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Highly fluorescent ethyl cellulose nanoparticles containing embedded semiconductor nanocrystals

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ABSTRACT

Highly luminescent organic nanoparticles were formed by embedding hydrophobic and hydrophilic (CdSe)ZnS quantum dots with core/shell structure into ethyl cellulose nanoparticles. The nanoparticles were prepared from oil-in-water nanoemulsions by a phase inversion process at constant temperature, followed by a solvent evaporation. The obtained fluorescent ethyl cellulose nanoparticles were functionalized by immobilization of a specific antibody, and applied in rapid agglutination test for detection of *Yersinia pestis* F1-antigen.

1. Introduction

Nanoparticles are of a great interest in a variety of scientific fields such as cell biology, biotechnology, diagnostics, analytics, and pharmaceutics [1–3]. In medicine, for example, functionalized nanoparticles find applications in sensing and diagnostics on a single-cell level [4]. Recent progress in polymer science allows preparation of mechanically stable, size- and shape-persistent polymer nanoparticles. The development of methods for producing fluorescent nanoparticles can open new fields for their application. Traditional fluorescent latex particles with organic dyes suffer from photobleaching and technical difficulties in multicolor analysis. Development of nanotechnological methods has made possible preparation of luminescent semiconductor nanocrystals, which are very promising alternative to organic dyes due to their excellent photostability, high quantum yield, and a narrow emission peak, the position of which depends only on the nanocrystal size (in the range of 2–8 nm) regardless of the excitation wavelength [5]. An example of such particles are (CdSe)ZnS nanocrystals containing the CdSe central core, which determines the fluorescence parameters, and a ZnS shell, which provides a high quantum yield of the fluorescence [6]. Semiconductor nanocrystals, or quantum dots (QD), are perspective for multicolor labeling and simultaneous identification of various biological objects [7]. They emit in a wide spectral range, from blue to red, depending on their size that allows tuning the fluorescence color with the use of one excitation source.

Among the polymers suitable for encapsulation of semiconductor nanocrystals, ethyl cellulose (EC) is very attractive. This hydrophobic polymer is non-toxic, stable, and has been widely used in pharmaceutics. This cellulose ether is a substantially water-insoluble polymer, and is excessively used in coatings of solid dosage forms to provide a controlled release profile of drug substances [8,9]. This polymer is used in many other drug delivery systems, such as matrices [10], microspheres [11], microcapsules [12], also in combination with other cellulose derivatives [13–15].

EC being primarily applied in organic solvents is now also available as 30% colloidal polymer particles of 200–500 nm in diameter, dispersed in an aqueous phase [16]. EC latexes can be cast or sprayed onto the surface of the desired dosage forms, the dispersion is exposed to gradual water evaporation and polymer deformation. Upon more complete evaporation of the aqueous phase or the organic solvent, the polymer chains are aligned to further coalescence to form a homogeneous, transparent film [16].

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Attractive colloidal systems are nanoemulsions with droplet size in the nanometric scale (typically in the range 20–200 nm), which are stable against flocculation and sedimentation. The droplets of the nanoemulsions can be used as "nanoreactors" for chemical reactions [17] or as dissolving medium for preformed polymers [18], drug delivery system [19], and cosmetics [20].

Nanoparticles containing diagnostic tests (qualitative, yes/no results) and assays (quantitative results) are usually based upon the specific interaction of antigen (Ag) with antibody (Ab). Sub-micron sized nanoparticles are used as the solid support for immobilization of Ab or Ag onto them. These "sensitized" nanoparticles then act to magnify or amplify the Ag–Ab reaction that takes place when they are mixed with a sample containing an opposite reactant. In addition, the use of fluorescent-labeled nanoparticles allows increasing the analysis sensitivity and specificity [21].

When antigens are present in the analyte, the interactions of antigens with the immobilized antibodies result in the aggregation of nanoparticles. Aggregation can be observed with the naked eye or monitored quantitatively by any physicochemical method sensitive to the size of aggregates. In simple particle agglutination test, a positive test results when uniformly dispersed milky-appearing Ab-coated particles in a drop of buffer on a glass slide react with Ag in a drop of sample (body fluids) to cause particle agglutination (clumping of the nanoparticles to look like curdled milk). These so called "glass slide tests" are portable, rapid, efficient, and useful even under the most poorly controlled conditions. Ideal for point-of-care use in the field, ambulance, or bedside, they can be performed quickly and simply (5 min from sample preparation) [2].

In this work we present a new process for preparation of EC nanoparticles containing embedded fluorescent probes: two types of (CdSe)ZnS QD with size of 3.5 and 6 nm emitting green and red light, respectively. Semiconductor nanocrystals embedded into EC particles can potentially be used not only as fluorescent markers, but additionally as luminescence sensors and ion probes.

The EC nanoparticles were produced by a solvent evaporation method from oil-in-water nanoemulsion prepared by phase inversion at constant temperature [22]. The possibility to use the fluorescent EC particles conjugated with antibody as a bioanalytical reagent in the particle agglutination test was also evaluated.

2. Material and methods

The following materials were purchased from Sigma and used without further purification: ethyl cellulose with a viscosity of 45 cPs for a 5% solution in 4:1 toluene:ethanol at 25 °C, sorbitan monolaurate (Span 20), mercaptoacetic acid, mercaptoethanol, *N*-hydroxysuccinimide, 1-ethyl-3-(3-dimetylaminopropyl) carbodiimide hydrochloride, Na-borate buffer, NaCl. Decaglycerol monolaurate was obtained by the courtesy of Sakamoto Yakuhin Kogyo Co. Ltd., Japan. Toluene, ethanol, methanol, dimethyl sulphoxide, chloroform were of analytical grade and purchased from Aldrich.

Semiconductor nanocrystals CdSe/ZnS with core/shell structure (CdSe central core which determines parameters of fluorescence and ZnS-shell which provides a high quantum yield of fluorescence and prevents quenching) stabilized with tri-*n*-octylphosphine oxide (TOPO) were synthesized as described previously [23]. In the present work hydrophobic nanocrystals of diameters of 3.5 nm with emission peak 546 nm and of 6.0 nm with emission peak 620 nm were used and excited with λ_{ex} = 380 nm.

Anti-*Y. pestis* monoclonal mouse antibody (clone F-19) and *Y. pestis* F1-antigen of the plague bacterium, *Yersinia pestis* which is fibrillar protein collapsed into an antiphagocytic capsule-like structure on the surface of the bacterium were gratefully donated by Prof. Sveshnikov P.G., RCMDT, Moscow.

The size of particles was measured by photon correlation spectroscopy (Coulter N4-MD), optical and fluorescent characteristics were measured with the use of Zeiss Axiovert 200 microscope, Shimadzu RF-551 spectrofluorimeter, BioDoc-IT System UV-Transilluminator. High resolution SEM measurements were performed with the use of Sirion electron microscope (FEI Company).

2.1. Preparation of EC nanoparticles

The EIP method is based on dropwise addition of water to a mixture of oil and surfactants while stirring that results in formation of o/w nanoemulsion with small and uniform droplet size [22]. More specifically, the surfactants (decaglycerol monolaurate and Span 20) were added to a 10% (w/w) solution of ethyl cellulose in toluene. Then the aqueous phase (10 mM NaCl) was dropwise added, at a constant rate of 0.5 ml/min. The addition of the aqueous phase was performed under continuous stirring at room temperature. The final nanoemulsion contained 20% (w/w) toluene and 5% (w/w) surfactant mixture. After the nanoemulsion was formed, the toluene was evaporated under reduced pressure (1 mmHg) at 45 °C for 30 min, resulting in the formation of ethyl cellulose nanoparticles. The samples before and after evaporation were analyzed by gas chromatograph for the presence of toluene. Toluene was extracted from the samples using dichloromethane prior to analysis. GC analysis was performed using a HP 5890 gas chromatograph with a 5% diphenyl, 95% dimethylpolysiloxane column (30/0.25 m).

2.2. Swelling of EC nanoparticles in water–ethanol medium

The study of EC particles swelling allows optimization of the procedure of QD embedding. Selection of the water–ethanol mixture was based on its ability to effectively disperse the fluorescent nanocrystals and to "loosen" EC nanoparticles to make easier the nanocrystal penetration into particles while preserving their colloidal stability.

Swelling of EC nanoparticles was carried out in glass cuvette by dropwise addition of ethanol to the dispersion of EC nanoparticles in water, stirring and incubation as described below.

The swelling experiments were performed as follows. Aqueous dispersion of EC nanoparticles (200 μl, 2% w/w) was diluted with water (2 ml). Then, to this sample (sample 1), ethanol was added (three times in 0.05 ml) and after each addition, the dispersion was incubated for 30 min (sample 2), or 0.15 ml of ethanol was added at once (sample 3). The total incubation time for samples 2 and 3 was 90 min.

2.3. Embedding of hydrophobic nanocrystals into EC nanoparticles

Ethanol (100 μl) was added dropwise to EC dispersion (1 ml). QDs (0.06 mg) with diameter of 3.5 nm and emission maximum at 546 nm stabilized with TOPO were purified by dispersing in chloroform and precipitating with methanol. Purified QDs were dispersed in ethanol (10 μl), and the fluorescence intensity was measured. Then the dispersion of QDs was added to 200 μl of EC nanoparticles dispersion in water–ethanol, and the mixture was stirred vigorously, sonicated for 5 min and incubated for 20 min while stirring (this procedure was repeated three times), shaken for 2 h at room temperature and centrifuged at 10,000 rpm for 10 min (to remove free QDs). To remove ethanol, the obtained EC nanoparticles containing embedded QDs (EC-QDs) were dialyzed against water. The fluorescence of the resulting dispersion containing EC-QDs was measured, and the obtained intensity was corrected taking into account the dilution during the embedding procedure.