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Application of silver nanoparticles and CdSe quantum dots sensitized with of C-like lectin for detection of *St. aureus*. Comparison of various approaches.

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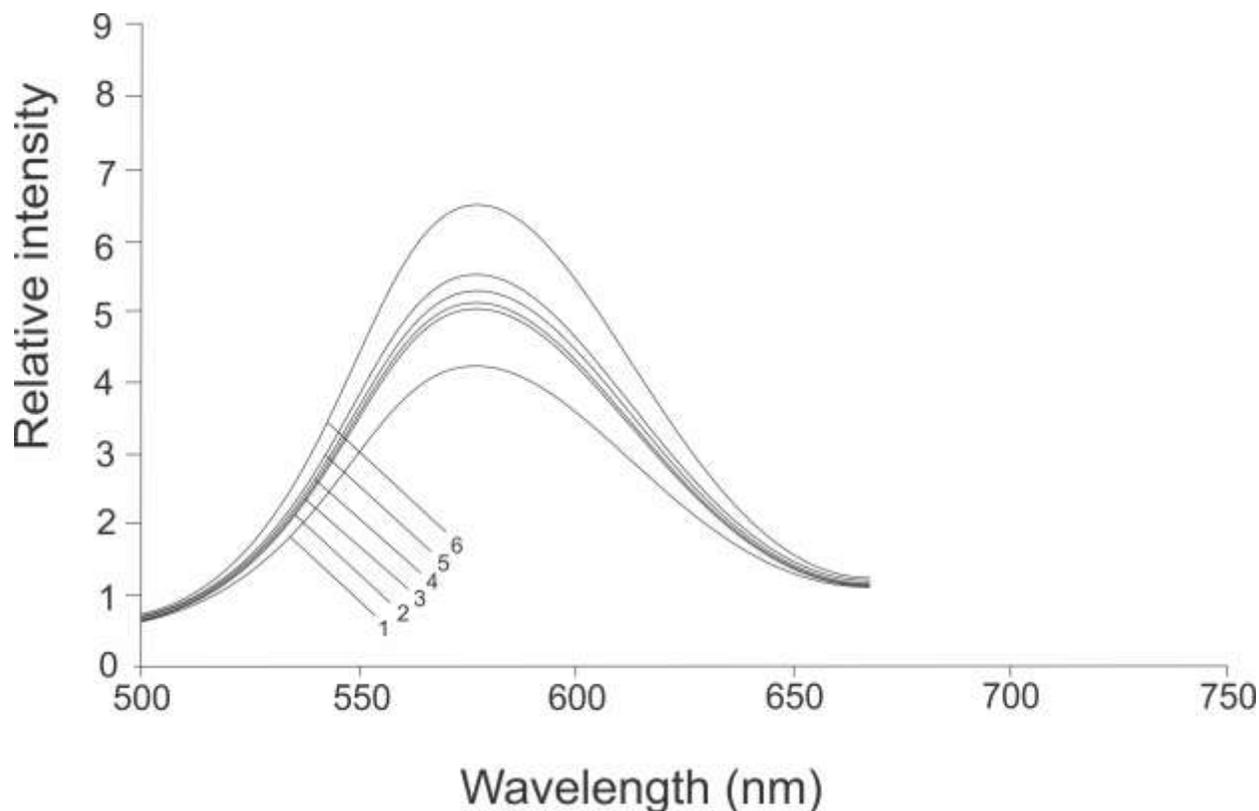
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Abstract

C-type lectin from hen egg shell as a recognition ligand for detection of *St. aureus* was applied..Three approaches for detection of bacteria were used and the sensitivities of the assays were compared. Two of them included spherical and anisotropic silver nanoparticles sensitized by lectin. In these cases the optical changes as a result of interaction of sensitized nanoparticles with bacteria were measured. In the third approach hybrid system of CdSe quantum dots-anisotropic silver nanoparticles sensitized by lectin was applied. Here fluorescent changes as a result of resonance energy transfer between nanoparticles as consequence of their interaction with bacteria were measured. The data demonstrate that assays with spherical silver nanoparticles permit to detect *St. aureus* in the range of $6 \times 10^4/\text{mL}$ - $2 \times 10^7/\text{mL}$, anisotropic silver nanoparticles in the range of $2 \times 10^5/\text{mL}$ - $1 \times 10^8/\text{mL}$, CdSe-Ag hybrid system in the range of $6 \times 10^3/\text{mL}$ - $2 \times 10^7/\text{mL}$. The data demonstrate that hybrid system CdSe-Ag with resonance energy transfer provides the best sensitivity.

Graphical abstract

Changes in fluorescent spectra of lectin sensitized CdSe quantum dots as a result of resonance energy transfer between nanoparticles during their interaction with *S. aureus* in hybrid system of quantum dot-silver nanoparticles .

Keywords: C-type lectin, silver nanoparticles, quantum dots, bacteria detection.

1. Introduction

Infectious diseases are serious public health problem especially in developing countries. They kill over 2 million children every year [1-3]. The great majority of these deaths occur in regions where bacteria are widespread in drinking water and food and therefore the detection of pathogenic bacteria in biological fluids, food products and water is extremely important task in medical diagnosis and in food security estimations. Traditional methods for detection of bacteria include cultivation, various types of immunoassay and molecular biology methods (PCR) [4-8].

Each of these methods has its advantages; however the utility of these methods is generally limited by their high cost and the requirement of trained staff. Moreover all of these methods are directed on detection of specific types of bacteria and they cannot be applied for detection of pathogens in general. Bacteria screening procedures are also very important because in many cases there is no necessity to detect specified pathogen and it is necessary to detect the presence of bacteria without their identification. In particular such situations arise in certain cases such as analysis of quality of water and milk [9-10]. Moreover in some kidney infections assay is directed for detection of infection without its identification as in the case of bacteriuria kit test and consequent treatment is based on the assay of antibiotic sensitivity of the pathogen for choosing of most effective antibiotic. Now in fact there are only few such screening tests. So bacteriuria test strips for detection of various bacteria in urine were developed and they are widely applied in clinical practice [11-12]. The test is based on chemical azo-reaction of nitrite and therefore it can be applied only for the detection of nitrate reducing bacteria. However many of pathogens have no nitrate reducing ability and therefore they cannot be detected and moreover the sensitivity of the assay is low. Another approach for bacteria detection was based on the measurement of bacterial ATP by luciferin–luciferase, ATP dependent reaction had been developed and marketed [13-14]. This assay was applied in particular for the control of drinking water and milk quality. It is well known that the total number of heterotrophic bacteria is the better indicator of their quality. But in these ATP assays the presence of other cells can induce false positive results (like the one observed in milk). Moreover here preliminary procedure for bacteria destroying is necessary. Therefore it is desirable to create other test systems which would be able to provide screening tests for various viral and bacterial pathogens. It is apparent that the approach must be based on detection of such molecules that are present in bacteria and viruses and it is desirable that they will be absent in other types of cells or are presented in trace amounts. Pathogens contain some conservative structures peculiar for many of them. So, peptidoglycans (mucopeptides, glycopeptides, mureins) are the structural elements of almost all bacterial cell walls. They constitute almost 95% of the cell wall in some Gram positive bacteria and 5–10% of the cell wall in Gram negative bacteria [15]. **Therefore it can be anticipated that** ligands that recognize such carbohydrate containing structures can be applied as markers for the detection of bacteria. In this respect, lectins being the carbohydrate binding proteins can be proposed as recognition elements in biosensors for detection of bacteria via binding with their

carbohydrate structures. The recent studies have demonstrated that lectins, in particular mannose binding lectin participates in antimicrobial innate immunity by binding bacterial pathogens and therefore it plays critical role in host defense [16-17]. Such antimicrobial properties were shown for some other lectins also [18-20]. It should be noted that lectins are specific for various carbohydrate structures and therefore a library of various lectins can be applied for detection of wide spectrum of bacteria. These proteins were applied successfully by various authors for detection of bacteria [21]. So a functional mannose self-assembled monolayer (SAM) in combination with lectin (Con A) was used as molecular recognition element for the detection of *E. coli* W1485 using Quartz Crystals Microbalance (QCM) as a transducer [22]. In this investigation concanavalin A, lectin from jack bean, with specificity to terminal α -D-mannosyl and α -D-glucosyl groups immobilized onto gold plated quartz crystal was able to detect *E. coli* in the range between 5.0×10^6 and 2.0×10^7 CFU/mL by measuring frequency changes of crystal. In the study of Townsend et al. [23] mannose binding lectin (MBL) was applied as a specific ligand for detection of various bacteria by flow cytometry. In studies of Disney et al [24] reverse procedure was applied for detection of bacteria. Generally bacteria use carbohydrates on cell surface as receptors for binding and consequent invasion. Here bacterial lectins are applied as binding molecules. Authors have used a carbohydrate-functionalized fluorescent polymer, which displays many carbohydrate ligands on a single polymer chain to allow multivalent detection of pathogens. These results showed that carbohydrate-functionalized fluorescent polymers are a versatile detection method for bacteria.

Nowadays nanomaterials open new pathways in medical diagnostics. Application of various nanoparticles allows to develop methods for rapid detection of various analytes by simple photometric methods [25-29]. Unique optical properties of nanoparticles, in particular gold and silver, and sensitivity of these properties to processes taking place on their surface enable application of these nanoparticles for detection of various biorecognition interactions (between antigen and antibody, lectin and carbohydrate, complementary oligonucleotides etc. In our earlier works we applied spheric silver nanoparticles for sensitive immunoassay of human IgG [30], anisotropic silver nanoparticles sensitized by potato lectin for detection of Gram positive as well as Gram-negative bacteria [31] and combination of silver nanoparticles-CdSe quantum dots for detection of human albumin by a simple one step photometric method [32].

In the article we applied these three approaches to compare their sensitivity for detection of *St. aureus*.

2.Methods.

Optical measurements were conducted on Spectrophotometer Hitachi 150-40, Fluorescent measurement were conducted on Spectrofluorimeter Perkin Elmer MPF 44A.

C-type lectin from egg shell was prepared according to [20] with minor modifications. In short hen egg shells were extracted by 10 volumes of 1 N HCl for 1 hour and supernatant was treated by ammonium sulphate of 30% saturation for removal of formed CaSO_4 . The protein was further precipitated by adding ammonium sulphate to 100% of saturation. The precipitate was suspended in 0.1 M acetate buffer pH 4.5 and dialyzed against the same solution. The dialyzed preparation was applied on column with CM-52 cellulose equilibrated by the same solution. The column was washed by buffer until absorbance in eluent was less than 0.02 at 280 nm. Then washing was conducted by 0.3 M acetate buffer pH 4.5 until absorbance of eluate was less than 0.02. The protein was eluted by 1 M acetate buffer pH 4.5. The preparation was electrophoretically homogeneous in 15% PAAG conducted in non-denaturing conditions according to [33]. Concentration of the protein was determined by optical method by comparison of absorbances at 224 nm and 236 nm according to [34].

Spherical silver nanoparticles were prepared by reduction of 4mM of silver nitrate by mixture of sodium citrate and tannin as described in [30]. Anisotropic silver nanoparticles were prepared by two-stage procedure as described in [35]. Water soluble CdSe quantum dots were prepared by reduction of CdCl_2 (4 mM) by freshly prepared NaHSe according to [36].

2.1. Sensitization of silver nanoparticles by lectin

Initially the protein was dialyzed against 1 mM of glycine NaOH buffer to prevent effects of salts on aggregation of nanoparticles. To 1mL of silver nanoparticles various amounts of dialyzed protein were added. Low amounts of protein are inadequate for sensitive assay whereas the high amounts of protein bring to aggregation of nanoparticles and to drastic changes in optical spectra. The amount of protein that brings to minimal changes in optical spectrum is chosen for consequent investigations. After addition of appropriate amount of protein to silver nanoparticles incubation for 2-3 hours was carried out then bovine serum albumin (BSA) in final concentration of 0.1 % was added and after an additional incubation for 1 hour glycine NaOH buffer (1 M) was added to final concentration of 0.05 M.

2.2 Sensitization of CdSe quantum dots

Initially CdSe quantum dots were treated for 24 hours by mercaptoethylamine to provide monolayer of thiol with exposed amine groups. In this procedure to 1 mL of nanoparticles 10 μ L of mercaptoethylamine (1 μ g/mL) was added. The protein 50 μ g/mL was treated by glutaric aldehyde in final concentration of 0.1% for 24 hours. Then treated protein was added to thiol modified CdSe nanoparticles and incubated for 24 hours. The final concentration of protein was 1 μ g/mL. Glycine–NaOH buffer and BSA in final concentrations of 2 mM and 0.05% appropriately were added.

2.3 Bacteria

Staphylococcus aureus 5233 (RCDM NAS RA), (All Russian Collection of Industrial Microorganisms, NSAP SRI). All strains are kept in the microorganisms' collection of the Department of Microbiology, Plant and Microorganism Biotechnology, Yerevan State University. The strains were grown on the skew MPA (meat–peptone agar, Obolensk, Russia) pH 7.2–7.6 at 37° C for 20 h. The bacteria were extracted by 0.02 M glycine–NaOH buffer, pH 8.3, containing 3 mM of CaCl₂. The amounts of bacteria were estimated by absorbance at 650 nm and these values were compared with turbidity standard that was prepared according to [15]. Initial amount of bacteria that was applied in experiments has turbidity at 650 nm equal to 1.

3. Results

3.1 Bacteria assay by spherical silver nanoparticles sensitized by egg shell lectin

Optical spectrum of spherical silver nanoparticles is presented in Fig 1. Spectrum is characterized by a single peak around 400 nm. Optimal amount of lectin for sensitization of these nanoparticles was 5 μ g/ml of nanoparticles. To 1.0 mL of such lectin sensitized silver nanoparticles 0.2 mL of bacterial suspension (*St.aureus*) of various concentrations and 0.1 mL of CaCl₂ solution (50 mM) were added. The solution was incubated for 2 hours and then optical spectra were recorded. Optical changes as a result of bacteria addition are presented in Fig 2.

3.2 Bacteria assay by anisotropic silver nanoparticles sensitized with egg shell lectin

Optical spectrum of anisotropic silver nanoparticles is presented in Fig 3. Optical spectrum is characterized by two peaks at 400 nm and 580 nm. It should be noted that long-wave peak is very sensitive to any process that takes place on the surface of nanoparticles and therefore the

assay was based on changes of band at 580 nm by bacteria binding. Here optimal concentration of lectin was 0.2 $\mu\text{g}/\text{mL}$ of nanoparticles. To 1.0 mL of such lectin sensitized silver nanoparticles 0.2 mL of bacterial suspension (*St. aureus*) of various concentrations and 0.1 mL of CaCl_2 solution (50 mM) were added. The solution was incubated for 2 hours and then optical spectra were recorded. The data obtained are presented in Table 1.

3.3 Bacteria assay by hybrid system of silver nanoparticles-CdSe quantum dots

Here the principle of resonance energy transfer between these nanoparticles upon their aggregation was applied. Anisotropic silver nanoparticles and CdSe quantum dots have strong overlapping in their spectra. Optical spectrum of silver nanoparticles is overlapped with spectrum of fluorescence of CdSe quantum dots (Fig 4). Therefore upon aggregation of these nanoparticles distances between them are decreased and resonance energy transfer takes place. As a result fluorescence of QDs will be quenched. Here silver nanoparticles as well as QDs were sensitized by egg shell lectin as presented in Materials and Methods. Interaction of bacteria with lectins on the surface of nanoparticles will bring to formation of complex Ag-bacteria-CdSe and as a result the distance between nanoparticles will be decreased and quenching of fluorescence will take place. At first stage we tested the effect of nonsensitized silver nanoparticles on nonsensitized QDs. We have expected that here also Stern –Volmer type quenching can take place as a result of dynamic interaction of the nanoparticles. And really the addition of various amounts of silver nanoparticles to CdSe quantum dots brings to quenching of fluorescence of quantum dots. These experiments demonstrate that in energy transfer studies it is preferential to apply ratio of QD/Ag=5. This ratio permits to obtain maximal sensitivity of the assay. In our studies we have added 0.2 mL of bacteria to 1 mL of lectin sensitized CdSe containing 0.2 mL lectin sensitized anisotropic silver nanoparticles and CaCl_2 in final concentration of 5 mM. The system was incubated for 2 hours and then fluorescence spectra were recorded. The data obtained are presented in Fig 5.

4. Discussion

Comparison of data obtained by application of various types of assays demonstrates that spheric silver nanoparticles provide more sensitivity in comparison to anisotropic silver nanoparticles. At first view it is unexpected result. It is well known that optical spectrum of spherical nanoparticles

is changed only upon strong aggregation processes (37). In this respect anisotropic silver nanoparticles are very sensitive to any subtle process that takes place on their surface and we could expect that the assay with anisotropic silver nanoparticles will be more sensitive in comparison to spherical nanoparticles. However spherical nanoparticles have certain advantages. At first it is possible to synthesize concentrated solution of nanoparticles and moreover they are more stable to action of sensitizer. It provides higher sensitivity. On the other hand anisotropic silver nanoparticles have much more lower concentration in comparison to spherical nanoparticles and moreover they are very sensitive to sensitization by lectin. Only 0.2 $\mu\text{g/mL}$ of nanoparticles conserve their stability whereas 5 $\mu\text{g/mL}$ of nanoparticles is a result for spherical nanoparticles. On the other hand hybrid system of Ag-QD provides higher sensitivity in comparison with spherical and anisotropic silver nanoparticles since in this case subtle changes in fluorescence can be detected. Thus assays with spherical silver nanoparticles permit to detect *St. aureus* in the range from $6 \times 10^4/\text{mL}$ - $2 \times 10^7/\text{mL}$, anisotropic silver nanoparticles in the range from $2 \times 10^5/\text{mL}$ - $1 \times 10^8/\text{mL}$, CdSe-Ag hybrid system from $6 \times 10^3/\text{mL}$ - $2 \times 10^7/\text{mL}$.

5. Conclusion

C-like lectin from hen egg shell sensitized with various types of silver nanoparticles and CdSe quantum dots were applied in various assays for detection of *St. aureus*. Sensitivities of these assays were compared. The best results were obtained by application of hybrid system of anisotropic silver nanoparticles-CdSe quantum dots. This approach permits to detect *St aureus* in the range of $6 \times 10^3/\text{mL}$ - $2 \times 10^7/\text{mL}$.

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Fig. 1. Optical spectrum of spherical silver nanoparticles

Fig. 2. Changes in optical spectra of lectin sensitized spherical silver nanoparticles during their interaction with *St. aureus*. Concentrations of added bacteria: 1- 2×10^8 /mL, 2- 4×10^7 /mL, 3- 8×10^6 /mL, 4- 1.6×10^6 /mL, 5- 3×10^5 /mL, 6- 6×10^4 /mL.

Fig 3 Optical spectrum of anisotropic silver nanoparticles.

Fig.4 Fluorescence spectrum of CdSe quantum dots (1) and long wave band of optical spectrum of anisotropic silver nanoparticles (2).

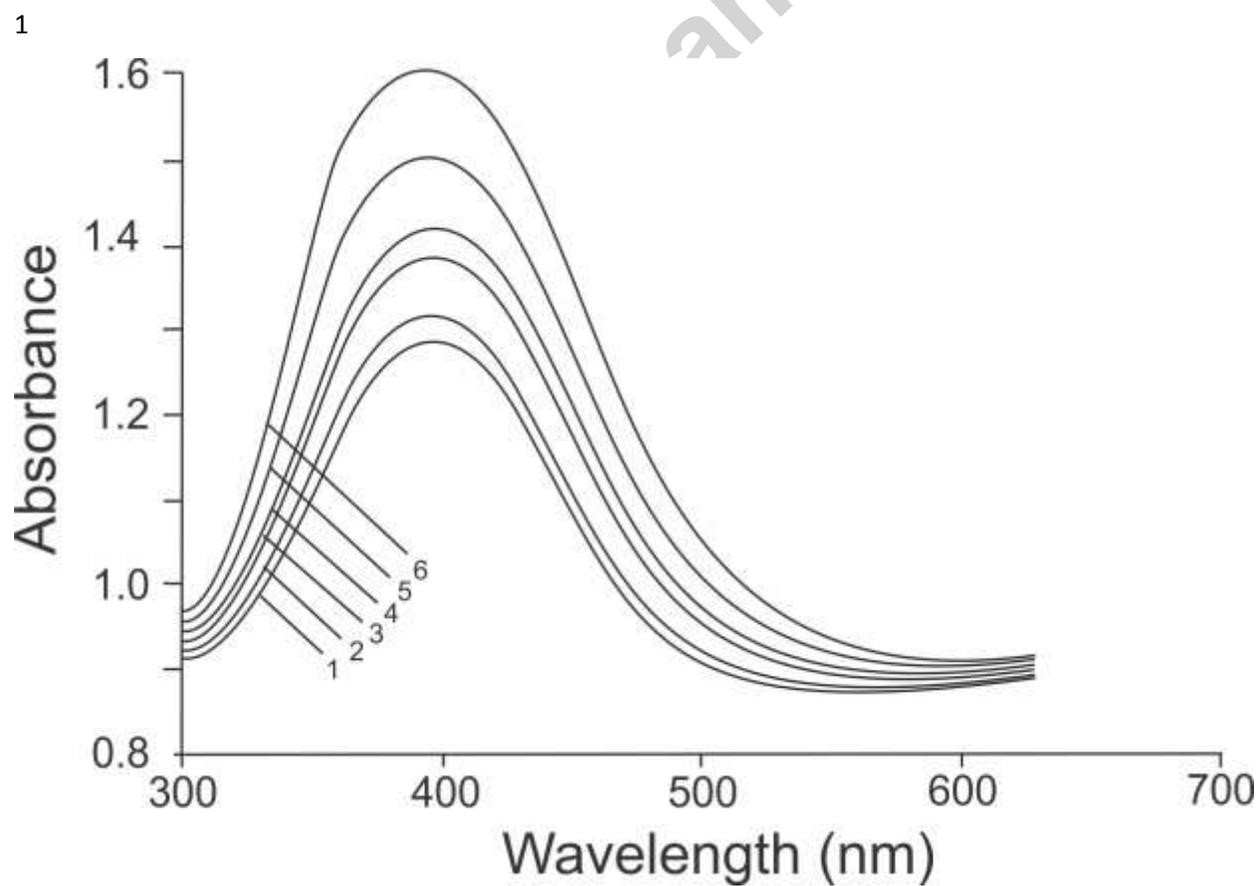
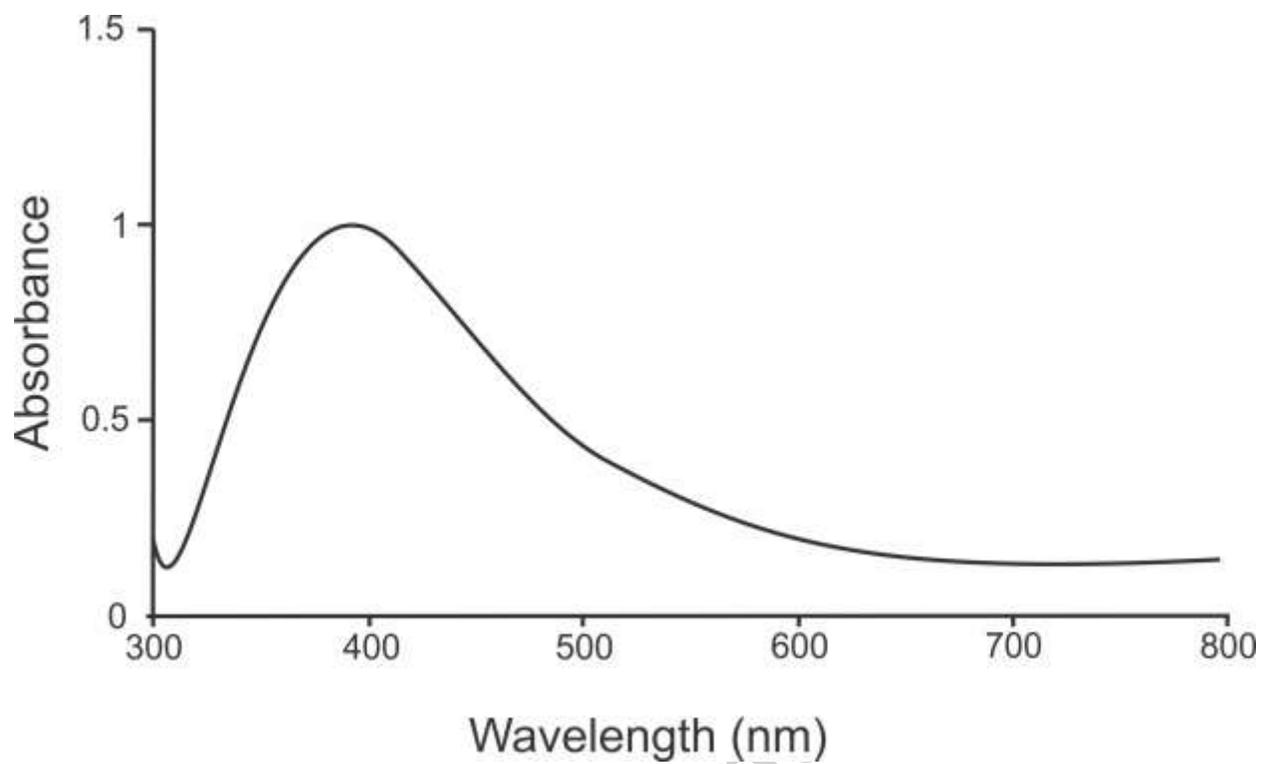
Fig 5 Changes in fluorescent spectra of lectin sensitized CdSe quantum dots during their interaction with *St. aureus* in hybrid system of quantum dot-silver nanoparticles (see Principle of assay in Results and Discussion). Concentrations of added bacteria: 1- 2×10^7 /mL, 2- 4×10^6 /mL, 3- 8×10^5 /mL, 4- 1.6×10^5 /mL, 5- 3×10^4 /mL, 6- 6×10^3 /mL.

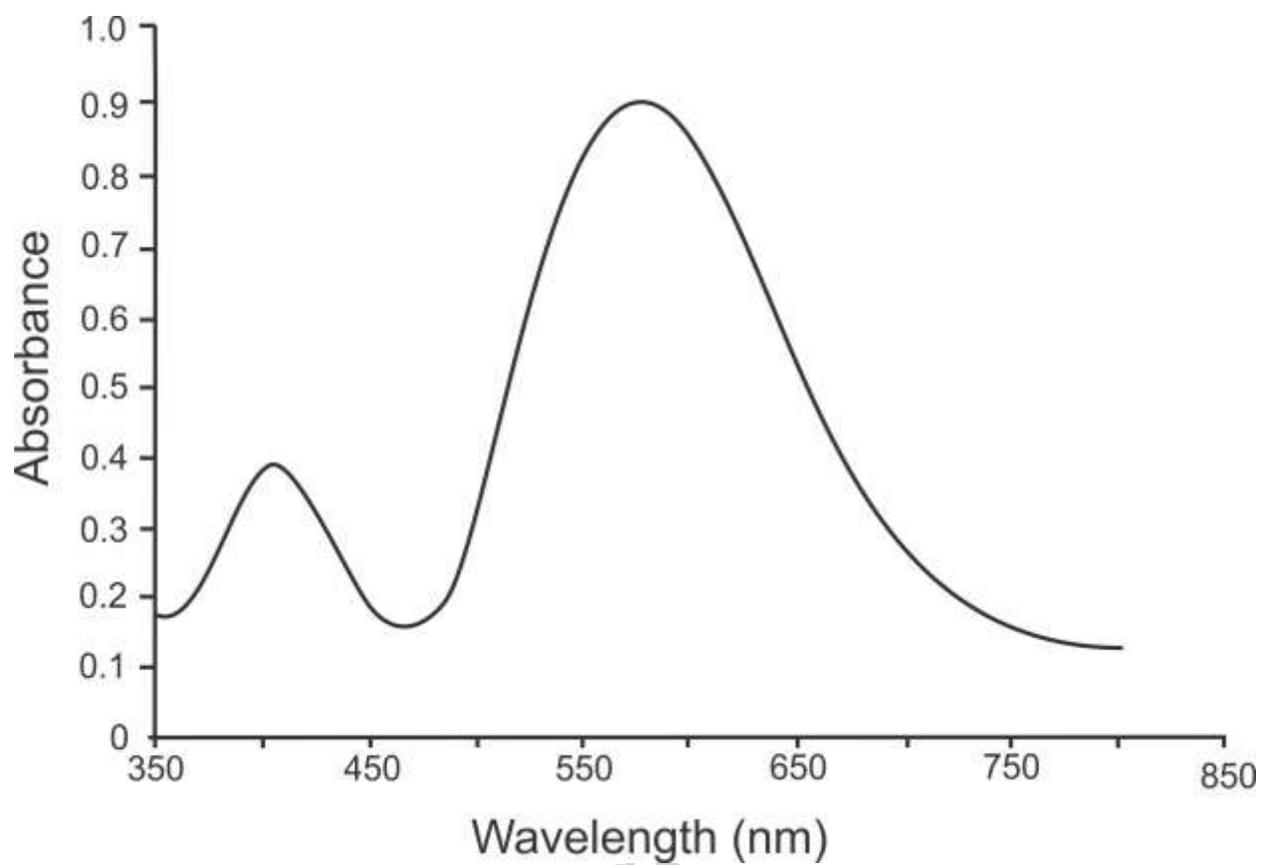
Table 1. Changes in optical spectra of lectin sensitized anisotropic silver nanoparticles during their interaction with *St. aureus*.

<i>St. aureus</i>	
ΔA at 570 nm	Concentration of bacteria/mL
0.221	1×10^8
0.086	2×10^7
0.027	5×10^6
0.024	1×10^6
0.016	2×10^5
0	4×10^4
0	0

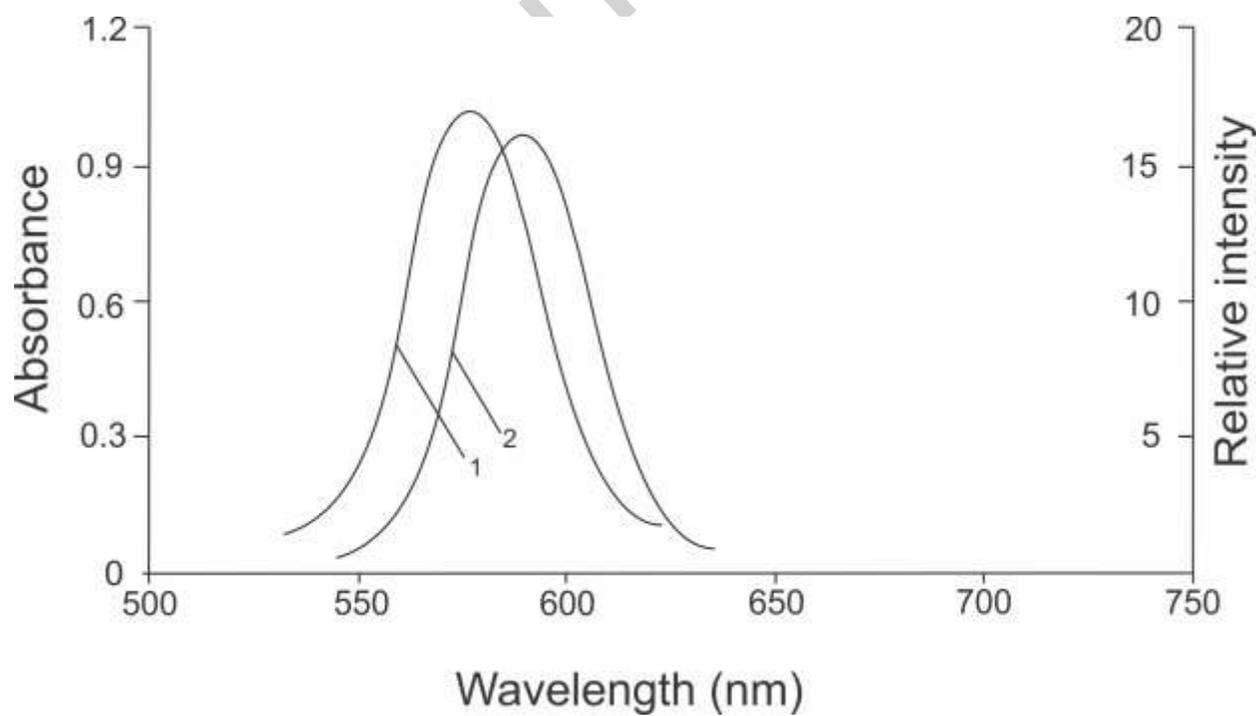
Highlights

- C-like lectin from hen egg shell was applied as recognition ligand for bacteria detection
- Spherical and anisotropic silver nanoparticles sensitized with lectin were applied for quantitative detection of *St. aureus*
- Hybrid system CdSe quantum dots-anisotropic silver nanoparticles sensitized by lectin provide higher sensitivity in comparison to previous approaches

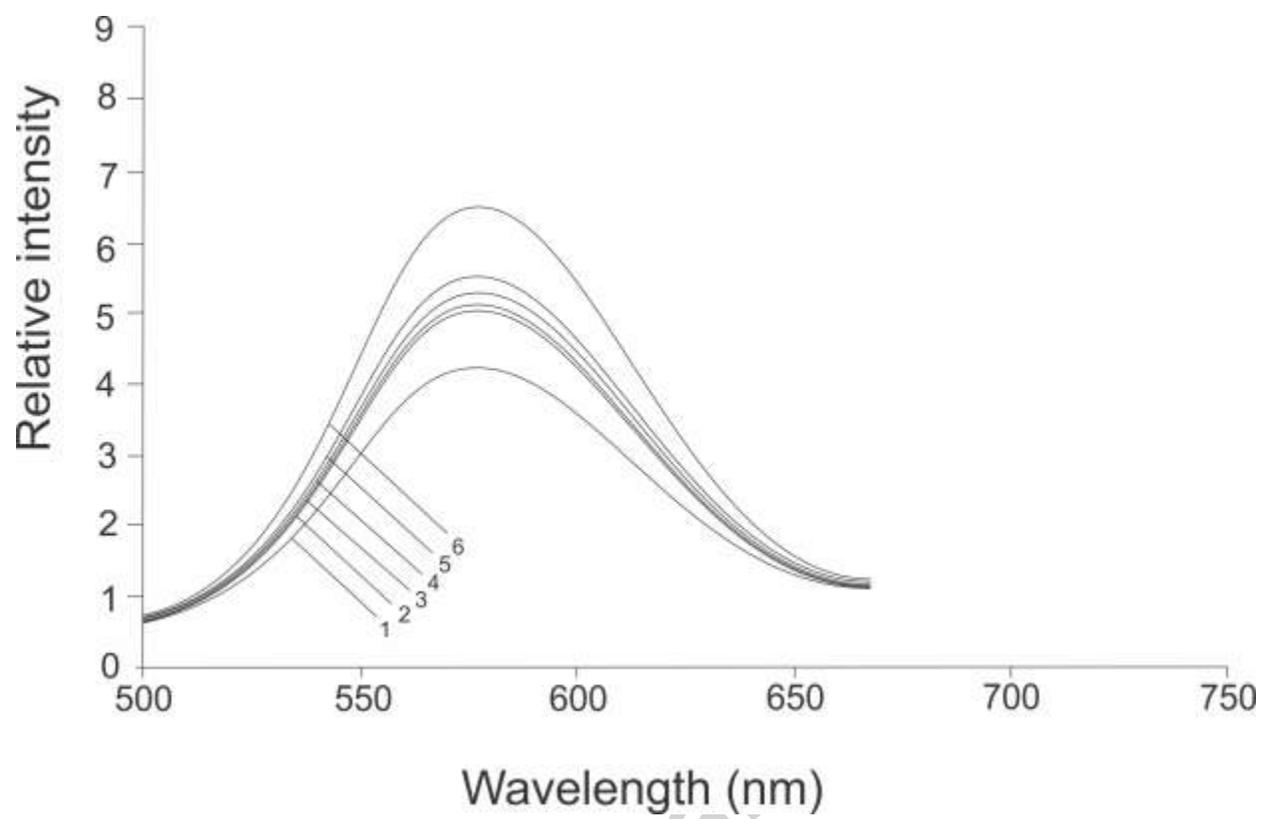




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