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Fluorometric immunoassay for human serum albumin based on its inhibitory effect on the immunoaggregation of quantum dots with silver nanoparticles

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ABSTRACT

Quantitative determination of HSA was conducted by competitive immunoassay. Inhibition of aggregation of antibody conjugated quantum dots (QD) with albumin conjugated silver nanoparticles (AgNPs) in the presence of HSA was conducted. If antibody-loaded CdSe QDs aggregate with HSA-coated silver nanoparticles the distance between the two kinds of nanoparticles will be reduced enough to cause fluorescence resonance energy transfer (FRET). In this case the yellow fluorescence of the Ab-QDs is quenched. However if HSA (antigen) is added to the Ab-QDs their surface will be blocked and they cannot aggregate any longer with the HSA-AgNPs. Hence, fluorescence will not be quenched. The drop of the intensity of fluorescence (peaking at 570 nm) is inversely correlated with the concentration of HSA in the sample. The method allows to determine HSA in the 30–600 ng·mL^{-1} concentration range.

1. Introduction

Various types of nanoparticles have been successfully applied in different assay procedures. In particular gold and silver nanoparticles have been applied in various assay systems for quantitative detection of diverse analytes and bacteria [1–3]. The principle of detection is based on the color changes of ligand conjugated nanoparticles upon their interaction with the appropriate analyte. As a result of this interaction red shift of plasmon resonance takes place and these changes correlate with analyte concentration. However these methods have drawbacks. The optical changes observed when these particles aggregate are the result of not only agglutination but also of subtle changes in refractive index on the particle surface so these combined effects can bring to interference of the results [4]. Moreover for spherical nanoparticles such optical changes upon aggregation are less pronounced and therefore for sensitive analysis it is necessary to synthesize nanoparticles of other forms [5]. QDs (fluorescent semiconductor nanoparticles) are another class of nanomaterials that are applied not only in the assay but also in cellular visualization procedures [6,7]. Fluorescence bands of these nanocomposites depend on their composition, sizes and thickness of shell. Until now multiple QDs with various fluorescent features have been synthesized and applied in various procedures for detection of various analytes [7–10]. One of the attractive features of QDs is the possibility to excite the diverse particles with light of the same wavelength and detect narrow emission bands from many particles simultaneously. It permits applying these nanoparticles in multiple assays [11]. However QDs themselves cannot be applied in various types of immunoassay because the interaction of antibody conjugated nanoparticles with appropriate ligands usually does not change fluorescent signals. Therefore for application of these nanoparticles in assay procedures they must be used with other types of nanoparticles [12,13]. One of the approaches in these immunoassays is the measurement of resonance energy transfer (FRET) between various types of nanoparticles for quantitative determination of analytes. [14] In particular such combined application of conjugated gold nanoparticles and quantum dots were applied in homogeneous immunoassay based on FRET for detection of carcinoembryonal antigen (CEA) [15]. In this assay goat anti-CEA antibodies labeled by luminescent CdTe quantum dots as energy donors were applied on one side and monoclonal goat anti-CEA antibody labeled gold nanoparticles as acceptor on the other side. In the presence of CEA as a result of interaction of these antibodies with antigen the distance between nanoparticles is decreased and energy transfer takes place so the resulting fluorescence is quenched. This approach provides to determine CEA in a range from 1 to 110 ng·mL^{-1} with detection limit of 0.3 ng·mL^{-1}. A similar approach but by application of inhibition of aggregation between QDs and gold nanoparticles was applied for determination of avidin [16]. This method permits detecting avidin in concentration range of 0.3–20 μg·mL^{-1}. Resonance energy transfer between CdSeCdS QDs and gold nanorods was applied in immunoassay for detection of viral antigens [17]. Similar approach was used for detection of DNA hybridization and mutations in epidermal growth factor receptor gene [18,19]. Here authors functionalized the surface of gold nanoparticles and quantum dots by different probes of DNA that were designed to provide complementary to an in-frame deletion mutation. The

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presence of mutation had brought to quenching of fluorescence of QDs. This method permitted to detect two mutations simultaneously. A versatile nanoprobe was developed for trypsin quantification with fluorescence resonance energy transfer (FRET). Here, fluorescence graphene quantum dot is utilized as a donor while a well-designed coumarin derivative, CMR2, as an acceptor. The approach permits detecting trypsin in concentration up to 0.7 $\mu g \cdot mL^{-1}$ [20]. An immunoassay based on FRET between terbium and quantum rods was applied in bioassays with picomolar sensitivity [21]. Here time resolved and steady state fluorescence was applied to analyze the effects of various factors on the sensitivity of assay. A similar approach with FRET between Rhodamine G and gold nanoparticles was successfully applied for specific determination of metallothioneins [22]. In this assay the presence of metallothionein in solution has brought to its immobilization with gold nanoparticles and as a result it blocked the interaction of Rhodamine G with nanoparticles preventing resonance energy transfer between Rhodamine G and gold nanoparticles and vice versa the absence of the protein in solution has brought to resonance energy transfer between Rhodamine G and gold nanoparticles. The rate of energy transfer correlated with concentration of metallothionein in media. Epitope imprinted polymeric nanoparticles doped with QDs were applied recently for recognition of albumin [23]. Carbon dot labeled antibodies and appropriate aptamers were applied in fluorescence aggregation assay for detection of mucin 1 [24]. All of these approaches demonstrate their efficacy for detection of various analytes. In the article we have applied anisotropic AgNPs and CdSe QDs pair for resonance energy transfer in agglutination inhibition assay for detection of human albumin.

2. Material and methods

Optical measurements were conducted on spectrophotometer Hitachi 150-40. Fluorescent measurements in ratio mode were conducted on spectrofluorimeter MPF 44A. Fluorescence spectra were recorded at excitation wavelength of 470 nm, excitation and emission slits were 8 mm. Anisotropic AgNP were prepared by two-step procedure according to [25]. The nanoparticles obtained then were dialyzed against 1 mM of tri-sodium citrate and stored at 2–8 °C until application. Morphology of AgNPs was estimated by transmission electron microscopy (TEM). Water soluble CdSe quantum dots were prepared according to [26] In brief the following procedure was applied. 140 mg of NaBH4 was dissolved in 1 mL of N_2 bubbled cold water and the solution was added to 90 mg of Se powder. After 1 h of reaction in ice bath the selenium powder was completely reduced by sodium borohydride and white precipitate was formed. Clear supernatant was separated, diluted by N_2 bubbled distilled water for 100 times and then it was applied for preparation of CdSe. 100 mL of $CdCl_2$ (5 mM) bring to boiling in two-necked retort under steam of N_2 sodium citrate in final concentration of 2 mM was added and then rapid injection of NaHSe solution is conducted. The solution is boiled for additional 30 min in backflow condenser,

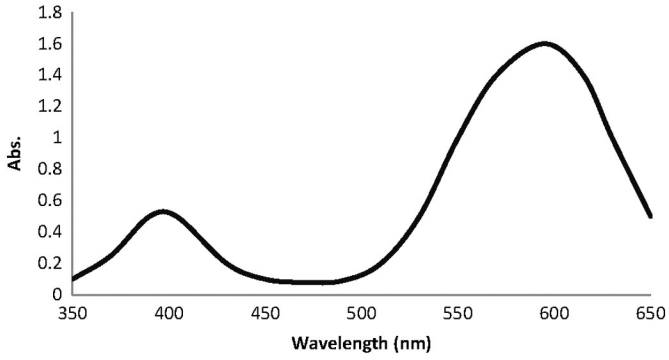

Fig. 1. Optical spectrum of AgNP.

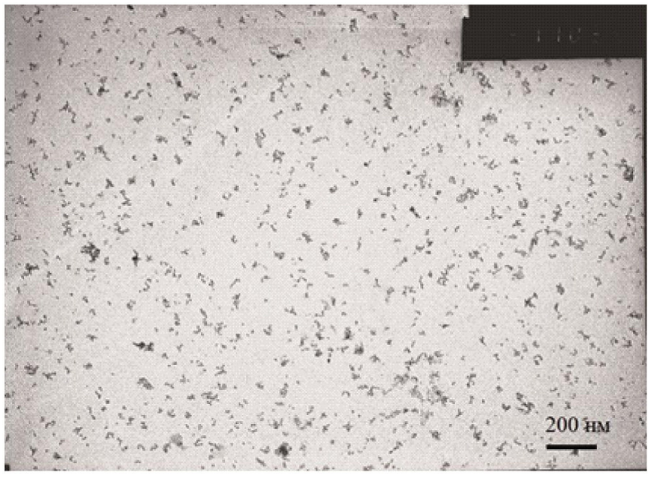

Fig. 2. TEM image of CdSe quantum dots.

cooled and dialyzed for removal of any side products. Human albumin was supplied by BIO-VAR Ltd (www.bio-varbiotechnology.am) and was applied without consequent purification. Rabbit polyclonal antibodies to human albumin were developed according to schedule described in [27]. IgG fraction was purified by fractionation with ammonium sulphate followed by anion-exchange chromatography on DEAE Toyopearl 650. The preparation was homogeneous by electrophoresis in 6% PAAG conducted in non-denaturating conditions.

2.1. Conjugation of nanoparticles

2.1.1. Conjugation of anisotropic silver nanoparticles

To 2.5 mL of AgNP nanoparticles 2.5 μg of human albumin dissolved in 1 mM borate buffer pH 8.2 was added. After incubation for 24 h at 2–8 °C AgNP were blocked by bovine serum albumin to final concentration of 0.1% and after an additional incubation for 1 h borate buffer pH 8.2 was added to final concentration of 10 mM.

2.1.2. Conjugation of quantum dots

Initially QDs were treated with 2-mercaptoethylamine (MEA) to form monolayer of thiol with exposed NH_2 groups for consequent covalent immobilization of antibodies. Here to 20 mL of QDs was added 1 mL of MEA (100 μM). The QDs were then incubated for 24 h at 2–8 °C and dialyzed against 0.5 mM trisodium citrate. Antibodies to human albumin were treated by sodium m-periodate. The protein was dissolved in 0.05 M acetate buffer pH 3.7 and to 1 mg of antibodies sodium m-periodate was added in molar ratio of 1/300. The solution was incubated for 15 min at 2–8 °C and then dialyzed against distilled water. To 10 mL of MEA treated QDs were added periodate oxidized antibodies (8 μg)

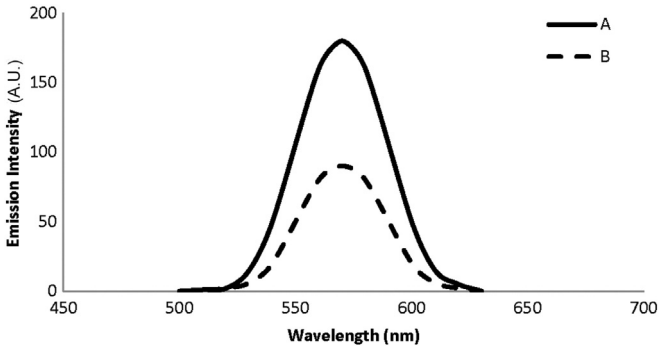

Fig. 3. Fluorescence spectra of CdSe QDs after illumination with sunlight for 72 h (solid line); before illumination (dotted line). Spectra acquired at an excitation wavelength of 470 nm.