

## Short Communication

## Low Potential Detection of NADH at Titanium-Containing MCM-41 Modified Glassy Carbon Electrode

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**Abstract**

Titanium-containing MCM-41 (Ti-MCM-41) modified glassy carbon electrode (GCE) can exhibit an excellent electrocatalytic activity towards the oxidation of  $\beta$ -Nicotinamide adenine dinucleotide (NADH). A dramatic decrease in the over-voltage of NADH oxidation reaction is observed at 0.28 V (vs. SCE). The modified electrode is found to be stable and reproducible. The electrode shows a linear response for a wide range of 10–1200  $\mu$ M NADH and the detection limit is 8.0  $\mu$ M. Ti-MCM-41 mesoporous molecular sieves provide an efficient matrix for development of NADH biosensors and the prepared electrode not only can be used to detect the concentration of NADH in biochemical reaction, but also as the potential matrix of the construction of dehydrogenases biosensor.

**Keywords:** NADH, Ti-MCM-41, Electrocatalytic, Biosensors, Dehydrogenases

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$\beta$ -Nicotinamide adenine dinucleotide (NADH) is involved as a cofactor in several hundred enzymatic reactions of  $\text{NAD}^+/\text{NADH}$ -dependent dehydrogenases. The electrochemical oxidation of NADH has thus been the subject of numerous studies related to the development of amperometric biosensors [1, 2]. Problems inherent to such anodic detection are the large overvoltage encountered for NADH oxidation at ordinary electrodes [3] and surface fouling associated with the accumulation of reaction products [4]. The formal potential of  $\text{NAD}^+/\text{NADH}$  in neutral pH is significant overpotential as large as 1.0 V for the direct oxidation at bare electrodes [5, 6]. Consequently, considerable effort has been devoted toward the goal of identifying new electrode materials and new methods that will reduce the overpotential for NADH oxidation and minimize surface passivation effects [7, 8]. More traditional methodology incorporates the use of mediators to catalyze the NADH reaction. Several mediators have been studied to date including ortho- and para-quinones, phenylenediamines, phenoxazines, alkylphenazines, phenothiazines, neutral red and phenothiazine dyes [9–13]. But the fast electron transfer between the electrode and the mediators and between the mediators and NADH and the stability and toxicity of the mediators limit their *in vivo* applications. Furthermore, some new materials such as carbon nanotube [7], carbon fiber [14, 15] and nanotube chitosan [16] et al. and some immobilized methods [17, 18] have been used to decrease the overvoltage of NADH and used to detect NADH concentration which promote the biosensor development greatly. Recently, the unique structural and catalytic

properties of mesoporous molecular sieves for structuring an electrochemical/electron transfer environment and resistance to biodegradation have attracted considerable attention [19]. Mesoporous molecular sieves have a large specific surface area, high mechanical, thermal, and chemical stability, good adsorption and penetrability. With the appropriate dimensions and functionalizations, it can act as current nanocollectors and as electron relays to an electrode [20] which brings new capabilities to electrochemical devices and has many potential sensing applications [21–23]. However, to the best of our knowledge, up to now, there is no report on reducing the overpotential for NADH oxidation on mesoporous materials. In this communication we report the oxidation of NADH at glassy carbon electrodes (GCEs) modified with titanium-containing MCM-41 (Ti-MCM-41) coating and the application in NADH biosensor. Ti-MCM-41 modified GCE offers a marked decrease in the overvoltage for NADH oxidation reaction (compared to bare GCE) to be observed at 0.28 V (vs. SCE; pH 6.2) and to circumvent NADH surface fouling effects. The sensor exhibits a good electrocatalytic behavior and a good stability to NADH.

The characterization of the prepared Ti-MCM-41 has been done with UV-visible spectra, energy dispersive spectroscopy (EDS) and  $\text{N}_2$  adsorption isotherms. In the UV-visible spectra of Ti-MCM-41, the band at 220 nm which has been assigned to Ti in tetrahedral coordination [24] is observed indicating Ti is incorporated into the framework of MCM-41. EDS shows that the atom ratio of Si to Ti is about 1:0.01.  $\text{N}_2$  adsorption isotherm shows the mesopore diam-

eter is about 2.81 nm and the specific surface area is about  $1050 \text{ m}^2 \text{ g}^{-1}$ . The parameters of Ti-MCM-41 are listed in Table 1.

Table 1. Pore Characterization of Ti-MCM-41.  $A_{\text{BET}}$ , specific surface area;  $d_{100}$ , XRD  $d_{100}$  space;  $a_0$ , lattice parameter;  $D$ , mesopore diameter;  $L$ , wall thickness; Si:Ti, the atom ratio of Si to Ti

$A_{\text{BET}}$ ( $\text{m}^2 \text{ g}^{-1}$ )	$d_{100}$ (nm)	$a_0$ (nm)	$D$ (nm)	$L$ (nm)	Si:Ti
1050	3.84	4.43	2.81	1.62	1:0.01

The morphologies of Ti-MCM-41/PVA and PVA films were characterized by scanning electron micrograph (SEM). From SEM images, we can see Ti/MCM-41/PVA film displayed a chemically clean uniform structure while bare PVA film did not display any distinct feature (Fig. 1). The aggregates of the Ti-MCM-41 matrix on modified electrode surface were well distributed.

The main objective of the present investigation was whether Ti-MCM-41 modified GCE improve the oxidation of NADH. It was found that no peak was observed at a bare GCE. Upon addition of NADH, the oxidation resulted in a peak with the potential of +0.70 V (Figures not shown). No peak was observed at Ti-MCM-41 modified GCE without the addition of NADH (curve a in Figure 2), which showed Ti-MCM-41 was electroinactive in the potential window. Upon addition of NADH, Ti-MCM-41 modified GCE exhibited a dramatic enhancement in the anodic peak current at 0.28 V (curve b in Figure 2). The enhanced anodic current in the presence of NADH came from the oxidation of NADH to  $\text{NAD}^+$  and the value of anodic peak potential of NADH oxidation of 0.28 V obtained at present case was more negative than 0.33 and 0.36 V at multi-wall CNTs and single-wall CNTs modified GCEs, respectively [7]. These results indicated that Ti-MCM-41 was more effective for electron transfer from NADH. From Figure 2, it was also found the magnitude of the catalytic current was proportional to the solution concentration of NADH, which

covered the values of a great relevance in biosensor design and application.

The presence of regular porous structure has already showed its benefits on the analytical performance and has been used to improve NADH oxidation or to reduce the overpotential [25–27]. In the present case, the decrease of the potential of NADH oxidation might be related to the higher hydrophilicity induced by Ti-MCM-41 at the modified electrode. The hydration water of Ti-MCM-41 also proffered to the electrode surface a better contact with the solution containing NADH and consequently could participate in increasing the electron transfer rate. Furthermore, regular mesoporous structure has much larger specific surface area ( $1050 \text{ m}^2 \text{ g}^{-1}$ ) than amorphous silica gels ( $300 \text{ m}^2 \text{ g}^{-1}$ ) which leads to the increase of the signal response and can be expressed by the lowering of the overvoltage for NADH oxidation. For comparison, cyclic voltammograms of amorphous silica gels doped with  $\text{TiO}_2$  with addition of NADH is shown as curve d in Figure 2. The anodic potential of NADH oxidation is 0.35 V under the

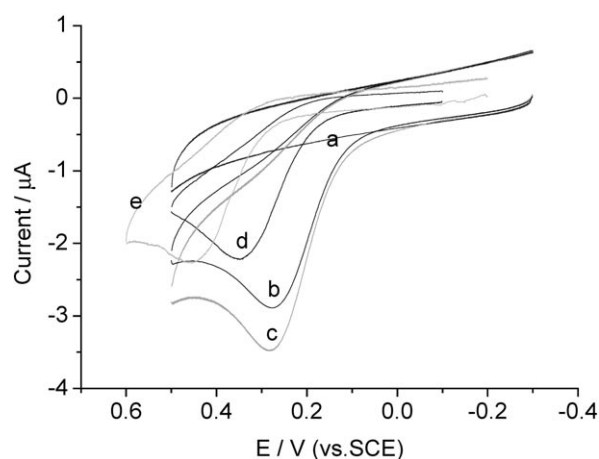


Fig. 2. Cyclic voltammograms of Ti-MCM-41 modified GCE in the absence (a), the presence of 0.10 (b), 0.15 mM (c) NADH and amorphous silica gels doped with  $\text{TiO}_2$  (d) and MCM-41 (e) modified GCEs in the presence of 0.10 mM NADH in 0.1 M pH 6.2 PBS at  $0.050 \text{ V s}^{-1}$ .

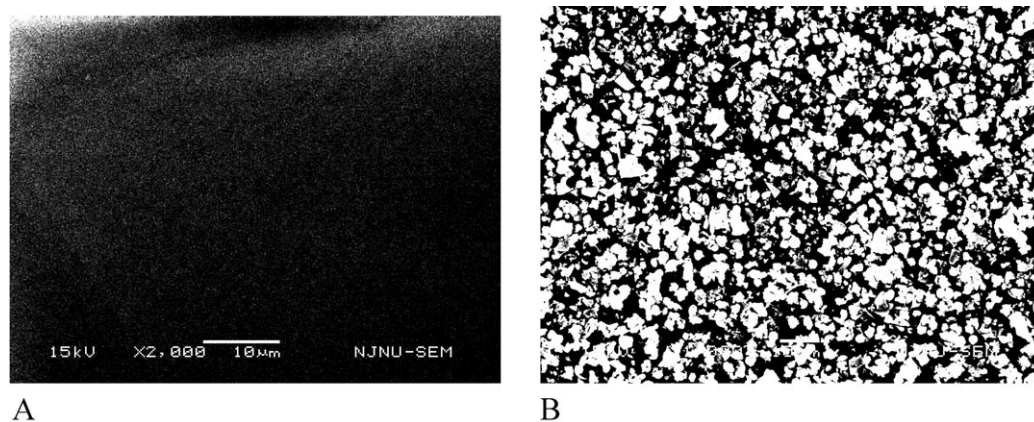


Fig. 1. SEM images of the PVA film before (A) and after (B) dropping on Ti-MCM-41.

same experimental conditions which is the same value as that of amorphous silica gels doped with Nb<sub>2</sub>O<sub>5</sub> [28].

The oxidation of NADH on MCM-41 modified GCE was also shown in curve e Figure 2. It was obviously that upon addition of NADH to phosphate buffer solutions (PBS), the anodic peak also appeared, but it appeared at 0.45 V which was more positive than 0.28 V of Ti-MCM-41 modified GCE (curve b in Figure 2). The substantial decrease in potential observed at Ti-MCM-41 was attributed to acceleration of the proton-transfer step by the metal oxide component of the composite [29].

The amperometric responses of the Ti-MCM-41 modified GCE upon successive additions of 10 mM NADH to 0.1 M pH 6.2 PBS at an applied potential of 0.28 V were shown in Figure 3. Upon addition of an aliquot of NADH to the buffer solution, the oxidized current decreased steeply to reach a stable value. The enzyme electrode achieved 95% of the steady-state-current in less than 10 seconds. The results demonstrated clearly that the electrocatalytic response was very fast, which could be used as an efficient sensor for NADH detection.

The calibration curve of the sensor under the optimized experimental conditions was shown in inset A Figure 3. The calibration range of NADH was done from 10 to 1500 μM. The linear response range of the sensor to NADH concentration was from 10 to 1200 μM with a correlation coefficient of 0.9989. The linear range was wider than that of 3–50 μM from GCEs modified with transition metal complexes containing 1,10-phenanthroline-5,6-dione ligands [30] and 10<sup>-4</sup>–10<sup>-2</sup> M from enzyme modified by electropolymerization of aminobenzene isomer and PPQ on electrode [31]. The sensitivity of the electrode was found to be 0.0018 μA μM<sup>-1</sup> and the detection limit of 8.0 μM were obtained at a signal to noise ratio of 3. The detection limit was lower than

5 × 10<sup>-4</sup> from electrode modified by PPQ [31] which was due to the large specific surface area and high enzyme loading on the porous materials.

An extremely attractive feature of the prepared sensor is whether Ti-MCM-41 modified GCE is stable. The electrode can keep 85% of its initial current response when successively sweeps for 60 min, which suggests it can circumvent NADH surface fouling effects.

The detection reproducibility of the developed sensor in the on-line assay for NADH was studied by establishing a flow injection analysis (FIA) system with a thin-layer cell. The flow rate used for NADH measurements was an important parameter since the process involves the enzymatic reaction kinetics and the diffusion of NADH. An optimal flow rate of 1.2 mL min<sup>-1</sup> was obtained by evaluating the analytical performance of the sensor, peak width and the measurement reproducibility. The reproducibility ascertained by monitoring the current response for ten replicate injections of 0.10 mM NADH with an applying potential of 0.28 V was shown in inset B Figure 3. The relative standard deviation (RSD) was 4.2%, indicating a good reproducibility of the sensor for FIA. Thus NADH did not leak out of the film in the process of FIA, and the sensor could repeatedly be used for FIA or on-line determination of NADH. The fabrication reproducibility of six electrodes, made independently, showed an acceptable reproducibility with a RSD of 7.2% for the current determined at 0.10 mM NADH.

In summary, we present a new material of NADH detection. Ti-MCM-41 modified GCE can exhibit an excellent electrocatalytic activity towards the oxidation of NADH. It can reduce the overvoltage of NADH oxidation reaction dramatically and can circumvent NADH surface fouling effects. NADH displays a good reproducibility and a

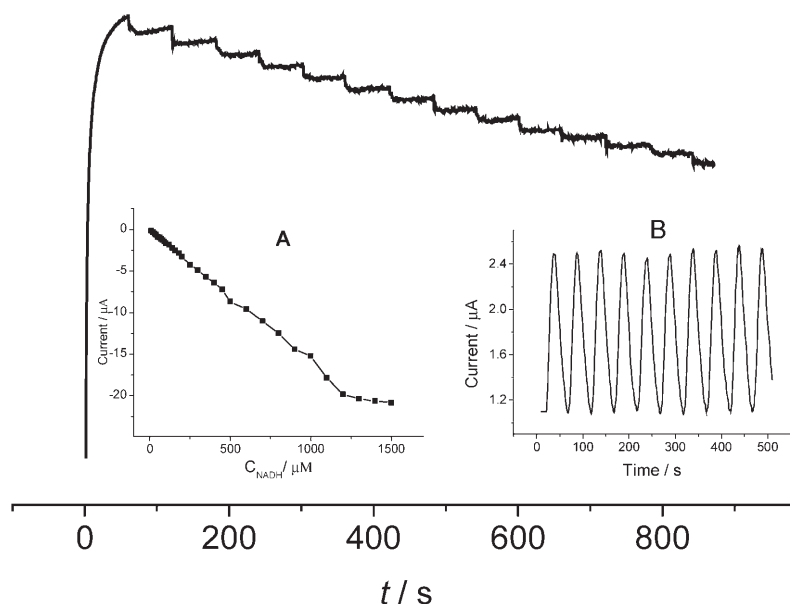


Fig. 3. Amperometric responses of Ti-MCM-41 modified GCE at 0.28 V upon successive additions of 5 μL 10 mM NADH to 5.0 mL 0.1 M pH 6.2 PBS. Inset A: Plot of electrocatalytic currents vs. NADH concentration. Inset B: FIA for 10 replicate injections of 0.10 mM NADH at the Ti-MCM-41 modified GCE at 0.28 V. Carrier solutions: 0.1 M pH 6.2 PBS with a flow rate of 1.2 mL min<sup>-1</sup>.

good stability. Ti-MCM-41 modified electrode can be used in biosensors to study the electrocatalytic reaction of biological important systems because the method of preparation is simple and the Ti-MCM-41 can present a good matrix for efficient redox reactions of biomolecules.

### Experimental

NADH was purchased from Sigma and used as received. PVA (average degree of polymerization,  $1800 \pm 100$ ) was purchased from Shanghai Laize Factory of Fine Chemicals. Tetrabutyl titanate was purchased from Shanghai Reagent Factory. All other chemicals were of analytical grade and were used without further purification. All solutions were made up with twice-distilled water. The solution of NADH was prepared freshly before each experiment. The 0.1 M PBS, which were made up of  $\text{Na}_2\text{HPO}_4$ ,  $\text{NaH}_2\text{PO}_4$  and  $\text{H}_3\text{PO}_4$ , were employed as a supporting electrolyte.

Ti-MCM-41 was prepared following a recipe similar to that reported by Tatsumi [32]. 30 mg Ti-MCM-41 was dispersed into 10 mL twice-distilled water and stirred for 3 hours to obtain  $3 \text{ mg mL}^{-1}$  Ti-MCM-41 suspension, then mixed with  $5 \mu\text{L}$  3% PVA solution of ethanol/water (V:V 1:1) to produce Ti-MCM-41/PVA that was used for the following work.

The GCE (3 mm in diameter) were polished to a mirror-like finish with 1.0, 0.3 and 0.05  $\mu\text{m}$  alumina slurry (Beuhler) followed by rinsing thoroughly with doubly distilled water. The electrodes were successively sonicated in 1:1 nitric acid, acetone and doubly distilled water, and then allowed to dry at room temperature.  $3 \mu\text{L}$  Ti-MCM-41/PVA colloid was dropped on the pretreated GCE surface and allowed to dry under ambient condition for 3 hours. After the modified electrode was rinsed with doubly distilled water twice or thrice, Ti-MCM-41 modified GCE was obtained. When not in use the electrode was stored in 0.1 M pH 6.2 PBS at  $4^\circ\text{C}$ .

UV-visible spectra were recorded on a Hitachi 340 spectrometer. Nitrogen adsorption measurements were performed on a Belsorp 28SA analyzer. EDS was performed on Thermo Noran analyzer. SEM was obtained using a JEOL JSM-5610LV Scanning Electron Microscope. Cyclic voltammetric and amperometric measurements were performed on CHI 660 electrochemical workstation (CH Instruments, USA). All electrochemical experiments were carried out in a cell containing 5.0 mL 0.1 M pH 6.2 PBS and using a platinum wire as auxiliary, a saturated calomel electrode as reference and the Ti-MCM-41 modified GCE as working electrodes. Amperometric measurements were done by successive additions of NADH solution into a continuously stirred PBS. The sensor responses were measured as the difference between total and residual currents. Two pumps of Luminescence Analyzer (IFFM-D) were used for FIA to deliver flow streams. The flow rate was optimized at  $1.20 \text{ mL min}^{-1}$ . All experiments were carried out at  $(25 \pm 2^\circ\text{C})$ .

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