Research Article

Near-Infrared Indocyanine Materials for Bioanalysis and Nano-TiO₂ Photoanodes of Solar Cell

Liqiu Wang, Pengjun Wang, Yang Liu, Weiwei Nie, Xiaofei Lv, Yue Wei, Yangyang Hao, Hailing Yu, Liang Tian, Lihui Zheng, and Liuqing Yang

College of Environmental and Chemical Engineering, Yanshan University, Qinhuangdao, Hebei 066004, China

Correspondence should be addressed to Liqiu Wang; liqiuwang@tom.com

Received 30 March 2013; Revised 25 May 2013; Accepted 3 June 2013

Academic Editor: Xinqing Chen

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Two near-infrared fluorescence N-p-carboxybenzyl indocyanines with the maxima fluorescence emission wavelengths of 565 nm and 641 nm in water, respectively, were synthesized by the ultrasonic method. Compared with those in water, the fluorescence intensity of the dyes became weaker in the solution of an alkali, alkaline-earth, or transition metal ion, except in Ag⁺ or Zn²⁺; in the solution of α-cyclodextrin or β-cyclodextrin, the fluorescence intensity of them was increased, even under strong acidic condition. The indocyanines were converted into their succinimidyl active ester for conjugating with NH₂ groups of lysine or BSA. Porous nano-TiO₂ film with 68~75 nm thickness was prepared on ITO conductive glass from tetrabutyltitanate by sol-gel. FESEM showed that the diameter of the nano-TiO₂ particle was 30~50 nm and the pore size was 200~300 nm. The indocyanines with or without the presence of the alkali, alkaline-earth, transition metal ion, or cyclodextrin could make the nano-TiO₂ films sensitized, and the products could be used as anodes for dye-sensitized solar cells.

1. Introduction

Cyanines have attracted a lot of attention as functional molecules in various high-tech fields [1–3], especially as photosensitizers in solar cell [4–6], and fluorescence probes in biosensor [7–9] or ions detection [10, 11]. Many cyanines have been developed, and the development of stable near-infrared cyanines was the key to avoid the influence of base ground of biomass on detectivesensitivity in bioanalysis [12]. We had designed and synthesized series of novel fluorescence indocyanines containing N-p-carboxybenzyl groups on the N atoms in the heterocyclic rings from 2,3,3-trimethyl-3H-indoleninium-5-sulfonate before the papers in [13, 14]. This paper synthesized symmetric N-p-carboxybenzyl trimethine sulfoindocyanine (dye 1) and N-p-carboxybenzyl squaraine (dye 2) by ultrasonic method, and it also investigated their spectral properties, especially in presence of an alkali, alkaline-earth, or transition metalion, α-cyclodextrin, or β-cyclodextrin. The dyes could combine with NH₂ groups of lysine or bovine serum albumin (BSA), and they were expected to be used as fluorescence probes in bioanalysis. Nano-TiO₂ film was prepared from tetrabutyl titanate by sol-gel and sensitized by the dyes and the dye composites. They could be used as photoanodes in dye-sensitized solar cell. Structures of dye 1 and dye 2 were shown in Scheme 1.

2. Experimental

Mass spectral determinations were recorded on HPI100APIES mass spectrometry. ¹H NMR spectra were made in D₂O on a VARIAN 400 MHz spectrometer. The detection of microstructure of products was performed by S-4800 field-emission scanning electron microscopy and D-max-2500 X-ray diffraction, and the thickness was performed by XP-2 step profiler measurement. Fluorescence measurements were performed by a PTI-C-700 Felix (USA), and UV-Vis spectra were measured on an HP-8453 spectrophotometer. Squaric acid was purchased from ACROS ORGANICS (USA), and
The intermediate was dissolved in pyridine, then a solution of triethyl orthoformal in pyridine was added slowly. After the mixture was kept at 85–90°C for 1.5 h under the ultrasonic method, dye 1 was formed. The crude product was separated and purified by C18 reverse-phase chromatography using water-methanol (0–4:1) as the eluent.

The intermediate was dissolved in pyridine with squaric acid in butanol/toluene (1:1) for 5 h at 85–90°C under the ultrasonic method. The formed crude product dye 2 was purified by C18 reverse-phase chromatography using water-methanol (0–4:1) as the eluent.

Dye 1 (or Dye 2) reacted with N-hydroxysuccinimide and DCC in DMF for 24 h at room temperature. The succinimidyl active ester solid of the dye was filtered and triturated, and it reacted with lysine or BSA in bicarbonate buffer (pH 9.5) at room temperature, respectively, and the conjugate was formed.

Tetrabutyl titanate reacted with absolute ethyl alcohol at room temperature for 20 min. Diethanolamine was added slowly. Twenty minutes later, ethyl alcohol with little water was trickled slowly into the mixture, then saturated PEG solution was added. Two hours later, stir was quit, and the mixture was left in stillness for three hours. A conductive glass was stood in the sol-formed gel, and it was pulled out at speed 3 cm/min by TL0.01 vertical membrane machine. After repeating the operation 4 times, a 4-layer TiO₂ film was obtained. The film was heated for 30 min at 100°C first, then the temperature was elevated to 550°C at the speed of 5°C/min, keeping it at the temperature for 1 h. The nano-TiO₂ film on the conductive glass obtained was impregnated into solution of the dye, the dye in the solution of the alkali, alkaline-earth, transition metal ion, or cyclodextrin for 4 h, and then the dye-sensitized nano-TiO₂ film was obtained.

3. Results and Discussion

Ultrasonic method as a modern synthetic technology has been widely used in organic synthesis due to its convenience and efficiency [15, 16]. Various organic reactions have been carried out under the ultrasonic method with high yields within a short reaction time. This paper synthesized the two dyes by condensation of the intermediate N-p-carboxybenzyl-2,3,3-trimethyl-3H-indoleninium-5-potassium sulfonate with triethyl orthoformate or squaric acid under ultrasonic irradiation, respectively. Compared with traditional method, ultrasonic technique had the advantages of high yields, short reaction time, and mild reaction conditions. The product mixture was easy to separate to get purified dye products due to less by-products especially coke material formed at low reaction temperature. The structures of the dye products were confirmed by 1H NMR and MS. Dye 1: ESI-MS: [M-K]⁻ (m/z = 755.7), [M-K-2H+Na]⁺ (m/z = 388.0), [M-K-H]⁻ (m/z = 377.0), [M-K-2H]⁻ (m/z = 251.4); 1H NMR (400 MHz, D₂O, δ ppm): 88.34 (m, 1H, β-proton of the bridge), 7.93 (s, 2H, 4,4′-H), 7.79–7.77 (d, 2H, J = 8.0 Hz, 6,6′-H), 7.69–7.67 (d, 4H, J = 7.2 Hz, γ₁, γ₂-H), 7.26–7.24 (d, 2H, J = 8.0 Hz, 7,7′-H), 7.11–7.09 (d, 4H, J = 7.2 Hz, β₁, β₂-H), 6.18–6.15 (d, 2H, J = 13.5 Hz, αα′-protons of the bridge), 5.30 (s, 4H, α,α′-H), 1.62 (s, 12H, C(CH₃)₃). Dye 2: ESI-MS: [M+Na-H]⁻ (m/z = 922.5), [M-K]⁻ (m/z = 861.0), [M-2K+Na]⁻ (m/z = 846.2), [M-2K]⁻ (m/z = 411.3), [M-2K-H]⁻ (m/z = 273.9), [M-2K-2H]⁻ (m/z = 205.1); 1H NMR (400 MHz, D₂O, δ ppm): 7.88 (s, 2H, 4,4′-H), 7.84–7.82 (d, 4H, J = 8.0 Hz, γ₁, γ₂-H), 7.76–7.74 (d, 2H, J = 8.0 Hz, 6,6′-H), 7.33–7.31 (d, 4H, J = 8.0 Hz, β₁, β₂-H), 7.25–7.23 (d, 2H, J = 8.0 Hz, 7,7′-H), 5.87 (s, 2H, αα′-protons of the bridge), 5.39 (s, 4H, α,α′-H), 1.69 (s, 12H, C(CH₃)₃). Dye 2 had the maxima UV-Vis absorption and fluorescence emission wavelengths of 631 nm and 641 nm in water, respectively. Figures 1 and 2 showed the UV-Vis absorption and fluorescence emission wavelengths of dye 1 were 551 nm and 565 nm in water, respectively. Cyclodextrins (CDs) are water soluble and have remarkable ability to form inclusion complexes with a wide variety of guest molecules to let them have special properties [17]. Compared with those in water, the maxima absorption and fluorescence emission wavelengths of the dye in the solution of α- or β-cyclodextrin were hardly changed, but the weaker absorption intensity at the maxima absorption wavelength and the fluorescence intensity became stronger due to the increase of rigidity of the dye molecules and the viscosity of the dyes solution, the decrease of rotating energy of the dye molecules, and the formation of the dye-cyclodextrin
inclusion. The changes of those in β-CD solution were the same as those in α-CD, even in the strong acidic aqueous solution of α- or β-CD. These indicated that the CDs could not increase quantity of the conjugated π electrons of the dye excited by photon, but they could let the excited π electrons be easy to emit fluorescence so that the fluorescence intensity of the dye was increased. The intensity of UV-Vis absorption at 235 nm of the dye in β-CD was smaller than that in α-CD. But the intensity of fluorescence emission at 636 nm of the dye in β-cyclodextrin was nearly 2 folds larger than that in α-CD. It demonstrated that the size of inner cavity of α-CD was suitable for combining with N-p-carboxybenzyl group in the molecule of the dye, while β-CD was suitable for the π-conjugated system of the dye. In the presence of Na⁺, K⁺, Ca²⁺, Mg²⁺, Ni²⁺, Ag⁺, Zn²⁺, Cu²⁺, or Cr³⁺, the changes in the maxima absorption wavelengths and fluorescence emission wavelengths were similar to those in CDs, but the fluorescence intensity was reduced except in the presence of Ag⁺ or Zn²⁺. Figure 3 showed the maxima UV-Vis absorption spectra of dye 2 in buffer, the solution of lysine, or BSA in buffer. It indicated that compared with those absorption spectra of dye 2 in buffer, the UV-Vis absorption intensity of dye 2 in the solution of lysine or BSA in buffer was reduced, and the absorption wavelength in lysine was not changed, but in BSA it was slightly redshift and the absorption peak got wider.

The dyes could be converted to their succinimidyl active ester and conjugated with free NH₂ groups in lysine, or BSA in bicarbonate buffer (pH 9.5) at room temperature, respectively. SDS-PAGE is the preferred electrophoretic system, and it is commonly used to separate different proteins [18]. This paper applied SDS-PAGE to investigate the dye-protein conjugates. Figure 4 showed the SDS-PAGE of BSA (lanes a, a’) and dye 2-BSA conjugates (lanes b, b’) unstained (lanes a, b) or stained (lanes a’, b’) by Coomassie Brilliant Blue. It indicated that the carboxyl groups in the molecule of dye 2 had reacted with BSA and that the color of the conjugates

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**Figure 1:** UV-Vis absorption spectra of dye 1.

**Figure 2:** Fluorescence emission spectra of dye 1.

**Figure 3:** UV-Vis absorption spectra of dye 2 in buffer, solution of lysine, or BSA in buffer.

**Figure 4:** SDS-PAGE of BSA and dye 2-BSA conjugates.
Optimized molecular structure, polarizability, dipole moment, and electrostatic potential of the dyes were obtained at level of B3LYP/6-31G* by DFT method of Gaussian 03, and they were analyzed by visual software, Gaussview, HyperChem, and Chem3D. The optimized molecular structure of dye 1 was shown in Figure 5. The data would be the foundation for further research for the structures of the dyes synthesized and other cyanines. Figure 6 showed the crystal shape of the dye 1-β-cyclodextrin inclusion under microscope. The crystal shapes of the dyes included in cyclodextrin were polygon.

Semiconductive metal oxides are sensitized by dyes to form the anodes of DSSC [19,20]. Porous nano-TiO₂ film was prepared on ITO conductive glass from tetrabutyl titanate by sol-gel technique. The microstructure of the film was investigated by XRD and FESEM, as shown in Figures 7 and 9. In Figure 7, diffraction peaks 1, 2, and 3 (30.4°, 35.4°, and 50.8°) belong to ITO conductive glass, and diffraction peaks 4, 5, and 6 (25.2°, 37.5°, and 60.5°) belong to TiO₂ crystallite. The results indicated that crystalline phase of the nano-TiO₂ was anatase, but the characteristic peaks of anatase TiO₂ were partly covered by those of ITO conductive glass due to the nano-TiO₂ film being thinner on the glass substrate. As shown in Figures 8 and 9, the porous TiO₂ film was 68–75 nm thickness, 200–300 nm pore dimension, and 30–50 nm diameter of the nano-TiO₂ particle. The dyes and their alkali, alkaline-earth, transition metal ion, or CD composites could sensitize the porous nano-TiO₂ film by impregnation method, and they made the photo current and voltage of the nano-TiO₂ film improved.

4. Conclusions

Two near-infrared fluorescence dyes were synthesized by the ultrasonic method. Generally, alkali, alkaline-earth, transition metal ion, α-cyclodextrin, or β-cyclodextrin could influence the UV-Vis absorption and fluorescence emission spectra of the dyes; some made the fluorescence emission intensity weaker, and some made them stronger, but there were little changes in absorption and fluorescence emission wavelengths. They could be converted to their succinimidyld active ester to conjugate with NH₂ groups of biomass, and they could sensitize porous nano-TiO₂ film to improve the photo current and voltage of the film. They were expected to be used as fluorescence probes in bioanalysis and as photosensitizers for dye-sensitized solar cell.
Acknowledgments

The authors would like to thank the National Natural Science Foundation of China (Grant no. 20776122), the Hebei Natural Science Foundation (Grant no. B2011203231), and the Key Laboratory of Applied Chemistry (Yanshan University) for providing the financial support for this research paper.

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