STUDY OF DIFFUSION PROPERTIES OF CHITOSAN-BASED NANOCOMPOSITES WITH AG-IN-S2 QUANTUM DOTS, AND TETRAPHENYLPORPHYRIN MOLECULES

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Chitosan is a widespread substance of natural origin. Chitosan nanoparticles can be used as drug delivery systems. During the study, methods for the synthesis of chitosan-based nanocomposites with Ag-In-S2 quantum dots (AIS), and tetraphenylporphyrin molecules and the spatial distribution of photoluminescent (PL) were analyzed. Potentially, such particles shall be able to get into cells without causing nanotoxicity associated with the small size of the objects. The preparation of a solution of chitosan nanoparticles (NPs) was carried out by the method of ionic gelation for water solutions. Two different types of nanoparticles were prepared; in one of the cases, sodium tripolyphosphate was used to crosslink chitosan molecules. The particle sizes were estimated from their diffusion parameters using fluorescence recovery after photobleaching (FRAP) technique. The experiments were performed with laser scanning confocal microscope Zeiss LSM 710 with 405 nm diode laser. Also results of FRAP investigations were compared to those of dynamic light scattering (DLS) measurements. Comparison of the data of two hydrodynamic measurements demonstrated the successful formation of nanoparticles with a diameter of about 100–300 nm. Comparison of sizes obtained by different methods showed the absence of individual molecules and quantum dots in the samples, which indicates the successful formation of nanocomposites.

Key words: chitosan, nanocomposites, photoluminescence, FRAP, DLS, diffusion.

Biocompatible nanoparticles based on chitosan are proposed as an object of study in this work. Planar and spherical formations with a diameter of about 100–300 nm made of chitosan can be used as drug delivery systems. Also, chitosan can encapsulate molecular sensitizers of reactive oxygen species (ROS) such as tetraphenylporphyrin (TPP). So, there are three types of nanoobjects in the solution: molecules of chitosan (diameter 100–300 nm), AIS quantum dots (5–10 nm) and molecules of tetraphenylporphyrin (1–2 nm). These samples are interesting not only in the context of their own usefulness and potential biological applicability, but also in the context of the study of complex solutions, since there is a unique opportunity to study samples that contain nanoobjects together, the sizes of which differ by several orders of magnitude using hydrodynamical measurements. For this purpose, dynamic light scattering (DLS) and fluorescence recovery after photobleaching (FRAP) were performed. DLS method is based on measuring the elastic scattering of light by particles in solution, so the effective hydrodynamic diameter of the scattering centers is calculated. The main aspect of FRAP technique lies in bleaching of small region of luminescent sample with high intensity laser pulse and observation of a diffusion speed during recovery.

The preparation of a solution of chitosan nanoparticles was carried out by the method of ionic gelation using two different techniques.

The first of them is based on the article [1] and includes the preparation of a 1% solution of acetic acid and the dissolution of powdered chitosan in it. Then the resulting sample was placed on a magnetic stirrer for 60 minutes. After that, with continued stirring, a 0.5% sodium tripolyphosphate solution was added dropwise to the sample, which provides crosslinking of the molecules.

The second method is in many aspects such as the one described above and is based on the study [2]. Firstly, a 1% mass fraction solution of acetic acid (15 ml) was prepared, in which 7.5 mg of powdered chitosan was dissolved. The resulting mixture was placed on a magnetic stirrer for 20 hours. A 1-N NaOH solution was also prepared by adding 4 g of dry matter to 100 ml of deionized water, which was then used to equalize the pH of the medium. The resulting pH was 5. Polyethylene glycol (PEG) prepared in a volume fraction of 0.01% as a replacement for 0.05% Tween-80 used by the authors of the article [2] was taken as an antiaggregation agent.

2 ml of PEG was added to a solution of chitosan in acetic acid while stirring continued. Separately, aqueous solutions of sodium tripolyphosphate (STPP) of different mass fractions were prepared, each of which was filtered through a 0.22 μm hydrophilic filter. Then 3.5 ml of tripolyphosphate, acting as a crosslinker for polymerized chitosan, at a concentration of 1 mg/ml were added to the chitosan solution in a ratio of 1:2.5.

After the initial solution of chitosan was prepared before its crosslinking, its mixtures with QD AIS solutions were also prepared without the formation of a nanocomposite system (which is achieved by crosslinking). To synthesize nanocomposites, the colloidal solution of quantum dots and molecules was added to the chitosan mixture at the beginning of 24-hour mixing in a ratio of 1:10 by volume. The resulting sample of nanocomposites was centrifuged at a speed of 4000 rpm for 15 minutes [2].

The study of the obtained solutions for the effective hydrodynamic size of nanocomposites was carried out according to the intensity profile of the spatial distribution of luminescence as a result of scanning with a laser beam using the method of fluorescence recovery after photobleaching (FRAP). The experiments were carried out on a Zeiss LSM-710 laser scanning confocal microscope using a semiconductor laser with a wavelength of 405 nm. Objectives 5x/0.13, 20x/0.4 and 20x/0.75 were used.

Further, as a result of which the dependences of the squared half-width of the Gaussian distribution on the time elapsed after bleaching were plotted. Then, using the Stokes-Einstein equation, the hydrodynamic radii of the particles were calculated:

$$r = \frac{k_B T}{6\pi\eta D},$$

where D is the diffusion coefficient obtained from experimental data [3].

The dynamic light scattering (DLS) method, implemented on a Malvern Zetasizer Nano ZS instrument, was also used to measure hydrodynamical sizes.

Experiments both with a mixture of chitosan/AIS/tetraphenylporphyrin and with nanoparticles formed because of crosslinking of chitosan revealed the presence of formations whose sizes are typical for nanoparticles (200–300 nm in diameter). Otherwise, the smaller particles were not observed. This means that if a certain amount of free QDs and/or molecules is present in such a solution, then it is insignificant, therefore, biocompatible nanocomposites have been successfully formed.

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