An Evanescent Wave Fiber Optic Biosensor Based on Metal-Enhanced Fluorescence (MEF)

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Abstract

Fiber-optic biosensors play a significant role in the development of biosensors because they can provide miniaturized and low-cost systems. Recently, there have been explosive developments in the Metal-Enhanced Fluorescence (MEF) technology to favorably modify the spectral properties and to alleviate photo-physical constraints. We report the development of core/shell nanoparticles with the silver core and silica shell for potential applications in fiber optic biosensor. The fluorescence intensity of the fluorescence probe doped core/shell nanoparticles is approximately 40-fold higher than that without core/shell nanoparticles doping. In addition, the enhanced emission of fluorescence intensity in different solvents changes from 1.2-to 100-fold as compared to the control sample.

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1. Introduction

Fiber-optic biosensors are widely used to measure biological species, because they are efficiency, accuracy, low-cost, and convenience than traditional immunological methods [1]. For use in optical sensor, the end result must be a change in an optical property induced by interaction of the recognition element with the target. In order to amplify sensitivity and selectivity, biosensors are being developed with various optical transduction mechanisms, including refractive index, absorption, fluorescence, and surface plasmon resonance [2].

Fluorescence sensing has become one of the dominant sensing technologies in medical diagnostics and biotechnology. The detection of a fluorophore is usually limited by its quantum yield, auto-fluorescence of the samples and the photo-stability of the fluorophores. Recently, there have been explosive developments in the Metal-Enhanced Fluorescence (MEF) technology. The presence of nearby metallic nanostructures can alter the free-space condition. Metal surfaces can increase or decrease the radiative decay rates of fluorophores and can increase the extent of resonance energy transfer [3].

Following excitation a fluorophore in free space can either emit a photon with a radiative deactivation rate (Γ) or return to the ground state by a non-radiative rate (k_{nr}). For simplicity we are omitting the transitions to the triplet
state and chemical processes leading to photodegradation. The quantum yield ($Q_0$) and lifetime ($\tau_0$) of the fluorophore in the free-space condition are given by:

$$Q_0 = \frac{\Gamma}{(\Gamma + k_{nr})}$$  \hspace{1cm} (1)

$$\tau_0 = \frac{1}{(\Gamma + k_{nr})}$$  \hspace{1cm} (2)

The presence of a nearby metal surface increases the radiative rate by addition of a new rate $\Gamma_m$. In this case, the quantum yield ($Q_m$) and lifetime of the fluorophore ($\tau_m$) near the metal surface are given by:

$$Q_m = \frac{(\Gamma + \Gamma_m)}{(\Gamma + \Gamma_m + k_{nr})}$$  \hspace{1cm} (3)

$$\tau_m = \frac{1}{(\Gamma + \Gamma_m + k_{nr})}$$  \hspace{1cm} (4)

These equations result in unusual predictions for a fluorophore near to a metallic surface. From Equations, we can see that as the value of $\Gamma_m$ increases, the quantum yield $Q_m$ increases, while the lifetime, $\tau_m$, decreases Fig. 1[4].

![Fig.1. Metal-Enhanced Fluorescence](image)

The fluorescence of fluorophores can be enhanced or quenched due to the presence of nearby metallic nanoparticles. The strength of the enhancement or quenching is influenced by many factors, such as size and shape of the metal nanoparticles, the orientation of the fluorophore dipole moments, the distance between a fluorophore and a nanoparticle, the organic solvents and quantum yield of the fluorophore. However, metallic nanoparticles are usually failed to yield fluorescence enhancement according to fluorophore–metal distances. At shorter distances, the local field could be extremely high as observed in surface enhanced Raman scattering; longer distance condition is the interaction of the excited molecule with metal which results in a rapid radiation of the excitation energy; when placed at an appropriate distance, can effectively enhance the fluorescence by transferring the free electrons of the fluorophore [5-7].

The core/shell nanoparticles mean that the core or the shell should contain metallic component. The core of structures can be a metal or metal oxide or inorganic while the shell can also be any inorganic material or can be a metal or metal oxide. The most widely used core/shell nanocomposites are gold or silver core with silica shell. Metallic nanoparticles find application in biosensing and the silica coat obtains the optimum fluorophore–metal distance. Most of all, the silica coat makes the metallic nanoparticles biocompatible. Therefore, core/shell nanoparticles have provided the best solution for MEF technology [8].

Evanescent wave fluorescent sensors have developed for 30 years. Nonetheless, the technology continues to evolve with new breakthroughs in optics, biochemistry, and chemical engineering. In this paper, we develop evanescent wave fluorescence biosensor with core/shell nanoparticles.

2. Methodology

2.1. Measurement System

The measurement system has three major components: (1) the optics, including the waveguide (optical fiber, JTFLH6006301040, Polymicro Technologies), the light source (488 nm solid-state laser 10mw, Melles Griot), filter (530nm long-pass filter, CVI), objective lens (N.A. 0.4, Kyowa) and the spectrometer (SP-150, Princeton Instrument); (2) the fluids for delivering sample and setting fiber optic probe; and (3) computer processor for analyzing the signal.
The optical setup for the measurement system involves the use of a 488 nm solid-state Laser. In order to achieve the best coupling efficiency of the laser beam to the optical fiber, an objective lens was used. In addition, a multimode optical fiber was chosen in order to generate the evanescent wave on the fiber surface. Then, in order to separate the laser beam from the fluorescence signal, a long-pass filter was attached in front of the spectrometer in order to obtain a high SNR for the fluorescence signal. The set-up schematic diagram is shown in Fig. 2.

After finishing the preparation of the silver core-silica shells, fluorophores (FITC, Sigma-Aldrich) were simply mixed with core/shell nanoparticles. In order to show the benefits of using core/shell nanoparticles, we prepared another controlled sample (pure FITC solution without sliver silica core shell) in the same fluorophore concentration (1 µg/mL).

2.2. Preparation of fiber probe

About 2.5 cm of the center part of a 600 µm multimode fiber (JTFLH6006301040, Polymicro Technologies) was burned away the outside polymer jacket and cladding. The bare optical fiber probe was cleaned by the piranha solution (mixture solution of H2SO4 and H2O2, volume ratio 7:3) for 1 day. The cleaned fiber probe was rinsed with deionized water before using.

2.3. Core/shell Nanoparticles

Metallic nanoparticles, e.g. Au or Ag, have been previously studied for coupling the electrons involved in the self quenching to their strong surface plasmon polariton fields (SPPF), upon photoexcitation. For this electron transfer, it is very important that the fluorophore is placed at a particular distance from a nanoparticle. In this study, we employ the core/shell nanoparticles to control this condition. The schematic diagram of core/shell nanoparticle is shown in Fig. 3.

2.3.1. Silver colloids

Silver colloids were prepared by adding dropwise 2mL of 38.8mM sodium citrate (Sigma-Aldrich) aqueous solution to 98mL of boiling aqueous solution containing 18 mg of silver nitrate (Sigma-Aldrich), under vigorous stirring. After boiling for 10 minutes the solution was cooled to room temperature. The as-prepared silver colloid solution was centrifuged at 500 rpm for 1 hour to remove larger colloids.
2.3.2. Core/shell nanoparticles

Under vigorous stirring, 0.4mL of silver colloid solution was mixed with 100mL of iso-propanol (Sigma-Aldrich) and 10mL of deionized water. Immediately after the addition of 2mL of 30% ammonium hydroxide (Fluka), different amounts of Tetraethyl orthosilicate (TEOS, Sigma-Aldrich) were added to the reaction mixture. To obtain different silica layer thicknesses, 40μL of the same amount of TEOS solution with a concentration between 50% and 100%, was added to the suspension. The reaction was stirred at room temperature for 30 minutes.

2.3.3. Purification of core/shell nanoparticles

Each suspension of core/shell nanoparticles was washed and centrifuged (at 3500 rpm for 30 min) three times with a water ethanol mixture (5:4) for 30 min, followed by resuspension in water. In addition, not only water but also four solvents were compared for solvent effect experiments.

3. Results and Discussion

3.1. Core/shell Nanoparticles

Transmission Electron Microscope analysis shown in Fig. 4 of the core/shell nanoparticles has shown that the silver core was about 50 ±10 nm diameter, while the thickness of the shell could vary from 15 to 27 nm dependent on the controlled procedures. The surface plasmon resonance peak of the silver expended wavelengths as the thickness of the silica shell increased. The importance of using the core/shell Nanoparticles is 3-fold: 1) silica layers offer chemical inertness and the versatility needed for the conjugation of biomolecules; 2) it protects the silver core from ions present in biological media and 3) it allows optimal distance for MEF phenomenon.

![Fig. 4](image_url)

Fig.4. TEM images of (A)Ag nanoparticles, (B) Ε, (C) Ε, (D) show the samples with different tickness of the SiO₂ coating at 15, 25, and 27nm, respectively. The diameter of the Ag is 50 nm ± 10 nm for all the samples.

3.2. Distance Effect

The fluorescent emission intensity of the FITC-doped core/shell nanoparticles was approximately 40-fold higher than that of the solution without core/shell nanoparticles, shown in Fig. 5. In this sensing platform, the core/shell nanoparticles allows for the distance dependent MEF phenomenon, which we have determined the optimum shell thicknesses are about 25 nm. We also considered concentration of core/shell nanoparticles in order to find optimal purpose.
Fig. 5. Fluorescence emission spectrum of FITC-doped core-shell nanoparticles and form the corresponding sample.

The relation between the fluorescence signals and the different FITC concentrations measured in the same nanoparticles shell diameter is shown in Fig. 6. The fluorescence intensity increases with increasing FITC-doped concentration. The increasing gradient also increases with FITC-doped concentration.

Fig. 6. Fluorescence signal of FITC at different concentrations (shell-25nm)

3.3. Solvent Effect

Various physical/chemical properties of organic solvents, such as viscosity, polarity, etc., may also affect the fluorescence intensity [5]. Four solvents which are considered not too harsh to bio-molecules are used in this study. They are 0.01M PBS (phosphate buffered saline, Sigma-Aldrich), ethanol (Shimakyu), methanol (Fluka) and 0.05% tween 20 (Sigma-Aldrich). Fig. 7 summarizes the experimental results measured from these four prepared solution to distinguish the solvent effect. We found that 1.2 times, 1.6 times, 14 times and over 100 times fluorescent intensity enhancements in respective organic solvents compared to that of the controlled sample. The 0.05% tween 20 is found to have the most significant effect among all.

Organic solvents affect the fluorescence intensity in biosensing, possibly by the shifting excitation/emission spectrums of the fluorophore, by the isomerization of the fluorophore, by shrinking fluorophore tagged proteins.
4. Conclusions

Fiber-optic biosensors play a significant role in a variety of application fields, e.g. medical, pharmaceutical, environmental, defense, bioprocessing, or food technology. Nonetheless, the technology continues to evolve with new breakthroughs in optics, biochemistry, and chemical engineering.

There has been an explosion in the use of core/shell nanoparticles to favorably modify the spectral properties of fluorophores and to alleviate some of these fluorophore photo-physical constraints. There is an optimal fluorescent molecule to metal distance for fluorescence enhancement provided by silica layers of core/shell nanoparticles. In addition, these layers also offer the versatility need for the conjugation of biomolecules and protect the silver core from ions present in biological media. We conclude that core/shell nanoparticles have capability to enhance the fiber optic sensing through amplifying fluorescence intensity and expect that their potential applications are used in a variety of biological applications.

References

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