

Review

## Electroanalysis of NADH Using Conducting and Redox Active Polymer/Carbon Nanotubes Modified Electrodes-A Review

S. Ashok Kumar and Shen-Ming Chen \*

Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, No. 1, section 3, Chung-Hsiao East Road, Taipei 106, Taiwan (ROC)

\* Author to whom correspondence should be addressed. Fax: +886 2270 25238; Tel: +886 2270 17147.

E-mails: smchen78@ms15.hinet.net (S.-M. C.); sakumar80@gmail.com (S. A. K.)

Received: 31 December 2007 / Accepted: 30 January 2008 / Published: 6 February 2008

---

**Abstract:** Past few decades, conducting and redox active polymers play a critical role in the development of transducers for biosensing. It has been evidenced by increasing numerous reports on conducting and redox active polymers incorporated electrodes for assay of biomolecules. This review highlights the potential uses of electrogenerated polymer modified electrodes and polymer/carbon nanotubes composite modified electrodes for electroanalysis of reduced form of nicotinamide adenine dinucleotide (NADH). In addition, carbon electrodes modified with organic and inorganic materials as modifier have been discussed in detail for the quantification of NADH based on mediator or mediator-less methods.

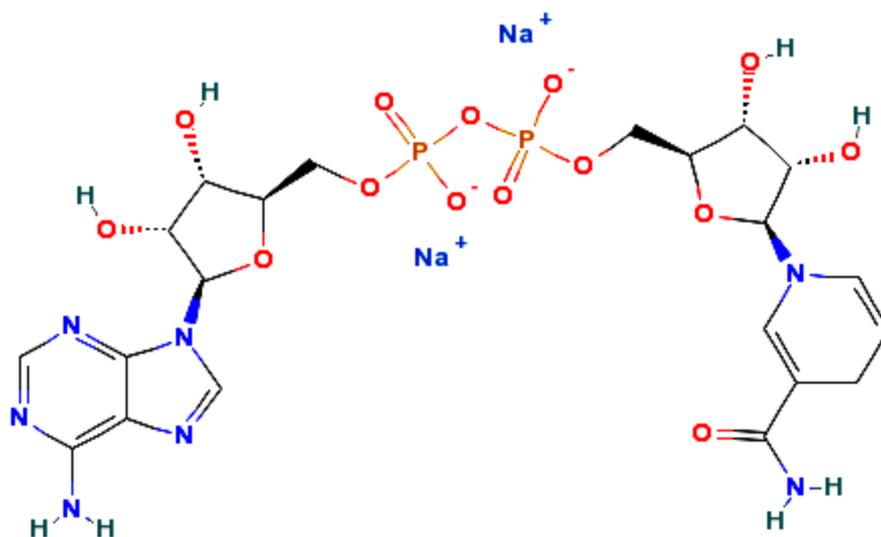
**Keywords:** electroanalysis of NADH, conducting polymer modified electrodes, electrocatalysis, polymer carbon nanotube nanocomposite, modified electrodes, sensors, chemically modified electrodes

---

### 1. Introduction

Reduced form of nicotinamide adenine dinucleotide (NADH) (Scheme 1) is an important coenzyme found in cells. It plays key biochemical roles as a carrier of electrons and a participant in metabolic redox reactions, as well as in cell signaling [1, 2]. The analysis of NADH coenzymes remains important in enzyme assays, since over 500 dehydrogenase enzymes use the oxidized form of these

ubiquitous coenzymes as cofactors to participate in organism metabolism. In past few decades, analytical chemist proposed a variety of analytical methods for NADH analysis [3, 4]. Among the other analytical methods, electrochemical methods are more familiar for detection of NADH because they have several advantages such as easy handling, small sampling volume, can be automated, less-interferences, user friendly, easily applicable for field analysis and less-cost.



**Scheme 1.** Chemical structural formula of NADH.

Direct oxidation of NADH takes place at bare electrodes (For ex. Au, glassy carbon electrode etc.) in high over potential ( $>1V$ ). Most of the electroactive biomolecules such as catecholamine, ascorbic acid and uric acid oxidized at nearly potential close to NADH oxidation. In addition, the direct oxidation of NADH at bare electrodes results electrode surface contamination (fouling) due to the adsorption of oxidized products of NADH which results poor sensitivity, poor selectivity and unstable analytical signals [4, 5].

To avoid the above obstacles in electroanalytical methods, chemically modified electrodes have been developed many decades ago. What is the meaning of chemically modified electrode? One typically modifies an electrode when the electrode materials given by nature will not allow the desired electron transfer reaction to occur under the selected experimental conditions. To overcome such obstacles, the region near the electrode surface can sometimes be altered to make the desired reaction more facile. There are many different types of applications of modified electrodes, among them particularly electrocatalysis has been attracted much attention among the researchers. Electrodes surface can be modified in order to facilitate a reaction which is kinetically unfavorable at an unmodified surface. Readers, those are unfamiliar with modified electrodes recommended to read the reviews in [5-7]. There are excellent reviews on modified electrodes for electrocatalytic oxidation of NADH by pioneer author Gorton et al. [8, 9].

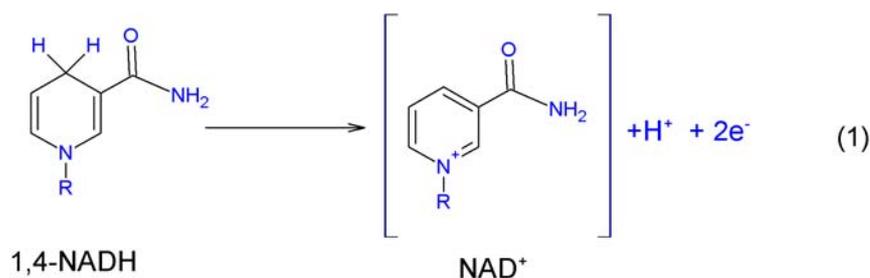
Conducting polymers have potential applications in various fields such as fuel cells, biosensors, electroanalysis, electrocatalysis, electrochromic displays and others [10-12]. In recent years, electrochemically synthesized polymer films on electrode surface considered for potential applications in electroanalysis of biological molecules. A recent review by Cosnier [13], deals recent

advances and scientific progress in electrochemical immobilization procedures for biological macromolecules on electrodes via electrogenerated polymer films. Electrochemical synthesis and some properties of nanostructured conducting polymers, and nanocomposites derived from conducting polymers and metals, carbon, and inorganic and organic materials and their potential applications in batteries, supercapacitors, energy conversion systems, corrosion protection, and sensors have been reviewed by Malinauskas et al [10].

Instantly, conducting polymers are a natural choice for preparing arrays of voltammetric sensors because they have a rich electrochemical behavior and their electrochemical properties can be modulated by introducing chemical modifications in the sensitive materials [14, 15]. Electropolymerization is a good approach to prepare polymer modified electrodes (PMEs) as adjusting electrochemical parameters can control film thickness, permeation and charge transport characteristics. PME's have many advantages in the detection of analytes because of its selectivity, sensitivity and homogeneity in electrochemical deposition, strong adherence to electrode surface and chemical stability of the film [16-18]. Thus, the electrochemically generated conducting PME's were employed for electroanalysis of ascorbate anions ( $pK_a$  4.17) in physiological condition. The various existing interactions of ascorbate anions with polymers films have been discussed in detail with the huge collections of earlier literature reports for assay of ascorbate anions using conducting PME's [19]. Recently, the recent advances in conducting polymer-based electrochemical sensors, including chemical sensors and biosensors have been reviewed [20].

Since their discovery [21-23], carbon nanotubes (CNTs) have profound applications in various fields of science and nanotechnology. They have attracted much attention because of their high electrical conductivity, mechanical strength, and chemical stability [24, 25]. The unique properties of CNTs make them extremely attractive for electrochemical sensors and biosensors [26–28]. Recent investigations demonstrated that CNTs show strong electrocatalytic activity and minimization of surface fouling if employed to improve the electrochemical response of some important bioactive molecules. Recently, methods based on electrochemical deposition [29] and electrochemical polymerizations [30-34] have been employed for loading CNTs onto electrodes. CNT-based electrodes have been demonstrated to reduce the overpotential significantly [26, 29]. The ability of carbon nanotubes to promote electron-transfer reactions suggests great promise for amperometric sensors [26]. Chemically modified carbon nanotubes for use in electroanalysis have been reviewed in detail by Gregory et al [35].

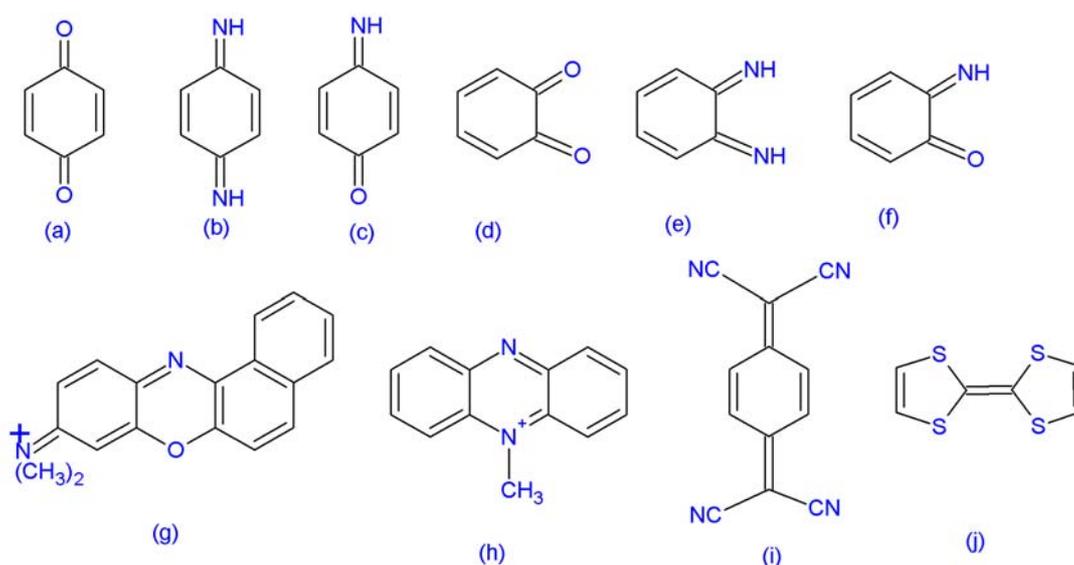
The overpotentials required for NADH oxidation at pH 7.0 are about +1.1 V for carbon [36] and +1.3 V for platinum [37] electrodes. In addition, the direct oxidation of NADH is often accompanied by electrode fouling and interfering background currents in real samples [8, 9] and sometimes leads to the formation of enzymatically inactive forms of  $NAD^+$  [38].



**Scheme 2.** Electrochemical oxidation reaction of NADH.

The electrochemical oxidation reaction of NADH is shown in scheme 2. A good mediator for efficient electrocatalytic oxidation of NADH should be (i) electrochemically active, (ii) formal potential ( $E^0$ ) of redox mediator should be less than oxidation potential of NADH, (iii) higher electron transfer rate constant ( $k_s$ ), (iv) formation of enzymatically active  $\text{NAD}^+$ , (v) anti-fouling effect, (vi) long-term stability, (vii) reduction of considerable overpotential etc

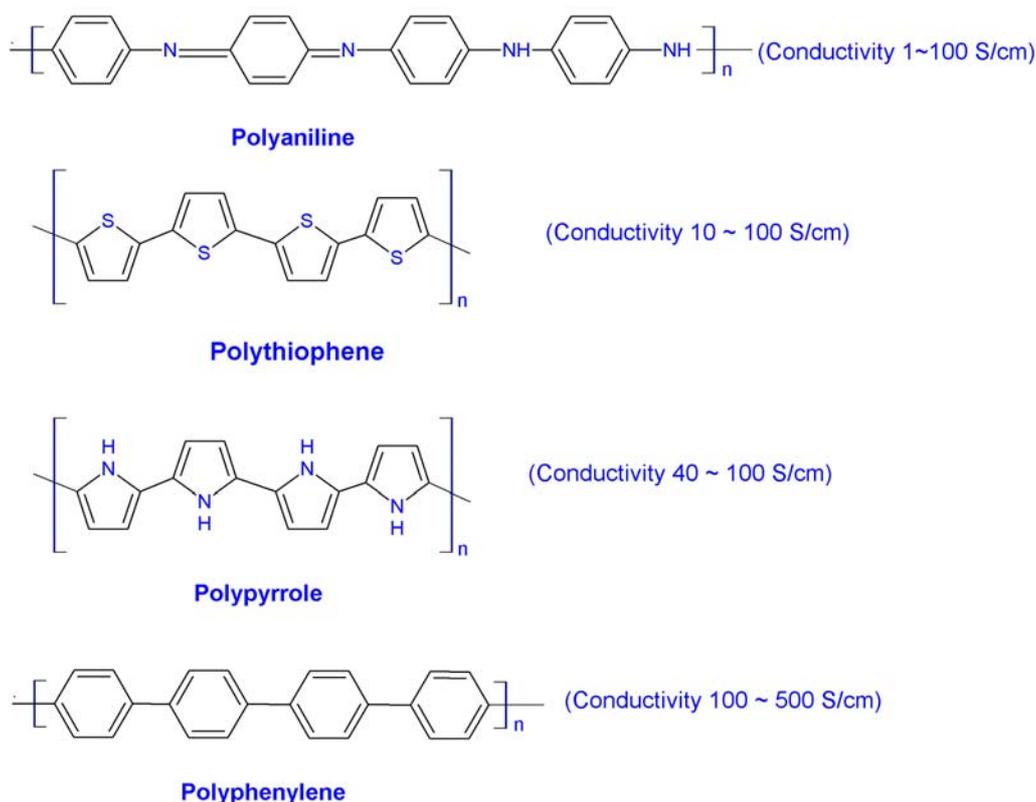
Examples of some of the most common mediators employed for NADH oxidation are presented in Scheme 3. All of the electrogenerated PME's have one of the basic functionality which can mediate the oxidation of NADH at lower potential [9]. Positively charged mediators are favorable for efficient electrocatalysis of NADH. When designing modified electrodes for NADH oxidation, it is not only important to identify suitable redox groups, which can catalyse NADH oxidation and can be attached to the electrode surface; it is also important to ensure facile charge transport between the immobilised redox sites in order to ensure that, in multilayer systems, the whole of the redox film contributes to the catalytic oxidation. One way to achieve this is by the use of electronically conducting polymers such as poly(aniline)–poly(anion) composite [39].



**Scheme 3.** Basic catalytic functionalities of many organic 2-electron-proton acceptors efficient for catalytic NADH oxidation (a-f). Structural formulae of some commonly used mediators for catalytic NADH oxidation: (g) Meldola blue (*p*-phenylenediimine), (h) *N*-methylphenazinium(*o*-phenylenediimine), (i).TCNQ (tetracyanoquinodimethane), (j) TTF (tetrathiofulvalene).

The electronically conducting polymers (ECPs) which covers several types of polymeric materials with electronic and/or ionic conductivity, including (i) doped conjugated polymers, (ii) redox polymers, (iii) polymer composites, and (iv) polymer electrolytes. ECPs as ion selective membrane and their applications in solid-state ion selective electrodes have been reviewed [40]. The most popular members of ECPs such as polyaniline (PANI), polythiophene, polypyrrole and polyphenylene are p-type semiconductors (Scheme 4) [42, 42]. Stability of polymers, conductivity and doping nature has been discussed in several reviews [43-50]. Conducting polymers are promising materials for a large variety of chemical sensors, their preparation and applications as chemical sensors in several aspects have been discussed in many reviews [51–76].

In this review, we summarized the recent updates on chemically modified electrodes (glassy carbon, carbon paste, graphite and pyrolytic carbon) proposed for detection of NADH using PME (conducting and redox active), carbon nanotubes/polymer nanocomposites, electrochemically polymerized dye modified electrodes, dye immobilized with the aid of matrices and other biomolecules etc. Detection limit, selectivity, linear range, effect of interferences on detection of NADH has been discussed here in detail.



**Scheme 4.** Some popular conducting polymers.

## 2. Electrocatalysis of NADH

### 2.1. Electrocatalytic oxidation of NADH at polyaniline (PANI)-co-polymer modified electrodes.

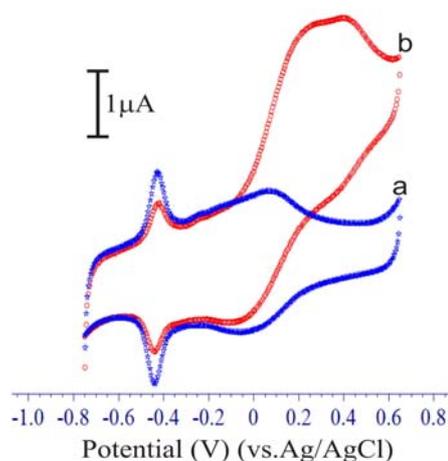
The catalytic activity of parent PANI is limited by the pH value, since PANI has little electrochemical activity at  $\text{pH} > 4$ ; the potential range of the electroactivity for PANI decreases with increasing pH value and its redox peaks disappears in the cyclic voltammetry for  $\text{pH} > 5$  [77-79]. Thus, in general, the redox potential of species to be oxidized and reduced by PANI is within the potential range in which PANI itself is electroactive, which restricts its application in electrocatalysis. To overcome this problems, self-assembly multilayer films of PANI were prepared using the layer-by-layer (LBL) method with a series of poly(anions), ie., sulfonated polyaniline (SPANI), poly(acrylic acid), poly(vinyl sulfonate), and poly(styrene sulfonate). Their electrochemical and their efficiency to electrocatalyze the oxidation of NADH in neutral solutions were investigated in detail by electrochemical techniques. PANI retained its reversibility, electrocatalytic activity and stability in neutral buffer ( $\text{pH} 7.1$ ) solution. PANI/SPANI multilayer films showed very good electroactivity in 0.1M phosphate buffer solution [80].

Toh et al., found that addition of  $\text{Ca}^{2+}$  ions increases the electrocatalytic response towards NADH at poly(aniline)-poly(vinylsulfonate) and poly(aniline)-poly(styrenesulfonate) modified electrodes by up to 12 and 25 times, respectively. This enhancement effect is reproducible and reversible. Measurement of the amount of charge passed through the polymer film in the presence of  $\text{Ca}^{2+}$  indicates a correlation between the total amount of  $\text{Ca}^{2+}$  incorporated within the polymer film and the magnitude of the enhancement of the electrocatalytic current for NADH oxidation at the polymer film. In addition,  $^{31}\text{P}$ -NMR studies showed that the amount of NADH bound within the poly(aniline) film is increased significantly when  $\text{Ca}^{2+}$  is present. These results suggest that the enhancement of the NADH oxidation current at poly(aniline) modified electrodes caused by  $\text{Ca}^{2+}$  is due to an increase in the partition of NADH into the polymer film and/or an increase in the binding affinity of the polymer for NADH [81].

Chemical reversibility of the NADH oxidation reaction was achieved by means of poly(1,2-diaminobenzene) conducting nanotubule coated glassy carbon electrode (GCE). The NADH amperometric response of the conducting nanotubule modified GCEs was shown to be extremely stable, with 98% of the initial response remaining after 48 h of stirring in the presence of  $1 \times 10^{-4}$  M NADH solutions (compared to 14% at the poly(1,2-diaminobenzene) modified GCEs). The nonconventional conducting polymer nanotubule-coated electrodes, when tested in amperometric mode for NADH electrochemical oxidation at an applied potential of 450 mV, showed a sensitivity of 99 nA/mM, an operational stability for 2 days, a storage stability of 2 weeks at 4 °C, a linearity from  $5 \times 10^{-5}$  to  $1 \times 10^{-3}$  M, and good NADH chemical reversibility, all of which make them useful tools for dehydrogenase enzyme probe assembly [82].

Electroanalysis of NADH has been studied using poly(p-amino benzenesulfonic acid)/flavin adenine dinucleotide film modified glassy carbon electrode (PABS/FAD/GCE) in phosphate buffer solution ( $\text{pH} 6.4$ ). Fig. 1 shows the electrocatalytic activity of PABS/FAD/GCE towards NADH, the highly amplified anodic peak current of polymer peak and decrease of cathodic peak indicates the mediated oxidation of NADH at lower potential. PABS/FAD/GCE has a fast response to NADH and a good linear response observed in the range from 10 to 300  $\mu\text{M}$ . The detection limit is estimated to be

1  $\mu$ M (S/N = 3) [83]. However, poly(p-amino benzenesulfonic acid) film modified electrode also could be useful for simultaneous detection of dopamine and ascorbic acid [84].



**Figure 1.** poly(p-amino benzenesulfonic acid)/flavin adenine dinucleotide film modified GCE in the absences (curve a) and presences of 50  $\mu$ M NADH (curve b) in 0.1M phosphate buffer solution (pH 6.4).

## 2.2. Electrocatalysis of NADH at polymerized dye film modified electrodes

Cyclic voltammetry (CV) was used to study 3,4-dihydroxybenzaldehyde (3,4-DHB) electropolymerization processes on carbon paste electrodes. The characteristics of the electropolymerized films were highly dependent on pH, anodic switching potential, scan rate, 3,4-DHB concentrations and number of cycles. Film stability was determined in citrate/phosphate buffer solutions at the same pH used during the electropolymerization process. The best conditions to prepare carbon paste modified electrodes were pH 7.8;  $0.0 \leq E_{\text{ap1}} \leq 0.25$  V;  $10 \text{ mV s}^{-1}$ ;  $0.25 \text{ mmol L}^{-1}$  3,4-DHB and 10 scans. These carbon paste modified electrodes were used for NADH detection at 0.23 V in the range  $0.015 \leq [\text{NADH}] \leq 0.21 \text{ mmol L}^{-1}$  [85]. Polymerization of luminol in strongly acidic aqueous solution generated thin electrochemically active films on GCE. This film also can be produced on platinum, gold and transparent semiconductor tin (IV) oxide electrodes. An electrochemical quartz crystal microbalance and CV were used to study in situ growth of the polyluminol film. This modified film is electrocatalytically active for NADH oxidation in acidic and neutral aqueous solutions, and the polyluminol mediated electrocatalytic oxidation of NADH [86].

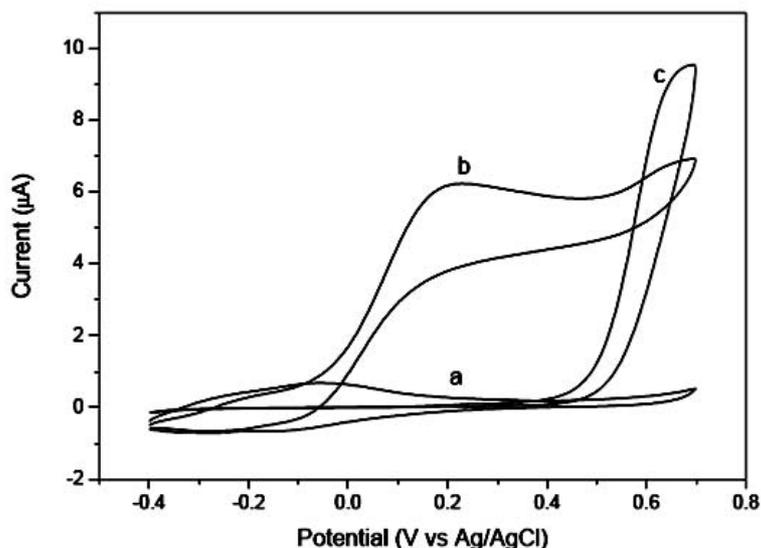
Poly(azure blue II) thin film was successfully assembled on the surface of a GCE by means of electrochemical polymerization, which was carried out with cyclic voltammetric sweeping in the potential range of - 0.6 to + 1.3 V (vs. SCE) in Britton-Robinson buffer solution (pH = 9.8) containing  $1.25 \times 10^{-4} \text{ mol/L}$  azure blue II. The effect of pH on the polymerization process of azure blue II and the electrochemical characteristics of the polymer-modified electrodes were studied in detail. The experimental results indicated that the electropolymerization of azure blue II could take place in basic or neutral media. The cyclic voltammograms of poly (azure blue II) thin film modified electrode showed the presence of two couples of redox peaks. The film modified electrode exhibited potent and persistent electrocatalysis for oxidation of NADH in phosphate buffer media with a diminution of the

overpotential of about 410 mV and an increase in peak current. The presence of some divalent cations in an electrolyte can greatly enhanced the electrocatalytic current for oxidation of NADH. The electrocatalytic current increased linearly with NADH concentration from  $1.0 \times 10^{-5}$  to  $8.0 \times 10^{-3}$  mol/L in the presence of  $4.0 \times 10^{-2}$  mol/L  $Mg^{2+}$  cation. The detection limit ( $3s_{bl}/S$ ) was  $5.0 \times 10^{-6}$  mol/L, and the relative standard deviation of determination results was 4.2% for six successive determinations of  $5.0 \times 10^{-4}$  mol/L NADH in the presence of  $Mg^{2+}$  cation [87].

A poly(thionine) modified screen-printed carbon electrode has been prepared by an electrooxidative polymerization of thionine in neutral phosphate buffer. The modified electrodes are found to give stable and reproducible electrocatalytic responses to NADH and exhibited good stability. Several techniques, including CV, X-ray photoelectron spectroscopy and scanning electron microscopy have been employed to characterize the poly(thionine) film. This modified electrode showed a dynamic range of 5-/100  $\mu$ M. The resulting calibration plot had a slope of 1.14  $\mu$ A/mM and correlation coefficient, 0.999. A detection limit of 3  $\mu$ M can be estimated on the basis of the signal-to-noise ( $S/N=3$ ) [88]. Meldola blue dye (MB) is one of the classic mediators for mediated oxidation of NADH [89]. Poly (MB) modified screen printed carbon paste electrode showed the good electrocatalytic activity in presence of  $5 \times 10^{-3}$  M NADH. Poly(MB) exhibited linear detection range from  $8 \times 10^{-6}$  to  $5 \times 10^{-4}$  M. Additionally, poly (MB) sensors allows lower detection limit as low as 2  $\mu$ M NADH [90].

Poly-Toluidine Blue (poly-TB) film was prepared by electro-oxidative polymerization on GCE in phosphate buffer solution. Poly-TB modified electrode exhibited excellent electrocatalysis toward the oxidation of NADH with decrease 450 mV of the overpotential compared with that at a bare GCE. Obviously, the catalytic current at the poly-TB modified GC in the flow-injection analyzer increased with increasing the NADH concentration and showed linear dependence of the catalytic current response to the NADH in the range of 5  $\mu$ M to 3.2 mM. The detection limit was 0.1  $\mu$ M. In this method L-ascorbic acid interfered with the determination of NADH in practical analysis [91].

The electrooxidation polymerization of phenothiazine derivatives, including azure A and toluidine blue O, has been studied at screen-printed carbon electrodes in neutral phosphate buffer. Both compounds yield strongly adsorbed electroactive polymer with reversible behavior and formal potential-closed to 0.04V at pH 6.9. Figure 2 shows typical voltammograms demonstrating the electrocatalytic activity of the poly(azure A) modified screen-printed carbon electrode for the oxidation of NADH. The reversible redox response of the poly(azure A) film can be observed over a wide range of potentials ca.  $-0.2 - 0.2$ V (versus Ag/AgCl) and its  $E^{0'}$  was located at  $-0.04$ V (versus Ag/AgCl) as shown in voltammogram (curve a). Voltammograms (curve b) observed in the presence of NADH showed a enhanced oxidation current and a large negative shift in the anodic peak potential of about 500mV, compared with that obtained at the bare screen-printed carbon electrode (curve c). These results demonstrated the electrocatalytic oxidation of NADH by the poly(azure A) film as described in equation 2. Further, the modified screen-printed carbon electrodes were found to be promising as an amperometric detector for the flow injection analysis (FIA) of NADH, typically with a dynamic range of 0.5–100  $\mu$ M [92].



**Figure 2.** Cyclic voltammograms of (a and b) poly(azure A) modified screen-printed carbon electrode in the absence (a) and presence (b and c) of 1mM NADH. Voltammogram (c) was obtained at a bare screen-printed carbon electrode. Scan rate:  $5\text{mVs}^{-1}$ . (Reproduced with permission from Gao et al., *Talanta* 2004, 62, 477–482)



The poly(azure B) modified electrodes exhibited excellent electrocatalysis and stability for NADH oxidation in phosphate buffer (pH 6.9), with an overpotential of more than 400mV lower than that at the bare electrodes. FIA of the poly(azure B)-modified screen-printed carbon electrode showed linear detection of NADH solution of increasing concentration from 0.5 to 100  $\mu\text{M}$ . A detection limit of  $2.0 \times 10^{-7}\text{M}$  for the poly(azure B)-modified electrode can be estimated on the basis of the signal-to-noise (S/N=3) ratio [93].

Electrochemical characteristics of poly(methylene blue) electrolytically deposited on GCE and examined the electrocatalytic activity of the polymer toward oxidation of the coenzyme NADH. Redox active properties of the cationic polyelectrolyte arose from both electrons self-exchange between electroactive sites and a high ionic film-conductivity. The diffusion coefficient of charge carriers in the film increased with decreasing solution pH, indicating the pH dependence of the electron diffusion coefficient. The electrocatalytic oxidation of NADH at the polymer-modified electrode proceeded via an intermediate charge-transfer complex of the reduced polymer with the oxidized coenzyme. The complex dissociated more rapidly into the oxidation products as the reduced polymer protonated. Thus, the rate constant for the cross-exchange reaction rose with a decrease in pH. For NADH oxidation, the polyelectrolyte exhibited an electrocatalytic activity higher than the monomeric dye because of a stronger oxidizing power of the second oxidized form of the polymer [94].

Electrocatalysis of NADH oxidation with the poly-toduiline blue films is reported by the method of CV and the catalytic reaction constant between NADH and poly-toduiline blue is determined by rotation disc electrode measurement. Their experiments proved that the poly-toduiline blue is an

excellent catalyst for NADH oxidation, shifted anodic peak potential negatively to 450 mV compared with unmodified GCE, and the rate constant of NADH oxidation on the GCE modified with the poly-touline blue films is exponentially greater than on modified GCE [95].

The modification of a GCE surface with an *o*-amino phenol (*o*-AP) film was achieved by electrochemical reduction of the in situ generated nitrophenyl diazonium cation. The functionalized electrode shows an efficient electrocatalytic activity towards the oxidation of NADH with reduction of overpotential, which is ca. 350 mV lower than that at the bare electrode. The formation of an intermediate charge transfer complex is proposed for the charge transfer reaction between NADH and adsorbed *o*-AP. The second-order rate constant for electrocatalytic oxidation of NADH,  $k_{\text{obs}}$ , and the apparent Michaelis–Menten constant  $K_m$ , at pH 7.0 were evaluated with rotating disk electrode (RDE) experiments, using the Koutecky–Levich approach. Using the *o*-AP-GCE, at an applied potential of +150 mV (vs. Ag/AgCl) with amperometric detection of NADH, a calibration range from  $7.5 \times 10^{-7}$  to  $2.5 \times 10^{-6}$  with a detection limit of  $1.5 \times 10^{-7}$  M was obtained, and excellent reproducibility was demonstrated with an RSD% = 2.1,  $n = 10$  using a concentration of  $1.0 \times 10^{-6}$  M NADH [96].

Electrochemical polymerization of acriflavine (AF) was carried out onto GCE from the aqueous buffer solution containing  $1.5 \times 10^{-3}$  M AF monomer (pH 3.5) which produced a thin electrochemically active film. This modified electrode was shown a stable reversible redox couple centered at +0.22 V in pH 3.5 buffer solutions. PAF/GCE was found to be more stable in acidic solutions and its formal potential was found to be pH dependent with a slope close to -60 mV/pH. The electrochemical deposition kinetics of poly(AF) onto gold coated quartz crystal was studied by using electrochemical quartz crystal microbalance (EQCM) combined with CV. PAF/GCE was found to be good mediator for electrochemical oxidation of NADH in pH 5 buffer solutions. The anodic current increased linearly with the additions of NADH concentration over the range from 80 to 720  $\mu\text{M}$  [97].

Electrochemically polymerized meldola blue on screen printed carbon electrode have been used for detection of NADH in the range from 8 - 500  $\mu\text{M}$  with detection limit of 2.5  $\mu\text{M}$  and a sensitivity of 3713  $\mu\text{A mol}^{-1}$  in amperometric determinations at 0 V vs. Ag/AgCl [98]. The redox mediators, potassium hexacyanoferrate (II), Meldola's Blue (MB), dichlorophenolindophenol (DCPIP), *p*-benzoquinone (*p*-BQ), *o*-phenylenediamine (*o*-PDA) and 3,4-dihydroxybenzaldehyde (3,4-DHB) incorporation on electrode surface by different strategies (in solution, entrapped in epoxy-composites, adsorbed or electropolymerized on the electrode surface) have investigated, several aspects regarding repeatability and reproducibility were considered and compared. It can be concluded, that electropolymerizing the mediator on epoxy–graphite composite electrodes is a valuable strategy to produce chemically modified sensors. This approach generates reproducible surfaces, and minimizes the surface fouling of the electrodes, principally when using DCPIP and *o*-PDA. It also permits high sensitivities, which are vital for the detection of low concentrations of NADH. Nonetheless, this method does not reduce significantly the working potentials, which limit the selectivity of these sensors [99]. Hybrid films composed of electropolymerized acriflavine–flavin adenine dinucleotide (AF/FAD) film modified electrodes have been prepared electrochemically on GCE, which used for electroanalysis of NADH [100].

The oxidation of NADH on electro-polymerized methylene green (MG)-modified GCE is described. This modified electrode shows an excellent electrocatalytic activity toward NADH oxidation, reducing its overpotential by about 650 mV and exhibits a wide linear range of 5.6 – 420

$\mu\text{M}$  NADH with the detection limit of  $3.8 \mu\text{M}$ . The electrode displays a good reproducibility and stability and the coexisting species does not affect the determination of NADH [101]. Electrocatalytic oxidation of NADH at a GCE modified with electrodeposited film derived from pyrocatechol violet has been studied. This modified electrode showed excellent electrocatalytic activity towards NADH and reduced overpotential of about 447 mV lower than that of the bare electrode and the linear concentrations ranging from  $2.5 \mu\text{M}$  to  $40 \mu\text{M}$ . In an amperometric determination, the limit of detection of NADH was estimated to be of the order of  $1.0 \mu\text{M}$  [102].

### 2.3 Photoelectrocatalytic Oxidation of NADH with Electropolymerized Toluidine Blue O

The poly-TBO modified GCE shows electrocatalytic activity toward NADH oxidation in phosphate buffer solution at pH 7.0, with an overpotential of ca. 350 mV lower than that at the bare electrode. When the modified electrode surface was irradiated with a 250W halogen lamp, a photoelectrocatalytic effect was observed for NADH oxidation and the current was increased about 2.2 times. The applied potential was selected at +100 mV for amperometric and photoamperometric detection of NADH. A linear calibration graph for NADH was obtained in the range between  $1.0 \times 10^{-5}$  and  $1.0 \times 10^{-3}$  M and between  $5.0 \times 10^{-6}$  and  $1.0 \times 10^{-3}$  M for amperometric and photoamperometric studies, respectively [103]. Electro-analytical applications of electrogenerated polymer coated electrodes are summarized in Table 1. All of these polymer modified electrodes have their own advantages and limitations for practical applications.

**Table 1.** Electroanalytical determinations of NADH using electrogenerated polymer modified electrodes

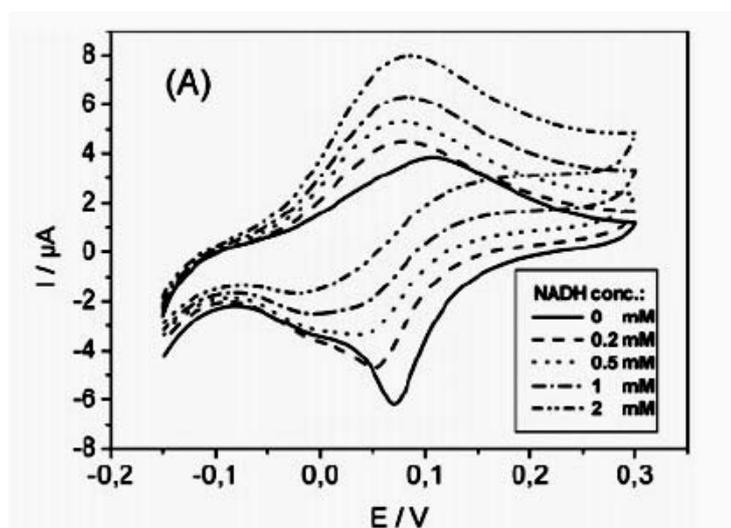
Polymer, copolymer or Poly(dye) films	Electroanalytical applications and characteristics	Ref.
PANI/SPANI multilayer (Layer by layer method)	Electrocatalysis of NADH in pH 7.1 phosphate buffer solution.	[80]
poly(aniline)–poly(vinylsulfonate) and poly(aniline)–poly(styrenesulfonate)	Catalytic current increases up to 12 and 25 times in the presences of $\text{Ca}^{2+}$ .	[81]
poly(1,2-diaminobenzene) conducting nanotubule coated GCE	Electrochemical oxidation of NADH at an applied potential of 450 mV, showed a sensitivity of 99 nA/mM, an operational stability for 2 days, a storage stability of 2 weeks at $4 \text{ }^\circ\text{C}$ , a linearity from $5 \times 10^{-5}$ to $1 \times 10^{-3}$ M	[82]

**Table 1.** Cont.

poly(p-amino benzenesulfonic acid)/FAD film modified GCE	This electrode has a fast response to NADH and a good linear response observed in the range from 10 to 300 $\mu\text{M}$ in pH 6.4 PBS. The detection limit is estimated to be 1 $\mu\text{M}$ (S/N = 3)	[83]
Poly(3,4-dihydroxybenzaldehyde) modified carbon paste electrodes	It has used for NADH catalytic detection at 0.23 V in the range $0.015 \leq [\text{NADH}] \leq 0.21 \text{ mmol L}^{-1}$	[85]
Poly(luminal) modified GCE	Electroanalysis of NADH oxidation in acidic and neutral aqueous solutions	[86]
Poly(azure blue II) film modified GCE	The electrocatalytic current increased linearly with NADH concentration from $1.0 \times 10^{-5}$ to $8.0 \times 10^{-3}$ mol/L in the presence of $4.0 \times 10^{-2}$ mol/L $\text{Mg}^{2+}$ cation. The detection limit ( $3s_{bl}/S$ ) was $5.0 \times 10^{-6}$ mol/L	[87]
poly(thionine) modified screen-printed carbon electrode	Detection of NADH within dynamic range of 5-/100 $\mu\text{M}$ . The resulting calibration plot had a slope of 1.14 $\mu\text{A}/\text{mM}$ and correlation coefficient, 0.999. A detection limit was 3 $\mu\text{M}$ (S/N=3).	[88]
Poly (Meldola) modified screen printed carbon electrode	Exhibited linear range between $8 \times 10^{-6}$ and $5 \times 10^{-4}$ M NADH. Poly (MB) sensors allow detecting as low as 2 $\mu\text{M}$ NADH.	[90]
Poly-Toluidine Blue modified GCE	It has reduced the over a 450mV of the overpotential for NADH. The linear range was 5 $\mu\text{M}$ to 3.2mM. The detection limit was 0.1 $\mu\text{M}$ . In this method L-ascorbic acid interfered with the determination of NADH in practical analysis	[91]
Poly(azure A) and Poly(toluidine blue O) modified screen-printed carbon electrodes	Reduced overpotential of more than 500 mV, promising as an amperometric detector for the flow injection analysis of NADH, typically with a dynamic range of 0.5–100 $\mu\text{M}$ .	[92]
poly(azure B) modified screen-printed carbon electrode	Detection of NADH in the concentration range from 0.5 $\mu\text{M}$ to 100 $\mu\text{M}$ . A detection limit was $2.0 \times 10^{-7}$ M (S/N=3).	[93]
Poly(methylene blue) modified GCE	This study examines NADH oxidation on monomeric dye and poly(methylene blue) modified electrode. Polymeric films showed enhanced electrocatalytic activity.	[94]
Poly-toduiline blue modified GC electrode	This electrode shift peak potential negatively to 450 mV and showed higher electrocatalytic current towards NADH.	[95]
Poly(acriflavine) modified GCE	Poly(acriflavine) found to be good mediator for electrochemical oxidation of NADH in pH 5 buffer solutions. The anodic current increased linearly with the additions of NADH concentration over the range from 80 to 720 $\mu\text{M}$ .	[97]

Poly(meldola blue)/screen printed carbon electrode	Detection of NADH in the range from 8 - 500 $\mu$ M with detection limit of 2.5 $\mu$ M and a sensitivity of 3713 $\mu$ A/ mol in amperometric determinations at 0 V vs. Ag/AgCl	[98]
Poly(methylene green)modified GCE	Showed an excellent electrocatalytic activity toward NADH oxidation, reducing its overpotential by about 650 mV and exhibits a wide linear range of 5.6 – 420 $\mu$ M NADH with the detection limit of 3.8 $\mu$ M.	[101]
Electrodeposited film derived from pyrocatechol violet on GCE	This electrode reduced overpotential of about 447 mV and the linear concentrations ranging from 2.5 $\mu$ M to 40 $\mu$ M. Limit of detection was 1.0 $\mu$ M	[102]
poly-TBO modified GCE with irradiation of 250W halogen lamp	A linear calibration graph for NADH was obtained in the range between $1.0 \times 10^{-5}$ and $1.0 \times 10^{-3}$ M and between $5.0 \times 10^{-6}$ and $1.0 \times 10^{-3}$ M for amperometric and photoamperometric studies at +100 mV.	[103]

#### 2.4. Electrocatalysis of NADH at polymer/carbon nanotubes composite modified electrodes



**Figure 3.** Cyclic voltammograms of 6 bilayers of PANI/PABS-SWNTs measured in 0.1 M PBS buffer (pH 7.2) containing different concentrations of NADH. Scan rate was 5mV/s. (Reproduced with permission from Liu et al., Langmuir 2005, 21, 5596-5599).

A conducting polymer, polyaniline, was successfully assembled with commercially available poly(aminobenzenesulfonic acid)-modified single-walled carbon nanotubes (PABS-SWNTs) via the simple layer-by-layer method. PABS-SWNTs inside the multilayer film can dope PANI effectively and shift its electroactivity to a neutral pH environment, pointing to their potential biological applications. The obtained PANI/PABS-SWNTs multilayer films are very stable and showed a high electrocatalytic ability toward the oxidation of NADH at a much lower potential (about +50 mV vs. Ag/AgCl), which makes it an ideal substrate for NADH detection and offers great promise for developing dehydrogenase-based biosensors depending on NADH as a cofactor. Figure 3 shows the cyclic voltammograms of a 6-bilayer PANI/PABSSWNTs film measured in 0.1MPBS buffer (pH 7.2) in the absence and presence of different amounts of NADH. For a six-bilayer sample, the detection

limit was  $1 \times 10^{-6}$  M as detected by the simple CV method, with a linear detection range for NADH at concentrations between  $5 \times 10^{-6}$  and  $1 \times 10^{-3}$  M. The substrate can be used repeatedly for consecutive detection cycles of NADH with a very stable signal [104].

A new type of poly(toluidine blue O)/multiwall carbon nanotube (PTBO/MWNTs) composite nanowires was fabricated by two steps: first, the TBO monomer was assembled onto multiwall carbon nanotubes (MWNTs) to form TBO/MWNTs adduct. Then, the obtained TBO/MWNTs adduct was electropolymerized on a GCE surface by CV. The nanowires modified GCE was characterized with scanning electron microscope (SEM), CV and electrochemical impedance spectroscopy (EIS). The PTBO/MWNTs composite nanowires modified electrode was applied to determinate NADH. Compared with bare GCE and MWNTs modified GCE, the PTBO/MWNTs composite nanowires modified GCE decreased the NADH oxidization overpotential by about 650 mV and 260 mV, respectively, with a much low peak potential at about 0.0 V (vs. SCE). The peak current response at PTBO/MWNTs composite nanowires modified GCE was about 4.5 times higher than that of ultrathin PTBO modified GCE, at the same peak potential. A linear range from 2.0  $\mu$ M to 4.5 mM was observed with fast response (within 5 s) and a low detection limit of 0.5  $\mu$ M (based on S/N = 3). The current diminution to 1.0 mM NADH is lower than 10% in a period over 70 min shows that the PTBO/MWNTs composite nanowires modified GCE is quite stable. The better electrocatalytic behavior toward NADH oxidation at PTBO/MWNTs composite nanowires modified GCE suggests that the composite nanowires is an excellent platform for electrochemical sensing and biosensing [105].

Electrocatalytic sensing of NADH using a hybrid thin film derived from MWNTs, Nafion (Nf) polymer and electrogenerated redox mediator is described. The redox mediator was electrochemically generated by the oxidation of serotonin on the hybrid thin film modified GCE (GC/Nf- MWNTs). Controlled potential electrolysis of serotonin at 0.1V in neutral solution results in the generation of the redox mediator 5,5'-dihydroxy-4,4'-bitryptamine (DHB) on the hybrid thin film. The electrogenerated DHB has redox active quinone-imine structure and was electrochemically characterized by studying the pH dependent redox response. The quinone-imine structure of DHB efficiently catalyzes the oxidation of NADH with a decrease in the over potential of about 500mV compared to the unmodified electrode. The MWNTs of the hybrid thin film facilitates the mediated electrocatalytic oxidation of NADH. The hybrid thin film modified electrode exhibits stable amperometric response and it linearly responds to NADH (0.5–400  $\mu$ M). This hybrid thin film modified electrode could detect NADH as low as 0.1  $\mu$ M at  $-0.05$ V with a sensitivity of 11.1 nA/ $\mu$ M in physiological pH [106].

The poly(1,2-diaminobenzene) was deposited onto the surface of a GCE modified with MWNTs via multipulse chronoamperometric electropolymerization (MCE) process. Compared with the composite prepared by conventional electropolymerization (CE), the electronic and ionic transport capacity of the MCE-based composite were significantly improved due to its unique nanoporous structure. The surface of the composite-modified GCE was characterized with SEM and CV. The nanoporous MCE-based electrode was applied to determination of NADH at low potential of 70mV, and a linear range from 2.0  $\mu$ M to 4.0 mM was observed with fast response (within 5 s) and a lower detection limit of 0.5  $\mu$ M (based on S/N = 3). In comparison, a narrow linear range from 5.0  $\mu$ M to 2.0 mM, slower response (up to 15 s) and a higher detection limit of 3.0  $\mu$ M (based on S/N = 3) was obtained with the electrode prepared by CE [107].

A novel nanocomposite of quinone-amine polymer and multiwalled carbon nanotubes was synthesized from iodateoxidation/Michael addition reaction of 1,2-dihydroxybenzene with *o*-phenylenediamine, which was characterized by transmission electron microscope, Fourier transform electron microscope, and UV-visible spectra. The nanocomposite modified Au electrode with well-defined quinone redox peaks effectively mediated the oxidation of NADH in pH 7.0 phosphate buffer, with an overpotential decrease by ca. 470 mV (vs. bare Au), a limit of detection of 6.4 nmol L<sup>-1</sup> and good anti-interferent ability. The linear range was obtained from 0.04 to 300 mmol L<sup>-1</sup> for nanocomposite modified Au electrode [108].

The preparation of poly-(3-methylthiophene)—multi-walled carbon nanotubes hybrid composite electrode shows a synergic effect of the electrocatalytic properties, and high active surface area of both the conducting polymer and carbon nanotubes, which gives rise to a remarkable improvement of oxidation of NADH with respect to PMEs, and CNTs-modified electrodes. The amperometric NADH detection at +300mV provided fast responses, a range of linearity between  $5.0 \times 10^{-7}$  and  $2.0 \times 10^{-5}$  mol l<sup>-1</sup>, and a detection limit of  $1.7 \times 10^{-7}$  mol l<sup>-1</sup> [109]. The nanocomposite was fabricated by the functionalization of SWNTs with poly(nile blue A), which was formed by electropolymerizing an nile blue A (Nb) monomer through the use of CV. The cyclic voltammetric results indicated that PNB-SWNTs were able to electrocatalyze the oxidation of NADH at a very low potential (ca. -80mV versus SCE) and lead to a substantial decrease in the overpotential by more than 700mV compared with the bare GCE [110]. Nanocomposites of electrogenerated polymer/carbon nanotubes modified electrodes and their applications for electroanalytical determinations of NADH are summarized in Table. 2.

**Table 2.** Electroanalysis of NADH using nanocomposites of polymer with carbon nanotube.

Polymer/CNT composites	Electroanalytical applications and characteristics	Ref.
Polyaniline successfully assembled with poly(aminobenzenesulfonic acid)-modified single-walled carbon nanotubes (Layer by layer method)	This film shifts electrocatalytic activity of polyaniline to neutral pH. For a six-bilayer sample, the detection limit can go down to $1 \times 10^{-6}$ M as detected by the simple CV method, with a linear detection range for NADH at concentrations between $5 \times 10^{-6}$ and $1 \times 10^{-3}$ M.	[104]
poly(toluidine blue O)/multiwall carbon nanotube composite nanowires modified GCE	This electrode decreased the NADH oxidization overpotential by about 650 mV. A linear range from 2.0μM to 4.5 mM was observed with fast response (within 5 s) and a low detection limit of 0.5μM (based on S/N = 3).	[105]

Table 2. Cont.

MWCNTs/Nafion/oxidation product of serotonin modified GCE	This hybrid thin film modified electrode exhibits stable amperometric response and it linearly responds to NADH (0.5–400 $\mu\text{M}$ ). Detection limit as low as 0.1 $\mu\text{M}$ at $-0.05\text{V}$ with a sensitivity of 11.1 $\text{nA}/\mu\text{M}$ in physiological pH.	[106]
poly(1,2-diaminobenzene)/MWNTs/GCE	This nanoporous MCE-based electrode was applied to determination of NADH at low potential of 70mV, and a linear range from 2.0 $\mu\text{M}$ to 4.0mM was observed with fast response (within 5 s) and a lower detection limit of 0.5 $\mu\text{M}$ (based on S/N = 3).	[107]
Nanocomposite of quinone-amine polymer and multiwalled carbon nanotubes modified Au electrode	This film mediated the oxidation of NADH in pH 7.0 phosphate buffer, with an overpotential decrease by ca. 470 mV (vs. bare Au), a limit of detection of 6.4 $\text{nmol L}^{-1}$ and good antiinterferent ability. The linear range was obtained from 0.04 to 300 $\text{mmol L}^{-1}$	[108]
Poly-(3-methylthiophene)—multi-walled carbon nanotubes hybrid composite electrode	Amperometric NADH detection at +300mV provided fast responses, a range of linearity between $5.0 \times 10^{-7}$ and $2.0 \times 10^{-5}$ $\text{mol l}^{-1}$ , and a detection limit of $1.7 \times 10^{-7}$ $\text{mol l}^{-1}$	[109]
SWNTs with poly(nile blue A)/GCE	Electrocatalyze the oxidation of NADH at a very low potential (ca. $-80\text{mV}$ versus SCE) and lead to a substantial decrease in the overpotential by more than 700mV	[110]

### 2.5 Other carbon nanotube based NADH transducers

A substantial (490 mV) decrease in the overvoltage of the NADH oxidation reaction (compared to ordinary carbon electrodes) is observed using single-wall and multi-wall carbon-nanotube coatings, with oxidation starting at ca. 0.05 V (vs. Ag/AgCl; pH 7.4). Furthermore, the NADH amperometric response of the coated electrodes is extremely stable, with 96% and 90% of the initial activity remaining after 60 min stirring of  $2 \times 10^{-4}$  and  $5 \times 10^{-3}$  M NADH solutions, respectively (compared to 20% and 14% at the bare surface). The CNT-coated electrodes thus allowed highly sensitive, low-potential, stable amperometric sensing. Such ability of carbon nanotubes to promote the NADH electron-transfer reaction suggests great promise for dehydrogenase-based amperometric biosensors [111]. The cyclic voltammetric responses for phenothiazine dyes, adsorbed onto CNT have been assessed as a means of promoting the low potential, sensitive and stable determination of NADH. The analytical signal of the toluidine blue modified carbon nanotubes (TB-CNT) has been evaluated. When using amperometric detection, the linear range was observed between 50 - 500 $\mu\text{M}$  NADH at  $-0.2\text{V}$ . Finally, the bulk modification of the CNT has allowed the development of an inexpensive and facile electrode design, in which the CNT are packed into a small cavity. The electrode was found to be stable and gave greatly improved signals [112].

Meldola's blue (MB) functionalized carbon nanotubes (CNT) nanocomposite film (MB/CNT) electrode was prepared by non-covalent adsorbing MB on the surface of a carbon nanotubes modified

glassy carbon electrode (CNT/GCE). The heterogeneous electron transfer rate constant ( $k_s$ ) of MB/CNT was calculated to be about three times larger than that of MB/Graphite powder modified electrode. The accelerated electron transfer kinetics was attributed to the unique electrical and nanostructural properties of CNT supports as well as the interaction between MB and CNT. NADH calibration curves at MB/CNT/GCE obtained by chronoamperometry in the range of 50 - 500  $\mu\text{M}$  with detection limit of 48nM NADH [113].

Screen-printed electrodes (SPEs) modified with Variamine blue (VB), covalently attached to the oxidized single-walled carbon nanotubes (SWCNTs-COOH), were developed and used as chemical sensors for the detection of the NADH. The VB redox mediator was covalently linked to the SWCNTs-COOH by the *N,N'*-dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (NHS) chemistry. At pH 7.0, the linear range of detection was  $7.5 \times 10^{-6} - 5 \times 10^{-2}$ . The limit of detection and limit of quantification were 1.7  $\mu\text{M}$  and 5.7  $\mu\text{M}$ , respectively. Applied potential was +100mV [114]. Through a new and simple ion-exchange route, two-electron redox mediator thionine has been deliberately incorporated into the carbon nanotubes (CNTs)/Nafion composite film due to the fact that there is strong interaction between any of two among the three materials (ionexchange process between thionine and Nafion, strong adsorption of thionine by CNTs, and wrapping and solubilizing of CNTs with Nafion). The resulting integrated films exhibited high and stable electrocatalytic activity toward NADH oxidation with the significant decrease of high overpotential, which responds more sensitively more than those modified by thioine or CNTs alone. Such high electrocatalytic activity facilitated the low potential determination of NADH (as low as  $-0.1$  V), which eliminated the interferences from other easily oxidizable species. The composite film electrode displayed a sensitive ( $4.70\text{mA}\text{M}^{-1}$ ) and fast response (ca. 10 s) to NADH. The linear range of NADH is from 2 to 400  $\mu\text{M}$  with a dynamic range up to 10 mM. This modified electrode provided a low-detection limit of 1  $\mu\text{M}$  estimated at a signal-to-noise ratio of 3 [115].

### 2.6 Biomolecules based NADH transducers

The oxidation of 5'-polyadenylic acid (poly-A) in neutral and alkaline solutions gives rise to redox-active products strongly adsorbed on the graphite electrode surface, which exhibit catalytic activity toward NADH oxidation. The amount and properties of the catalyst are remarkably influenced by the pH of the oxidation medium. Electrodes modified from 0.1 M NaOH solutions containing the polynucleotide allow carrying out the amperometric detection of NADH at 50mV (vs. Ag/AgCl), at pH 9, within a wide concentration range,  $2.5 \times 10^{-8} - 1 \times 10^{-4}$  M, and with a detection limit of  $1.1 \times 10^{-8}$  M. These electrodes also exhibit very good stability and reproducibility [116].

s-Adenosyl-L-methionine (SAMe) (an adenosine analogue with therapeutical activity against affective disorders and liver dysfunctions) can be oxidized on graphite electrode yielding a strongly adsorbed electroactive oxidation product for which a quinone-imine structure is proposed. This compound is capable of electrocatalyzing the NADH oxidation at low potentials, lowering the overvoltage by about 300 mV. An amperometric method for NADH determination at +0.1 V (Ag/AgCl/ KCl sat) is developed using an oxidized-SAMe-modified electrode in pH 9. Linear calibration plots were obtained in the range from 42.4 to 424 nM with a detection limit of 2.4 nM [117].

Redox properties of two coenzymes, flavin adenine dinucleotide (FAD) and coenzyme A (CoA) are studied on pyrolytic graphite electrodes. Both of them exhibited an oxidation process at about 1.2 V in pH 9, associated with the oxidation of their adenine moiety. The resulting adsorbed oxidation products have a common structure, an electroactive quinone-imine that acts as efficient catalyst of the oxidation of NADH at low potentials. Finally, a modified electrode prepared with the oxidation product of CoA was used to determine NADH in a wide linear range ( $1 \times 10^{-8}$  –  $2.43 \times 10^{-5}$  M) with a detection limit of 3 nM [118].

Fisetin is a natural product of the flavonoid family, ubiquitous in some plants and fruits. A new modified electrode, FMGCE, were prepared by electrodeposition of fisetin at the surface of a preactivated GCE. FMGCE was used as voltammetric and amperometric sensors for determination of NADH in quiet and moving solutions respectively. The calculated limit of detection of NADH by voltammetry was 0.06 mM. While, these values decrease to 2.4 and 1.8 mM, respectively for an amperometric procedure [119]. Catechin is a polyphenolic flavonoid that can be isolated from a variety of natural sources, including tea leaves, grape seeds, and the wood and bark of trees such as acacia and mahogany. Catechin was immobilized on PEDOT/GCE (poly(3,4-ethylenedioxythiophene)/GCE)-modified electrodes and used as a mediator for NADH oxidation. The heterogeneous electron transfer rate constant for NADH oxidation at the catechin/PEDOT/GCE was determined using the rotating disk electrode technique and found to be  $9.88 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ . The sensitivity of the electrode was 19 nA/ $\mu\text{M}$  [120].

### 2.7 Mediators immobilized on inorganic materials surface for NADH electrocatalysis

The immobilization of meldola blue (MB) on silica gel coated with niobium oxide and electrochemical investigation of its behavior as well as the electrocatalytical oxidation of NADH is reported. A linear response range between  $1.0 \times 10^{-5}$  and  $7.50 \times 10^{-4}$  mol L<sup>-1</sup> NADH concentration at pH 7.0 was observed. The heterogeneous electron transfer rate,  $k_{\text{obs}}$ , was  $1852 \text{ M}^{-1} \text{ s}^{-1}$  and the Michaelis-Menten constant, was 0.27 mM at pH 7.0 with the electrode coverage of about  $4.4 \times 10^{-9}$  mol cm<sup>-2</sup>, evaluated from the rotating disk electrode experiments [121]. A study of modified carbon paste electrode employing Nile blue adsorbed on silica gel modified with niobium oxide for electrocatalytic oxidation of NADH is described. A linear response range for NADH between  $1.0 \times 10^{-5}$  and  $5.2 \times 10^{-4}$  mol l<sup>-1</sup>, at pH 7.0, was observed at this electrode, with an applied potential of -/200 mV versus SCE. The formation of an intermediate charge transfer complex was proposed between NADH and adsorbed Nile blue. The heterogeneous electron transfer rate,  $k_{\text{obs}}$ , was  $1400 \text{ M}^{-1} \text{ s}^{-1}$  and the apparent Michaelis-Menten constant, was 0.21 mM at pH 7.0 evaluated from rotating disk electrode experiments with an electrode coverage of about  $5.2 \times 10^{-9}$  mol cm<sup>-2</sup> [122].

O-phenylenediamine adsorbed onto silica gel modified with niobium oxide for electrocatalytic oxidation of NADH was reported. A linear response range for NADH between  $4.0 \times 10^{-5}$  and  $8.0 \times 10^{-4}$  mol l<sup>-1</sup>, at pH 7.0, was observed for the electrode, with an applied potential of -50 mV vs. SCE. The formation of an intermediate charge transfer complex is proposed for the charge transfer reaction between NADH and adsorbed PDA. The heterogeneous electron transfer rate,  $k_{\text{obs}}$ , was  $5480 \text{ mol}^{-1} \text{ l s}^{-1}$  and the apparent Michaelis -/Menten constant,  $1.04 \times 10^{-4}$  mol l<sup>-1</sup> at pH 7.0, evaluated with rotating disk electrode experiments with an electrode with coverage of PDA of  $5.7 \times 10^{-9}$  mol cm<sup>-2</sup> [123]. The

basic electrochemistry of carbon paste electrodes modified with two new redox mediators, nitrofluorenone derivatives, one neutral and one negatively charged, adsorbed on zirconium phosphate has been studied as well as their ability to oxidize NADH electrocatalytically [124].

Disperse Blue 1 as mediator was immobilized by physical adsorption onto graphite electrodes. The electrochemical behavior was studied using CV. The modified electrodes were mounted in a flow injection manifold, and a catalytic current due to the oxidation of NADH was measured. The sensor exhibited good operational and storage stability. Good linearity was achieved within the concentration range 0.002 to 0.1 mM NADH with a detection limit (signal/noise ratio=3) 0.002 mM NADH [125]. Carbon paste electrode modified using the electron mediator bis(1,10-phenanthroline-5,6-dione) (2,2'-bipyridine)ruthenium(II) ( $[\text{Ru}(\text{phend})_2\text{bpy}]^{2+}$ ) exchanged into the inorganic layered material zirconium phosphate. The intercalated mediator remained electroactive within the layers showed electrocatalytic effect towards NADH [126].

The carbon ceramic electrode prepared with sol-gel technique is modified by a thin film of chlorogenic acid. By immersing the carbon ceramic electrode in aqueous solution of chlorogenic acid at less than 2 s a thin film of chlorogenic acid adsorbed strongly and irreversibly on the surface of electrode. This modified electrode showed excellent electrocatalytic activity toward NADH oxidation and it also showed a high analytical performance for amperometric detection of NADH. The catalytic rate constant of the modified carbon ceramic electrode for the oxidation of NADH is determined by CV measurement. Under the optimized conditions the calibration curve is linear in the concentration range 1–120  $\mu\text{M}$ . The detection limit ( $S/N = 3$ ) and sensitivity are 0.2  $\mu\text{M}$  and 25  $\text{nA } \mu\text{M}^{-1}$  [127].

Thin toluidine blue and zinc oxide (ZnO) hybrid films have been grown on GCE and indium tin oxide coated ( $\text{SnO}_2$ ) glass electrodes by using CV. TBO/ZnO hybrid films modified electrode is electrochemically active, dye molecules were not easily leached out from the ZnO matrix and the hybrid films can be considered for potential applications as sensor for amperometric determination of reduced NADH at 0.0 V. A linear correlation between electrocatalytic current and NADH concentration was found to be in the range between 25  $\mu\text{M}$  and 100  $\mu\text{M}$  in phosphate buffer. In addition, dopamine, ascorbic acid and uric acid are not interference in amperometric detection of NADH in this proposed method. In addition, TBO/ZnO hybrid film modified electrode was highly stable and its response to the NADH also remained relentless [128]. Hybrid films made of ZnO and Meldola's blue (MB) using CV has been reported. MB/ZnO hybrid films modified GCE showed one reversible redox couple centered at formal potential ( $E^0$ )  $-0.12\text{V}$  (pH 6.9). The surface coverage ( $\Gamma$ ) of the MB immobilized on ZnO/GCE was about  $9.86 \times 10^{-12} \text{ mol cm}^{-2}$  and the electron transfer rate constant ( $k_s$ ) was determined to be  $38.9 \text{ s}^{-1}$ . The MB/ZnO/GCE electrode acted as a sensor and displayed an excellent specific electrocatalytic response to the oxidation of NADH. The linear response range between 50 and 300  $\mu\text{M}$  NADH concentration at pH 6.9 was observed with a detection limit of 10  $\mu\text{M}$  ( $S/N = 3$ ). The electrode was stable about 1 month without a notable decrease in current. Indeed, dopamine, ascorbic acid, acetaminophen and uric acid did not show any interference during the detection of NADH at this modified electrode [89].

### 3. Conclusions

Last few decades, the electrochemist play a prominent role in sensor technology and they have been contributed their ideas in the fabrication of suitable sensors for the detection of NADH. Electrochemical technique is one of the promising methods for preparation of polymer modified electrodes; thickness and functionalities of polymer surface can be easily controlled by adjusting the parameters such as monomer concentration and applied anodic potential. The essential futures required for best sensors are higher selectivity, good sensitivity, and lower detection limit, less interferences, longer shelf-life, easily renewable surface and less-cost. All of the above requirements can be achieved by combining polymers with carbon nanotubes because of their exceptional electrical, chemical, and mechanical properties, which make them an attractive candidate for diverse applications such as nanoelectronics and biosensors. Continuous efforts of electrochemist on polymer and polymer/CNT nanocomposite will lead new developments in the fabrication of NADH transducers with fulfilling the above requirements for best sensors.

In the same way, inorganic materials such as niobium oxide, ZnO have been used for modification of electrode surface with adsorption of suitable mediator for electrocatalytic oxidation of NADH. These trend will continue, new ideas will come up for selective detection of NADH based on polymers (electronically conducting and redox active), polymer/CNT nanocomposite, inorganic materials (ex. ZnO).

### Acknowledgements

This project work supported by the National Science Council and the Ministry of Education of the Taiwan (ROC). National Taipei University of Technology assisted in meeting the publication costs of this article.

### References

1. Belenky, P.; Bogan, K.L.; Brenner, C. NAD<sup>+</sup> metabolism in health and disease. *Trends Biochem. Sci.* **2007**, *32*, 12-19.
2. Pollak, N.; Dölle, C.; Ziegler, M. The power to reduce: pyridine nucleotides – small molecules with a multitude of functions. *Biochem. J.* **2007**, *402*, 205-18.
3. Bergmer, H.U., Ed.; *Methods of Enzymatic Analysis*, Third Edition, Weinheim: Verlag Chemie, Weinheim, Deerfield Beach, Florida, Basel, 1983; Vol. 3, p. 42.
4. Turner, A.P.F., Karube, I., Wilson, G.S., Eds.; *Biosensors-Fundamentals and Applications*. Oxford University Press, 1987; p.21.
5. Murray, R.W.; in Bard, A.J., Ed.; *Electroanalytical Chemistry*; Marcel Dekker: New York, 1984; Vol. 13, pp. 191-397.
6. Murray, R.W.; Chemically Modified Electrodes. *Accts. Chem. Res.* **1980**, *13*, 135-141.
7. Redepenning, J.G. Chemically modified electrodes: a general overview. *Trends in analytical chemistry* **1987**, *6*, 18-22.

8. Gorton, L.; Dominguez, E. Electrochemistry of NAD(P)<sup>+</sup>/NAD(P)H, encyclopedia of electrochemistry. In *Bioelectrochemistry*; Wilson, G.S., Ed.; Weinheim: Wiley-VCH, 2002; Vol. 9.
9. Gorton, L.; Dominguez, E. Electrocatalytic oxidation of NAD(P)/H at mediator-modified electrodes. *Reviews in Molecular Biotechnology* **2002**, *82*, 371-392.
10. Malinauskas, A.; Malinauskiene, J.; Ramanavicius, A. Conducting polymer-based nanostructured materials: electrochemical aspects. *Nanotechnology* **2005**, *16*, R51-R62.
11. Kossmehl, G., Engelmann, G. In: *Handbook of Oligo and Polythiophenes*; Fichou, D., Ed.; Wiley-VCH: New York, 1999 (Chapter 10).
12. Lamy, C., Leger, J.M., Garnier, F. In: *Handbook of Organic Conductive Molecules and Polymers*; Nalwa, H.S., Ed.; John Wiley & Sons: New York, 1997; Vol. 3 (Chapter 10).
13. Cosnier, S. Biosensors based on electropolymerized films: new trends. *Anal. Bioanal. Chem.* **2003**, *377*, 507-520.
14. Skotheim, T., Ed.; *Handbook of Conducting Polymers*, Marcel Dekker: New York, 1986; Vols. 1 and 2.
15. MacDiarmid, A.G. Synthetic Metals: A Novel Role for Organic Polymers (Nobel Lecture), *Angew. Chem.* **2001**, *40*, 2581-2590.
16. Kumar, S.A.; Chen, S.M. Electrocatalytic reduction of oxygen and hydrogen peroxide at poly(*p*-aminobenzene sulfonic acid)-modified glassy carbon electrodes. *Journal of Molecular Catalysis A: Chemical* **2007**, *278*, 244-250.
17. Kumar, S.A.; Tang, C.F.; Chen, S.M. Poly(4-amino-1-1'-azobenzene-3, 4'-disulfonic acid) coated electrode for selective detection of dopamine from its interferences, *Talanta* **2008**, *74*, 860-866.
18. Kumar, S.A.; Chen, S.A.; Electrochemical, microscopic, and EQCM studies of cathodic electrodeposition of ZnO/FAD and anodic polymerization of FAD films modified electrodes and their electrocatalytic properties, *J Solid State Electrochem.* **2007**, *11*, 993-1006.
19. Malinauskas, A.; Garjonytė, R.; Mažeikienė, R.; Jurevičiūtė, I. Electrochemical response of ascorbic acid at conducting and electrogenerated polymer modified electrodes for electroanalytical applications: a review *Talanta* **2004**, *64*, 121-129.
20. Rahman, Md. A.; Kumar, P.; Park D.-S., Shim, Y.-B. Electrochemical Sensors Based on Organic Conjugated Polymers. *Sensors* **2008**, *8*, 118-141.
21. Iijima, S. Helical microtubules of graphitic carbon. *Nature* **1991**, *354*, 56-58.
22. Wiles, P.G.; Abrahamson, J. Carbon fibre layers on arc electrodes—I : Their properties and cool-down behavior. *Carbon*, **1978**, *16*, 341-349.
23. Abrahamson, J.; Wiles, P.G.; Rhoades, B.L. Structure of carbon fibres found on carbon arc anodes. *Carbon* **1999**, *37*, 1873-1874.
24. Baughman, R.H.; Zakhidov, A.; de Heer, W.A. Carbon Nanotubes--the Route Toward Applications. *Science* **2002**, *297*, 787-792.
25. Ajayan, P.M. Nanotubes from carbon, *Chem. Rev.* **1999**, *99*, 1787-1799.
26. Wang, J. Carbon-Nanotube Based Electrochemical Biosensors: A Review. *Electroanalysis* **2005**, *17*, 7-14.

27. Chen, R.S.; Huang, W.H.; Tong, H.; Wang, Z.L.; Cheng, J.K. Carbon fiber nanoelectrodes modified by single-walled carbon nanotubes. *Anal. Chem.* **2003**, *75*, 6341-6345.
28. Banks, C.E.; Davies, T.J.; Wildgoose, G.G.; Compton, R.G. Electrocatalysis at graphite and carbon nanotube modified electrodes: edge-plane sites and tube ends are the reactive sites. *Chem. Commun.* **2005**, 829-841.
29. Luo, X.L.; Xu, J.J.; Wang, J.L.; Chen, H.Y. Electrochemically deposited nanocomposite of chitosan and carbon nanotubes for biosensor application, *Chem. Commun.* **2005**, 2169-2171.
30. Guo, M.L.; Chen, J.H.; Li, J.; Tao, B.; Yao, S.Z. Fabrication of polyaniline/carbon nanotube composite modified electrode and its electrocatalytic property to the reduction of nitrite. *Anal. Chim. Acta* **2005**, *532*, 71-77.
31. Gao, M.; Huang, S.; Dai, L.; Wallace, G.; Gao, R.; Wang, Z. Aligned coaxial nanowires of carbon nanotubes sheathed with conducting polymers. *Angew. Chem.* **2000**, *112*, 3810-3813;
32. Yao, X.; Wu, H.; Wang, J.; Qu, S.; Chen, G. Carbon Nanotube/Poly(methyl methacrylate) (CNT/PMMA) Composite Electrode Fabricated by In Situ Polymerization for Microchip Capillary Electrophoresis. *Chem. Eur. J.* **2007**, *13*, 846-853.
33. Yogeswaran, U.; Thiagarajan, S.; Chen, S.M. Pinecone shape hydroxypropyl- $\beta$ -cyclodextrin on a film of multi-walled carbon nanotubes coated with gold particles for the simultaneous determination of tyrosine, guanine, adenine and thymine. *Carbon* **2007**, *45*, 2783-2796.
34. Yogeswaran, U.; Chen, S.-M. Electrocatalytic properties of electrodes which are functionalized with composite films of f-MWCNTs incorporated with poly(neutral red). *J. Electrochem. Soc.* **2007**, *154*, E178-E186.
35. Gregory, G.; Wildgoose, G.G.; Banks, C.E.; Leventis, H.C.; Compton, R.G. Chemically Modified Carbon Nanotubes for Use in Electroanalysis, *Microchim Acta* **2006**, *152*, 187-214.
36. Moiroux, J.; Elving, P.J. Effects of adsorption, electrode material, and operational variables on the oxidation of dihydronicotinamide adenine dinucleotide at carbon electrodes, *Anal. Chem.* **1978**, *50*, 1056-1062.
37. Jaegfeldt, H. Adsorption and electrochemical oxidation behaviour of NADH at a clean platinum electrode, *J. Electroanal. Chem.* **1980**, *110*, 295-302.
38. Bartalits, L.; Nagy, G.; Pungor, E. Determination of enzyme activity in biological fluids by means of electrochemical oxidation of NADH at a modified glassy carbon electrode, *Anal. Lett.* **1984**, *17*, 13-41.
39. Bartlett, P.N.; Simon, E.; Toh, C.S. Modified electrodes for NADH oxidation and dehydrogenase-based biosensors, *Bioelectrochemistry* **2002**, *56*, 117-122.
40. Bobacka, J. Conducting Polymer-Based Solid-State Ion-Selective Electrodes, *Electroanalysis* **2006**, *18*, 7-18.
41. Kanazawa, K.K.; Diaz, A.F.; Geiss, R.H.; Gill, W.D.; Kwak, J.F.; Logan, J.A.; Rabolt, J.F.; Street, G.B. Organic metals: polypyrrole, a stable synthetic metallic polymer. *J. Chem. Soc., Chem. Commun.* **1979**, *19*, 854-855.
42. Persaud, K.C. Polymers for chemical sensing, *Materials Today* **2005**, *8*, 38-44.
43. Shirakawa, H.; Louis, E.J.; MacDiarmid, A.G.; Chiang, C.K.; Heeger, A.J. Synthesis of electrically conducting organic polymers: halogen derivatives of polyacetylene, (CH)<sub>x</sub>, *J. Chem. Soc., Chem. Commun.* **1977**, 578-580.

44. Shirakawa, H. The discovery of polyacetylene film: The dawning of an era of conducting polymers, *Synth. Met.* **2001**, *125*, 3-10.
45. MacDiarmid, A.J. Synthetic metals: a novel role for organic polymers. *Synth. Met.* **2001**, *125*, 11-22.
46. Heeger, A.J. Semiconducting and metallic polymers: the fourth generation of polymeric materials, *Synth. Met.* **2001**, *125*, 23-42.
47. Skotheim, T., Ed.; *Handbook of Conducting Polymers*, Marcel Dekker: New York, 1986; Vols. 1 and 2
48. Nalwa, H.S., *Handbook of Organic Conductive Molecules and Polymers*; Wiley: Chichester 1997.
49. Skotheim, T.A., Elsenbaumer, R.L., Reynolds, J.R., Eds.; *Handbook of Conducting Polymers*, Second Edition; Marcel Dekker: New York, 1998.
50. Lyons, M.E.G., *Electroactive Polymer Electrochemistry*; Plenum Press: New York, 1994.
51. Ivaska, A. Analytical applications of conducting polymers, *Electroanalysis* **1991**, *3*, 247-254.
52. Imisides, M.D.; John, R.; Riley, P.J.; Wallace, G.G. The use of electropolymerization to produce new sensing surfaces: A review emphasizing electrode position of heteroaromatic compounds, *Electroanalysis* **1991**, *3*, 879-889.
53. Bidan, G. Electroconducting conjugated polymers: New sensitive matrices to build up chemical or electrochemical sensors, *Sens. Actuators B* **1992**, *6*, 45-56.
54. Zotti, G. Electrochemical sensors based on polyconjugated conducting polymers. *Synth. Met.* **1992**, *51*, 373 -382.
55. Bartlett, P.N.; Birkin, P.R. The application of conducting polymers in biosensors. *Synth. Met.* **1993**, *61*, 15 -21.
56. Teasdale, P.R.; Wallace, G.G. Molecular recognition using conducting polymers: basis of an electrochemical sensing technology—Plenary lecture. *Analyst* **1993**, *118*, 329-334.
57. Barlett, P.N.; Cooper, J.M. A review of the immobilization of enzymes in electropolymerized films, *J. Electroanal. Chem.* **1993**, *362*, 1-12.
58. Josowicz, M. Applications of conducting polymers in potentiometric sensors, *Analyst*, **1995**, *120*, 1019-1024.
59. Emr, S.A.; Yacynych, A.M. Use of polymer films in amperometric biosensors *Electroanalysis*, **1995**, *7*, 913-923.
60. Adeloju, S.B.; Wallace, G.G. Conducting polymers and the bioanalytical sciences: new tools for biomolecular communications. A review, *Analyst*, **1996**, *121*, 699-703.
61. Gcpel, W.; Schierbaum, K.D. in *Handbook of Organic Conductive Molecules and Polymers*, Vol. 4, *Conductive Polymers: Transport, Photophysics and Applications* (Ed: H. S. Nalwa), Wiley, Chichester, 1997, pp. 621-659.
62. Fabre, B.; Simonet, J. Electroactive polymers containing crown ether or polyether ligands as cation-responsive materials, *Coord. Chem. Rev.* **1998**, *178-180*, 1211-1250.
63. Giuseppi-Elie, A.; Wallace, G.G.; Matsue, T. In *Handbook of Conducting Polymers*, Second Edition; Skotheim, T.A., Elsenbaumer, R.L., Reynolds, J.R., Eds.; Marcel Dekker: New York, 1998; pp. 963-991

64. Lewis, T.W.; Wallace, G.G.; Smyth, M.R. Electrofunctional polymers: their role in the development of new analytical systems. *Analyst*, **1999**, *124*, 213-219.
65. Wallace, G.G.; Smyth, M.; Zhao, H. Conducting electroactive polymer-based biosensors, *Trends Anal. Chem.* **1999**, *18*, 245-251.
66. McQuade, D.T.; Pullen, A.E.; Swager, T.M. Conjugated Polymer-Based Chemical Sensors, *Chem. Rev.* **2000**, *100*, 2537-2574.
67. Kane-Maguire, L.A.P.; Wallace, G.G. Communicating with the building blocks of life using organic electronic conductors. *Synth. Met.* **2001**, *119*, 39-42.
68. Fabre, B. In *Handbook of Advanced Electronic and Photonic Materials and Devices*, Vol. 8.: *Conducting Polymers*, Nalwa, H.S.; Academic Press: San Diego, 2001; pp. 103-129.
69. Sensors Update, Vol. 8 (Eds: M. Leclerc, in H. Baltes, W. Gcpel, J. Hesse), Wiley-VCH, Weinheim, 2001; pp. 21-38.
70. Ramanaviciene, A.; Ramanavicius, A. Application of Polypyrrole for the Creation of Immunosensors, *Crit. Rev. Anal. Chem.* **2002**, *32*, 245-252.
71. Janata, J.; Josowicz, M. Conducting polymers in electronic chemical sensors. *Nature Materials* **2003**, *2*, 19-24.
72. Trojanowicz, M. Application of Conducting Polymers in Chemical Analysis. *Microchim. Acta* **2003**, *143*, 75-91.
73. Bobacka, J.; Ivaska, A.; Lewenstam, A. Potentiometric Ion Sensors Based on Conducting Polymers. *Electroanalysis* **2003**, *15*, 366 -374.
74. Adhikari, B.; Majumdar, S. Polymers in sensor applications, *Prog. Polym. Sci.* **2004**, *29*, 699-766.
75. Ramanavičius, A.; Ramanavičienė, A.; Malinauskas, A. Electrochemical sensors based on conducting polymer—polypyrrole, *Electrochimica Acta* **2006**, *51*, 6025-6037.
76. Bai, H.; Shi, G. Gas Sensors Based on Conducting Polymers, *Sensors* **2007**, *7*, 267-307.
77. Diaz, A.F.; Logan, J.A. Electroactive polyaniline films. *J. Electroanal. Chem.* **1980**, *111*, 111-114.
78. Ohsaka, T.; Ohnuki, Y.; Oyama, N.; Katagiri, K.; Kamisako, K. IR absorption spectroscopic identification of electroactive and electroinactive polyaniline films prepared by the electrochemical polymerization of aniline. *J. Electroanal. Chem.* **1984**, *161*, 399-405.
79. Cui, S.Y.; Park, S.M. Electrochemistry of conductive polymers XXIII: polyaniline growth studied by electrochemical quartz crystal microbalance measurements. *Synth. Met.* **1999**, *105*, 91-98.
80. Tian, S.; Baba, A.; Liu, J.; Wang, Z.; Knoll, W.; Park, M.K.; Advincula, R. Electroactivity of Polyaniline Multilayer Films in Neutral Solution and Their Electrocatalyzed oxidation of  $\beta$ -Nicotinamide Adenine dinucleotide. *Adv. Funct. Mater.* **2003**, *13*, 473-479.
81. Toh, C.S.; Bartlett, P.N.; Mano, N.; Aussenac, F.; Kuhn, A.; Dufour, E.J. The effect of calcium ions on the electrocatalytic oxidation of NADH by poly(aniline)-poly(vinylsulfonate) and poly(aniline)-poly(styrenesulfonate) modified electrodes. *Phys. Chem. Chem. Phys.* **2003**, *5*, 588-593.

82. Valentini, F.; Salis, A.; Curulli, A.; Palleschi, G. Chemical Reversibility and Stable Low-Potential NADH Detection with Nonconventional Conducting Polymer Nanotubule Modified Glassy Carbon Electrodes. *Anal. Chem.* **2004**, *76*, 3244-3248.
83. Kumar, S.A.; Chen, S.M. Electrochemically polymerized composites of conducting poly(*p*-ABSA) and flavins (FAD, FMN, RF) films and their use as electrochemical sensors: A new potent electroanalysis of NADH and NAD<sup>+</sup>. *Sensors and Actuators B* **2007**, *123*, 964-977.
84. Jin, G.; Zhang, Y.; Cheng, W. Poly(*p*-aminobenzene sulfonic acid)-modified glassy carbon electrode for simultaneous detection of dopamine and ascorbic acid. *Sensors and Actuators B: Chemical* **2005**, *107*, 528-534.
85. Delbem, M. F.; Baader, W.J.; Serrano, S.H.P. Mechanism of 3,4-dihydroxybenzaldehyde electropolymerization at carbon paste electrodes - Catalytic detection of NADH, *Quimica Nova* **2002**, *25*, 741-747.
86. Chen, S.M.; Lin, K.C. The electrocatalytic properties of biological molecules using polymerized luminol film-modified electrodes, *J. Electroanal. Chem.* **2002**, *523*, 93-105.
87. Li, N. B.; Duan, J. P.; Chen, G. N. Electrochemical Polymerization of Azure Blue II and Its Electrocatalytic Activity toward NADH Oxidation. *Chin. J. Chem.* **2003**, *21*, 1191-1197.
88. Gao, Q.; Cui, X.; Yang, F.; Ma, Y.; Yang, X. Preparation of poly(thionine) modified screen-printed carbon electrode and its application to determine NADH in flow injection analysis system. *Biosensors and Bioelectronics* **2003**, *19*, 277-282.
89. Kumar, S.A.; Chen, S.M. Fabrication and characterization of Meldola's blue/zinc oxide hybrid electrodes for efficient detection of the reduced form of nicotinamide adenine dinucleotide at low potential. *Analytica Chimica Acta* **2007**, *592*, 36-44.
90. Vasilescu, A.; Noguer, T.; Andreescu, S.; Calas-Blanchard, C.; Bala, C.; Marty, J.L. Strategies for developing NADH detectors based on Meldola Blue and screen-printed electrodes: a comparative study. *Talanta* **2003**, *59*, 751-765.
91. Chen, Y.; Yuan, J.; Tian, C.; Wang, X. Flow-Injection analysis and voltammetric detection of NADH with a Poly-Toluidine Blue modified electrode, *Analytical Sciences* **2004**, *20*, 507-511.
92. Gao, Q.; Wang, W.; Ma, Y.; Yang, X. Electrooxidative polymerization of phenothiazine derivatives on screen-printed carbon electrode and its application to determine NADH in flow injection analysis system. *Talanta* **2004**, *62*, 477-482.
93. Sha, Y.; Gao, Q.; Qi, B.; Yang, X. Electropolymerization of Azure B on a Screen-Printed Carbon Electrode and its Application to the Determination of NADH in a Flow Injection Analysis System. *Microchim. Acta* **2004**, *148*, 335-341.
94. Komura, T.; Niu, G.Y.; Yamaguchi, T.; Asano, M.; Matsuda, A. Coupled Electron-Proton Transport in Electropolymerized Methylene Blue and the Influences of Its Protonation Level on the Rate of Electron Exchange with  $\beta$ -Nicotinamide Adenine Dinucleotide, *Electroanalysis* **2004**, *16*, 1791-1800.
95. Yuan, J.; Chen, Y.; Wang, X.; Tian, C. Electrocatalysis of  $\beta$ -nicotinamide adenine dinucleotide oxidation with electropolymerized films of toluidine blue. *Fenxi Huaxue* **2004**, *32*, 53-55.

96. Nassef, H.M.; Radi, A.; O'Sullivan, C.K. Electrocatalytic sensing of NADH on a glassy carbon electrode modified with electrografted o-aminophenol film, *Electrochem. Commun.* **2006**, *8*, 1719-1725.
97. Chen, S.M.; Liu, M.I.; Kumar, S.A. Electrochemical Preparation of Poly(acriflavine) Film-Modified Electrode and Its Electrocatalytic Properties Towards NADH, Nitrite and Sulfur Oxoanions. *Electroanalysis* **2007**, *19*, 999-1007.
98. Vasilescu, A.; Andreescu S.; Bala, C.; Litescu, S.C.; Noguier, T.; Marty, J.L. Screen-printed electrodes with electropolymerized Meldola Blue as versatile detectors in biosensors, *Biosensors and Bioelectronics* **2003**, *18*, 781-790.
99. Prieto-Simón, B.; Fàbregas, E. Comparative study of electron mediators used in the electrochemical oxidation of NADH, *Biosensors and Bioelectronics* **2004**, *19*, 1131-1138.
100. Lin, K.C.; Chen, S.M. Characterization of Hybrid Poly(acriflavine)/FAD Films and Their Electrocatalytic Properties with NAD<sup>+</sup> and NADH, *Journal of The Electrochemical Society* **2006**, *153*, D91-D98.
101. Dai, Z.H.; Liu, F.X.; Lu, G.F.; Bao, J.C. Electrocatalytic detection of NADH and ethanol at glassy carbon electrode modified with electropolymerized films from methylene green, *J Solid State Electrochem* **2008**, *12*, 175-180.
102. Golabi, S.M.; Zare, H.R.; Hamzehloo, M. Electrochemistry and Electrocatalytic Activity of Pyrocatechol Violet (PCV) Film on a Glassy Carbon Electrode Towards the Oxidation of Reduced Nicotinamide Adenine Dinucleotide(NADH), *Electroanalysis* **2002**, *14*, 611-618.
103. Dilgin, Y.; Gorton, L.; Nisli, G. Photoelectrocatalytic Oxidation of NADH with Electropolymerized Toluidine Blue O, *Electroanalysis* **2007**, *19*, 286-293.
104. Liu, J.; Tian, S.; Knoll, W. Properties of Polyaniline/Carbon Nanotube Multilayer Films in Neutral Solution and Their Application for Stable Low-Potential Detection of Reduced  $\beta$ -Nicotinamide Adenine Dinucleotide, *Langmuir* **2005**, *21*, 5596-5599.
105. Zeng, J.; Wei, W.; Wu, L.; Liu, X.; Liu, K.; Li, Y. Fabrication of poly(toluidine blue O)/carbon nanotube composite nanowires and its stable low-potential detection of NADH. *J. Electroanal. Chem.* **2006**, *595*, 152-160.
106. Raj, C.R.; Chakraborty, S. Carbon nanotubes–polymer–redox mediator hybrid thin film for electrocatalytic sensing, *Biosens. Bioelectron.* **2006**, *22*, 700-706.
107. Zeng, J.; Gao, X.; Wei, W.; Zhai, X.; Yin, J.; Wu, L.; Liu, X.; Liu, K.; Gong, S. Fabrication of carbon nanotubes/poly(1,2-diaminobenzene) nanoporous composite via multipulse chronoamperometric electropolymerization process and its electrocatalytic property toward oxidation of NADH. *Sensors and Actuators B* **2007**, *120*, 595-602.
108. Tu, X.; Xie, Q.; Huang, Z.; Yang, Q.; Yao, S. Synthesis and Characterization of Novel Quinone-Amine Polymer/Carbon Nanotubes Composite for Sensitive Electrocatalytic Detection of NADH. *Electroanalysis* **2007**, *19*, 1815-1821.
109. Agui, L.; Pena-Farfal, C.; Yanez-Sedeno, P.; Pingarron, J.M. Poly-(3-methylthiophene)/carbon nanotubes hybrid composite-modified electrodes. *Electrochimica Acta* **2007**, *52*, 7946-7952.
110. Du, P.; Liu, S.; Wu, P.; Cai, C. Single-walled carbon nanotubes functionalized with poly(nile blue A) and their application to dehydrogenase-based biosensors. *Electrochimica Acta* **2007**, *53*, 1811-1823.

111. Musameh, M.; Wang, J.; Merkoci, A.; Lin, Y. Low-potential stable NADH detection at carbon-nanotube-modified glassy carbon electrodes. *Electrochemistry Communications* **2002**, *4*, 743-746.
112. Lawrence, N.S.; Wang, J. Chemical adsorption of phenothiazine dyes onto carbon nanotubes: Toward the low potential detection of NADH, *Electrochemistry Communications* **2006**, *8*, 71-76.
113. Zhu, L.; Zhai, J.; Yang, R.; Tian, C.; Guo, L. Electrocatalytic oxidation of NADH with Meldola's blue functionalized carbon nanotubes electrodes, *Biosensors and Bioelectronics* **2007**, *22*, 2768-2773.
114. Radoi, A.; Compagnone, D.; Valcarcel, M.A.; Placidi, P.; Materazzi, S.; Moscone, D.; Palleschi, G. Detection of NADH via electrocatalytic oxidation at single-walled carbon nanotubes modified with Variamine blue. *Electrochimica Acta* **2008**, *53*, 2161-2169.
115. Huang, M.; Jiang, H.; Zhai, J.; Liu, B.; Dong, S. A simple route to incorporate redox mediator into carbon nanotubes/Nafion composite film and its application to determine NADH at low potential, *Talanta* **2007**, *74*, 132-139.
116. De-los-Santos-Alvarez, P.; Molina, P.G.; Lobo-Castanfon, M.J.; Miranda-Ordieres, A.J.; Tunfon-Blanco, P. Electrocatalytic Oxidation of NADH at Polyadenylic Acid Modified Graphite Electrodes. *Electroanalysis* **2002**, *14*, 1543-1549.
117. De-los-Santos-Alvarez, N.; Lobo-Castanfon, M.J.; Miranda-Ordieres, A.J.; Tunfon-Blanco, P. Electrocatalytic Oxidation of NADH by Oxidized s-Adenosyl-LMethionine (SAME): Application to NADH and SAME Determinations. *Electroanalysis* **2004**, *16*, 881-887.
118. De-los-Santos-Alvarez, N.; Lobo-Castanfon, M.J.; Miranda-Ordieres, A.J.; Tunon-Blanco, P. Electrochemical and Catalytic Properties of the Adenine Coenzymes FAD and Coenzyme A on Pyrolytic Graphite Electrodes. *Electroanalysis* **2005**, *17*, 445-451.
119. Golabi, S.M.; Irannejad, L. Preparation and Electrochemical Study of Fisetin Modified Glassy Carbon Electrode. Application to the Determination of NADH and Ascorbic Acid, *Electroanalysis* **2005**, *17*, 985-996.
120. Vasantha, V.S.; Chen, S.M. Synergistic effect of a catechin-immobilized poly(3,4-ethylenedioxythiophene)-modified electrode on electrocatalysis of NADH in the presence of ascorbic acid and uric acid. *Electrochimica Acta* **2006**, *52*, 665-674.
121. de S. Santos, A.; Gorton, L.; Kubota, L.T. Electrocatalytic NADH Oxidation Using an Electrode Based on Meldola Blue Immobilized on Silica Coated with Niobium Oxide. *Electroanalysis* **2002**, *14*, 805-812.
122. de S. Santos, A.; Gorton, L.; Kubota, L.T. Nile blue adsorbed onto silica gel modified with niobium oxide for electrocatalytic oxidation of NADH, *Electrochimica Acta* **2002**, *47*, 3351-3360.
123. Ce'sar Pereira, A.; de Santana Santos, A.; Kubota, L.T. o-Phenylenediamine adsorbed onto silica gel modified with niobium oxide for electrocatalytic NADH oxidation, *Electrochimica Acta* **2003**, *48*, 3541-3550.
124. Munteanu, F.D.; Mano, N.; Kuhn, A.; Gorton, L. NADH electrooxidation using carbon paste electrodes modified with nitro-fluorenone derivatives immobilized on zirconium phosphate. *Journal of Electroanalytical Chemistry* **2004**, *564*, 167-178.

125. Stergiou, D.V.; Prodromidis, M.I.; Veltsistas, P.G.; Evmiridis, N.P. Study of the Electrochemical Behavior of Disperse Blue 1-Modified Graphite Electrodes. Application to the Flow Determination of NADH. *Electroanalysis* **2004**, *16*, 949-954.
126. Santiago, M.E.B.; Velez, M.M.; Borrero, S.; Diaz, A.; Casillas, C.A.; Hofmann, C.; Guadalupe, A.R.; Colon, J.L. NADH Electrooxidation Using Bis(1,10-phenanthroline-5,6-dione) (2,2'-bipyridine)ruthenium(II)-Exchanged Zirconium Phosphate Modified Carbon Paste Electrodes, *Electroanalysis* **2006**, *18*, 559-572.
127. Salimi, A.; Hallaj, R.; Ghadermazi, M. Modification of carbon ceramic electrode prepared with sol-gel technique by a thin film of chlorogenic acid: application to amperometric detection of NADH. *Talanta* **2005**, *65*, 888-894.
128. Kumar, S.A.; Chen, S.M. Electrocatalysis and Amperometric Detection of the Reduced Form of Nicotinamide Adenine Dinucleotide at Toluidine Blue/Zinc Oxide Coated Electrodes, *Electroanalysis* **2007**, *19*, 1952-1958.