

Research Article

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

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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 tetrabutyl titanate 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.

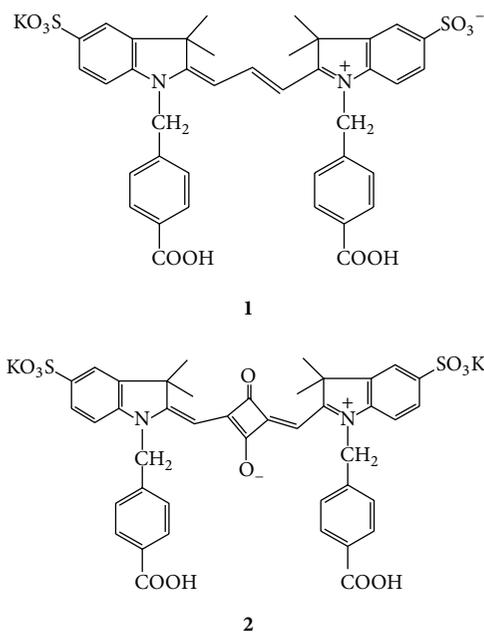
1. Introduction

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 detective sensitivity 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 metal ion, 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 HP1100API-ES 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



SCHEME 1: Structures of the indocyanines.

ITO conductive glass was purchased from Qinhuangdao Yaohua Glass Factory (China).

Intermediate *N*-*p*-carboxybenzyl-2,3,3-trimethyl-3H-indoleninium-5-potassium sulfonate was prepared by quaternization of 2,3,3-trimethylindoleninium-5-potassium sulfonate with *p*-chloromethyl benzoic acid; refer to our previous works in [13, 14].

The intermediate was dissolved in pyridine, then a solution of triethyl orthoformate 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 chromatograph using water-methanol (0–4 : 1) as the eluent.

The intermediate in pyridine reacted 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 chromatograph 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 laid 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]²⁻ (*m/z* = 388.0), [M-K-H]²⁻ (*m/z* = 377.0), [M-K-2H]³⁻ (*m/z* = 251.4); ¹H NMR (400 MHz, D₂O, δppm): δ8.34 (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,γ₁'-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,β₁'-H), 6.18–6.15 (d, 2H, *J* = 13.5 Hz, α,α'-protons of the bridge), 5.30 (s, 4H, α₁,α₁'-H), 1.62 (s, 12H, C(CH₃)₂). 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]²⁻ (*m/z* = 411.3), [M-2K-H]³⁻ (*m/z* = 273.9), [M-2K-2H]⁴⁻ (*m/z* = 205.1); ¹H NMR (400 MHz, D₂O, δppm): 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, α,α'-protons of the bridge), 5.39 (s, 4H, α₁,α₂-H), 1.69 (s, 12H, C(CH₃)₂).

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 spectra and fluorescence emission spectra of dye 1 in water and aqueous solution of α-cyclodextrin. The maxima 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

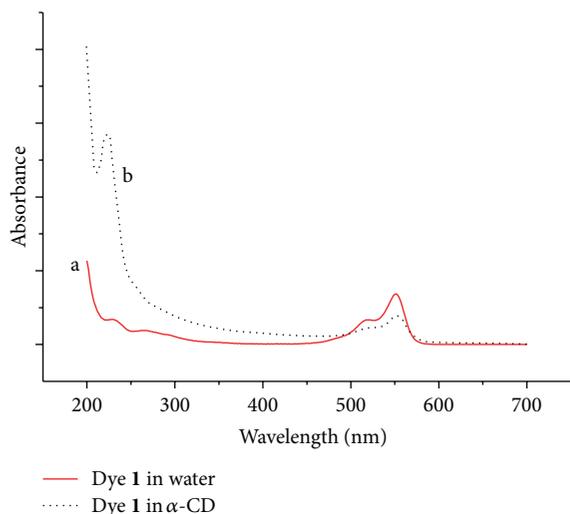


FIGURE 1: UV-Vis absorption spectra of dye 1.

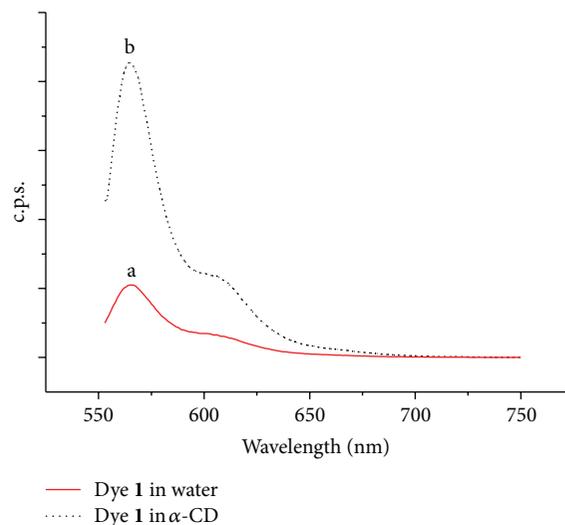


FIGURE 2: Fluorescence emission spectra of dye 1.

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^+ , Ca^{2+} , Mg^{2+} , Ni^{2+} , Ag^+ , Zn^{2+} , Cu^{2+} , or Cr^{2+} , 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 Zn^{2+} . 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 NH_2 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

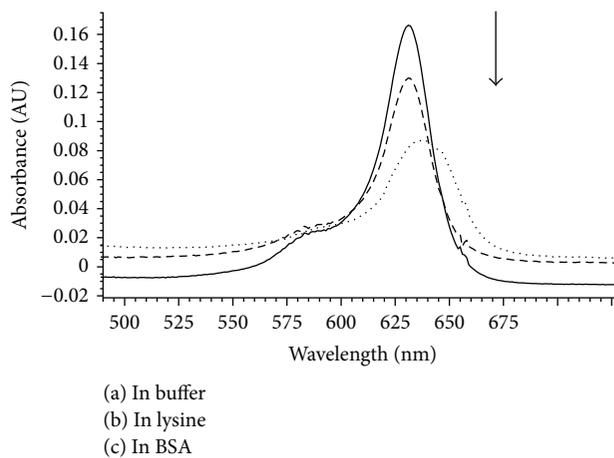


FIGURE 3: UV-Vis absorption spectra of dye 2 in buffer, solution of lysine, or BSA in buffer.

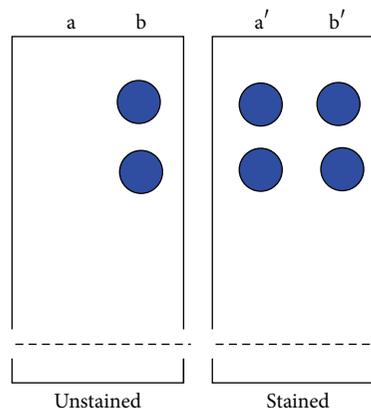


FIGURE 4: SDS-PAGE of BSA and dye 2-BSA conjugates.

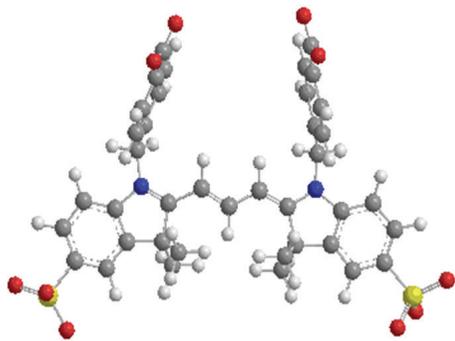


FIGURE 5: Optimized molecular structure of dye 1.

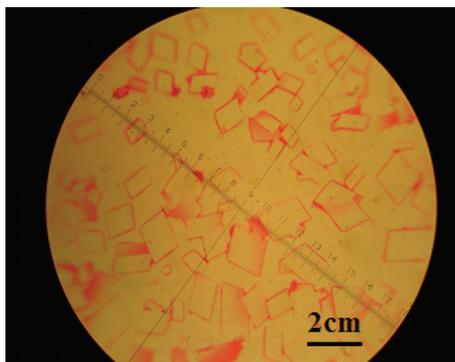
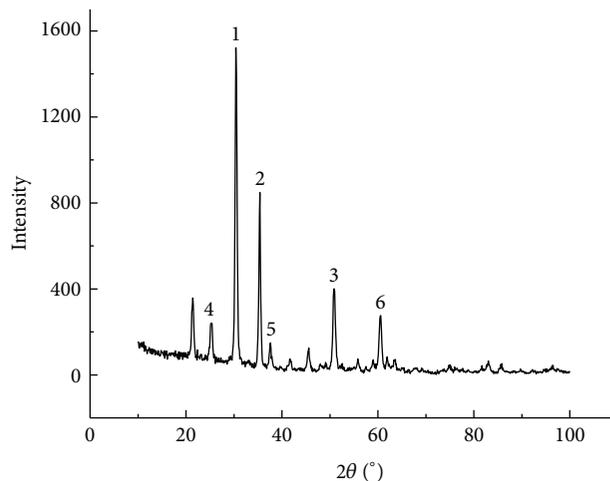
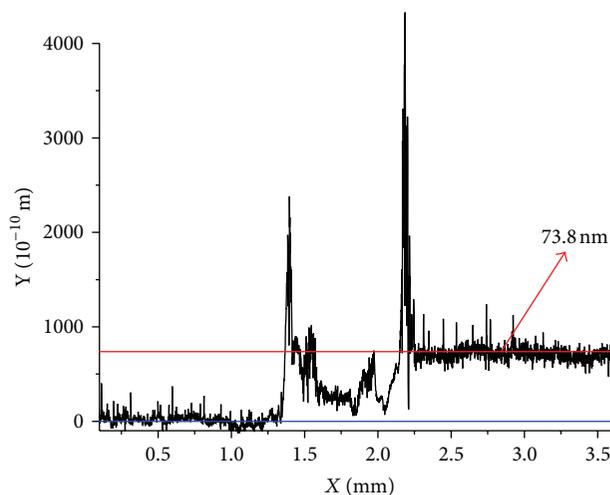


FIGURE 6: Crystal shape of dye 1-β-CD.

was blue due to the color of dye 2. They were also detected by HPLC and ESI-MS.

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

FIGURE 7: XRD pattern of the nano-TiO₂ film.FIGURE 8: Thickness of the nano-TiO₂ film.

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 succinimidyl 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.

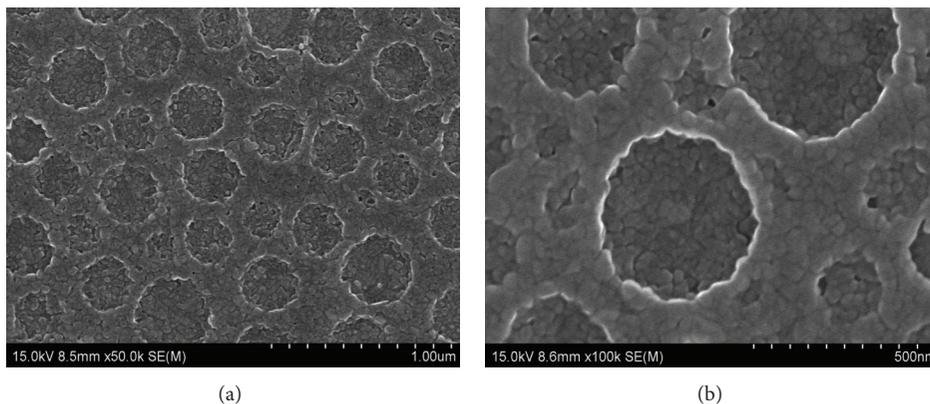


FIGURE 9: FESEM photographs of the nano-TiO₂ film.

Acknowledgments

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References

- [1] Z. Y. Li, Y. L. Fang, and S. Xu, "Squaraine dye sensitized TiO₂ nanocomposites with enhanced visible-light photocatalytic activity," *Materials Letters*, vol. 93, pp. 345–348, 2013.
- [2] Y. Prostota, O. D. Kachkovsky, L. V. Reis, and P. F. Santos, "New unsymmetrical squaraine dyes derived from imidazo[1,5-a]pyridine," *Dyes and Pigments*, vol. 96, pp. 554–562, 2013.
- [3] A. S. Tatikolov, Z. A. Krasnaya, L. A. Shvedova, and V. A. Kuzmin, "Effects of chromophore interaction in photophysics and photochemistry of cyanine dyes," *International Journal of Photoenergy*, vol. 2, no. 1, pp. 23–30, 2000.
- [4] B. O'Regan and M. Grätzel, "A low-cost, high-efficiency solar cell based on dye-sensitized colloidal TiO₂ films," *Nature*, vol. 353, no. 6346, pp. 737–740, 1991.
- [5] K. Sayama, K. Hara, N. Mori et al., "Photosensitization of a porous TiO₂ electrode with merocyanine dyes containing a carboxyl group and a long alkyl chain," *Chemical Communications*, no. 13, pp. 1173–1174, 2000.
- [6] P. V. Kamat and G. C. Schatz, "Nanotechnology for next generation solar cells," *Journal of Physical Chemistry C*, vol. 113, no. 35, pp. 15473–15475, 2009.
- [7] S. Z. Xiao, S. H. Turkyilmaz, and B. D. Smith, "Convenient synthesis of multivalent zinc(II)—dipicolylamine complexes for molecular recognition," *Tetrahedron Letters*, vol. 54, pp. 861–864, 2013.
- [8] K. C. Hannah and B. A. Armitage, "DNA-templated assembly of helical cyanine dye aggregates: a supramolecular chain polymerization," *Accounts of Chemical Research*, vol. 37, no. 11, pp. 845–853, 2004.
- [9] K. Endo and Y. Nakamura, "A binary Cy3 aptamer probe composed of folded modules," *Analytical Biochemistry*, vol. 400, no. 1, pp. 103–109, 2010.
- [10] J. Kang, O. Kaczmarek, J. Liebscher, and L. Dahne, "Prevention of H-aggregates formation in Cy5 labeled macromolecules," *International Journal of Polymer Science*, vol. 2010, Article ID 264781, 7 pages, 2010.
- [11] K. M. Shafeekh, M. K. A. Rahim, M. C. Basheer, C. H. Suresh, and S. Das, "Highly selective and sensitive colourimetric detection of Hg²⁺ ions by unsymmetrical squaraine dyes," *Dyes and Pigments*, vol. 96, pp. 714–721, 2013.
- [12] M. C. Basheer, U. Santhosh, S. Alex, K. G. Thomas, C. H. Suresh, and S. Das, "Design and synthesis of squaraine based near infrared fluorescent probes," *Tetrahedron*, vol. 63, no. 7, pp. 1617–1623, 2007.
- [13] L. Wang, X. Peng, R. Zhang, J. Cui, G. Xu, and F. Wang, "Syntheses and spectral properties of fluorescent trimethine sulfo-3H-indocyanine dyes," *Dyes and Pigments*, vol. 54, no. 2, pp. 107–111, 2002.
- [14] L. Wang, X. Peng, F. Song et al., "New near-infrared indocyanines and their spectral properties in SiO₂ sol-gel," *Dyes and Pigments*, vol. 61, no. 2, pp. 103–107, 2004.
- [15] H. Fillion and J. L. Luche, *Synthetic Organic Sonochemistry*, Plenum Press, New York, NY, USA, 1998.
- [16] L. Buriol, T. S. München, C. P. Frizzo et al., "Resourceful synthesis of pyrazolo [1, 5-a] pyrimidines under ultrasound irradiation," *Ultrasonics Sonochemistry*, vol. 20, no. 5, pp. 1139–1143, 2013.
- [17] T. Tedeschi, S. Sforza, S. Ye et al., "Fast and easy colorimetric tests for single mismatch recognition by PNA-DNA duplexes with the diethylthiadicyanin dye and succinyl-β-cyclodextrin," *Journal of Biochemical and Biophysical Methods*, vol. 70, no. 5, pp. 735–741, 2007.
- [18] F. Y. Hao and X. W. Zhou, *Biochemistry and Molecular Biology Experiments*, Higher Education Press, Beijing, China, 2009.
- [19] B. K. Kılıç, E. Gur, and S. Tuzemen, "Nanoporous ZnO photoelectrode for dye-sensitized solar cell," *Journal of Nanomaterials*, vol. 2012, Article ID 474656, 7 pages, 2012.
- [20] S. Kathirvel, H. S. Chen, C. C. Su, H. H. Wang, C. Y. Li, and W. R. Li, "Preparation of smooth surface TiO₂ photoanode for high energy conversion efficiency in dye-sensitized solar cells," *Journal of Nanomaterials*, vol. 2013, 8 pages, 2013.



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