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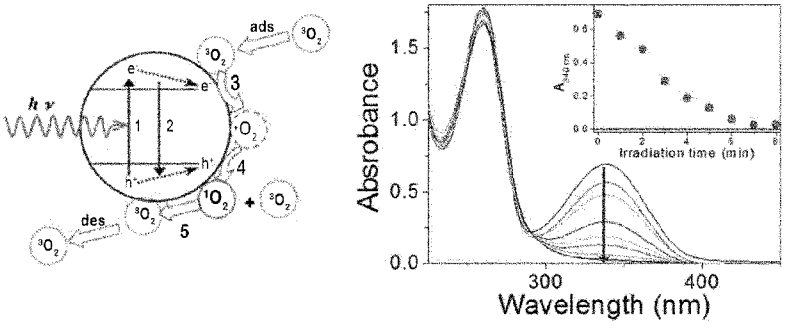
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(54) Title: APPLICATION OF NADH SPECTRUM TO THE PHOTOCATALYTIC ACTIVITY ASSAY

[Fig. 2]



(57) Abstract: Provided is a method for measuring photocatalytic activity of a photocatalyst, more particularly, a method for easily measuring the photocatalytic activity of a photocatalyst based on the change of absorbance or fluorescence intensity of NADH resulting from the photoactivation of the photocatalyst. The disclosed method for measuring photocatalytic activity of a photocatalyst is advantageous in that the photocatalytic activity of a photocatalyst can be measured in a short time using a small amount of sample and the speed and accuracy of measurement can be improved over the existing methods via a simple analysis procedure of measuring fluorescence intensity in a mixture solution of the photocatalyst and NADH without special pretreatment. In addition, objective comparison is possible because the photocatalytic activity of a photocatalyst can be quantified by measuring the change of the fluorescence intensity of NADH with time in the presence of the photocatalyst and calculating the reaction rate.

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Description

Title of Invention: APPLICATION OF NADH SPECTRUM TO THE PHOTOCATALYTIC ACTIVITY ASSAY

Technical Field

[0001] The present disclosure relates to a method for measuring photocatalytic activity of a photocatalyst, and more particularly, to a method for easily measuring photocatalytic activity of a photocatalyst based on the change in absorbance or fluorescence intensity of NADH in a suspension of the photocatalyst in powder form.

[0002]

Background Art

[0003] A photocatalyst refers to a material that can induce chemical changes of various materials when illuminated with light (particularly, UV light). A variety of photocatalysts including TiO_2, SiO_2, WO_3 and ZnO are currently available. Among them, TiO_2-based photocatalysts with high photocatalytic activity and low manufacturing cost have been widely used. Various methods of manufacturing these photocatalysts have been patented. However, due to the lack of a concrete method for evaluating the photocatalytic activity of these photocatalysts, different manufacturers or researchers have been using different methods of measuring the activities.

[0004] For example, Japanese Patent Publication No. 2001-183359 discloses a conventional method of evaluating the photocatalytic activity, based on the contact angle measurement with eyes or using a microscope. However, the above method is still not sufficient to be an absolute evaluation technique because it is far from providing a precise and quantitative analysis data on the change of contact angle between water and a base material. Further, the above method involves the steps of the formation of a photocatalyst layer and an organic layer followed by UV irradiation, and the analysis result depends on the coat layer materials making it difficult to compare the photo activities of different catalysts.

[0005] Japanese Patent Publication No. 2000-162129 discloses a method of evaluating a photocatalytic activity whereby, after a photocatalyst is coated on a base material, the base material is painted with methylene blue and absorbance is measured after drying. However, according to this method, when the photocatalyst is coated on the base material, the coating surface morphology can be formed irregularly. As a result, the dye on the photocatalyst may also be painted with irregular thickness. This may cause difference in the absorbance of the painted dye layer depending on the measurement area. Further, since the absorption property of photocatalyst changes along with the drying condition, accurate measurement of photocatalytic activity cannot be expected.

[0006] Korean Utility Model No. 20-230249 discloses a kit for evaluating photocatalytic activity based on FT-IR. However, it requires an expensive instrument and relative or absolute comparison is very difficult because the result varies greatly depending on the sealing sate of the kit and the contamination of the material to be tested.

[0007] In the photocatalytic activity measurement method disclosed in *Applied Catalysis B: Environmental* 45, 23-38 (2003) and others, a photocatalyst suspension mixed with an analyte (solution volume > 100 mL) is irradiated by UV light with repetitive sampling of a small portion of solution at predetermined time intervals. After separating the photocatalyst by centrifugation, absorbance of the sampled analyte is measured. This method is disadvantageous in that a large volume (> 100 mL) of sample and a long time (> 1 hour) are required to measure the photocatalytic activity at each concentration.

[0008] Examples of other photocatalytic activity evaluation methods include the oil reduction method whereby an oil is applied on a coat layer and then the weight decrease of the oil with time is monitored and the microbial observation method whereby the capacity to kill microbes is determined. According to the oil reduction method, an organic oil in liquid phase such as salad oil is applied uniformly and thinly over a photocatalytic functional film and the weight decrease of the oil caused by photolysis is monitored after UV irradiation. However, this method requires a long time of more than 10 hours and the accuracy is not good because the small weight change of less than 1 mg must be monitored over a long period of time. According to the microbial observation method, after addition of a microbial broth of a predetermined amount, the number of surviving microbes is counted over time. However, this method is not advantageous in that it requires culturing of the microbes, which needs a relatively long time, and the counting of the microbes is usually performed manually, which may result in an inaccurate result.

[0009] Accordingly, there is a need for the development of a method that allows the analysis of photocatalytic activity of a photocatalyst in a short period of time using a small quantity of sample without being affected by varying conditions and materials.

[0010]

Summary of Invention

Technical Problem

[0011] The inventors of the present disclosure found that photocatalytic activity of a photocatalyst can be measured easily based on the fact that the fluorescence intensity of NADH changes upon UV irradiation as it is oxidized by reacting with reactive oxygen species (ROS) produced by the photocatalyst.

[0012] The present disclosure relates to a method for measuring photocatalytic activity of a

photocatalyst in an aqueous suspension, which is initially in powder form, using NADH.

[0013]

Solution to Problem

[0014] In one general aspect, the present disclosure relates to a method for measuring photocatalytic activity of a photocatalyst, including: (a) adding NADH and a photocatalyst to a container and measuring the fluorescence intensity of NADH before photoactivation of the photocatalyst; (b) irradiating UV light to the container to activate the photocatalyst and then measuring the fluorescence intensity of NADH; and (c) determining the photocatalytic activity of the photocatalyst from the change in the fluorescence intensity of NADH before and after the photoactivation of the photocatalyst.

[0015]

Advantageous Effects of Invention

[0016] The method for measuring photocatalytic activity of a photocatalyst presented by the present disclosure is advantageous in that the photocatalytic activity of a photocatalyst can be measured short time using a small amount of sample and the speed and accuracy of measurement can be improved over the existing methods via a simple analysis procedure of measuring fluorescence intensity in a mixture solution of the photocatalyst and NADH without pretreatment for separation. In addition, objective comparison is possible because the photocatalytic activity of a photocatalyst can be quantified by calculation of the reaction rate from the fluorescence intensity change of NADH with time in the presence of the photocatalyst.

[0017]

Brief Description of Drawings

[0018] The above and other objects, features and advantages of the present disclosure will become apparent from the following description of certain exemplary embodiments given in conjunction with the accompanying drawings, in which:

[0019] Fig. 1 illustrates a reaction where NADH reacts with reactive oxygen species (ROS) produced by a photocatalyst and is converted to NAD^+, the absorption and fluorescence spectra of NADH, and the absorption spectrum of NAD^+;

[0020] Fig. 2 illustrates a procedure whereby ROS are produced on the surface of a photocatalyst and shows the decrease in the absorbance of NADH at 340 nm as the result of reaction with ROS;

[0021] Fig. 3 shows the region of NADH concentration where the increase of fluorescence intensity shows linear dependence;

[0022] Fig. 4 shows the pattern of NADH fluorescence intensity change over the near-UV irradiation time in the presence or absence of TiO_2;

[0023] Fig. 5 shows the linear increase of NADH reaction rate along with the increase in TiO_2concentration up to 67 μg/mL;

[0024] Fig. 6 shows the relationship between the absorbance and fluorescence intensity of NADH depending on near-UV irradiation time in the presence of TiO_2;

[0025] Fig. 7 shows the pattern of NADH fluorescence intensity change over the near-UV irradiation time in buffer solutions of various pHs without TiO_2;

[0026] Fig. 8 shows the pattern of NADH fluorescence intensity change over the near-UV irradiation time in buffer solutions of various pHs with a constant concentration of TiO_2; and

[0027] Fig. 9 shows the NADH photooxidation rate depending on pH of the reaction mixture.

[0028]

Best Mode for Carrying out the Invention

[0029] Hereinafter, the embodiments of the present disclosure will be described in detail with reference to accompanying drawings.

[0030] The present disclosure relates to a method for measuring photocatalytic activity of a photocatalyst, comprising: (a) adding NADH and a photocatalyst to a container and measuring the fluorescence intensity of NADH before photoactivation of the photocatalyst; (b) irradiating UV light to the container to activate the photocatalyst and then measuring the fluorescence intensity of NADH; and (c) determining the photocatalytic activity of the photocatalyst from the change in the fluorescence intensity of NADH before and after the photoactivation of the photocatalyst.

[0031] In the first step, NADH and a photocatalyst are added to a container and the fluorescence intensity of NADH is measured before the photocatalyst is activated. The measurement is made with the photocatalyst before activation by light. The fluorescence intensity may be measured as a spectrum using a spectrofluorometer or at a predetermined wavelength for many sample solutions in a multiple well plate by using a fluorescence microplate reader.

[0032] First, an NADH solution exhibiting maximum absorbance in the near-UV region, i.e. at 290 to 400 nm, is added to the container. The NADH solution is prepared by dissolving NADH in a solvent and diluting, for example, with DIW or pH buffer solution such that absorbance measured at 340 nm is between 0.75 and 1.5. Specifically, the concentration of NADH may be from 31 μM to 250 μM. To give a specific example, the NADH solution may be prepared by dissolving 33 mg of NADH in 10 mL of a solvent and then diluting to 1/40 to 1/10. The solvent may be one wherein NADH is dissolved well, such as deionized water or a pH 8 phosphate buffer solution.

[0033] After the prepared NADH solution is added to the container, the photocatalyst is added thereto. The photocatalyst may be any one capable of inducing chemical change of various materials upon irradiation of light (particularly UV), such as TiO_2, SiO_2, WO_3 or ZnO. The photocatalyst may be used alone or in combination of two or more. The photocatalyst may be in any form, including powder or sol. Specifically, photocatalyst nanopowder suspended in a solvent may be used to give a stable suspension of small-sized photocatalyst particles. The solvent for suspending the photocatalyst nanopowder may be an aqueous pH 8 sodium hydroxide (NaOH) solution, an aqueous pH 8 phosphate buffer solution, or the like.

[0034] The amount of the photocatalyst may be determined such that absorbance for a 1 cm-path is between 0.3 and 3.0 at the wavelength of photo activation. If the absorbance exceeds 3.0, the NADH photooxidation rate does not linearly depend on the amount of the photocatalyst. In contrast, if the absorbance is smaller than 0.3, the measurement may not be accurate because the change in fluorescence intensity of NADH is very small.

[0035] In addition, an adequate buffer solution may be prepared and added according to a known method in order to prevent the problem that the photocatalyzed ROS production rate depends on the pH of the solution. For example, a pH 8 phosphate buffer solution may be prepared by mixing an aqueous solution of 5 mM monobasic sodium phosphate and an aqueous solution of 5 mM dibasic sodium phosphate at about 94 : 6. For accurate pH adjustment, 100 mL of an aqueous solution of 5 mM monobasic sodium phosphate may be added to an Erlenmeyer flask and an aqueous solution of 5 mM dibasic sodium phosphate may be added in small amounts until the pH reaches 8, while continuously stirring the solution using a magnetic stirrer and measuring the pH of the solution using a pH electrode.

[0036] The measurement of fluorescence intensity may be carried out after keeping the photocatalyst sample under a light-blocked condition, e.g., in a dark room, for a predetermined time (about 10 minutes) so that the photocatalyst loses its activity.

[0037] After the pretreatment, the spectrofluorometer is set to a desired wavelength region and the fluorescence intensity of NADH before activation of the photocatalyst is measured at a region of 455 to 465 nm. Herein, the reliability of data is determined by the detection accuracy of the spectrofluorometer.

[0038] Second, UV light is irradiated to the container to activate the photocatalyst and then the fluorescence intensity of NADH is determined at predetermined time intervals. When the UV light is irradiated, ROS produced by the photocatalyst oxidize NADH to NAD^+, resulting in gradual decrease of the concentration of NADH. As a result, the fluorescence intensity of the maximum peak at 460 nm decreases. The decreasing pattern depends greatly on such factors as the photocatalytic activity of the photo-

catalyst, time, or the like.

[0039] The UV irradiation is performed using a UV lamp with a wavelength in the range of 290 to 400 nm. For the NADH fluorescence intensity measurement, excitation wavelength may be selected to be within 10 nm of the maximum absorption wavelength of NADH to obtain accurate fluorescence intensity in a short time. UV irradiation time and irradiation intensity may be adjusted as required.

[0040] Finally, the reaction rate is calculated based on the change in the fluorescence intensity of NADH before and after the photoactivation of the photocatalyst to quantitatively determine the photocatalytic activity of the photocatalyst. The photocatalytic activity per unit weight per unit time may be calculated from the measured fluorescence intensities according to Equations 1 and 2.

[0041] [Equation 1]

[0042] Photocatalytic activity = (Rate of change in fluorescence intensity × Initial NADH concentration) / (Concentration of photocatalyst)

[0043] In the above Equation 1, the rate of change in fluorescence intensity is the slope in the plot of relative fluorescence intensity over UV irradiation time.

[0044] [Equation 2]

[0045] Relative fluorescence intensity = (Fluorescence intensity of NADH after UV irradiation) / (Initial fluorescence intensity of NADH)

[0046] As described, the method for measuring photocatalytic activity according to the present disclosure allows quantitative measurement of the photocatalytic activity of a photocatalyst in a short time period and comparison of the photocatalytic activity of different photocatalysts. Thus, it may be used in the selection of efficiency photocatalyst products and related studies. Further, since the NADH used in the photocatalytic activity measurement is in itself not degraded by UV but is oxidized to NAD^+ in the presence of a photocatalyst, it is very useful in measuring the photocatalytic activity of the photocatalyst.

[0047] When fluorescence measurement is inapplicable, the photocatalytic activity of the photocatalyst may be measured by monitoring the change in the absorbance of NADH. The related procedure is the same as the fluorescence intensity measurement, except that absorbance of an NADH mixture solution is measured at 335 to 345 nm after removing a photocatalyst from the mixture solution, for example, by centrifugation. A specific experimental procedure will be described in Example 2. After monitoring the change in the absorbance of NADH after UV irradiation, the photocatalytic activity per unit weight per unit time may be calculated by Equation 3.

[0048] [Equation 3]

[0049] Photocatalytic activity = (Rate of change in absorbance) / (Absorption coefficient × Absorption path length) / (Concentration of photocatalyst)

[0050] In the above Equation 3, the rate of change in absorbance is the slope of a graph of absorbance over UV irradiation time.

[0051]

Mode for the Invention

[0052] The examples and experiments will now be described. The following examples and experiments are for illustrative purposes only and not intended to limit the scope of the present disclosure.

[0053] **<Preparation Example>**

[0054] [Preparation of TiO_2 suspension]

[0055] As the photocatalyst of which the photocatalytic activity will be measured using NADH, TiO_2 (Degussa P25 Aeroxide, Evonic Co., Ltd.) was used. In order to obtain a stable TiO_2 suspension of small-sized particles, TiO_2nanopowder (40 mg) and an aqueous NaOH solution (pH 10, 10 mL) were added to a 20 mL glass vial (4 mg/mL), and stirred at 900 rpm for 24 hours using a magnetic stirrer. Then, the suspension was kept for another 24 hours without stirring, and the supernatant (5 mL) was collected. It was confirmed through dynamic light scattering (DLS) that TiO_2 nanoparticles with a diameter of 160 to 190 nm were suspended in the supernatant. A particle size analyzer (ELS-Z, Otsuka Electronics Co. Ltd.) was used for the DLS measurement.

[0056] [Preparation of NADH solution]

[0057] An NADH (Cat. No. 43423, Fluka Co., Ltd.) solution was prepared using DIW (resistance> 18 MΩcm) produced in a Milli-Q Reference Water System Production Unit (Millipore Co.) as a solvent.

[0058] [Preparation of buffer solutions]

[0059] The following reagents were used to prepare pH buffer solutions.

[0060] An acetate buffer solution was prepared by mixing a 5 mM aqueous acetic acid solution with a 5 mM aqueous sodium acetate solution. A phosphate buffer solution was prepared by mixing a 5 mM aqueous monobasic sodium phosphate solution with a 5 mM aqueous dibasic sodium phosphate solution. A sodium carbonate buffer solution was prepared by mixing a 5 mM aqueous sodium carbonate solution with a 5 mM aqueous sodium bicarbonate solution. All the reagents used to prepare the buffer solutions were purchased from Sigma-Aldrich (ACS reagent grade).

[0061] **<Example 1> Measurement of photocatalytic activity at various NADH and TiO_2 concentrations**

[0062] The measurement of photocatalytic activity according to present disclosure is based on the quantification of NADH concentration through measurement of the fluorescence intensity of NADH. For this, the fluorescence intensity of NADH should be linearly proportional to the NADH concentration. Thus, before carrying out photocatalytic

activity measurement, the relationship between the fluorescence intensity and concentration of NADH was investigated. The result is shown in Table 1 and Fig. 3.

[0063] [Table 1]

Fluorescence intensity of NADH at various concentrations	
NADH concentration (μM)	Fluorescence intensity (arbitrary unit)
0	99.4
31.25	332
62.5	551
125	948
250	1,544
500	2,358
1,000	3,080
-Excitation: 340 ± 5 nm -Emission: 460 ± 5 nm	

[0064]

[0065] As seen from Table 1 and Fig. 3, the fluorescence intensity of NADH is linearly proportional to the NADH concentration in the range of 31 to 250 μM. At higher concentrations, a linearly increasing pattern was not observed under the given optical arrangement condition. Accordingly, when the photocatalytic activity measurement is performed through NADH fluorescence monitoring, the NADH concentration may be maintained to be 250 μM or lower.

[0066] In order to determine the proper concentrations of NADH and TiO_2 for the photocatalytic activity measurement, experiments were performed using four NADH solutions sequentially diluted 2-fold (from 500 μM to 62 μM) and seven TiO_2 suspensions sequentially diluted 2-fold (from 4 mg/mL to 62.5 μg/mL). In order to induce oxidation of NADH by the photocatalytic activity of TiO_2, a mixture solution of NADH and TiO_2 at each concentration was added to a 96-well plate (30096, SPL Co. Ltd.) and UV (wavelength: 312 nm) was irradiated using a transilluminator (WUV-M20, Daihan Scientific Co., Ltd.). In order to maintain the pH of the mixture solution constant, 104 μL of NADH solution and a 91 μL of 5 mM aqueous phosphate buffer at pH 8 was mixed and then a 13 μL of TiO_2 suspension was added thereto. While irradiating UV for 10 minutes, the absorbance or fluorescence intensity of NADH was measured at 1-minute intervals to monitor the decrease of NADH concentration over time. The fluorescence intensity of NADH was measured using a fluorescence microplate reader (Gemini EM, Molecular Devices Co., Ltd.) with 340 nm

excitation/ 460 nm emission adopting top read mode.

[0067] The results are shown in Tables 2 and 3 and Figs. 4 and 5.

[0068] [Table 2]

Relative fluorescence intensity of NADH after near-UV irradiation		
Length of UV irradiation (min)	Relative fluorescence intensity	
	NADH	NADH + TiO_2
0	1	1
1	0.968	0.908
2	0.966	0.777
3	0.932	0.522
4	0.931	0.347
5	0.926	0.231
6	0.934	0.0764
7	0.921	0.0252
8	0.938	0.0318
- NADH concentration : 125 μM - TiO_2 concentration : 100 μg/mL - pH 8 buffer solution was used.		

[0069]

[0070] [Table 3]

Reaction rates at various NADH and TiO_2 concentrations				
NADH concentration (μM)	31	62	125	250
TiO_2 concentration (μg/mL)	Reaction rate (μM/min)			
3.91	0.05	0.268	0.925	1.96
7.81	0.509	0.836	1.32	2.41
15.63	1.61	2.33	2.89	4.08
31.3	3.80	4.68	6.53	7.23
62.5	6.17	7.87	11.6	13.0
125	8.39	11.2	13.7	17.7
250	7.92	14.2	15.1	21.0
- pH 8 buffer solution was used.				

[0071]

[0072] The relative fluorescence intensities over UV irradiation time for the NADH solution or the suspension wherein NADH and TiO_2 were present together are summarized in Table 2 and Fig. 4. As seen from Fig. 4, there was little change in fluorescence intensity with time when near-UV was irradiated to the NADH solution, whereas the fluorescence intensity decreased linearly with time when near-UV was irradiated to the solution mixture of NADH and TiO_2. Through linear regression of the NADH fluorescence intensity over UV irradiation time shown in Fig. 4, the rate constant k_{app} (μM/min) of the reaction whereby NADH is oxidized by the photocatalyst can be calculated approximately.

[0073] Table 3 and Fig. 5 show the calculated results of rate constant of NADH oxidation at various NADH and TiO_2 concentrations. As seen from Table 3 and Fig. 5, without regard to the NADH concentration, a linear relationship was observed between the reaction rate and the TiO_2 concentration when the TiO_2 concentration was in the range of from 7.81 to 62.5 μg/mL (i.e., when the absorbance for 1 cm path length at 312 nm is between 0.3 and 3.0). At higher concentrations, a linear relationship was not observed because of the self-filtering effect caused by absorption or scattering by TiO_2. Thus, when measuring the activity of a photocatalyst according to the present disclosure, an accurate photocatalytic activity may be obtained when the concentration of the photocatalyst is determined such that the absorbance at the excitation wavelength is between 0.3 and 3.0. The above method is not limited to be applied to the present invention. That is, when determining the activity of a photocatalyst, a region should be selected as where the conversion rate of the substrate shows a linear relationship with the concentration of the photocatalyst.

[0074] **<Example 2> Comparison of decreasing pattern of absorbance and fluorescence intensity over UV irradiation time**

[0075] Experiment was carried out as follows in order to measure and compare the decrease of the absorbance and fluorescence intensity of NADH with UV irradiation time. First, in 36 wells of a well plate, NADH (250 μM; 104 μL), TiO_2(1.6 mg/mL; 13 μL) and a pH 8 phosphate buffer solution (91 μL) was mixed. Then, while irradiating near-UV (wavelength: 312 nm) using a transilluminator, the solutions in three wells were collected at 1 minute intervals. The solutions were then mixed and centrifuged at 6,000 rpm for 20 minutes to sediment TiO_2 nanoparticles. Then, the supernatant was taken and absorbance was measured at 335 to 345 nm. For the absorbance measurement, a UV/Vis spectrophotometer (UV-1700, Shimadzu Co., Ltd.) was used. In order to measure the initial NADH concentration before the UV irradiation, the solutions were taken from three wells before the UV irradiation and then subjected to the centrifugation and absorbance measurement. In addition, for comparison of the change of

fluorescence intensity with the change of absorbance, the solutions in three wells were collected and the fluorescence intensities from the wells were measured after each UV irradiation using a fluorescence microplate reader.

[0076] The result is shown in Table 4 and Fig. 6.

[0077] [Table 4]

Relative absorbance and fluorescence intensity of NADH depending on near-UV irradiation time		
UV irradiation time (min)	Relative intensity	
	Relative absorbance intensity	Relative fluorescence intensity
0	1	1
1	0.816	0.908
2	0.694	0.777
3	0.417	0.522
4	0.271	0.347
5	0.186	0.231
6	0.0855	0.0764
7	0.0304	0.0252
8	0.0420	0.0318
- NADH concentration : 125 μM - TiO_2 concentration : 100 μg/mL		

[0078]

[0079] It can be seen from the Table 4 and Fig. 6 that the absorbance and fluorescence intensities of NADH are related to each other. This shows that the decrease of NADH concentration due to oxidation by ROS can be monitored by measuring the fluorescence intensity of NADH. For absorbance measurement, a sample pretreatment process such as centrifugation is required. In particular, when many different kinds of samples are to be measured at once, much time is required for the pretreatment and the measurement accuracy may decrease during the separation step. In contrast, fluorescence intensity measurement can be made for multiple samples without pretreatment by repeating irradiation of near-UV and fluorescence measurement on a multiple-well plate. Thus, this example suggests that the photocatalytic activity measurement according to the present disclosure may be applied for accurate high-throughput screening.

[0080] **<Example 3> Photocatalytic activity measurement at various pH condition**

[0081] In order to measure the change in the photocatalytic activity of TiO_2nanoparticles depending on pH, reaction solutions were prepared by adding a buffer solution (95 μL) with pH ranging from 5 to 11 to a solution obtained by mixing NADH (250 μM; 100 μL) and TiO_2(4 mg/mL; 5 μL). Then, the decrease of NADH concentration after UV irradiation was observed at each pH. As the pH buffer solution, sodium acetate was used for pH 5, sodium phosphate was used for pH 6-8, and sodium carbonate was used for pH 9-11.

[0082] The result is shown in Tables 5 to 7 and Figs. 6 to 8.

[0083] [Table 5]

Decrease of NADH fluorescence after near-UV irradiation at various pH							
Time (min)	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	pH 11
0	1	1	1	1	1	1	1
1	0.987	0.995	0.997	1.01	0.985	0.988	1.00
2	0.985	0.995	0.993	0.999	0.988	0.979	0.992
3	0.964	0.982	0.983	0.996	0.985	0.979	0.991
4	0.951	0.988	0.978	1.00	0.981	0.977	0.984
5	0.935	0.967	0.977	0.988	0.975	0.980	0.982
6	0.920	0.959	0.966	0.976	0.974	0.979	0.975
7	0.905	0.953	0.955	0.974	0.963	0.969	0.967
8	0.893	0.949	0.951	0.973	0.961	0.964	0.967
9	0.888	0.943	0.951	0.968	0.956	0.955	0.957
10	0.869	0.942	0.946	0.960	0.956	0.948	0.955
- NADH concentration : 125 μM - pH 5: sodium acetate buffer solution - pH 6-8: sodium phosphate buffer solution - pH 9-11: sodium carbonate buffer solution							

[0084]

[0085]

[Table 6]

Decrease of NADH fluorescence due to photocatalyst at various pH							
Time (min)	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	pH 11
0	1	1	1	1	1	1	1
1	0.653	0.878	0.935	0.915	0.868	0.872	0.805
2	0.440	0.768	0.845	0.812	0.719	0.729	0.574
3	0.296	0.647	0.750	0.701	0.572	0.578	0.372
4	0.193	0.540	0.653	0.583	0.426	0.438	0.218
5	0.125	0.443	0.555	0.476	0.303	0.325	0.123
6	0.0831	0.348	0.454	0.371	0.203	0.216	0.0648
7	0.0607	0.262	0.364	0.271	0.118	0.136	0.0462
8	0.0484	0.193	0.278	0.190	0.0700	0.0868	0.0418
9	0.0460	0.132	0.198	0.122	0.0486	0.0536	0.0445
10	0.0468	0.0867	0.130	0.0745	0.0440	0.0466	0.0449
- NADH concentration : 125 μM - TiO_2 concentration : 80 μg/mL							

[0086]

[0087] [Table 7]

Rate of decrease of NADH fluorescence due to photocatalyst at different pH		
Buffer solution pH	Reaction rate (μM/min)	
	NADH only	NADH + TiO_2
5	1.701	35.0
6	0.846	14.0
7	0.754	11.3
8	0.654	13.1
9	0.500	17.7
10	0.476	17.3
11	0.648	25.0
- NADH concentration : 125 μM - TiO_2 concentration : 80 μg/mL		

[0088]

[0089] In Tables 5 and 6 and in Figs. 7 and 8, the measurement results of relative fluorescence intensities of NADH over UV irradiation time in pH buffer solutions with and without TiO_2, respectively, have been summarized. In the absence of the photocatalyst, TiO_2, the reaction rate rarely changes with pH since the photoinduced oxidation of NADH hardly occurs. In contrast, in the presence of TiO_2, the rate of NADH oxidation changes with pH since the production rate of ROS by the photocatalyst changes. The change in the rate of oxidation of NADH depending on pH is summarized in Table 7 and Fig. 9.

[0090] This result shows that, when measuring photocatalytic activity, it is important to maintain the pH of the corresponding solution constant using a buffer solution.

[0091] The present application contains a subject matter related to Korean Patent Application No. 10-2010-0100036, filed in the Korean Intellectual Property Office on October 13, 2010, the entire contents of which is incorporated herein by reference.

[0092] Those skilled in the art will appreciate that the conceptions and specific embodiments disclosed in the foregoing description may be readily utilized as a basis for modifying or designing other embodiments for carrying out the same purposes of the present disclosure. Those skilled in the art will also appreciate that such equivalent embodiments do not depart from the spirit and scope of the disclosure as set forth in the appended claims.

[0093]

[0094]

[0095]

Claims

[Claim 1] A method for measuring photocatalytic activity of a photocatalyst, comprising:
adding NADH and a photocatalyst to a container and measuring the fluorescence intensity of NADH before photoactivation of the photocatalyst;
irradiating UV light to the container to activate the photocatalyst and then measuring the fluorescence intensity of NADH; and
determining the photocatalytic activity of the photocatalyst from the change in the fluorescence intensity of NADH before and after the photoactivation of the photocatalyst.

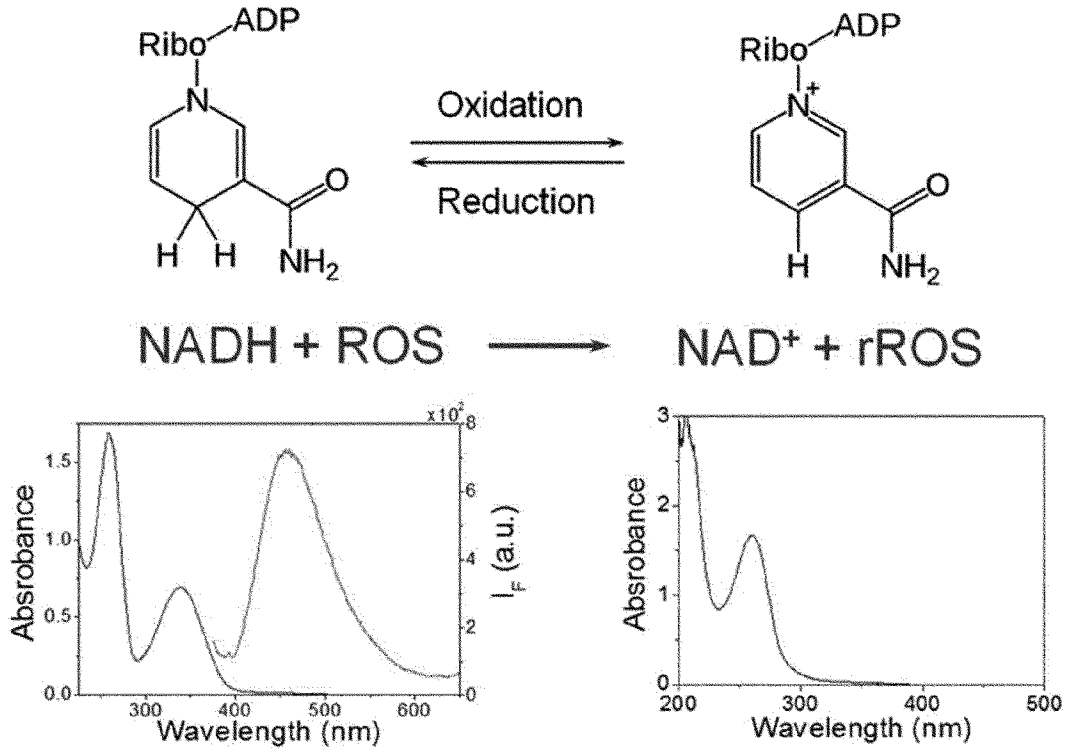
[Claim 2] The method for measuring photocatalytic activity of a photocatalyst according to claim 1, wherein the absorbance of NADH is measured instead of the fluorescence intensity of NADH.

[Claim 3] The method for measuring photocatalytic activity of a photocatalyst according to claim 1 or 2, wherein the photocatalyst is one or more selected from the group consisting of TiO_2, SiO_2, WO_3 and ZnO.

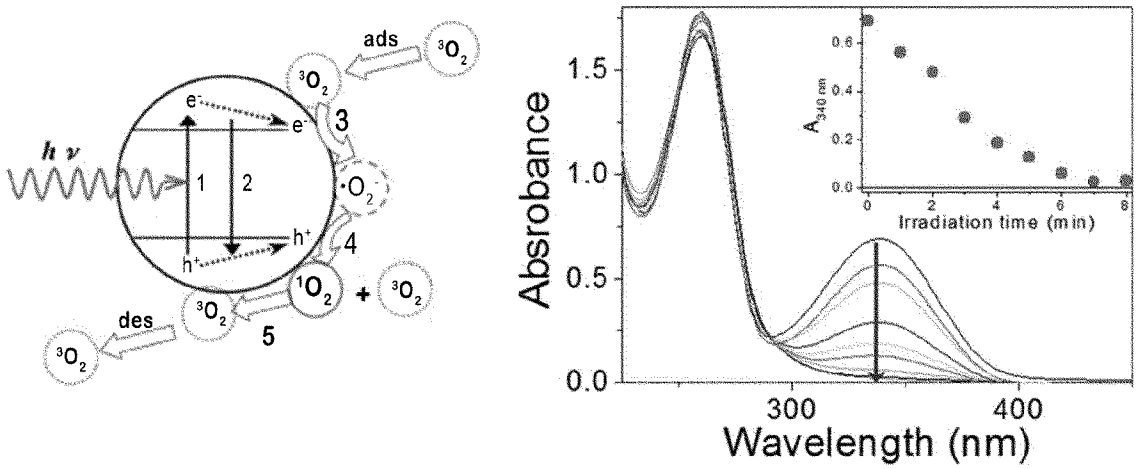
[Claim 4] The method for measuring photocatalytic activity of a photocatalyst according to claim 1 or 2, wherein the UV light has a wavelength in the range from 290 to 400 nm.

[Claim 5] The method for measuring photocatalytic activity of a photocatalyst according to claim 1 or 2, wherein the concentration of the NADH is in the range from 31 μM to 250 μM.

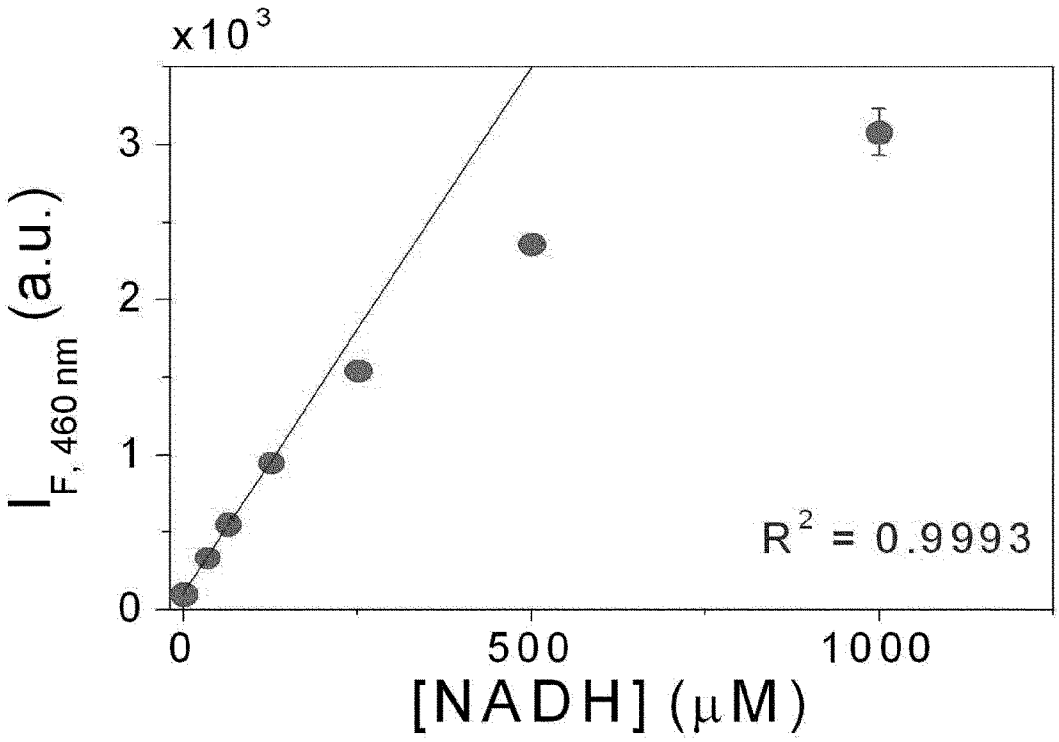
[Fig. 1]


Ribo
ADP
N
O
H
H
NH2
Oxidation
Reduction
Ribo
ADP
N+
O
H
NH2
NADH + ROS
NAD+ + rROS
Absrobance
Wavelength (nm)
I_F (a.u.)
x10^2
Absrobance
Wavelength (nm)

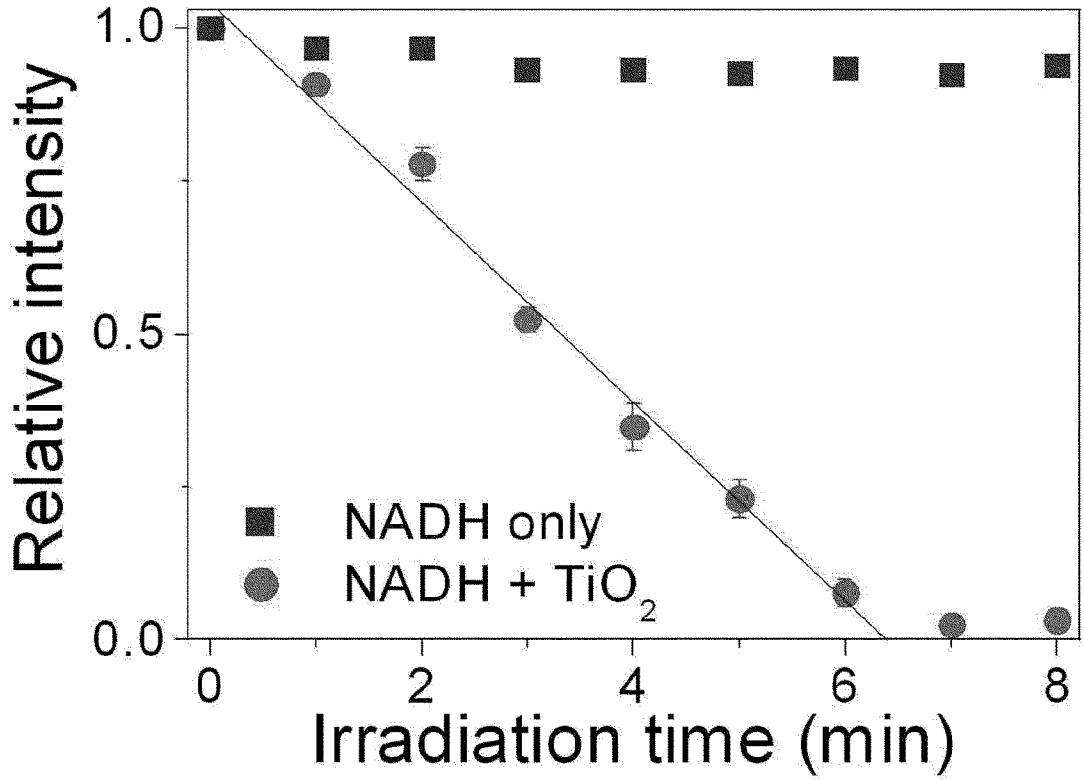
[Fig. 2]


ads
3O2
e-
e-
3
h ν
1
2
·O2-
h+
h+
4
1O2
+
3O2
des
3O2
3O2
5
Absrobance
Wavelength (nm)
A340 nm
Irradiation time (min)

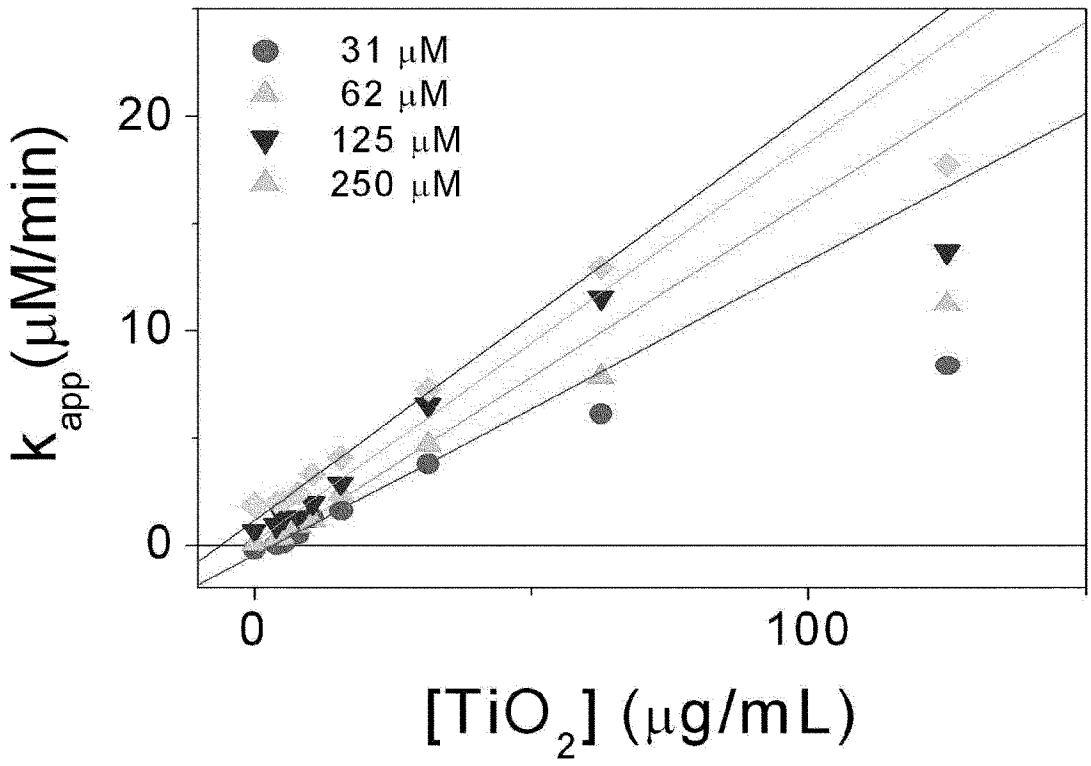
[Fig. 3]


x10^3
3
2
1
0
I_F, 460 nm (a.u.)
R^2 = 0.9993
0
500
1000
[NADH] (μM)

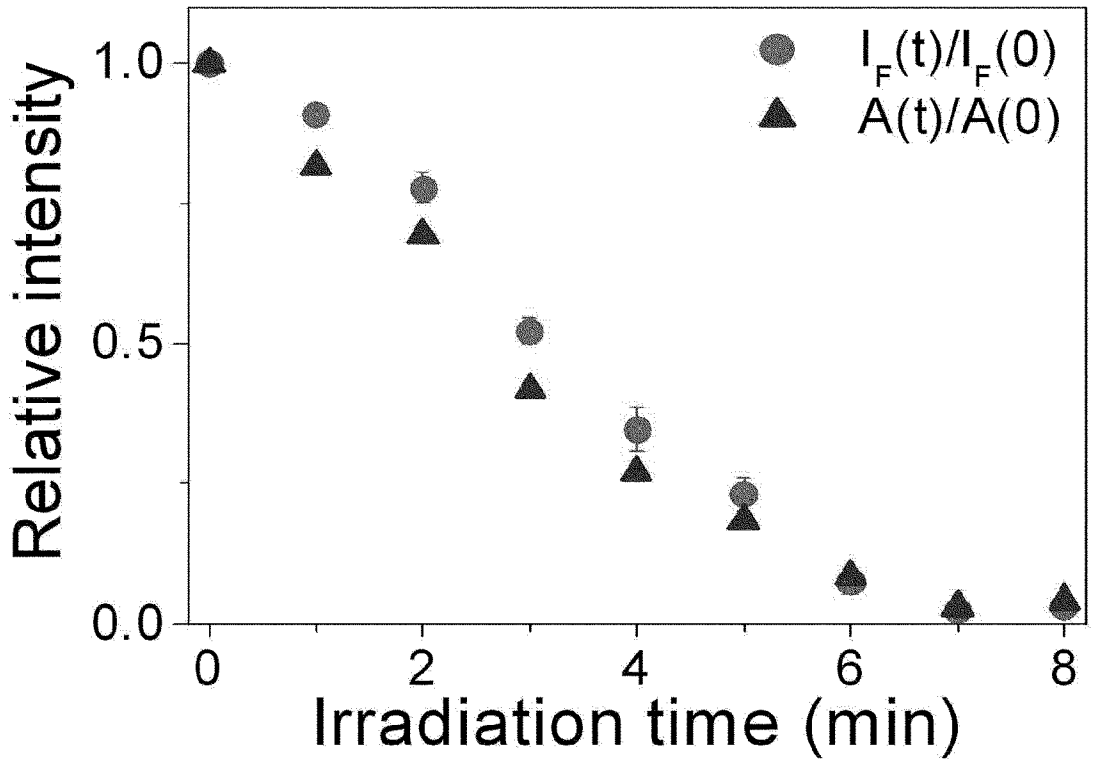
[Fig. 4]


1.0
0.5
0.0
Relative intensity
NADH only
NADH + TiO2
0
2
4
6
8
Irradiation time (min)

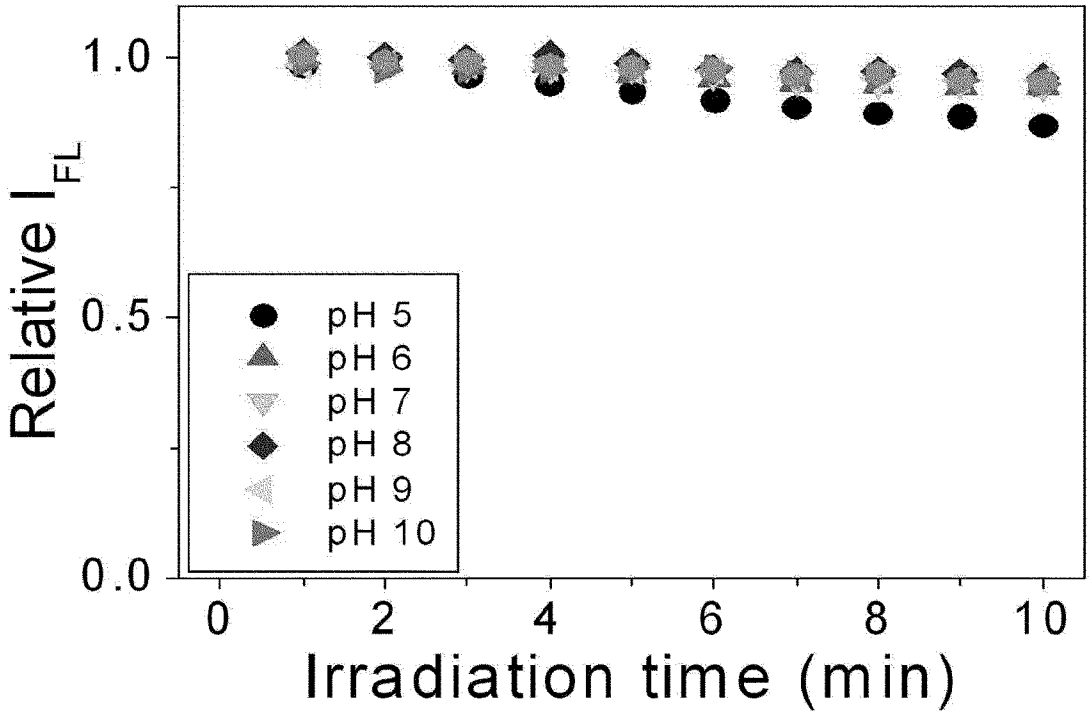
[Fig. 5]


31 μM
62 μM
125 μM
250 μM
20
10
0
0
100
k_{app}(μM/min)
[TiO_2] (μg/mL)

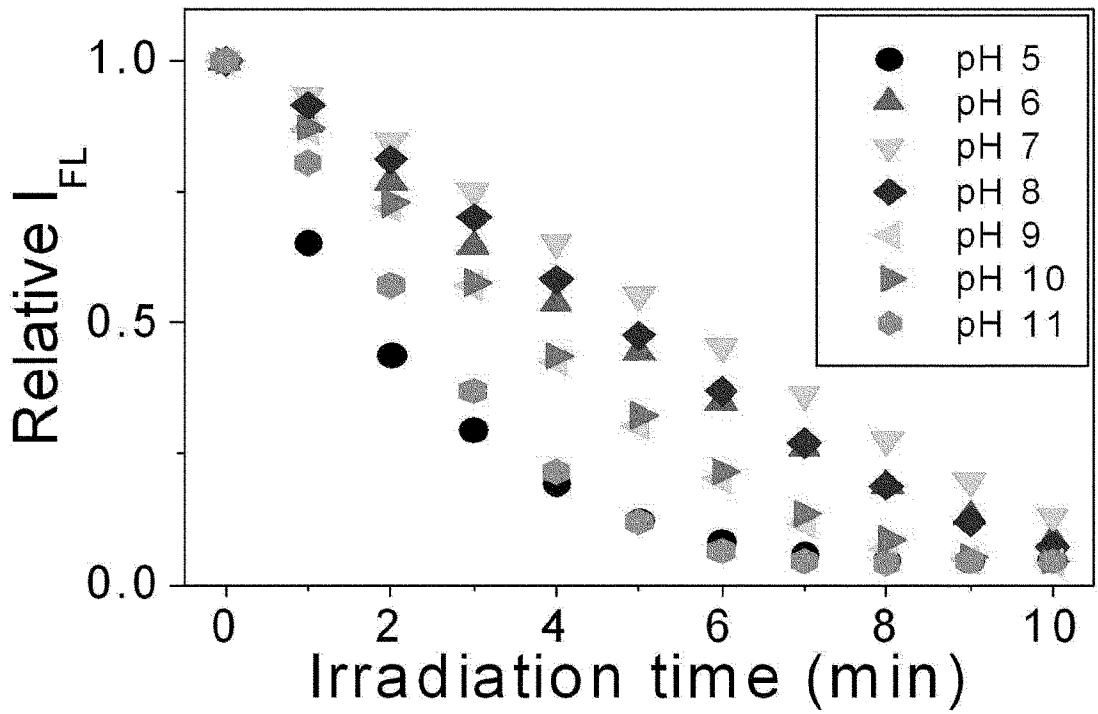
[Fig. 6]


$I_F(t)/I_F(0)$
A(t)/A(0)
1.0
0.5
0.0
0
2
4
6
8
Relative intensity
Irradiation time (min)

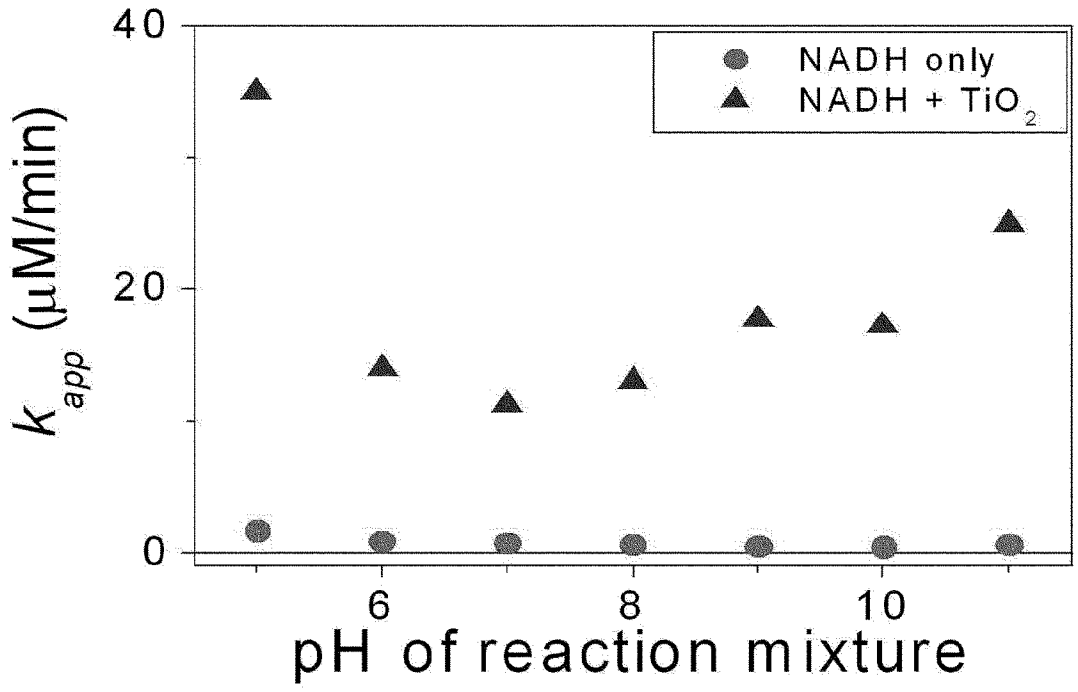
[Fig. 7]


1.0
0.5
0.0
Relative I_{FL}
pH 5
pH 6
pH 7
pH 8
pH 9
pH 10
0
2
4
6
8
10
Irradiation time (min)

[Fig. 8]


1.0
0.5
0.0
Relative I_{FL}
pH 5
pH 6
pH 7
pH 8
pH 9
pH 10
pH 11
0
2
4
6
8
10
Irradiation time (min)

[Fig. 9]


40
20
0
k_{app} (μM/min)
NADH only
NADH + TiO_2
6
8
10
pH of reaction mixture

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2010/007910

A. CLASSIFICATION OF SUBJECT MATTER

G01N 21/64(2006.01)i, G01N 21/33(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)

G01N 21/64; G01N 21/78; G01N 21/25; G01N 31/10; G01N 21/00

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) & Keywords: photocatal*,(fluore*,spectr*,absor*)+(uv,ultraviolet*,,activity*)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KR 10-2003-0053226 A (재단법인 포항산업과학연구원 et al.)	1-4
A	See page 3, line 20 - page 3, line 39, fig. 1.	5
Y	KEN-ICHI ISHIBASHI et al. `Quantum yields of active oxidative species formed on TiO2 photocatalyst` Journal of Photochemistry and Photobiology A: Chemistry 134 (2000) 139-142	1-4
A	See column 1, line 1 - column 2, line 11.	5
A	KR 10-2009-0051787 A (현대자동차주식회사 et al.) See page 4, line 29 - page 5, line 12, claim 1.	1-5
A	JP 2008-501937 A (NEUMANN et al.) 24 January 2008 See paragraph 17 - paragraph 14, claim 1.	1-5

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 NOVEMBER 2011 (23.11.2011)	**24 NOVEMBER 2011 (24.11.2011)**

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2010/007910

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
KR 10-2003-0053226 A	28.06.2003	None	
KR 10-2009-0051787 A	25.05.2009	None	
JP 2008-501937 A	24.01.2008	DE 102004027118 A1	29.12.2005
		DE 102004027118 B4	11.05.2006
		EP 1751524 A1	14.02.2007
		JP 2008-501937 T	24.01.2008
		WO 2005-119220 A1	15.12.2005