enzyme ratio and spatial location of each enzyme markedly improves the sensing capabilities of the microbiosensor towards the detection of purines. For instance, adenosine, an important agent of the peripheral and central nervous system was determined in a wide concentration range. The determination of glucose is one of the most frequently performed routine analysis in clinical laboratory as well as in microbiological and food industries. Diabetes is now a serious global problem that has attracted continuous interest for the development of an efficient glucose sensor. An artificial pancreas has come to a reality for a dynamically responding to glucose level and controlling insulin release based on the sensor's response.

Although the entrapment of glucose oxidase (GOX) in polypyrrole constitutes the most popular and "old" concept of enzyme electrode, criteria for biosensor fabrication were recently re-investigated [32]. Chou et al. compared galvanostatic and potentiostatic preparation of the host polypyrrole matrix. Considering the sensitivity and selectivity of the glucose biosensor, the optimal conditions were 50 mmol L^{-1} of pyrrole, 0.5 mg mL^{-1} glucose oxidase, 50 mC cm^{-2}, 382 μA cm^{-2} as applied current density [33]. Although this conventional electrochemical method of biomolecule entrapment was mainly focused on the immobilization of the enzymes (oxidases and dehydrogenases) few examples have been devoted to the immobilization of other biomolecule species such as coenzymes, antibodies, cells and erythrocytes [34–38]. Another example reported by Sung and Bae described the efficient and reproducible polypyrrole entrapment of bulky entities consisting of poly(2-acrylimido-2-methyl propane) sulphonic acid conjugated via polyethylene oxide without GOX molecules [39]. Immobilization of glucose oxidase in PTSA doped polypyrrole/polytetrahydrofuran graft copolymers were studied by Toppare et al. [40] where the conductivity was enhanced due to anisotropy.

In order to preserve the three-dimensional structure of the proteins and thereby their biological activity, there is a need to improve markedly the biocompatibility of the electrogenerated polymers. A possible and simple way of counterbalancing the hydrophobic nature of organic polymers is to incorporate hydrophilic functionalities within these films. The co-immobilization of enzymes and highly hydrophilic laptonite nanoparticles into polypyrrole matrices led to strong enhancement of biosensor activity for the amperometric detection of cholesterol [41] and lactate [42].

Tian and Zhu [43] developed a novel amperometric biosensor for the detection of glucose utilizing the two enzymes glucose oxidase (GOD) and horseradish peroxidase (HRP). They constructed this glucose biosensor by electrochemical deposition of a polypyrrole membrane in the presence of GOD on the surface of a HRP modified sol–gel derived mediated ceramic carbon electrode using ferrocene carboxylic acid (FCA) as mediator to transfer electron between the enzyme and electrode. In the hetero-bilayer configuration of the electrode, all the enzymes remained well immobilized in the electrode matrices and showed favourable enzymatic activities. They carried out the amperometric detection of glucose at +0.16 V versus SCE in 0.1 M phosphate buffer solution (pH 6.9), with a linear response range between 0.08 and 1.3 mM L^{-1} of glucose. Miniaturized disposable amperometric biosensors [44] were developed based on a polypyrrole film with immobilized glucose oxidase for glucose determination in serum. A commercially available three electrode system created on a planar corundum ceramic base was used as biosensor substrate, and a working Pt electrode was modified by a electrogenerated overoxidized polypyrrole film (PPyox). Glucose oxidase (GOD) was immobilized, either by enzyme entrapment into the electropolymerized film by electrochemical procedure (PPyox/GOD), or by gel-entrapment over the PPyox modified electrode by co-crosslinking with glutaraldehyde/bovine serum albumin (BSA) (PPyox/GOD-gel). A comparison of the glucose sensitivity, done by dropping 50 μL of the sample solutions onto the relevant electrode sys-

tems, showed that both sensors were linear upto l0 mM, though the Pt/PPyox/GOD-gel sensor is the more sensitive of the two (168 ± 15 nA/mM versus 53 ± 7 nA/mM). These two sensors showed remarkable anti-interference selectivity, moreover the PPyox/GOD-gel sensor also had good stability and could be used for FIA of glucose with linearity range extending upto 25 mM. The effects of the polyacrylamide–polypyrrole ratio and crosslinking on the biosensor response have been investigated, as well as the influence of analytical parameters such as pH and enzymatic loading. The polyacrylamide–polypyrrole (PAPPy) biosensor is free from interferences arising out of ascorbic and uric acids, which allows its use for quantitative analysis of glucose in human blood serum.

A hetero-bilayer configuration for in situ biochemical reducing the interference in the amperometric glucose biosensor was constructed by electrochemically formed polypyrrole in the presence of horseradish peroxidase (HRP), on top of an immobilized glucose oxidase (GO) film [45]. A simple electropolymerization process is described by Adeloju and Moline [46] fabricated an ultrathin polypyrrole (PPy)–glucose oxidase (GOD) film (∼55 nm) for potentiometric biosensing of glucose, using 0.1 M pyrrole and 55–110 U/mL GOD. Current density was 0.05 mA/cm^2 [an electrical charge of 25 mC cm^{-2}]. Long-term storage of the biosensor in acetate buffer improved the sensitivity of the biosensor by a factor of ∼2. They reported that the biosensor could be repeatedly used for over 2 months with little or no loss in sensitivity. Komaba et al. [47] reported a urea biosensor by immobilizing urease enzyme in an electropolymerized overoxidized polypyrrole (PPy) on a Pt electrode. The urease-immobilized polypyrrole electrode showed a stable potential response to urea based on pH response of the overoxidized polypyyrole film electrode. This biosensor showed a Nernstian response, with a slope of 31.8 mV decade^{-1} over concentration range of 1×10^{-4} to 0.3 mol/dm^3 urea. Osaka et al. [48] constructed a highly sensitive and rapid flow injection system for urea analysis with a composite film of electropolymerized polypyrrole (PPy) and a polyion complex incorporating urease. This system showed a sensitivity of 120 mV decade^{-1} and a lifetime of more than 80 assays. They attributed the high sensitivity of the system to an additional potential response of overoxidized polypyrrole to ammonia or ammonium ions superimposed on the response to pH change. If a concentrated buffer solution is injected immediately after the sample injection, the system shows a capability of assaying more than 15 samples per hour.

A general theoretical model was derived for the interpretation of the anionic, cationic and redox sensitivity of p-doped conducting polymers [49,50]. The model has been applied to PPy doped with metal complexing ligands [51–53]. The potentiometric selectivity of p-doped conducting polymers has also been considered theoretically [54]. The anion-selectivity of PPy seems to arise from the size of the anion incorporated as counterion in the electropolymerization step [55,56] and PPy was found to show an appreciable selectivity to nitrate [57,58]. But, apart from such molecular imprinting, the ionic response is generally nonselective unless specific ion-recognition sites (complexing ligands, ionophores) are included in the conducting polymer membrane, as was demonstrated in some articles [51,52,59,60].

The redox response of conducting polymers depends on whether the electron transfer is coupled to ion transfer or not [49,50,61]. In addition to redox potential measurements [62], the redox response of a conducting polymer is useful for potentiometric sensing of biomolecule [63] and species like oxygen [64,65]. The redox response also plays a role in the signal generation of the PPy based amperometric ammonia sensor [66–68]. Trivedi et al. studied on the application of conducting polyaniline as sensor material for ammonia [69].

Cholesterol biosensor was constructed [70] by entrapment of cholesterol oxidase (ChOx) within a composite of poly(2-hydroxyethyl methacrylate) (p(HEMA))/polypyrrole membrane. Platinum electrode-supported polymer films were prepared by UV polymerization of the hydrogel component containing dissolved enzyme, followed immediately by electrochemical polymerization of entrapped pyrrole monomer (Py) within the preformed hydrogel network. Cholesterol biosensors were also constructed [71] by entrapment of cholesterol oxidase within a polypyrrole (PPy) film electropolymerized in a flow system. This method involved in adjustment of the biosensor characteristics with low reagent consumption. The proposed cholesterol oxidase-based biosensor, designated as Pt/PPy–ChOx, was applied to the determination of cholesterol in reference serum samples. The results were consistent with certified values. Another approach [71,72] is the immobilization of the enzyme and laponite particles in a polypyrrole matrix in order to enhance the sensitivity and stability of a cholesterol oxidase-based biosensor. This type of biosensor was constructed by electropolymerization of a laponite nanoparticle–amphiphilic pyrrole derivative–enzyme mixture preadsorbed on the electrode surface. Compared to a similar biosensor without laponite, this biosensor sensitivity increased from 5.1 to 13.2 mA M^{-1} cm^{-2}. Yon Hin and Lowe [73] constructed a bienzyme electrode for the detection of total cholesterol by incorporating cholesterol esterase and cholesterol oxidase in polypyrrole films. This involves in situ deposition of both enzymes during the electrochemical polymerization of pyrrole. A fast amperometric response to cholesterol and good storage stability was achieved. Kajiya et al. [74] also incorporated cholesterol oxidase and ferrocenecarboxylate ions in polypyrrole films. Correlating the current response with the apparent enzymatic activity of polypyrrole films, this group obtained a remarkable amperometric response for cholesterol using above polymer films.

Garcia-Moreno et al. [75] prepared a biosensor by immobilization of the horseradish peroxidase (HRP) enzyme during the electropolymerization of *N*-methylpyrrole for use in the estimation of organic peroxides in reverse micelles. A detailed review on applications of polymers in biosensors has been given by Adhikari and Majumdar [76].

Polypyrrole modified electrodes are used for detection of DNA [77–79], Polypyrrole has also been used as immunosensors incorporating anti-human serum albumin [80] and antibodies [81–83].

Trivedi used a room temperature melt to electrosynthesize conducting polypyrrole. They electroynthesized polypyrrole films in lithium perchlorate/acetonitrile medium to get films

with conductivities around 10 S/cm. They anodized the undoped films obtained from the melt methodology [84] using the ClO_4^- ions by sweeping the potential between −0.2 and 0.8 V versus Ag/AgCl. The conductivity of redoped film was around 18 S/cm. This rise in conductivity is due to the layered structure [84–87]. Modified electrodes were made using the redoped films for which the biosensing property of glucose was tested. The amperometric experiments showed improved sensing capabilities. This may be due to the formation of ion gate channels created by a layered structure. Consequently, a spatially controlled biomolecule placement may be possible in the polymeric structure [88].

3. Drug delivery by polypyrrole

The current methods of drug delivery pose specific problems that scientists are attempting to address. For example, many drugs potencies and therapeutic effects are limited or reduced because of the partial degradation that occurs before they reach the target in the body. Further, injectable medications could be made less expensive and administered more easily if they are dosed orally. However, this improvement cannot take place until methods are developed for safe delivery of drugs through specific areas of the body (e.g. stomach, where low pH can destroy a medication) or through an area where healthy bone and tissue be badly affected.

The goal of all the drug delivery systems is to deploy medications intact to specifically targeted parts of the body through a medium that can control the therapy's administration by means of either a physiological or chemical trigger. To achieve this, researchers are putting enormous efforts in the fields of micro- and nanotechnology. During the past decade, polymeric microspheres, polymer micelles and hydrogel-type materials have all been shown to be effective in enhancing drug targeting specificity, lowering systemic drug toxicity, improving treatment absorption rates and providing protection for pharmaceuticals against biochemical degradation. In addition, several other experimental drug delivery systems show exciting signs of promise, including those composed of biodegradable polymers, dendrimers, electroactive polymers and modified C_{60} fullerenes.

Many sophisticated and potent drugs have been developed in recent years. Conventional dosage forms such as oral delivery and injection are unsuitable for many of these new protein and DNA based drugs. Unable to target a specific area, these delivery routes often require frequent administration of drugs in high dosages. Consequently, the body experiences a rapid release of drug, which renders these dosage forms unsuitable for many of the new drugs for which toxicity is observed for concentration spikes. The blood concentration and target area of these drugs must be therefore carefully controlled. Another important reason for the necessity to deliver drugs at a controlled rate is that the efficacy of many new and traditional drugs is strongly influenced by their temporal administration. For example, many therapeutic agents are most effective when delivered in a pulsatile release profile. Therefore new cost effective technology for controlled drug delivery is necessary for administering the new drugs. Progress has been made in recent years following advances in biomedical microelectromechanical systems (BioMEMS), such as microparticles, biocapsules, microneedles and micropumps [89]. The ideal drug delivery system that can self regulate the delivery rate in response to the patient's physiological condition to obtain optimal therapeutic effect has yet to be attained.

Smela reviewed for the reasons why conducting polymers are the ideal materials for biomedical actuators [90]. These light weight materials have large strain and high strength. They are able to work at room or body temperature and in body fluids. They only require low voltages for actuation (typically four or less). Many conducting polymers are also biocompatible and can be readily microfabricated with existing technology. Conducting polymers are chosen as the material of micropump drug delivery systems [91]. Experiments are being conducted to choose the polymer or polymer composites most suitable for the application. Literature indicates polypyrroles are good candidates due to their in vivo stability and biocompatibility. Different micropump designs are also being modeled by computer simulation. Conducting polymers exhibit reversible electrochemical response, where they expand upon oxidation and contract in reduction. The volume change originates from the insertion or expulsion of counter-ions from an electrolyte for charge balancing, together with the movement of solvent molecules [90]. When conducting electroactive polymers expand, they generate a force and movement in a particular direction. This electrochemically controlled actuation is studied for a wide range of applications, including artificial muscles, microfluidics and microrobotics.

Electrochemical switching of an electronically conductive polymer is accompanied by charge compensation through ion movement into or out of a membrane formed from such a polymer, which can be made to work as an ion gate. Burgmayer and Murray [92] demonstrated that polypyrrole functions very well as an ion gate membrane, which is positively charged in its oxidized state and neutral and hydrophobic in its reduced state. An important practical application of the ion gate membrane could be the controlled delivery of drugs, which can be accumulated in the membrane e.g. during oxidation and then released by electrochemically controlled reduction of the membrane. Polypyrrole (PPy) is particularly suitable for this purpose because the monomer is nontoxic and the electrosynthesis of the polymer membrane can be done in aqueous solution.

Miller et al. [93,94] showed that glutamate and dopamine can be released from a polypyrrole membrane using potential control. Pyo et al. [95] have demonstrated that controlled release of adenosine 5′-triphosphate (ATP) can be done using the same polymer membrane and recently Hepel and Mahdavi [96] have used a composite polypyrrole film as an ion gate membrane for the potential controlled release of a cationic drug, chlorpromazine.

The electrosynthesis conditions have great influence on the redox properties of the PPy membrane and this determine the ion gate function of the membrane. In a recent study [97] attempts were made for the electrochemical polymerization of polypyrrole conductive polymer in order to find the optimum conditions for producing a PPy membrane which could exchange anions.

Iseki et al. evaluated the efficiency of a PPy membrane as a device for drug delivery using model compounds, which in

aqueous solution produce anions with therapeutic activity. The anions chosen were salicylate, nicoside and naproxen. These anions have an aromatic structure and are of medium size [98]. The delivery of anions from the membrane was followed by using an electrochemical quartz crystal microbalance (EQCM) as well as high pressure liquid chromatography (HPLC) as an analytical tool.

4. Concluding remarks

Our survey of the literature shows that conducting polymers, in particular, polypyrrole, as a biocompatible macromolecule is the best candidate for biosensing and drug delivery. In the light of the discovery of many new protein, DNA based drugs, there is a need for new low cost and effective drug delivery systems to be fabricated and tested. It is believed that polypyrrole and their derivative based systems can play a significant role in the quest for such ideal systems. Studies in our laboratory have indicated that DNA from plant source can be incorporated in polypyrrole by electropolymerization and can be used as a biosensor for variety of molecules. Further study is in progress.

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