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Laser-induced fluorescence reader with a turbidimetric system for sandwich-type immunoassay using nanoparticles

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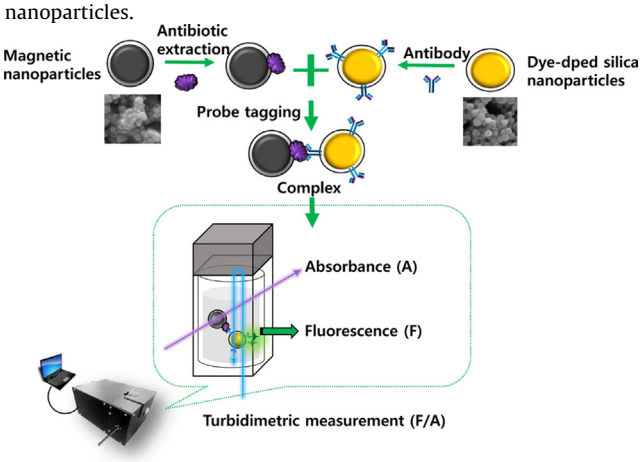
HIGHLIGHTS

- Laser-induced fluorescence system with ratiometric correction was developed.
- The system reduced experimental error caused by particle loss and aggregation.
- The detection limit of about 39 pg mL^{-1} for salinomycin was obtained.
- Calibration linearity and sensitivity were also significantly improved.
- The system has the potential for bioanalysis using various nanoparticles.

GRAPHICAL ABSTRACT

Laser-induced fluorescence reader with ratiometric correction for sandwich-type immunoassay using nanoparticles.



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ABSTRACT

A unique laser-induced fluorescence (LIF) reader equipped with a turbidimetric system was developed for a sandwich-type immunoassay using nanoparticles. The system was specifically designed to reduce experimental error caused by particle loss, aggregation and sinking, and to improve analytical performance through ratiometric measurement of the fluorescence with respect to the turbidimetric absorbance. For application to determine the concentration of salinomycin, magnetic nanoparticles (MNPs) and FITC-doped silica nanoparticles (colored balls) immobilized with antibody were synthesized for magnetic extraction and for tagging as a fluorescence probe, respectively. The detection limit of about 39 pg mL^{-1} was obtained, which was an improvement of about 2-fold compared to that obtained without employment of the turbidimetric system. Calibration linearity and sensitivity were also improved, with increase from 0.8601 to 0.9905 in the R^2-coefficient and by 1.92-fold for the curve slope, respectively. The developed LIF reader has the potential to be used for fluorescence measurements using various nanomaterials, such as quantum dots.

1. Introduction

Recently, immunoassay using magnetic nanoparticles has been extensively applied for the determination of various bio-targets, such as antibiotics, biomarkers, cells, etc. High-pressure liquid

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chromatography (HPLC) [1–3], liquid chromatography–mass spectrometry (LC–MS) [4,5], enzyme-linked immunosorbent assay (ELISA) [6–8], chemiluminescence (CL) [9–13], and fluorescence [14–18] are the common detection techniques employed in such immunoassays. Among them, HPLC and LC–MS are accompanied with the advantages of high sensitivity, low limit of detection (LOD) and high resolution. However, these methods require high cost for maintenance, in addition to sophisticated operation and expertized sample pre-treatment [16]. While chemiluminescence provides the merit of high sensitivity owing to extremely low background, it also suffers from poor stability [19–21]. Compared to these methods, ELISA provides the advantages of portability and speed, but suffers from relatively poor detection limit.

Recently, a new analytical platform for the determination of various antibiotics from food products was introduced, which employed nanoparticles and laser-induced florescence (LIF) spectrometry [16]. According to the measurement platform, MNPs were used to extract antibiotic targets by magnetic separation, after which tagging was carried out with colored balls as a probe for detection. The platform showed excellent sensitivity and selectivity owing to the signal amplification and immunoassay. In addition, the colored balls allowed the fluorescent measurement to be relatively free from quenching and photo bleaching [22,23]. However, this method suffered from poor particle stability and dispersion due to aggregation and sinking during the treatment and measurement process. Although many fluorescence techniques using various types of nanoparticles have been developed so far, no report has tackled these issues instrumentally.

In this work, an LIF reader equipped with a turbidimetric system was built in order to measure the concentration of an antibiotic by a sandwich-type immunoassay using nanoparticles. Since the immunoassay platform required centrifugation and magnetic separation, followed by washing, the analytical results suffered from particle loss which occurred during these steps. Therefore, the sample extraction process requires careful treatment throughout the experiment. Furthermore, as mentioned above, the extracted products dispersed in a sample cell can become aggregated or sink during the LIF measurement, causing signal reduction or fluctuation. Such issues can be minimized by a ratiometric measurement using the turbidimetric system attached to the LIF reader in the developed system, i.e., the fluorescence intensity divided by the turbidimetric absorbance would allow reflection of the number of particles effectively being measured. Although ratiometry is employed in various analysis methods, turbidimetric correction has not yet been introduced for the sandwich-type immunoassay using nanoparticles.

The performance of the developed system was demonstrated by determining the concentration of salinomycin, which is a polyether ionophore antibiotic effective for feed and growth facilitation [24]. Since its misuse induced serious intoxication, causing various clinical signs in humans and animals, such as nausea, diarrhea, vomiting, etc. [24], the EU officially prohibited its use. Therefore, the developed LIF system will be useful for market surveillance of food products, and for the study of pharmacokinetics and human toxicity [24].

2. Experimental

The fluorescence reader was designed to measure the fluorescence emitted from the colored nanoparticles tagged on the antibiotic target. For demonstration, salinomycin concentration was determined by a sandwich-type immunoassay platform using two synthesized nanoparticles, i.e., magnetic nanoparticles for target extraction, and dye-doped silica nanoparticles as probes. Improvement of the analytical performance was illustrated by obtaining the analytical figures-of-merit.

2.1. Instrumental design of fluorescence reader

The schematic diagram of the designed fluorescence reader is illustrated in Fig. 1. A DPSS diode laser (MBL-III-473-5 mW, CNI, China) was used as the excitation source for fluorescence, with a broadband dielectric mirror (BB1-E02, 400–750 nm, Thorlabs, USA). A cylindrical-shaped quartz detection cell equipped with a reflection cap was used so that the laser beam could multiply pass through the cell from bottom to top, resulting in increase of the cell pathlength. The photons emitted from the probe nanoparticles were detected by the photosensor (H10769PA-40, Hamamatsu, Japan), which was positioned at a right angle, and counted by a photon counting unit (C9744 and C8855-01, Hamamatsu, Japan) after passing through a plano-convex lens (LA1131, $f = 50$ mm, uncoated, Thorlabs, USA) and an interference filter (FF03-525/30-25, Semrock, USA) for wavelength selection (F). For the measurement of turbidimetric absorbance, a D_2 lamp (DO 650 TJ, Heraeus Noblelight) was used for the light source, with a monochromator (Lambda 25) for wavelength selection, and an Si photodiode detector (S1336-44BQ, Hamamatsu, Japan) (A). For data handling and operation, software was designed by Sensor Tech (Sungnam, Korea), through which the measured counts per second were divided by the absorbance for ratiometric treatment.

2.2. Preparation of amine-functionalized Fe_3O_4 magnetic nanoparticles (MNPs) for target extraction

Amine-functionalized Fe_3O_4 MNPs were synthesized through the alkaline co-precipitation of $FeCl_3 \cdot 6H_2O$ and $FeCl_2 \cdot 4H_2O$ [25],

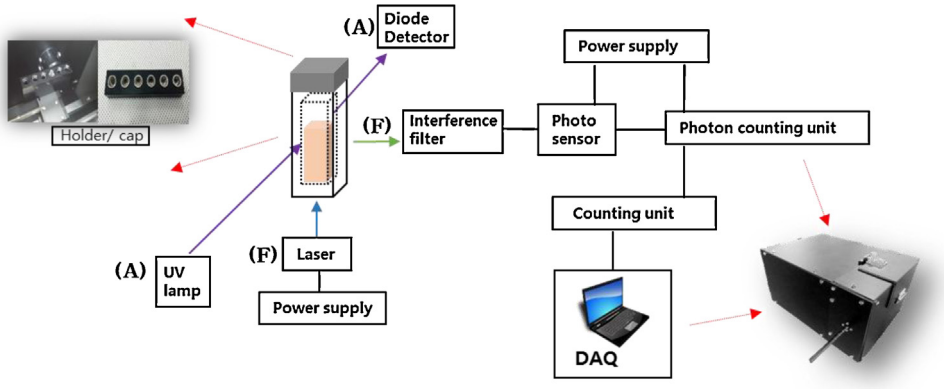

Fig. 1. Schematic diagram of the laser-induced fluorescence (LIF) reader system (F) combined with turbidimetric absorption measurement (A).