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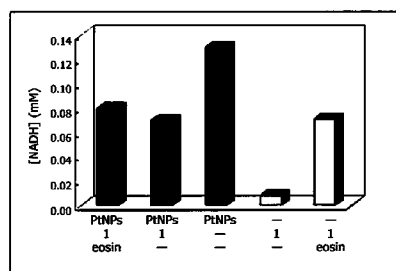
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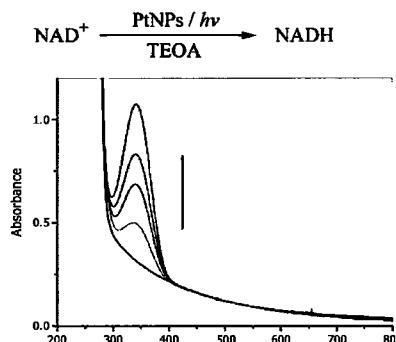
(54) Title: PHOTOCATALYTIC PRODUCTION METHOD OF OXIDOREDUCTASE COFACTORS USING PT NANOPARTICLES

[Fig. 1]

(A)



(B)



(57) Abstract: Provided is a method for photochemical regeneration of oxidoreductase cofactors including (a) adding oxidative oxidoreductase cofactors and platinum (Pt) nanoparticles to an oxidoreductase reactor, and (b) irradiating light to the resulting mixture of step (a), thereby photochemically regenerating the oxidoreductase cofactors by conversion into reductive oxidoreductase cofactors through photoreactions in the presence of the Pt nanoparticles as redox mediators. This method can be very useful in increasing efficiencies of various biocatalytic reactions using the oxidoreductase cofactors.

Description

Title of Invention: PHOTOCATALYTIC PRODUCTION METHOD OF OXIDOREDUCTASE COFACTORS USING PT NANOPARTICLES

Technical Field

- [1] The present invention relates to a method for photocatalytic regeneration of oxidoreductase cofactors, including (a) adding platinum (Pt) nanoparticles to oxidative oxidoreductase cofactors, and (b) irradiating light to the resulting mixture of step (a) to be converted into reductive oxidoreductase cofactors through photoreactions in the presence of Pt nanoparticles as redox mediators.
- [2] This work was supported by the SRC/ERC program of MOST/KOSEF (R11-2005-008-000-0) and National Research Foundation of Korea Grant funded by the Korean Government (2009-0087304).

Background Art

- [3] Great efforts for the development of alternative energy supplies have been made as fossil fuel reserves are depleting. There have been attempts to convert radiant electromagnetic energy into chemical energy in the form of reducing potential, i.e., dihydronicotinamide adenine dinucleotide (NADH) using a photochemical system.
- [4] Nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) are important cofactors found in cells, and reduced into NADH and NADPH, respectively.
- [5] NAD is used for glycolysis and a TCA cycle in cellular respiration, and the reducing potential storing in NADH is converted into ATP through an electron transport system or used for anabolism. NADPH provides reducing potential and is used for anabolism such as a synthesis of fatty acid and nucleic acid. NADP serves as a critical oxidizing agent in an initial reaction of photosynthesis, i.e., photolysis of water, and forms NADPH. NADPH provides reducing potential to a Calvin cycle of photosynthesis.
- [6] FAD and FMN are kinds of prosthetic groups of a flavin enzyme group, which involve hydrogen and electron transport in a redox system in a living body. FAD refers to riboflavin adenine dinucleotide, which is a nucleotide composed of a compound of riboflavin, two phosphates, and adenosine, and FMN refers to riboflavin phosphate, which corresponds to phosphate ester of riboflavin. FAD and FMN are critical materials transporting hydrogen and electrons in the redox system in the living body. They serve as cofactors of the flavin enzyme group, and involve electron transport to an electron receptor from a substrate.
- [7] In the nature system, photosynthesis is performed in chloroplasts in cells of green

plants. In light reactions, O_2 is produced, and in light-independent or dark reactions, glucose is synthesized and water is produced. More particularly, chlorophyll and electron transport systems are included at the thylakoid membrane at which the light reactions occur. The light reactions are classified into water photolysis and photophosphorylation. The water photolysis is a process of splitting water by light energy absorbed by the chlorophyll, thereby producing electrons (e^-), hydrogen ions, and oxygen. The photophosphorylation is a process of converting light energy absorbed by the chlorophyll into chemical energy to produce ATP. In the photophosphorylation, when light energy is absorbed by the chlorophyll, the chlorophyll is excited, and thus electrons are emitted. The electrons produce ATPs through the electron transport system. During the photophosphorylation, $NADPH_2$ is also produced to be used in the light-independent reaction, occurring at the stroma of the chloroplasts to synthesize glucose from carbon dioxide using ATP and $NADPH_2$ produced in the light reaction.

- [8] A system mimicking the natural photosynthesis typically contains three independent components of a photosensitizer, an electron transport, and a catalyst.
- [9] NADH and NADPH are used as cofactors in various biological reactions performed by many redox enzymes. The photocatalytic production of NADH and NADPH has attracted great interest due to its relevance to solar energy conversion and artificial photosynthesis. As the cofactor being used in stoichiometric amounts for the biological reactions is expensive for wide applications, efficient systems of carrying out solar energy capture and conversion are to be developed in the interest of regeneration of NADH and NADPH. Furthermore, attempts to develop visible light-driven systems have been carried out, because the visible light accounts for 46% of the total solar energy [Diwald, O. et al. Photochemical activity of nitrogen-doped rutile $TiO_2(110)$ in visible light. *J. Phys. Chem. B* **108**, 6004-6008 (2004)].
- [10] As examples of the three components for the NADH photoproduction by visible light, light-harvesting inorganic dyes were coupled with methyl viologen and dehydrogenases as an electron carrier/catalyst pair, in the presence of a sacrificial electron donor [Willner, I. & Mandler, D. Enzyme-catalysed biotransformations through photochemical regeneration of nicotinamide cofactors. *Enzyme Microb. Technol.* **11**, 467-483 (1989); Gratzel, M. Artificial photosynthesis: water cleavage into hydrogen and oxygen by visible light. *Acc. Chem. Res.* **14**, 376-384 (1981); Mandler, D. & Willner, I. Solar light induced formation of chiral 2-butanol in an enzyme-catalyzed chemical system. *J. Am. Chem. Soc.* **106**, 5352-5353 (1984)].
- [11] In a few attempts for non-enzymatic photochemical regeneration of NADH/NADPH, mononuclear Rh complexes were used as an electron transport and a hydride transport catalyst in the presence of $Ru(bpy)_3^{2+}$ as the photosensitizer [Wienkamp, R. & Steckhan, E. Selective generation of NADH by visible light. *Angew. Chem. Int. Ed.*

Engl. **22**, 497 (1983)]. In order to assemble all communicating components together for more practical applications, co-immobilization of photosensitizers, redox mediators, catalysts or enzymes has been also investigated. For example, hydrogenase immobilized on CdS particles as photosensitizers was reported to produce NADH. However, such integrated systems usually faced the problem of lower activities than those with comparable homogeneous ones.

- [12] While a method for electrochemical regeneration of oxidoreductase cofactors using metal nanoparticles has been disclosed in Korean Patent Publication No. 2010-0011160, this method requires a first redox mediator such as an Rh II complex, and has a low regeneration yield.

[13]

Disclosure of Invention

Technical Problem

- [14] The present invention is directed to a system for effective photochemical regeneration of oxidoreductase cofactors by introducing Pt nanoparticles (PtNPs).

Solution to Problem

- [15] One aspect of the present invention provides a method for efficient photochemical regeneration of oxidoreductase cofactors using Pt nanoparticles having excellent activities of a photosensitizer, an electron transport and a catalyst. When a visible light is irradiated to the Pt nanoparticle solution in the presence of electron donors, oxidative oxidoreductase cofactors are converted into reductive oxidoreductase cofactors in a good yield, and thus the present invention is completed.

Advantageous Effects of Invention

- [16] As described above, a method for photochemical regeneration of oxidoreductase cofactors using Pt nanoparticles and light irradiation is provided, which may be very useful in increasing efficiency of various biocatalytic reactions of the oxidoreductase cofactors using the Pt nanoparticles.
- [17] Particularly, the photochemical regeneration method regenerates NADH in a yield three times higher than that of a conventional electrochemical regeneration method.

Brief Description of Drawings

- [18] FIG. 1(A) shows comparison of the yield of NADH produced under different reaction conditions {PtNPs: 1 unit (92.5 μ M Pt atoms); **1**: 0.01 mM [Cp*Rh(bpy)(H₂O)]²⁺; and eosin: 0.05 mM} with 0.2 mM NAD⁺ and 0.4 M TEOA in a 0.15 M phosphate buffer (pH = 7.0) for 12 hours at 25 °C
- [19] FIG. 1(B) shows UV-vis absorbance spectra of NADH at time intervals (0, 3, 6, 9, and 12 h) of light irradiation ($\lambda > 400$ nm) with 1 unit of PtNPs, 0.2 mM NAD⁺, and 0.4 M TEOA in a 0.15 M phosphate buffer (pH = 7.0) at 25 °C

- [20] FIG. 2(A) shows rates of photochemical NADH formation under different concentrations of PtNPs [1 unit (■), 2 units (●), 3 units (▲), and 4 units (▼)] with 0.2 mM of NAD⁺ and 0.4 M of TEOA in a 0.15 M phosphate buffer (pH = 7.0).
- [21] FIG. 2(B) shows a plot of turnover numbers per hour obtained with PtNPs in 0.2 mM NAD⁺ and 0.4 M TEOA for 12 hours, the numbers being calculated with respect to the amount of PtNPs assumed as a nanoparticle containing 2800 Pt atoms;
- [22] FIG. 3 shows an effect of pH on the photoregeneration of NADH with 1 unit of PtNPs, 0.2 mM NAD⁺, and 0.4 M TEOA in a 0.15 M phosphate buffer after 9 hours of light irradiation ($\lambda > 400$ nm);
- [23] FIG. 4(A) shows comparison of NADH formation under different concentrations of TEOA with 1 unit of PtNPs and 0.2 mM NAD⁺ in a 0.15 M phosphate buffer;
- [24] FIG. 4(B) shows rates of NADH formation under different concentrations of NAD⁺ [0.1 mM (■), 0.2 mM (▲), and 0.3 mM (●)] with 1 unit of PtNPs and 0.4 M TEOA in a 0.15 M phosphate buffer (pH = 7.0);
- [25] FIG. 5 shows electron-transport communication among PtNPs, NAD⁺, and TEOA for catalytic cycles of NADH generation. Charge separation and electron transfer occur by the photoexcited PtNPs;
- [26] FIG. 6 shows absorbance spectral changes in NADH formation from a system composed of (A) 1 unit PtNPs, 0.01 mM [Cp*Rh(bpy)(H₂O)]²⁺, 0.05 mM eosin, 0.2 mM NAD⁺, and 0.4 M TEOA ; (B) 1 unit PtNPs, 0.01 mM [Cp*Rh(bpy)(H₂O)]²⁺, 0.2 mM NAD⁺, and 0.4 M TEOA in 0.15 M phosphate buffer after 12 h of irradiation ($\lambda > 400$ nm). The absorption band around 500 nm derived from eosin;
- [27] FIG. 7 shows fluorescence spectra of 0.5 μ M eosin in the absence of and after the addition of 4 unit PtNPs. The excitation wavelength is 524 nm;
- [28] FIG. 8 shows absorption spectral changes in NADH production under different concentrations of (A) 2 and (B) 3 units of PtNPs with 0.2 mM NAD⁺ and 0.4 M TEOA in a 0.15 M phosphate buffer at 0, 3, 6, 9 and 12 hours;
- [29] FIG. 9 shows effect of pH on the NADH photoregeneration from a system composed of 1 unit of PtNPs with 0.2 mM NAD⁺ and 0.4 M TEOA in a 0.15 M phosphate buffer at 0, 3, 6, 9 and 12 hours after irradiation;
- [30] FIG. 10 shows absorbance spectral changes in NADH regeneration under different concentrations of TEOA (A) 200mM and (B) 300 mM with 1 unit PtNPs, and 0.2 mM NAD⁺ in 0.15 M phosphate buffer at 0, 3, 6, 9, and 12 h;
- [31] FIG. 11 shows absorbance spectral changes in NADH regeneration using Au nanoparticles;
- [32] FIG. 12 shows absorbance spectral changes in NADH regeneration using Pd nanoparticles;
- [33] FIG. 13 shows electrochemical NADH production using PtNPs;

- [34] FIG. 14 shows absorbance spectral changes in NADH regeneration with 1 mM EDTA as an electron donor;
- [35] FIG. 15 shows absorbance spectral changes in NADH regeneration with 400 mM triethylamine (Et_3N) as an electron donor; and
- [36] FIG. 16 shows absorbance spectral changes in NADH regeneration with 100 mM sodium (Na)-formate as an electron donor.

[37]

Mode for the Invention

- [38] Hereinafter, exemplary embodiments of the present invention will be described in detail. However, the present invention is not limited to the exemplary embodiments disclosed below, but can be implemented in various types. Therefore, the present exemplary embodiments are provided for complete disclosure of the present invention and to fully inform the scope of the present invention to those ordinarily skilled in the art.
- [39] The present invention provides a method for photochemical regeneration of oxidoreductase cofactors, including: (a) adding PtNPs to oxidative oxidoreductase cofactors; and (b) irradiating light to the resulting mixture in step (a).
- [40] In the present invention, step (a) is to add PtNPs to oxidative oxidoreductase cofactors.
- [41] The oxidative oxidoreductase cofactors subject to reduction in the present invention may be nicotinamide cofactors such as nicotinamide adenine dinucleotide (NAD^+) or nicotinamide adenine dinucleotide phosphate (NADP^+), or flavin cofactors such as flavin adenine dinucleotide (FAD^+) or flavin mononucleotide (FMN^+), and preferably NAD^+ .
- [42] In the present invention, electron donors may be added to a reactor of the oxidoreductase of step (a), and triethanolamine is preferably used as the electron donor.
- [43] In the present invention, a concentration of the Pt nanoparticles added to the reactor of the oxidoreductase may be at least 1 unit (here, 1 unit contains $92.5 \mu\text{M}$ of Pt atoms), and preferably, 2 to 4 units.
- [44] In the present invention, the reaction in step (a) may be performed at pH 7 or more, and preferably 7 to 10.
- [45] The light irradiated to the mixture of step (a) may be a visible light.
- [46] In step (a), the reaction may be performed in a buffer, which may be, but is not limited to, a phosphate buffer, a Tris buffer, an ammonium phosphate buffer or an MES buffer.
- [47]
- [48] *Before the light irradiation to the mixture of step (a), air may be removed from the

mixture, for example, by evacuation.

[49] The photoregeneration of the oxidoreductase cofactors can be examined under an aerobic or anaerobic condition.

[50] In the present invention, the PtNPs may independently serve as light absorbing units. In detail, the PtNPs may act as a photosensitizer as well as an electron transport and a catalyst. In addition, the PtNPs may capture photons under a visible light.

[51] The present invention provides a device for photochemically regenerating oxidoreductase cofactors, including: a reactor including oxidative oxidoreductase cofactors and PtNPs; and a light irradiation device.

[52] The present invention also provides a method of reducing an enzyme using the oxidoreductase cofactors regenerated by the above method. To examine the function of NADH regenerated by the above method, production of L-glutamate by the regenerated NADH can be confirmed by the conversion of α -glutamate into L-glutamate (see Example 8).

[53] Examples of the convertible enzymes may include, but are not limited to, glutamate, formate dehydrogenase, and glucose-6-phosphate dehydrogenase.

[54] "NAD" refers to nicotinamide adenine dinucleotide, "NADP" refers to nicotinamide adenine dinucleotide phosphate, "FAD" refers to flavin adenine dinucleotide, "FMN" refers to flavin mononucleotide, "PtNP" refers to a platinum nanoparticle, "TEOA" refers to triethanolamine, "Cp*" refers to pentamethylcyclopentadienyl, and "bpy" refers to 2,2'-bipyridine.

[55] Hereinafter, specific configurations and actions of the present invention are described with reference to examples, but the scope of the present invention is not limited to the following examples.

[56] [Examples]

[57] [Preparation of Reagents]

[58] (1) Water was purified with a MilliQ purification system. All reagents purchased from Aldrich were used.

[59] (2) $[\text{Cp}^*\text{Rh}(\text{bpy})]^{2+}$ was prepared according to a known method[Kolle U., Kang B.-S., Infelta P., Comte P. and Gratzel M. Elektrochemische und Pulsradiolytische Reduktion von (Pentamethylcyclopentadienyl)(polypyridyl)

[60] rhodium-Komplexen, *Chem. Ber.*, **122**, 1869-1880 (1989); Steckhan, E., Herrmann, S., Ruppert, R., Dietz, E., Frede, M., Spika, E. *Organometallics***10**, 1568- (1991)].

[61]

[62] **Preparation Example 1: Preparation of PtNPs**

[63] PtNPs were prepared by reducing potassium tetrachloroplatinate (K_2PtCl_4) with polyvinylpyrrolidone (MW = 10k).

[64] In detail, a hot aqueous mixture of K_2PtCl_4 (11.5 mM, 120 mL) was reacted with

polyvinylpyrrolidone (3 g) for 4 hours. The reaction was performed by stirring at 90 to 100 °C for 4 hours, and the solution obtained by the reaction was dark brown or black.

[65] Here, 1 unit of PtNPs solution was a solution containing 92.5 µM of Pt atoms.

[66]

[67] **Comparative Preparation Example 1: Preparation of Au nanoparticles**

[68] Au nanoparticles were prepared by reducing H₂AuCl₄ with polyvinylpyrrolidone (MW = 10k).

[69] In detail, H₂AuCl₄ (11.5 mM, 120 mL) was reacted with polyvinylpyrrolidone (3 g) for 4 hours. The reaction was performed by stirring at 90 to 100 °C for 4 hours, and the solution obtained by the reaction was red.

[70]

[71] **Comparative Preparation Example 2: Preparation of Pd nanoparticles**

[72] Pd nanoparticles were prepared by reducing K₂PdCl₄ with polyvinylpyrrolidone (MW = 10k).

[73] In detail, K₂PdCl₄ (11.5 mM, 120 mL) was reacted with polyvinylpyrrolidone (3 g) for 4 hours. The reaction was performed by stirring at 90 to 100 °C for 4 hours, and the solution obtained by the reaction was light black.

[74]

[75] **Example 1: Size and Distribution of PtNPs**

[76] The size and distribution of PtNPs were examined on transmission electron microscopy (TEM) and dynamic light scattering instruments (Malvern, Zetasizer Nano ZS). The nanoparticles had a size of 5±2nm, and were formed in an almost circular shape.

[77]

[78]

[79] **Example 2: UV-vis Spectra and TEM Images**

[80] UV-vis spectra were recorded on a Hewlett Packard 8458 spectrophotometer. TEM images were recorded on a JEOL 2010FX electron microscope operating at 200 kV. Emission spectra were collected on a Perkin-Elmer LS55 luminescence spectrophotometer.

[81] UV-vis was performed to measure a concentration of NADH at 340 nm, and luminescent images were taken to show that luminance of eosin was extinct due to the PtNPs.

[82]

[83] **Example 3: Photochemical Regeneration of NADH**

[84] The photochemical regeneration of NADH was carried out in the presence of 1 unit of PtNPs, 0.4 M TEOA, and 0.2 mM NAD⁺ in 0.15 M phosphate buffer (1 mL, pH 5-9) at 25 °C. Photoreactions were performed in a 3 mL glass cuvette (vial) equipped

with a magnetic stirrer and a stopper.

- [85] Samples (1 mL) consisting of all the components were transported into the cuvette and de-aerated samples were prepared by repeated evacuation followed by Ar flushing. A 330 W Xe lamp equipped with a 420 nm cut-off filter was used as a light source. The production of NADH was determined by UV absorption at 340 nm ($\epsilon = 6220 \text{ cm}^{-1} \text{ M}^{-1}$).
- [86] In the photochemical reduction of NAD^+ with eosin/PtNPs/ $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})]^{2+}$ / TEOA in phosphate buffer (pH = 7.0) at room temperature, a 40% conversion of NAD^+ into NADH was obtained after 12 hours of light irradiation with a 420 nm cut-off filter [see FIGS. 1(A) and 6(A)]. The photochemical reaction was run in the absence of eosin, but surprisingly produced a similar amount of NADH (36%, see FIG. 6(B)). This result demonstrates that PtNPs alone can act as a light-absorbing unit.
- [87] Meanwhile, even exclusion of both $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})]^{2+}$ and eosin provided clean spectral changes around 340 nm during photolysis and produced 59% NADH under the same conditions (see FIG. 1). This result clearly demonstrates that visible light-driven electron flow occurred from PtNPs to NAD^+ at the expense of TEOA and PtNPs alone acted as a photosensitizer as well as an electron transport and a catalyst.
- [88] When both PtNPs and TEOA were removed from the system, no photosensitized formation of NADH was observed. It should be noted that the fluorescence of eosin was quenched by PtNPs (see FIG. 7), but energy transport from the excited eosin did not improve the yield of NADH.
- [89] In order to compare the photocatalytic activity between 1 unit PtNPs (the solution of 1 unit PtNPs contained $92.5 \mu\text{M}$ Pt atoms) and 0.01 mM $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})]^{2+}$, reactions with $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})]^{2+}$ and $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})]^{2+}$ /eosin were carried out for the NAD^+ reduction in the presence of TEOA. $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})]^{2+}$ and $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})]^{2+}$ /eosin resulted in 4 and 36% NADH, respectively. The photoexcited eosin also showed the activity to transport its energy to $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})]^{2+}$, but a negligible amount of NADH could be detected by $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})]^{2+}$ alone [Fig. 1(A)]. Consequently, the above observations demonstrate that PtNPs proved to be more active than $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})]^{2+}$ /eosin as the photocatalytic system to convert the photon energy and produce NADH.
- [90] The Au and Pd nanoparticles prepared in Comparative Examples 1 and 2, respectively, yielded insignificant changes in the amounts of NADH under the same conditions (see FIGS. 11 and 12).
- [91] Molecular oxygen was known to react readily with methyl viologen or
- [92] $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})]^{2+}$ reduced chemically or electrochemically [Hollmann, F., Witholt, B. & Schmid, A. $[\text{Cp}^*\text{Rh}(\text{bpy})(\text{H}_2\text{O})]^{2+}$: a versatile tool for efficient and non-enzymatic regeneration of nicotinamide and flavin coenzymes. *J. Mol. Cat. B: Enzym.*

19-20, 167-176 (2003); Rauwel, F. & Thevenot, D. Use of ring-disc electrodes and viologens for titration of cytochrome-C and oxygen and for study of their reduction kinetics. *J. Electroanal. Chem.* **75**, 579-593 (1977)]. It was also reported that the presence of oxygen severely limited the yield due to depolarization of Pt particles and reoxidation of a reduced relay in the study of hydrogen photoregeneration. To examine the O₂ effect on the photoexcited PtNPs, NADH regeneration with PtNPs/TEOA was examined under aerobic and anaerobic conditions. As a result, no difference in the formation rate of NADH was observed, indicating that the photoreaction is insensitive to oxygen and the activated surface of PtNP has a low affinity towards O₂ in the presence of NAD⁺ and TEOA. The regeneration method of the present invention exhibited excellent regeneration efficiency regardless of the aerobic or anaerobic condition.

[93]

[94] **Comparative Example 1: Electrochemical Regeneration of NADH**

[95] The electrochemical reaction was carried out under conditions including at -0.8 V for 12 hours under the same concentrations used in Example 3, such as 1 unit PtNPs (92.5 uM), and 0.2 mM NAD⁺ coenzyme, and 0.1 mM [Cp*Rh(bpy)(H₂O)]²⁺, resulting in an yield of 18% (see FIG. 13). This is even lower than the yield of NADH photochemical regeneration using the same concentrations of the PtNPs and coenzymes, which is 59%.

[96]

[97] **Example 4: Yield of NADH Photoproduction according to PtNPs Concentration**

[98] The initial rate of NADH photoproduction was found to depend on the PtNP concentration (see FIGS. 2(A) and 8). As the amount of PtNPs increased from 1 to 4 units, the initial rate for the NADH formation increased and the final yield obtained in the irradiation up to 12 hours increased from 59 to 86%. The plot appeared linear at a low concentration of PtNPs, but then curved at higher concentrations probably due to the limiting substrate.

[99] Further increases of the PtNPs concentration above 3 units led to a constant yield, indicating the photochemical steps become turnover-limiting. The maximum conversion was 86% obtained at 3 units of PtNPs. At 1 unit of PtNPs, the yield was 59%, which corresponds to ~ 300 turnovers per hour based on PtNPs, assuming that a 5 nm PtNP contains 2800 Pt atoms. As predicted, the turnover number decreased with increasing the PtNPs concentration [see FIG. 2(B)].

[100]

[101] **Example 5: NADH Photoproduction according to pH**

[102] The amounts of NADH formation were observed under various pH conditions of 5.6, 6.0, 7.0, 8.0 and 9.1. The degrees of NADH formation were examined using solutions

having different pHs (see FIG. 3).

- [103] While the maximum yield for NADH generation was observed at pH 7.0, significant amounts of NADH were also measured at pH 8.0 and 9.1 (see FIG. 9). At higher proton concentrations, the NADH generation was remarkably inhibited. The decrease in rate of NADH formation at acidic pH was known as a result of the protonation of TEOA which was transported into a less effective electron donor.

[104]

[105] **Example 6: Effect of TEOA Concentration on NADH Regeneration**

- [106] Examination of the concentration dependence of TEOA was carried out and is shown in FIG. 4(A). The production of NADH increases with increasing TEOA concentration under a condition of 1 unit of PtNPs. As the concentration of TEOA was varied 0.2 to 0.4 M, the NADH conversion was obtained as 36 - 55% in 9 hours (see FIG. 10). This reveals that the oxidation step of TEOA was also rate-limiting.

[107]

[108] **Comparative Example 2: Effect of Different Electron Donor on NADH Regeneration**

- [109] It was noted that substitution of TEOA with ethylenediamine tetraacetic acid (EDTA), triethylamine (Et₃N), and Na-formate as electron donors under different concentrations of 1, 400, and 100 mM, respectively, resulting in trace amounts of NADH formation in 12 hours (see FIGS. 14 to 16). The yields were 3, 2, and 5%, respectively.

[110]

[111] **Example 7: NADH Regeneration Rate according to NAD⁺ Concentrations**

- [112] If the surface of PtNPs is needed for the catalytic reaction, an increase in the concentration of NAD⁺ over a certain level would have no effect on the (regeneration) rate as the surface is fully saturated with the substrate. According to the observations, the (regeneration) rate was a constant above 0.2 mM NAD⁺, reminiscent of the zero-order reactions [see FIG. 4(B)].

- [113] Based on all observations described herein, it can be noted that PtNPs and TEOA were useful for visible light-driven reduction of NAD⁺ (see FIG. 5). The photoexcited PtNPs were capable of reducing and oxidative quenching NAD⁺ and TEOA, respectively. The light-driven PtNPs were capable of inducing charge separation and the excited electrons were transported to NAD⁺ for reduction, while valence band holes were reductively scavenged by TEOA. Consequently, it can be noted that PtNPs were able to capture incident photons under the visible light.

[114]

[115] **Example 8: Enzyme Reaction using Produced NADH**

- [116] The photoproduction carried out for 9 hours using 5 mM α -glutamate, 80 mM ammonium phosphate, and 2 units of glutamate dehydrogenase under the reaction

conditions (PtNP, NAD, TEOA, pH = 7) led to a conversion of 85%. The reaction product was L-glutamate, which was analyzed using HPLC using a 150 mm ODS-3V column and a 0.08% phosphate aqueous solution for separation at 210 nm.

[117] Reactions with other enzymes such as formate dehydrogenase and glucose-6-phosphate dehydrogenase are also possible.

[118] While the invention has been shown and described with reference to certain exemplary embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

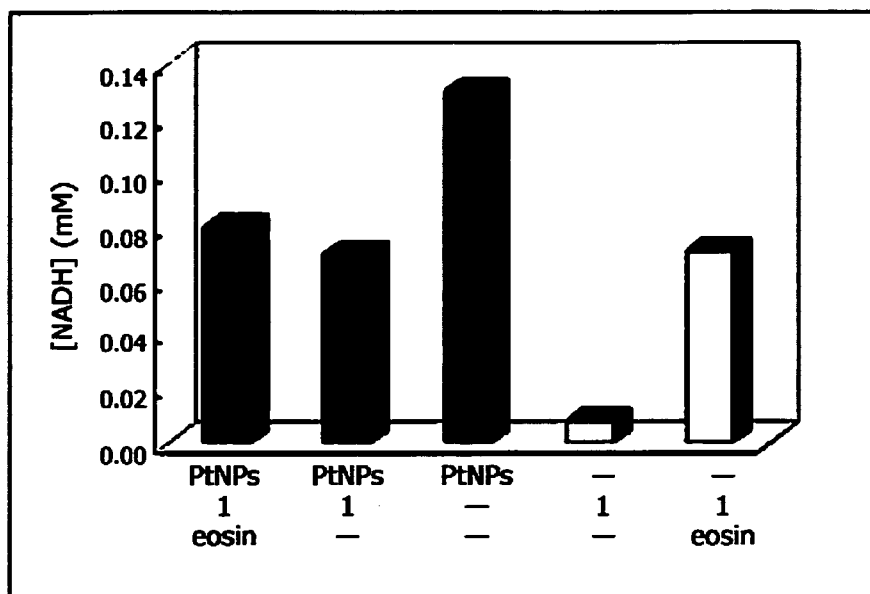
[119]

Claims

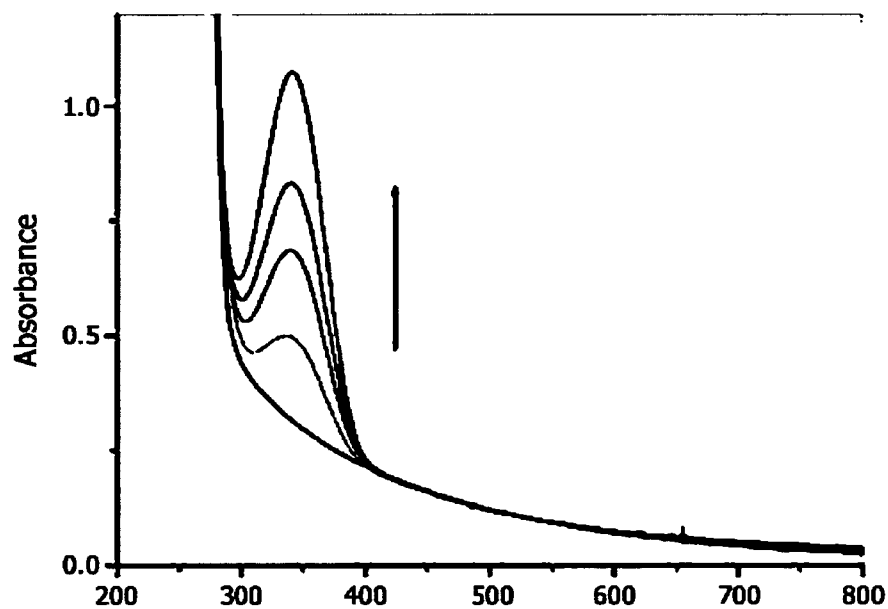
- [Claim 1] A method for photochemical regeneration of oxidoreductase cofactors, comprising:
(a) adding platinum (Pt) nanoparticles to oxidative oxidoreductase cofactors; and
(b) irradiating light to the resulting mixture of step (a).
- [Claim 2] The method of claim 1, wherein the oxidative oxidoreductase cofactors in step (a) are one selected from the group consisting of NAD⁺, NADP⁺, FAD⁺, and FMN⁺.
- [Claim 3] The method of claim 1, wherein step (a) further includes adding an electron donor.
- [Claim 4] The method of claim 3, wherein the electron donor is triethanolamine.
- [Claim 5] The method of claim 1, wherein the Pt nanoparticles in step (a) are added at a concentration of 1 unit or more, the 1 unit containing 92.5 M of Pt atoms.
- [Claim 6] The method of claim 5, wherein the Pt particles in step (a) are added at a concentration of 2 to 4 units.
- [Claim 7] The method of claim 1, wherein step (a) is carried out at pH 7 or more.
- [Claim 8] The method of claim 1, wherein the light in step (b) is a visible light.
- [Claim 9] The method of claim 1, wherein step (a) is carried out in a buffer.
- [Claim 10] A device for photochemical regeneration of oxidoreductase cofactors, comprising:
a reactor including oxidative oxidoreductase cofactors and Pt nanoparticles; and
a light irradiating device.
- [Claim 11] A method of reducing an enzyme using oxidoreductase cofactors re-generated according to any one of claims 1 to 9.

[Fig. 1]

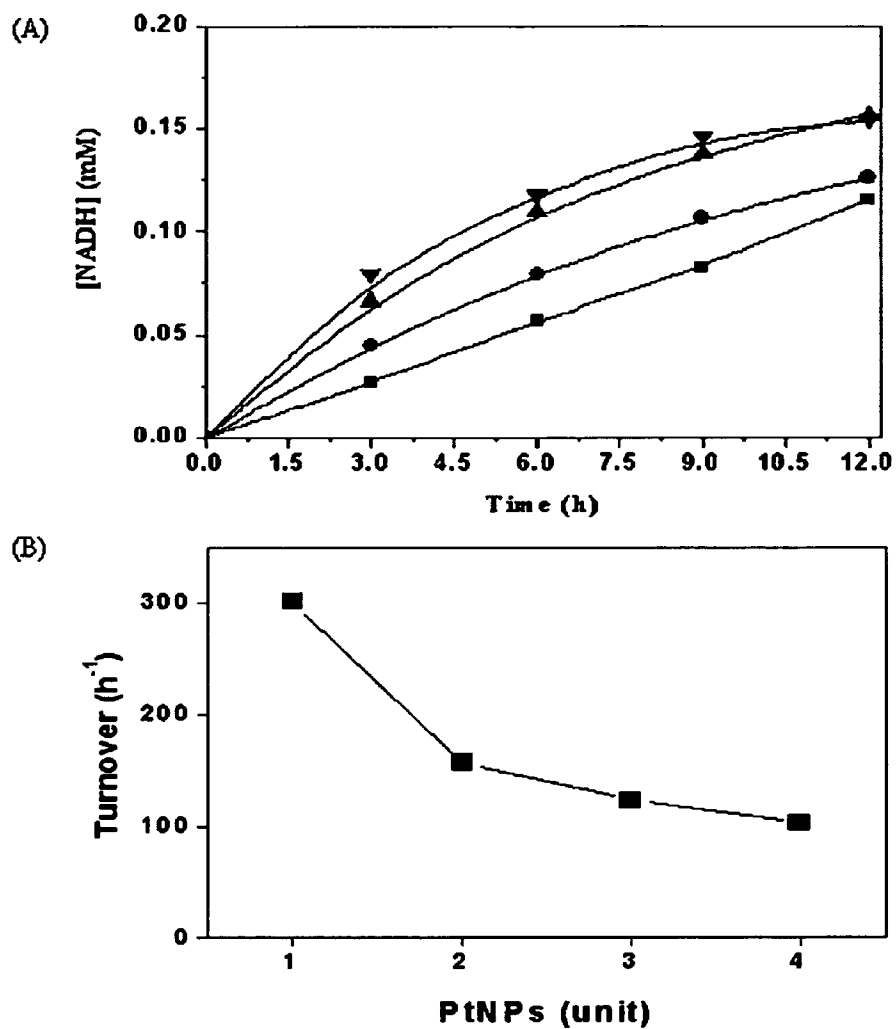
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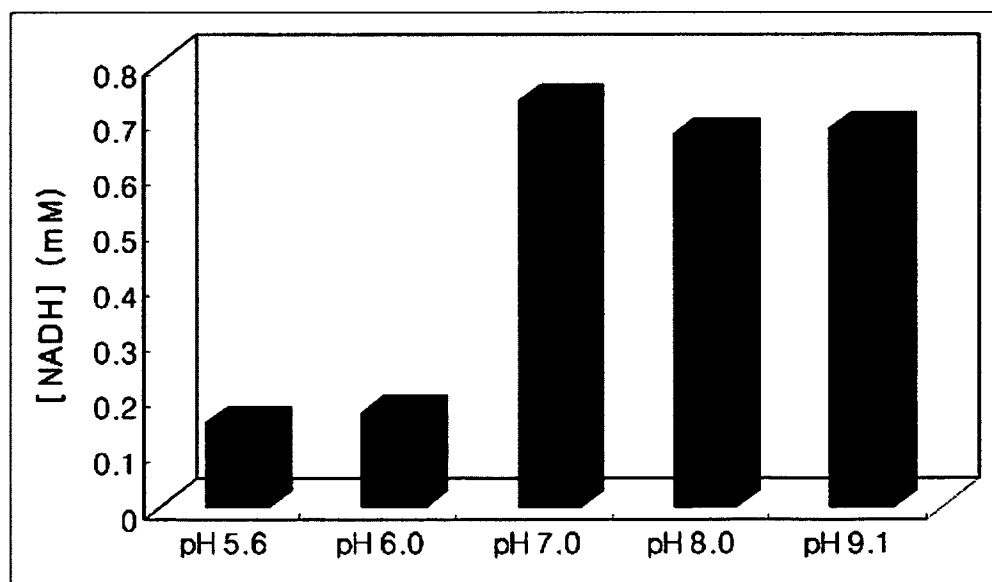
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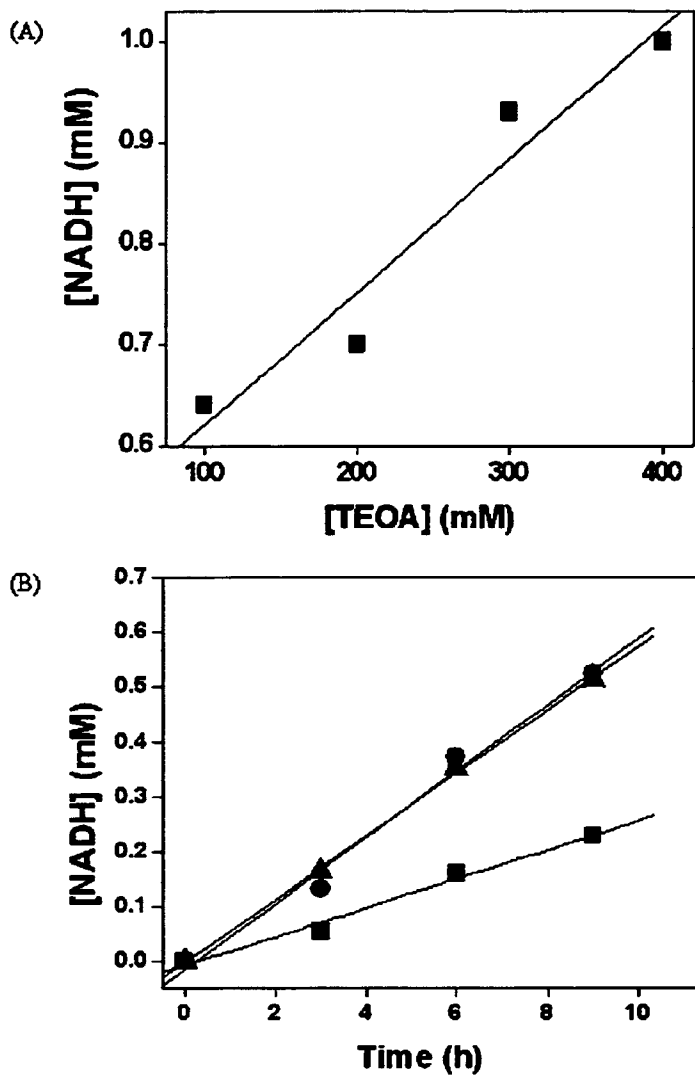
[Fig. 2]



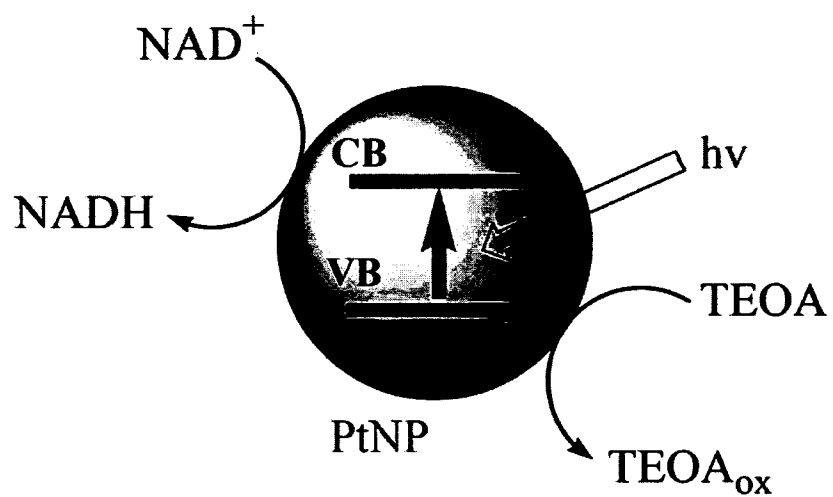
[Fig. 3]



[Fig. 4]

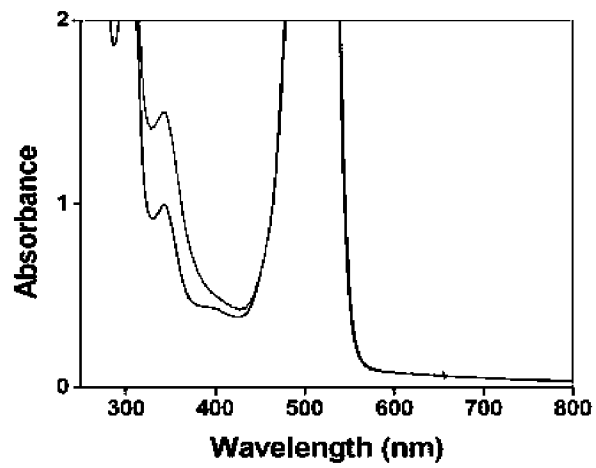


[Fig. 5]

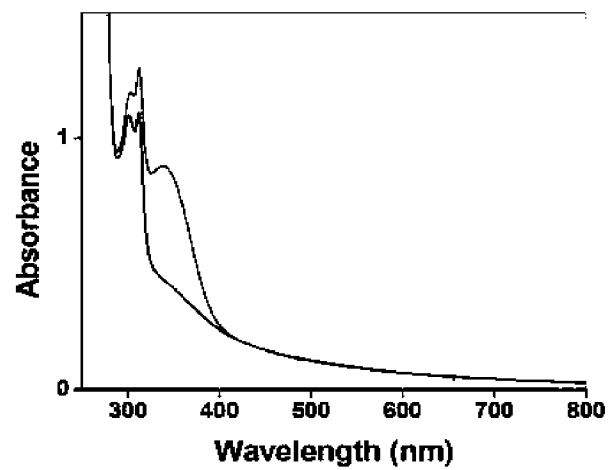


[Fig. 6]

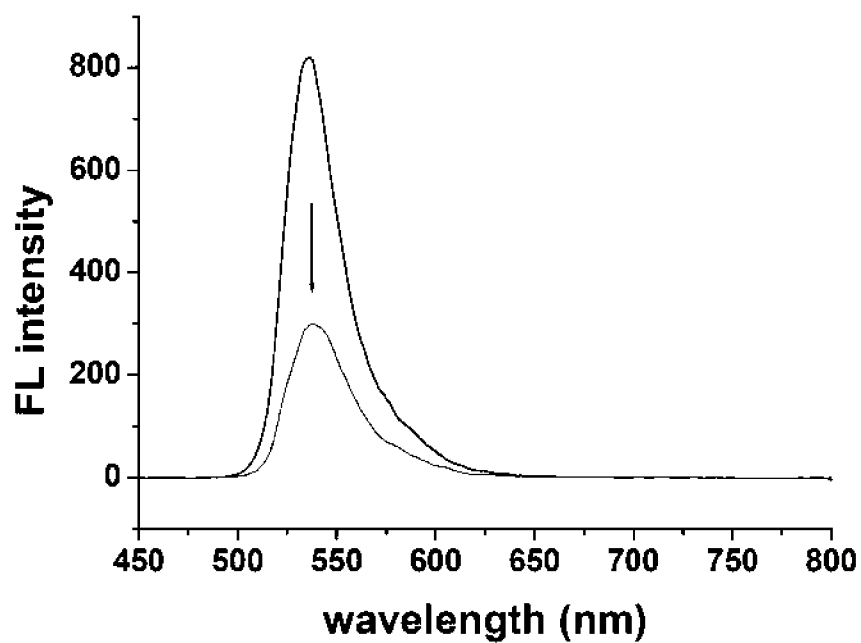
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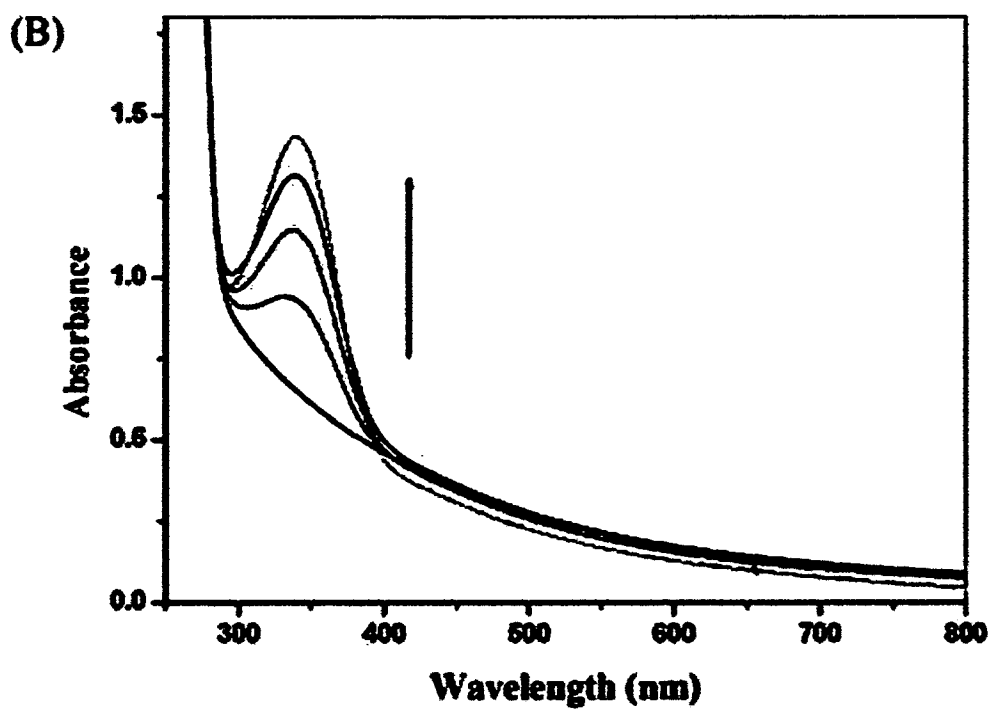
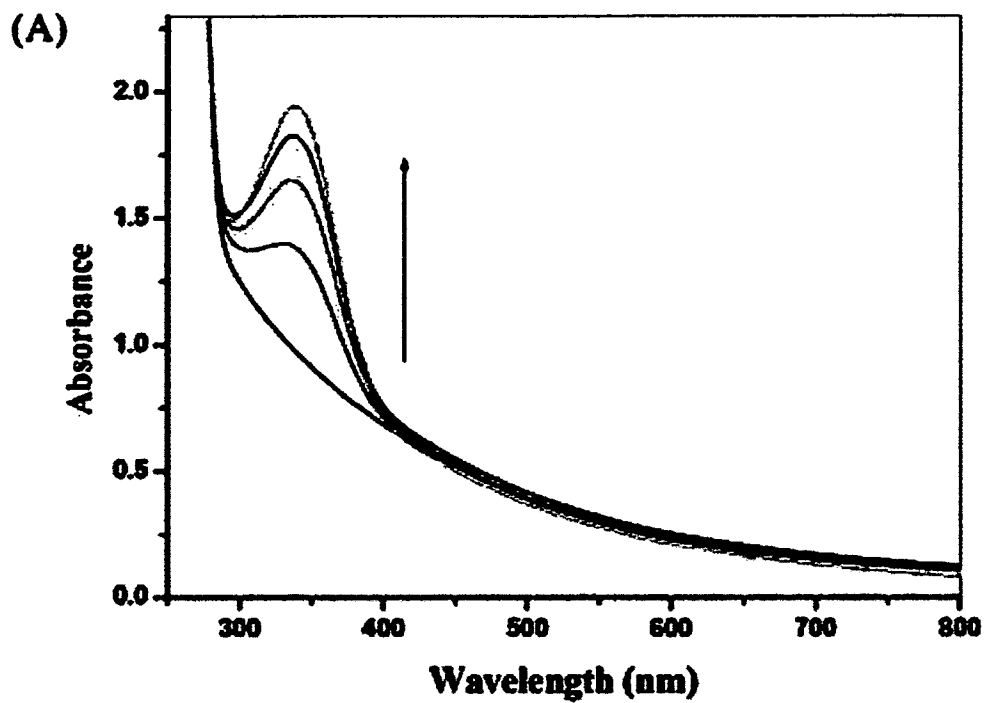
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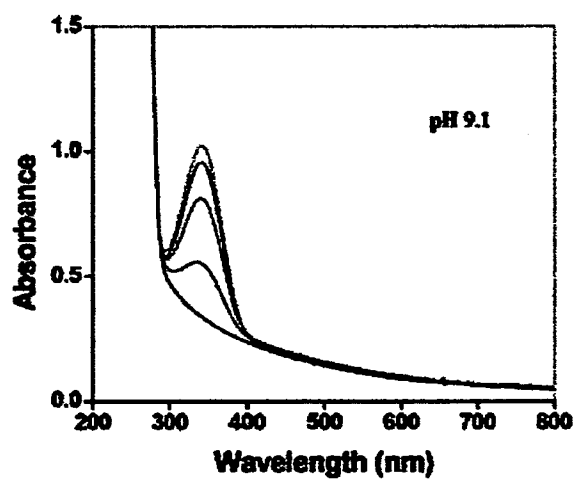
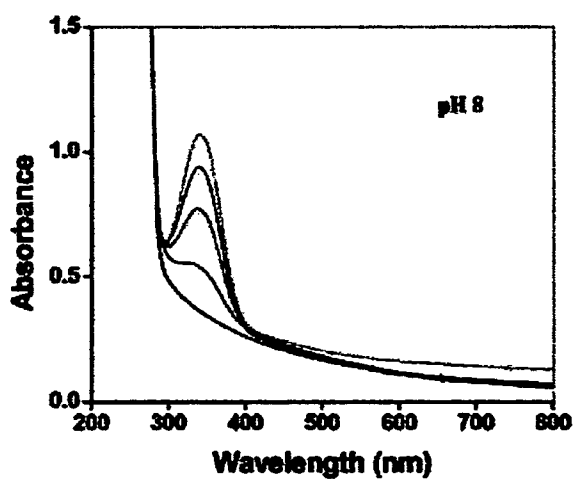
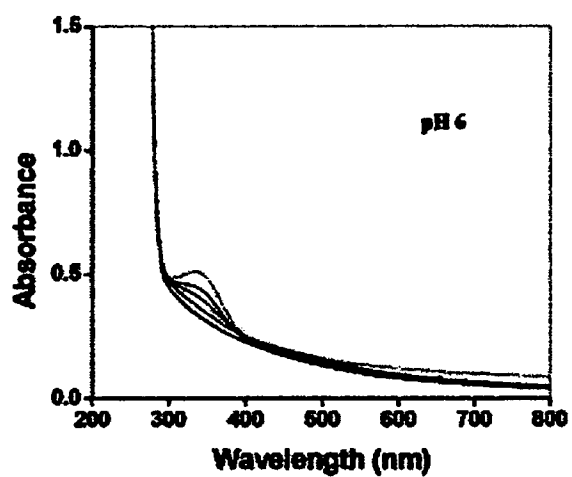
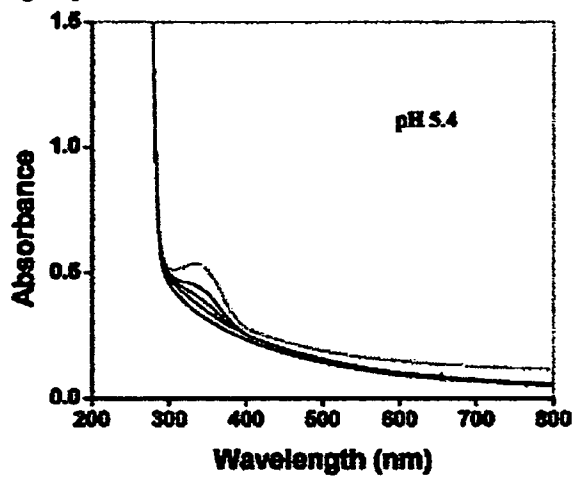
[Fig. 7]



[Fig. 8]

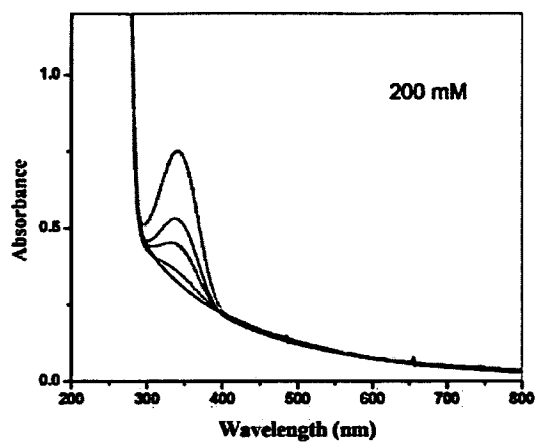


[Fig. 9]

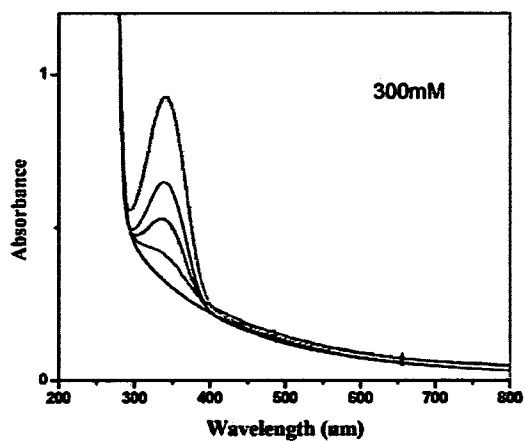


[Fig. 10]

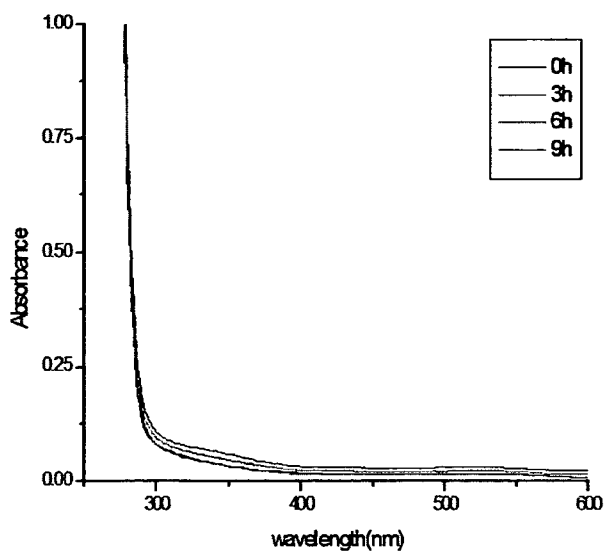
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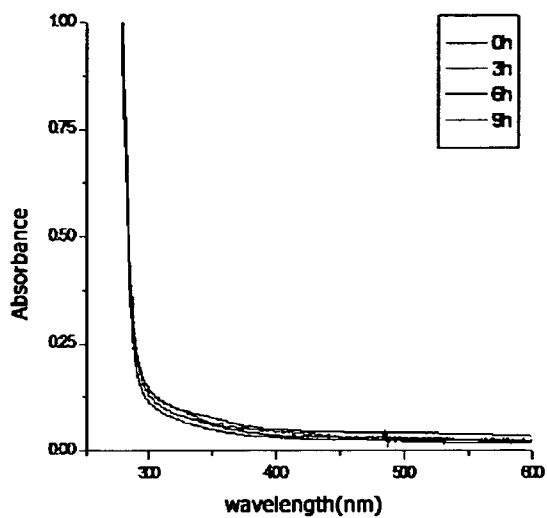
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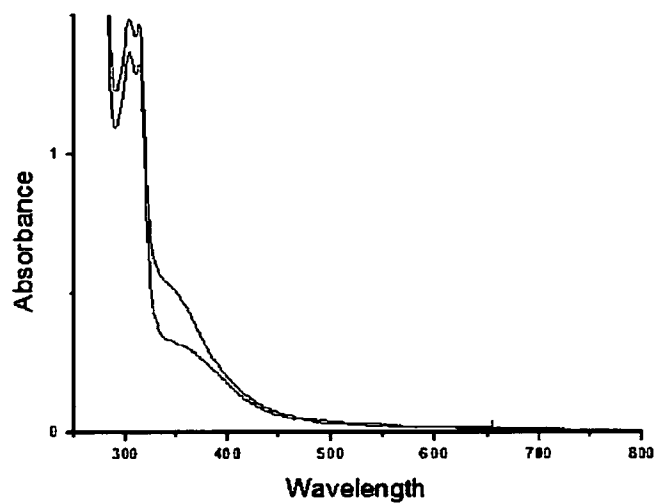
[Fig. 11]



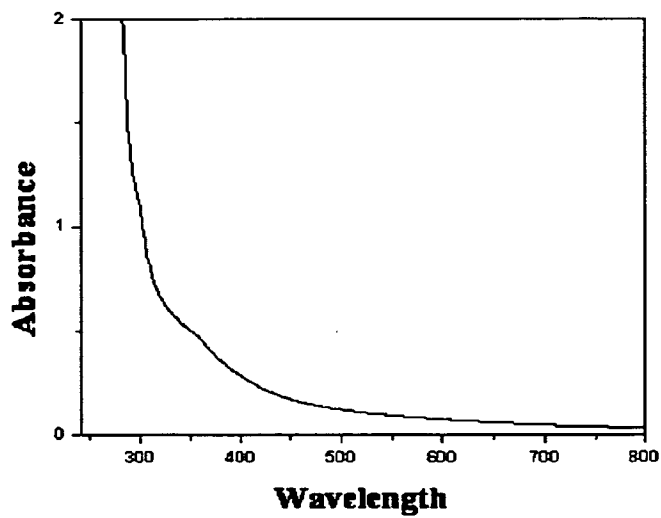
[Fig. 12]



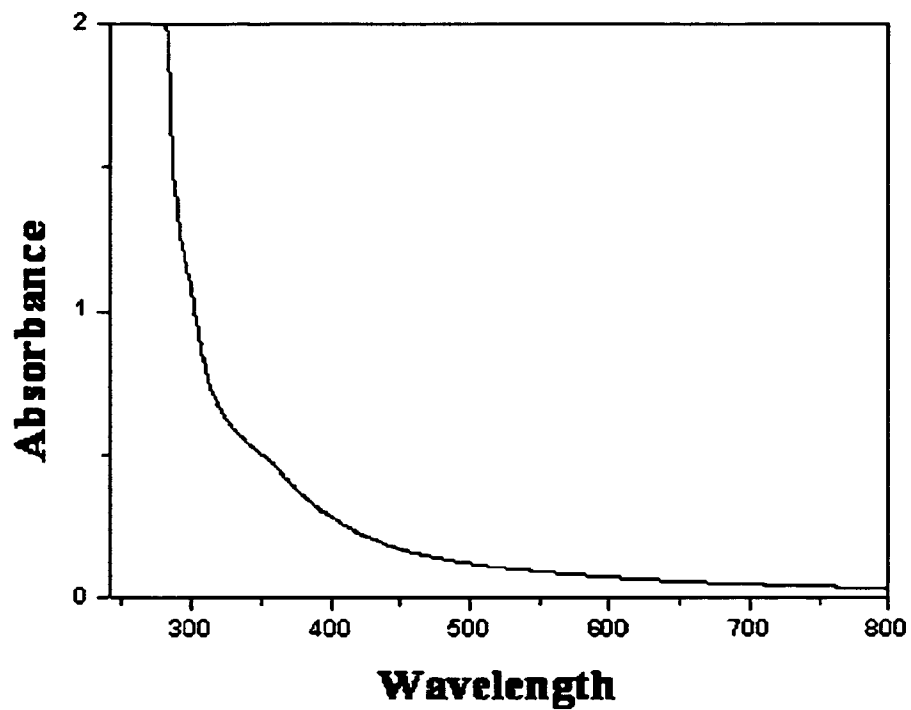
[Fig. 13]



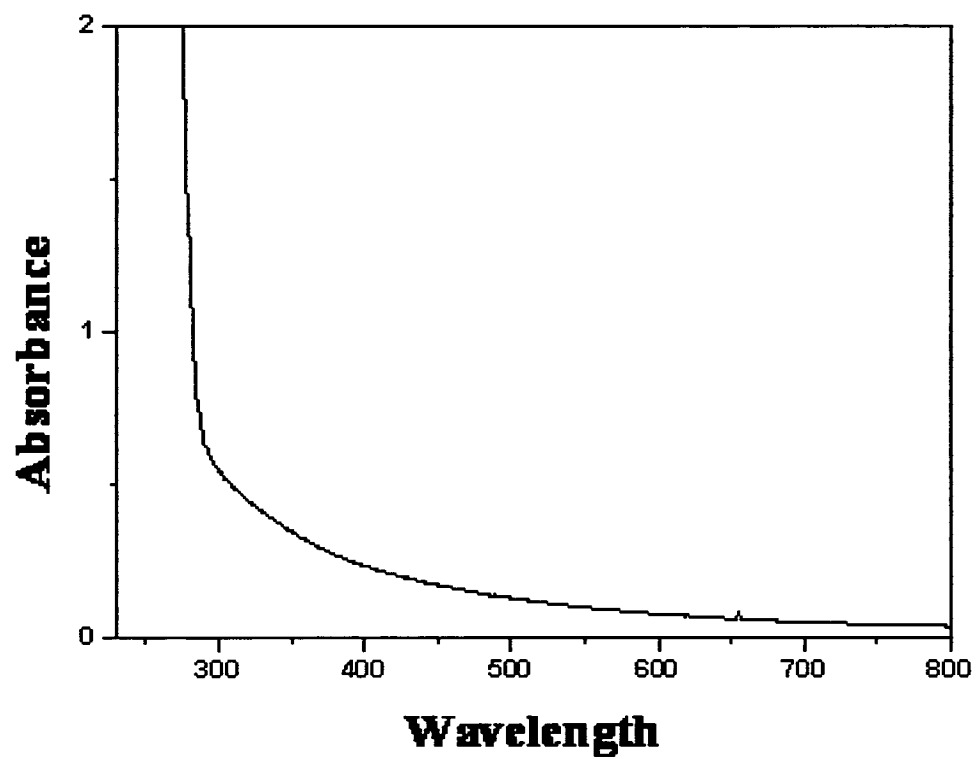
[Fig. 14]



[Fig. 15]



[Fig. 16]



A. CLASSIFICATION OF SUBJECT MATTER*C12N 9/00(2006.01)i*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N 9/00; B82Y 5/00; C12N 9/96; C12N 9/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal), Google Scholar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SONG, H. et al. 'Electrochemical regeneration of NADH enhanced by platinum nanoparticles' Angewandte Chemie. Vol. 120, pp. 1773-1776 (2008)	1-10
X	See p. 1773, left col. lines 1-10; Scheme 2.	11
A	KR 10-2010-0092749 A (한국과학기술원) 23 August 2010 See the abstract.	1-11
A	KR 10-2010-0011160 A (한국화학연구원 et al.) See the abstract.	1-11
A	SHI, Q. et al. 'Visible-light photocatalytic regeneration of NADH using P-doped TiO ₂ nanoparticles' Journal of Molecular Catalysis B: Enzymatic. Vol. 43, pp. 44-48 (1 August 2006) See the abstract.	1-11



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

20 JUNE 2011 (20.06.2011)

Date of mailing of the international search report

20 JUNE 2011 (20.06.2011)

Name and mailing address of the ISA/KR

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Telephone No. 82-42-481-8288



INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2010/006375Patent document
cited in search reportPublication
datePatent family
member(s)Publication
date

KR 10-2010-0092749 A

23.08.2010

None

KR 10-2010-0011160 A

03.02.2010

None