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Aggregation-induced emissive nanoparticles for fluorescence signaling in a low cost paper-based immunoassay

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ABSTRACT

Low cost paper based immunoassays are receiving interest due to their fast performance and small amounts of biomolecules needed for developing an immunoassay complex. In this work aggregation-induced emissive (AIE) nanoparticles, obtained from a diastereoisomeric mixture of 1,2-di-(4-hydroxyphenyl)-1,2-diphenylethene (TPEDH) in a one-step top-down method, are characterized through Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), and Zeta potential. By measuring the Zeta potential before and after labeling the nanoparticles with antibodies we demonstrate that the colloidal system is stable in a wide pH-range. The AIE-active nanoparticles are deposited on chitosan and glutaraldehyde modified paper pads overcoming the common aggregation-caused quenching (ACQ) effect. Analyte concentrations from 1000 ng and below are applied in a model immunocomplex using Goat anti-Rabbit IgG and Rabbit IgG. In the range of 7.81 ng–250 ng, linear trends with a high R^2 are observed, which leads to a strong increase of the blue fluorescence from the TPEDH nanoparticles.

1. Introduction

For the application of fluorescent molecules in Fluorescent Immunoassays (FIA), sensitivity and intensity of the light emission are important parameters [1]. In addition, controlling the number of fluorophores that are attached to the biomolecules, such as antibodies or oligonucleotides like DNA and RNA, is crucial for quantitative analytical applications [2]. Commonly, six to ten fluorescent molecules like fluorescein isothiocyanate (FITC) are bound to one single antibody [3]. A limitation for the maximum number of attached fluorophores generally exists as a result of aggregation-caused quenching (ACQ) [4]; namely, the non-radiative decay of the excited state by intermolecular interactions between the chromophoric moieties [5]. Therefore, the detection of small analyte quantities can be afflicted with a large error or even be impossible to detect. To overcome this limitation, we made use of a fluorophore with aggregation-induced emission (AIE) properties [6]. Inspired by previous reports on FIAs involving fluorescent nanoparticles made from gold [7], silica [8], polymers [9], and quantum dots [10], we furnished AIE-active nanoparticles from a diastereoisomeric mixture of 1,2-di-(4-hydroxyphenyl)-1,2-diphenylethene (TPEDH). TPEDH and its derivatives have been previously reported to act as a fluorescence turn-on probe when aggregated or bound to biomolecules, i.e. proteins [11]. The scaffold is based on tetraphenylethene (TPE) that has been extensively used in various applications not only due to its reliable performance as an AIEgen but also because of its facile synthesis allowing for various derivatizations [12,13]. In line with novel approaches for signal generation in immunoassays, several paper based immunoassays were published in recent years and enjoy more and more popularity due to the benefits of using paper as a low-cost and widely available platform rather than commonly used polystyrene plates [14,15]. Paper-based plates are functionally related to plastic well plates, but they widen the application scope by offering additional features [16]. For example, thin (~180 μm) paper-microzone plates, only require small sample volumes (5 μL per zone), and can be manufactured from inexpensive materials ($0.05 per plate) [17]. Commonly, enzymes are applied to paper based immunoassays due to their reliable signal generation. However, a disadvantage

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compared to chemical or instant signal generation lies in the longer enzymatic reaction time for an added substrate being converted into a measurable analyte [18]. The method of choice for producing the nanoparticles comprised of the AIEgen was a one-step top-down approach, using cavitation for breaking down larger particle clusters. Using the mentioned diastereoisomeric mixture of TPEDH gave AIE-active nanoparticles solely comprised of the AIEgen, which is novel in this field as previous reported AIEgen-containing nanoparticles required adjuvants [19–21]. In previous publications, top-down methods using milling processes, with a resulting nanoparticle diameter between 100 and 500 nm, were reported [22]. In the case of the applied cavitation, locally-lowered pressure induces a phase-transition from liquid to gas resulting in the formation of μm sized gas bubbles [23]. As the pressure in the surrounding medium increases, the gas bubbles implode, which results in rapid temperature and pressure changes providing enough energy to break down the amorphous TPEDH-aggregates into smaller, more homogeneous particles. Nanoparticles are getting more important in different bio-analytic devices due to their individual properties [24]. The herein reported TPEDH nanoparticles were well characterized regarding their protein adsorption capabilities, fluorescence intensities, and stability as a function of the pH. In this work, a model antibody-antigen complex was applied using polyclonal Goat anti-Rabbit IgG (Gt-α RbIgG) acting as capture antibodies and Rabbit IgG (RbIgG) as the antigen. Using cellulose paper pads for performing immunoassays saves costs, sample and antibody solution volumes, time, and facilitates the disposal afterwards. The synthesis of TPEDH nanoparticles with subsequent protein labeling and application to a paper based fluorescent immunoassay for signal generation is a novel approach in the field of low cost paper diagnostics. The benefit of TPEDH for signaling in paper immunoassays lies in the reliable signal generation without suffering from quenching effects of the fluorescence compared to most common colorimetric signaling methods suffering from ACQ.

2. Materials and methods

2.1. Materials and chemicals

All reagents were purchased from Sigma-Aldrich Chemical Co. and used as received without further purification. THF (Labscan) was dried over Na/benzophenone and distilled before use. All chemical reactions were carried out under a nitrogen atmosphere by using Schlenk techniques. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AV 400 spectrometer in deuterated chloroform. Goat anti-Rabbit Antibodies (Gt-α RbIgG) were used as capture antibodies, and Rabbit antibodies (RbIgG) as the analyte, which were purchased from Arista Biologicals Inc. Whatman® Chromatographic Paper 1Chr was purchased from Sigma-Aldrich Chemical Co. and used as received. Unless otherwise specified, ultrapure water obtained from a Millipore water purification system (MilliQ) was used to prepare aqueous solutions. 0.01 m pH 7.4 phosphate buffer solution (1x PBS) was used as buffer for antibody immobilization. A 1% solution of BSA in 1x PBS was used as blocking buffer to block residual sites on paper to minimize non-specific adsorption. An aqueous 0.5% sodium dodecyl sulfate (SDS) solution was used to wash away unbound reagents. An office puncher was purchased from a local shop in Hong Kong for paper pad cut out. Polystyrene plates were obtained from Axygen Scientific Pipette Tip boxes for 5–200 μL tips. For the top-down fabrication of the TPEDH nanoparticles a Brandson Sonifier 450 was used. BioRad Gel Doc XR for fluorescence image taking of the paper pads. Image analysis and nanoparticle characterization, ImageJ software was used. Zeta Plus BIC (Brookhaven Instruments Corporation) was used to measure the particle size distribution through dynamic light scattering (DLS) and the zeta potential. JEOL 7100F Scanning electron microscope (SEM) was used for TPEDH nanoparticle imaging.

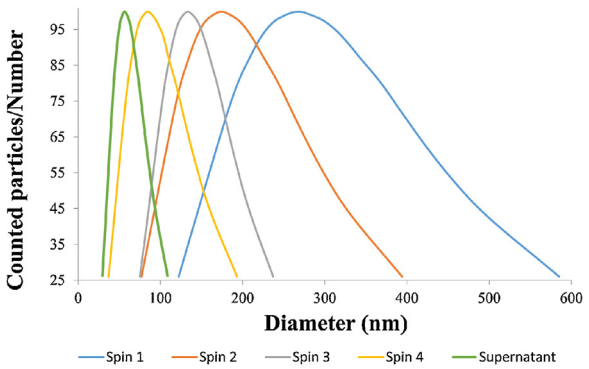

Fig. 1. Counted and measured TPEDH particles and their diameter with a BIC Zeta Plus from different centrifuged batches, 1st spin (3 min, 850 G), 2nd spin (3 min, 2350 G), 3rd spin (3 min, 6000 G), 4th spin (12 min, 20,250 G), and 4th spin supernatant.

2.2. Synthesis of 1,2-di-(4-hydroxyphenyl)-1,2-diphenylethene (TPEDH)

A stirred mixture of zinc dust (1.18 g, 18 mmol) and 4-hydroxybenzophenone (3 g, 15.1 mmol) in dry THF (100 mL) was treated with $TiCl_4$ (1 mL, 9 mmol) at 0 °C under an atmosphere of nitrogen. After stirring at 22 °C for 30 min, the mixture was heated at 65 °C for 18 h. Reactive species were quenched with an aqueous solution of K_2CO_3 (10%, 50 mL). The mixture was extracted with CH_2Cl_2 (3 × 100 mL), the combined organic phases washed with brine (1 × 50 mL), dried over anhydrous $MgSO_4$, and evaporated in vacuo. The residue was recrystallized from EtOH to afford TPEDH (89%, 2.45 g) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$), δ (TMS, ppm): 7.01 – 7.12 (m, 10H), 6.90 (t, 4H), 6.54 (d, 4H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (TMS, ppm): 154.11 (C—O), 144.20, 139.71, 135.52, 132.80, 131.45, 127.75, 126.36, 114.75; NMR data conform with the previous reported ones [25].

2.3. AIE nanoparticle production by top-down approach

Amorphous TPEDH powder (10 mg) and an aqueous 1% SDS (10 mL) solution were subsequently added into a glass beaker. After a homogenous mixture was formed by manually stirring with a glass rod, the beaker was placed into an ice bath. The sonicator tip (Brandson; tapered micro tip, $d = 3$ mm) was submerged into the TPEDH-SDS mixture. The solution was sonicated for 15 min at an amplitude of 300 μm. Subsequently, the sonicated TPEDH was transferred into 2 mL micro centrifuge tubes (Eppendorf) and centrifuged (14680 rpm, 12 min). Afterwards, a pellet-like precipitate containing TPEDH nanoparticles was formed and the supernatant discarded. The nanoparticles were washed once with MilliQ water (2 mL) and afterwards separated by its size through repeated centrifugation at different velocities (Fig. 1). After the separation process the TPEDH nanoparticles were suspended in 1x PBS and stored at 4 °C in the dark.

2.4. TPEDH nanoparticle labeling

To apply the synthesized TPEDH nanoparticles to immunoassays, they were labeled with capture antibodies. As a proof of concept, the TPEDH was labeled with Gt-α RbIgG through physisorption. Keeping the TPEDH nanoparticles in 1x PBS, they were washed once with MilliQ water before adding the