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Design and application of novel functional dyes containing polymers for biosensors and organic syntheses

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Abstract

We have synthesized polymers covalently attached with dye molecules such as Toluidine Blue O and Neutral Red. Those polymeric systems were studied for sensors (glucose, ethanol, vitamin K etc.) based on electrocatalytic oxidation of NADH. We also utilized the polymeric dye system as a mediator for the electrochemical regeneration of NAD+ to NADH in enantioselective reductions of ketone and aldehyde compounds.

Introduction

The nicotinamide adenin dinucleotide (NAD+/NADH) dependent dehydrogenase constitute the largest group of redox enzyme known today[1]. The electrochemistry of both the oxidized (NAD+) and reduced (NADH) forms of the cofactor at naked electrodes is complicated by large over voltage[2]. However the limitations for NADH oxidation can be solved by the introduction of redox mediators that can shuttle the electrons from NADH to the electrode at a substantially decreased overvoltage[3]. The electrochemical oxidation of NADH is catalyzed by the mediator and involves two steps ;

$$NADH + Med_{ox} \longrightarrow NAD^+ + Med_{red} \quad (1)$$

$$Med_{red} \longrightarrow Med_{ox} + H^+ + e^- \quad (2)$$

By immobilizing the mediating species directly on the electrode surface to produce a chemically modified electrode (CME), both reactions (1) and (2) will occur at the interface between the solution and the electrode. Once the NADH has reached the electrode surface, it reacts with the oxidized form of mediator (Med_{ox}), yielding NAD+ and the reduced form of the mediator (Med_{red}) (1). The reduced form of the mediator will be electrochemically reoxidized, if the applied potential (E_{appl}) is more positive than the formal potential E^0 value of the mediator (2). The mediator will thus dictate at what potential the electron transfer occurs. Consequently, the detection of NADH can take place at much lower applied potential, thus minimizing the risk of interferences and electrode fouling.

Positively charged derivatives of phenothiazine(i.e. Toluidine Blue O) adsorbed on graphite to produce chemically modified electrodes have been shown to be highly efficient mediators for a selective electrocatalytic oxidation of NADH producing enzymatically active NAD+ [3].

In order to present the mediating species from diffusing away from the electrode surface, we have synthesized various polymers bounded covalently with dye compound (Figure 1).

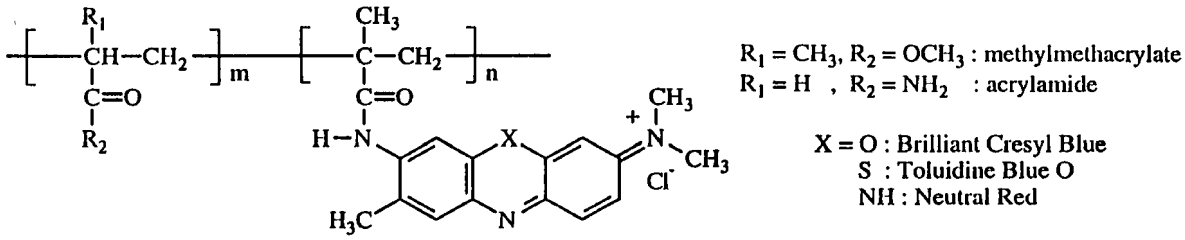


Figure 1 Dye containing polymers

Figure 2 shows typical voltammetric results for a carbon paste electrode containing polymer bound TBO dye compound. With no NADH present, the voltammogram displays low anodic currents. Upon addition of NADH, however, voltammetry changes dramatically with a large increase in the oxidation current and no increase in the reduction current.

This result showed that the polymeric mediator has high catalytic efficiency for electrocatalytic NADH oxidation at low potentials. Thus, we have applied the polymeric mediator as ethanol and glucose detections[4-6]. The sensoring system based on the oxidation of NADH is shown in Scheme 1.

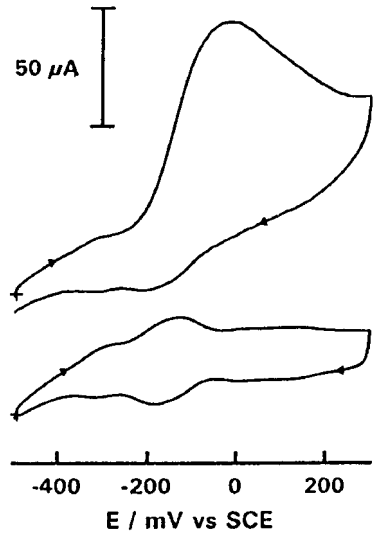
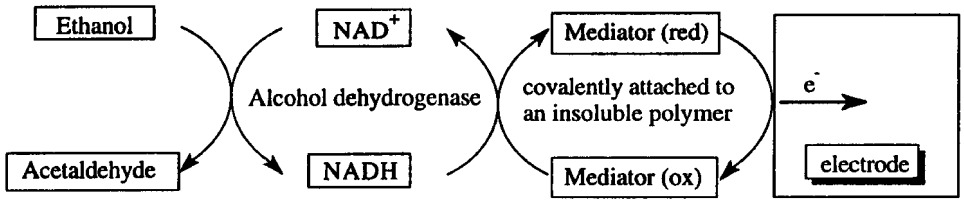


Figure 2 Cyclic voltammogram of carbon paste electrode consisting of TBO-containing polymer in testing 0.1M phosphate buffer solution (pH 7.0); (Lower) without NADH, and (Upper) presence of 7.5mM of NADH. The reference electrode; SCE and sweep rate; 5mV/sec.



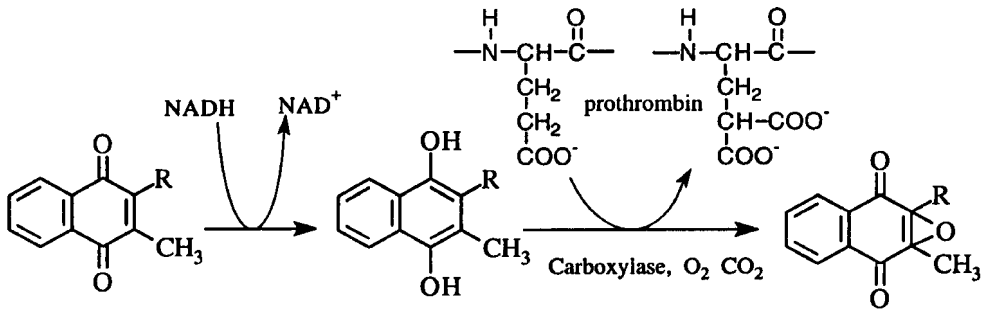
Scheme 1 Ethanol detection based on oxidation of NADH

We will report in this paper an electrochemical vitamin K_3 detection using the polymeric dye system.

1.Vitamin K_3 detection based on polymeric dye system

Vitamin K is known to be essential for blood clotting, its deficiency will result in a condition of hypoprothrombinemia which can cause hemorrhages throughout the body[7]. It is administered to heart patients using anticoagulant drugs to raise the prothrombin level. Although vitamin K can be synthesized by bacteria in the colon[8], large amount or prolonged use of antibiotics destroy these bacteria and result in vitamin deficiency. Therefore the detection of vitamin K levels in blood is of great interest.

Vitamin K is converted into the reduced form by a reductase in the presence of the coenzyme, NADH[9-11]. The reduced form of vitamin K reacts with carboxylase and descarboxyprothrombin. The resulting prothrombin is the blood coagulation factor II (shown in Scheme 2).



Scheme 2 The route for the formation of prothrombin from vitamin K

We have found that a fast electron transfer occurs between NAD+ and certain organic dye molecules such as phenazine derivatives. Therefore the electron transfer from vitamin K to organic dyes via NAD+/NADH proceeds in the following manner ;

$$\text{Vitamin K} + \text{NADH} \longrightarrow \text{Vitamin KH} + \text{NAD}^+$$

$$\text{NAD}^+ + \text{dye}_{red} \longrightarrow \text{NADH} + \text{dye}_{ox}$$

$$\text{dye}_{ox} + e^- \longrightarrow \text{dye}_{red}$$

In order to immobilized water soluble dyes, we have prepared poly(methyl methacrylate)(PMMA) bound Toluidine Blue O (TBO). The polymer obtained was repeatedly purified by precipitation from a dioxane solution into water. The modified carbon paste was prepared by thoroughly mixing 100mg of graphite powder with a measured amount of dye containing polymer in a THF solution. After evaporate solvent, NADH in water solution was added. The cyclic volytammogram of a carbon paste electrode consisting of PMMA bounded TBO is showm in Figure 3a. The broaded reduction peak of TBO is observed around -400mV (vs Ag/AgCl). The reduction current intensity increased rapidly after the addition of the water soluble vitamin K_3 solution (Figure 3b). Toluidine Blue O, which is oxidized by vitamin K_3 can be reduced electrochemically.

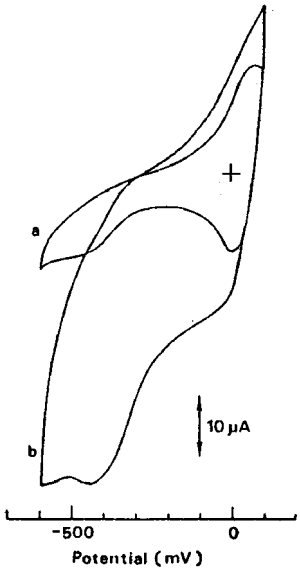


Figure 3 Cyclic voltammogram of carbon paste electrode consisting of TBO-PMMA in testing 0.1M phosphate buffer solution (pH 7.0); (a) without vitamin K, and (b) presence of 31.5mM of Vitamin K_3. The reference electrode; Ag/AgCl and sweep rate; 5mV/sec.

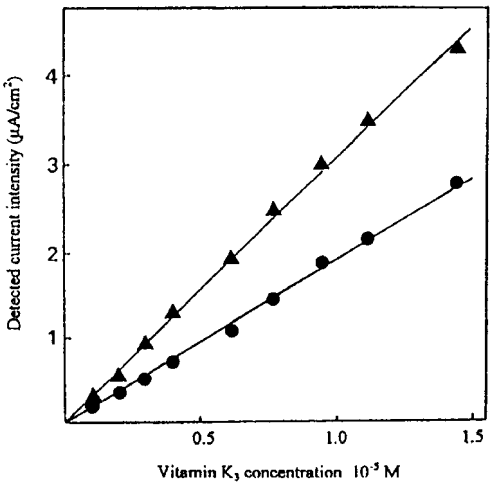
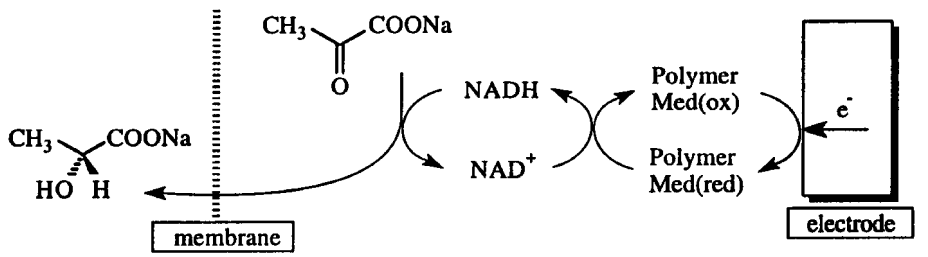


Figure 4 Vitamin K_3 calibration curves for the TBO-PMMA/NAD+/carbon paste electrodes at an apploed potential of -400mV (●) and -500mV (▲) vs. Ag/AgCl

It was founded that the carbon paste electrode can detect low concentration of vitamin K_3, as low as 1x10-6M, and the observed current intensity was proportional to the concentration of vitamin K_3. These results are plotted in Figure 4.

2 Electro-enzymatic reaction of pyruvic acid using polymeric dye system as an electrochemical regenerating NADH from NAD+

Enzymatic catalysis in organic synthesis has attracted a great deal interest for their high stereo and chemoselectives[12,13]. In order to utilize the enzyme system for organic synthesis, considerable efforts have been devoted in the regeneration of NADH from NAD+. Direct electrochemical methods for reduction of NAD+ to NADH suffers from high overvoltage and side reactions. Thus, several attempts have been made using various mediators to decrease the overvoltage, so that electrochemical reactions can be used to regenerate one-redox form of the cofactor when a dehydrogenase reaction is used in organic synthesis. Whitesides and coworkers first reported the enzyme-catalyzed reduction of sodium pyruvate with electrochemical regeneration of NAD(P)+ using flavoenzyme and methyl viologen as the electro-transfer mediator and then other also used various free mediators in enzyme-catalyzed organic synthesis[14]. As we discussed in above, a fast electron transfer occurs at low applied potential between NAD+ and reduced forms of phenothiazine derivatives such as TBO. Thus, we have initiated to investigate the enzymatic reduction of pyruvate using water soluble polyacrylamide bound covalently with TBO (Scheme 3).



Scheme 3 Enzymatic reduction of pyruvate using polyacrylamide-TBO

A typical reaction procedure is as follows; A 150mL beaker was used as a reaction vessel. In the cathodic chamber (a cellulose fabular membrane a flat width 32mm, molecular weight cutt off ~1400), 15mL solution containing tris/HCl (pH 8.0), 0.3g TBO-polymer (the molecular ratio of TBO and acrylamide was 20:1), K_2SO_4 (50mmol), 0.33g sodium pyruvate (3mmol), 2.3mg ß-mercaptoethanol, L-lactic dehydrogenase (5mg), and NADH (10mg) were placed. A platinum plate (1x3cm) as the cathode was immersed into the sodium. The analyte solution was consisted of 100mM tris/HCl, pH 8.0 buffer solution. An anodic platinum wire (1mm diameter) was coiled over the membrane tubing. A reference electrode (Ag/AgCl) was immersed into the solution. The solution was purged with nitrogen for 1hr to eliminate oxygen dissolved. The reaction was carried out under nitrogen atmosphere at ambient temperature (22-24°C) with confinously stirring by a magnetic stirrer. The potental of the platinum electrode was adjusted to -100mV (vs. Ag/AgCl). The reaction was completed in 7days, lactic acid produced was isolated by acidity of the solution followed by extraction with ethylacetate (yield 95.5%). We determined the absolute configuration of the enzymatically reduced product; L-lactic acid on the basis of the analysis of 1H NMR spectra of the MTPA derivatives. The lactic acid was methylated with diazomethane in ether to corresponding methyl ester, which has reacted with (s)-(+)MTPA-Cl in CCl_4 in the presence of 3-(dimethylamino) propylamine in yielding of MTPA derivatives[15]. ^{1}H-NMR spectra (300MHz) of MTPA ester of L-lactate ester was showed 99% ee based on the signal of the methoxyl groups.

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