J Fluoresc (2014) 24:1433–1438
DOI 10.1007/s10895-014-1426-8

ORIGINAL PAPER

Synthesis of Water-Soluble CdSe Quantum Dots With Various Fluorescent Properties and Their Application in Immunoassay for Determination of C-Reactive Protein

V. K. Gasparyan

Received: 9 April 2014 / Accepted: 7 July 2014 / Published online: 18 July 2014

Abstract Effects of various factors on synthesis and fluorescent properties of CdSe quantum dots were studied. It was shown that variation of pH, stabilizer and concentration of precursors brings to obtaining of quantum dots with various fluorescent properties. The nanoparticles prepared were conjugated with rabbit antibodies to C-Reactive protein and C-Reactive protein for competitive immunoassay for determination of CRP. It was shown that interaction of these dots as a result of antigen-antibody reaction brings to resonance energy transfer and these changes in fluorescence spectra correlate with concentration of CRP. This approach permits to determine CRP in range between 4–100 ng

Keywords CdSe quantum dots · Synthesis · C-Reactive protein · Competitive immunoassay · Resonance energy transfer

Introduction

The development of nanotechnology as a result of preparation and application of various nanomaterials and nanodevices brought to new revolutionary directions in biomedical investigations such as laboratory diagnostics, visualization of cellular processes, delivery of drugs to specific targets, therapy [1]. So, unique optical properties of gold and silver nanoparticles permit to use them in laboratory diagnostics for one-step detection of various antigens, antibodies, bacteria and viruses, and also in photodynamic therapy for cancer treatment [2–5]. Magnetic nanoparticles of Fe, Co were applied successfully for selective separation of various cell types [6]. Some other nanoparticles are applied for selective delivery of drugs and genes [7, 8]. Fluorescent semiconductor nanoparticles (quantum dots) have particular role in these processes. This class of nanoparticles has unique fluorescent properties that are able to change traditional approaches in medicine and in particular in medical diagnostics [9, 10]. These nanoparticles are distinguished sharply from traditional organic fluorophores by their properties. The general features that attract the attention of investigators to these type of nanoparticles are high quantum yield, high stability against photobleaching, broad excitation band and narrow bands of fluorescence [11, 12]. It permits to excite many dots simultaneously by application of only one excitation band. It opens new prospects for simultaneous multiplex assay of many compounds. Usually small particles have short-wave band of fluorescence whereas larger particles have band shifted in long-wave area. It permits to synthesize quantum dots with various fluorescent properties by variation of conditions of this process [13, 14]. At present time various dots with various composition and with various fluorescent properties such as CdSe, CdS, CdTe, ZnS, ZnSe and also other more complex nanocomposites [15-17] were obtained. Moreover the composition of nanoparticles, in particular the sizes of core and shell, also determine their fluorescent properties. For instance CdSe quantum dots with tunable fluorescent band ranging from 450 to 650 nm can be prepared. In case of CdTe quantum dots these bands can range from 450 to 750 nm [18]. Such nanoparticles are prepared by two general methods. In one case high temperature reaction is applied where organometallic precursors are destroyed and in presence of appropriate stabilizers they form monodisperse nanoparticles with high quantum yield of fluorescence. However, in this case, hydrophobic nanoparticles are formed and for their

V. K. Gasparyan (✉)
Institute of Biochemistry, National Academy of Sciences, P.Sevak str.5/1, Yerevan 0014, Georgia
e-mail: vgasparyan@excite.com

application in the biomedical investigations their surface must be functionalized. Moreover, large-scale production of such dots has serious technical problems [19, 20]. It should be noted also that stabilizers that are applied in these processes (trioctylphosphine (TOP) and trioctylphosphine oxide, (TOPO) are very toxic compounds. In the other case the dots are prepared immediately in water media as a result of chemical reaction between precursors. Although in the last case monodispersity and quantum yield of fluorescence get slightly worse, this method in some cases is more attractive because it permits the immediate conjugation of biomolecules to these nanoparticles. There are reports in literature about preparation and application of such water soluble quantum dots in various types of bioassays [21, 22], however data about factors that determine the fluorescent properties of these nanoparticles are scarce.

Data about preparation of water soluble CdSe quantum dots and the effects of various factors on their fluorescent properties are presented in this article. Moreover, we have applied these nanoparticles in competitive immunoassay for quantitative determination of C- Reactive protein (CRP). It is one of the more characteristic acute-phase proteins and is considered a reliable indicator of disease activity in various clinical conditions [23]. At this point CRP level in biological fluids is determined by several immune methods such as enzyme immunoassay, turbidimetry, latex agglutination etc. [24–26]. However, recently new approaches based on nanotechnology were applied successfully for the determination of diagnostically relevant substances and in particular for the determination of CRP. So CdSe and ZnSe quantum dots were applied for screening CRP in human plasma [27]. In this approach the authors coated these dots with O-phosphorylethanolamine that is ligand for CRP. The presence of CRP in plasma brings to its interaction with these sensitized dots. As a result of such interaction the intensity of fluorescence is decreased and it is correlated with the concentration of CRP in plasma. The author's note that the results obtained using ZnSe quantum dots were not acceptable because the estimated concentrations were much higher than the expected ones. The application of sensitized CdSe quantum dots showed the best results when the analytical procedure was applied in range of CRP concentrations between 1–4 mg/L. In the other case the authors applied quantum dots in microplate format for very sensitive detection of CRP [28]. Here the nanoparticles sensitized with monoclonal antibodies were used as a fluorescent labels for protein detection. In fact in this case the enzyme label was replaced by fluorescent label. It was reported that the proposed CRP assay provides a wide analytical range of 0.001–100 mg/L with a detection limit of 0.06 μg/L within 1.5 h. Assay used in our article is based on competitive immunoassay where interaction of antibody with appropriate antigen brings to resonance energy transfer between various dots. As a result the intensity of fluorescence is changed depending on the concentration of antigen in the sample.

Experimental Section

Cadmium chloride, sodium borohydride, Se powder, thioglycolic acid were purchased from Sigma –Aldrich. Solution of NaHSe was prepared by addition of fresh prepared solution of $NaHB_4$ to fine powder of Se according to [29] with some modifications. In particular, 140 mg of $NaBH_4$ 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 with periodic stirring 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. In this reaction, as noted in reference [32], the 0.7 M solution of NaHSe is formed. Solutions of $CdCl_2$ bring to boiling in two-necked retort under steam of N_2 and then rapid injection of NaHSe solution is conducted. The solution is boiled for additional 30 min in backflow condenser, cooled and dialyzed for removal of any side products. C-reactive protein (CRP) from human pleural fluid was purified according to [30]. Monospecific antibodies to the protein were raised according to schedule described in [31]. Concentration of CRP was determined from ratio $A^{1\%}_{1cm}=19$ [32]. Concentration of antibodies was determined from ratio $A^{1\%}_{1cm}=14.5$ [33]. Sensitization of proteins on nanoparticles was conducted by incubation of the appropriate protein with nanoparticles for 1 h in presence of 1 mM of Tris–HCl buffer pH=8.2. Optimal concentrations of the proteins were determined empirically. Then bovine serum albumin in final concentration of 0.1 % was added and after additional incubation for 30 min Tris –HCl buffer and NaCl was added to the final concentration of 0.05 M and 0.15 M respectively. For assay of free resonance energy transfer (FRET) between various dots 1 ml of dot with various amounts of sensitized antibodies to CRP were mixed with dots sensitized by fixed amount of CRP and after 10 min of incubation fluorescence spectra were measured. For quantitative determination of CRP

Table 1 The effects of concentrations of NaHSe on fluorescent properties of CdSe nanoparticles

N	$CdCl_2$ (1mM) mL	NaHSe (7 mM) mL	λ_{ex} (nm)	λ_{em} (nm)
1	50	1	420	506
2	50	6	420	512
3	50	10	420	540
4	50	15	420	590
5	50	20	420	590
6	50	40	-	-

Table 2 The effects of sodium citrate on fluorescent properties of CdSe nanoparticles

N	$CdCl_2$ (50 ml).	Na-citrate (mM)	NaHSe (7 mM) (mL)	λ_{ex} (nm)	λ_{em} (nm)
1	4 mM	5	1	470	563
2	3.6 mM	5	1	470	563
3	3,2 mM	5	1	-	-
4	2.8 mM	5	1	470	573
5	2.4 mM	5	1	470	575
6	1.6 mM	5	1	470	590
1	4 mM	5	2	470	569
2	3.6 mM	5	2	470	573
3	3,2 mM	5	2	470	575
4	2.8 mM	5	2	470	582
5	2.4 mM	5	2	470	595
6	1.6 mM	5	2	470	598

by competitive immunoassay to 0.7 ml of dot, sensitized by antibodies to CRP, 0.1 ml of solution with various amounts of CRP were added. After incubation for 30 min 0.7 ml of dots sensitized by CRP were added. Fluorescence spectra were recorded after 30 min.

Fluorescent measurements were conducted in stationary mode on spectrofluorimeter Perkin –Elmer MPF 44A.

Results and Discussions

At the first stage of our investigations we studied the effect of concentrations of NaHSe on the fluorescent properties of nanoparticles. In this case as a stabilizer the polyvinylpirrolidone with molecular weight 24 kDa was applied in final concentration of 0.2 %. The pH of mixture was brought to 11 by adding 1 M of NaOH. Table 1 presents the results of such investigations. The data demonstrate that rising amounts of NaHSe brought to shifting of fluorescence band in long-wave area but on the other hand its high concentrations brought to quenching of fluorescence. Although these nanoparticles were very stable for a few months, they were unsuitable for sensitization by proteins. Any attempts to dialysis of these nanoparticles against water or buffers for consequent sensitization by proteins brought to irreversible aggregation of nanoparticles. However, the nanoparticles conserve their stability by dialysis against 1 mM of thioglycolic acid. It is well known that quantum dots have affinity to thiol compounds and apparently in this case the stabilization of nanoparticles was associated with formation of monolayer of thiol compounds on the surface of nanoparticles with exposed carboxyl groups. It suggests that biomolecules could be immobilized on surface of nanoparticles via covalent bonding with thiol compounds. However, such covalent binding usually brings to some losses in immunological activity and as a result the sensitivity of assay is decreased. Moreover it is very important that thiol

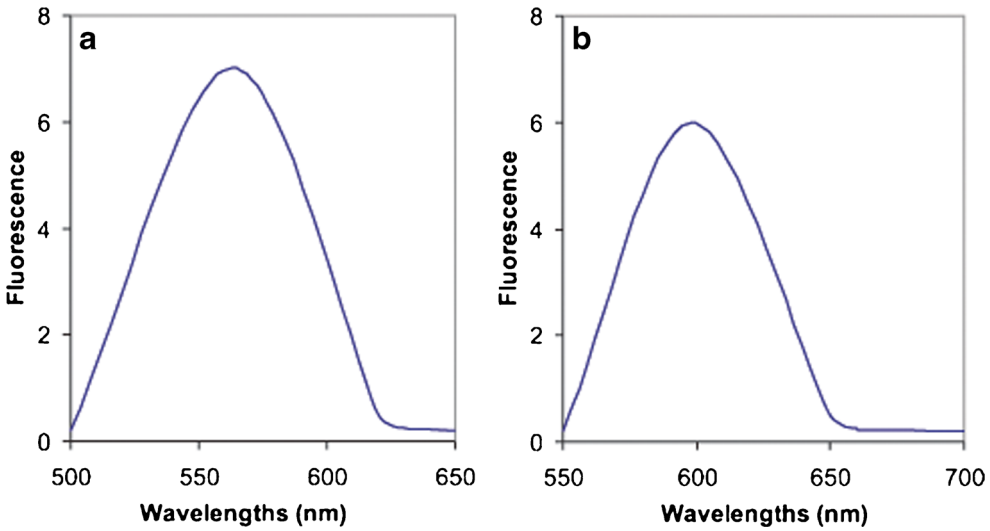

Fig. 1 A –quantum dot 1 with emission band at 563 nm, B-quantum dot 2 with emission band at 598 nm. In both cases excitation was conducted at 470 nm

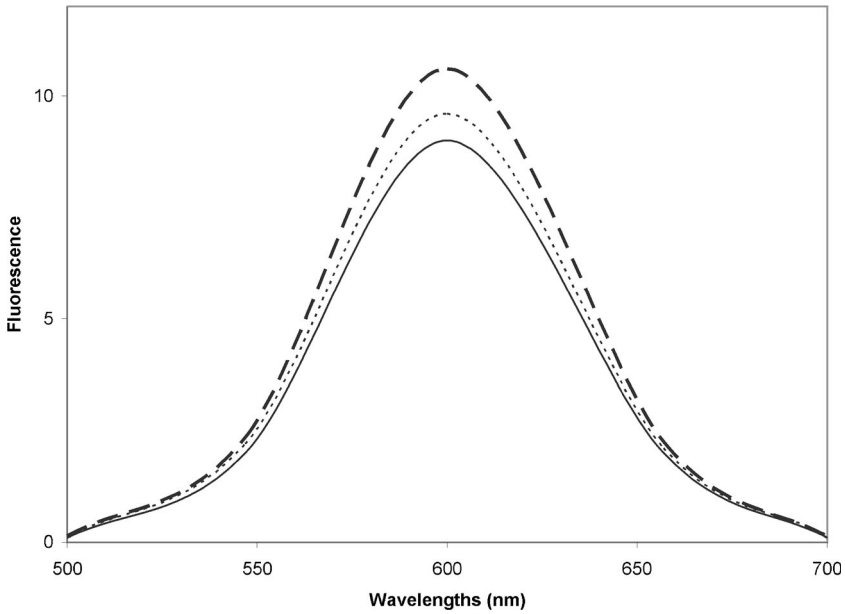

Fig. 2 The changes in spectra of fluorescence by interaction of dot 1 and dot 2_____1 ml of dot 1 (0.6 μg Ab to CRP) + 1 ml of dot 2 (1 μg CRP)…… 1 ml of dot 1 (3 μg Ab to CRP) + 1 ml of dot 2 (1 μg CRP)- - - - 1 ml of dot 1 (8 μg Ab to CRP) + 1 ml of dot 2 (1 μg CRP) In all cases excitation was conducted at 470 nm

compounds are strong quenchers of fluorescence of CdSe and CdTe quantum dots [34] and such treatment will worsen fluorescent features of the nanoparticles. Therefore, in the next set of our experiments we have tested sodium citrate as a stabilizer since this compound has such role in the synthesis of gold and silver nanoparticles [35, 36] and, moreover, we studied its effects on fluorescence bands. Moreover, here we have applied higher concentrations of $CdCl_2$ for preparation of more concentrated suspension of nanoparticles. Table 2 demonstrates data of these experiments. As can be seen, in this case the shifting of fluorescence bands is more limited (35 nm). In this case the prepared dots maintain aggregative stability and fluorescence properties for several months. These dots were applied further in experiments for determination of CRP by resonance energy transfer method. Fluorescent spectra of these dots are presented in Fig. 1. At the first stage we tested the ability of these dots to resonance energy transfer. For this purpose two dots with various emission bands were applied. Dot 1 has emission band at 563 nm and dot 2 has emission band at 598 nm. These nanoparticles were sensitized by antibodies to CRP and CRP. We expect, that interaction of these dots as a result of antigen –antibody reaction will bring to decreasing inter-particle distance and therefore to resonance energy transfer. In this case fluorescence at 563 nm must be decreased whereas that at 598 nm must be increased. Fig 2 in fact demonstrates that interaction of these dots brings to changes in fluorescence spectra. As can be seen the rising concentration of antibodies brings to increasing fluorescence with some

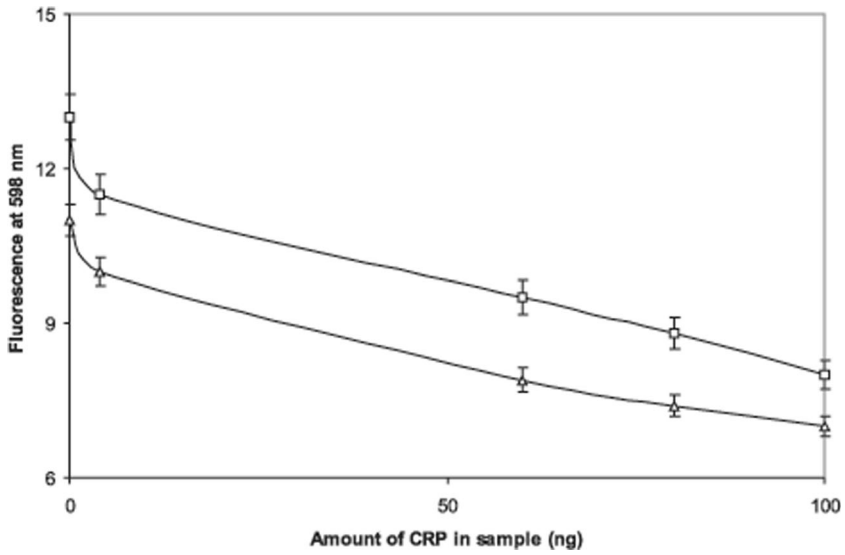

Fig. 3 The changes in intensit of fluorescence at 595 nm as a result of inhibition of agglutination by various concentrations of antigen. The data presents three experiments. CV for lower concentration of CRP was 7.3 % and 4.6 % for upper range of concentrations □ reaction in the presence of human serum (final concentration is 1%) Δ reaction without the serum

shifting of emission band in red-wave area. The obtained data were applied further for quantitative determination of CRP by method of inhibition of agglutination. This protein is a marker of inflammation and its determination is very important for diagnosis and prognosis of some diseases [37, 38]. In this case dot 1 and dot 2 were sensitized by antibodies to CRP and CRP as noted above. However, here dot 1, sensitized by antibodies, at first was incubated with various concentrations of CRP and then was mixed with CRP sensitized dot 2. Here agglutination of nanoparticles and therefore fluorescence intensity was determined by concentration of CRP in the solution. So high concentration of CRP in the solution will bring to its binding with dot 1 and as a result inhibition of interaction between dot1 and dot 2 will take place. In this case resonance energy transfer will be decreased and vice versa- low concentrations of CRP in the solution will improve agglutination and as a result will increase resonance energy transfer. The results of these experiments are presented in the Fig. 3. As can be seen from this picture this system permits to detect antigen in range from 4 to 100 ng. Since assays of various biologically relevant substances are conducted in serum we have conducted this assay in presence of human serum (final concentration 1 %) Fig. 3. The presence of serum brings to total rising of fluorescence without affecting the sensitivity of the assay. Apparently such increase of fluorescence can be associated with some energy transfer from fluorophores in serum to quantum dots. The obtained data demonstrate that such system can be applied effectively for determination of various antigen/antibodies in serum and other biological fluids.

References

1. Vo- Dinn T (2006) Nanotechnology in Biology and Medicine. CRC Press, London
2. Lee C, Gaston MA, Weiss AA, Zhang P (2013) Colorimetric viral detection based on sialicacid stabilized gold nanoparticles. Biosens Bioelectron 42:236–241
3. Saha K, Agasti SS, Kim S, Li X, Rotello VM (2012) Gold nanoparticles in chemical and biological sensing. Chem Rev 112:2739–2779
4. Raji V, Kumar J, Rejiya CS, Vibin M, Shenoi VN, Abraham A (2011) Selective photothermal efficiency of citrate capped gold nanoparticles for destruction of cancer cells. Expl Cell Res 317:2052–2058
5. Gasparyan VK, Bazukyan IL (2013) Lectin sensitized anisotropic silver nanoparticles for detection of some bacteria. Anal Chim Acta 766:83–87
6. Oh JK, Park JM (2011) Iron oxide-based superparamagnetic polymeric nanomaterials: Design, preparation, and biomedical application Prog Polymer Sci 36: 168-189
7. Shi J, Votruba AR, Farokhzad OC, Langer R (2010) Nanotechnology in drug delivery and tissue engineering: from discovery to applications. Nano Lett 10:3223–3230
8. Bharali DJ, Klejbor I, Stachowiak EK, Dutta P, Roy I, Kaur N, Bergey EJ, Prasad PN, Stachowiak MK (2005) Organically modified silica nanoparticles: A nonviral vector for in vivo gene delivery and expression in the brain. PNAS 102:11539–11544
9. Chan WCV, Maxwell DJ, Gao X, Bailey RE, Han M, Nie S (2002) Luminescent quantum dots for multiplexed biological detection and imaging. Curr Opin Biotech 13:40–46
10. Zhang B, Liang X, Hao L, Cheng J, Gong X, Liu X, Ma G, Chang J (2009) Quantum dots/particle-based immunofluorescence assay: Synthesis, characterization and application. J Photochem Photobiol B Biol 94:45–50
11. Alvisatos, AP (1996) Semiconductor clusters, nanocrystals and quantum dots Science 271: 933-937
12. Hines MA, Guyot-Sionnest P (1996) Synthesis and characterization of strongly luminescing ZnS-capped CdSe nanocrystals J. Phys Chem 100:468–471
13. Dabbousi BO (1997) (CdSe) ZnS-core-shell quantum dots: synthesis and characterization of a size series of highly luminescent nanocrystallites. J Phys Chem B 101:9463–9475
14. Thoma SG, Sanchez A, Provencio PP, Abrams BL, Wilcoxon JP (2005) Synthesis, optical properties, and growth mechanism of blue-emitting CdSe nanorods J. Am Chem Soc 127:7611–7614
15. Dethlefsen JR, Døssing A (2011) Preparation of a ZnS shell on CdSe quantum dots using a single-molecular ZnS precursor. Nano Lett 11: 1964–1969
16. Deng Z, Tong L, Flores M, Lin S, Cheng JX, Yan H, Liu Y (2011) High-quality manganese-doped zinc sulfide quantum rods with tunable dual-color and multiphoton emissions. J Am Chem Soc 133: 5389–5396
17. Pons T, Lequeux N, Mahler B, Sasnouski S, Fragola A, Dubertret B (2009) Synthesis of near-infrared-emitting, water-soluble CdTeSe/CdZnS core/shell quantum dots. Chem Materials 21:1418–1424
18. Murray CB, Norris DJ, Bawend MG (1993) Synthesis and characterization of nearly monodisperse CdE(E=S,Se,Te) semiconductor nanocrystals. J Am Chem Soc 115: 8706-8715-.
19. Hao E, Sun H, Zhou Z, Liu J, Yang B, Shen J (1999) Synthesis and optical properties of CdSe and CdSe/CdS nanoparticles. Chem Mater 11:3096–3102
20. Peng ZA, Peng XG (2001) Formation of high quality CdTe, CdSe and CdS nanocrystals using CdO as precursor. J Am Chem Soc 123: 183–184
21. Li H, Cao Z, Zhang Y, Lau C, Lu J (2011) Simultaneous detection of two lung cancer biomarkers using dual-color fluorescence quantum dots. Analyst 136:1399–1405
22. Tennico HH, Hutanu D, Koesdjojo MT, Bartel CM, Remcho VT (2010) On-chip aptamer-based sandwich assay for thrombin detection employing magnetic beads and quantum dots. Anal Chem 82: 5591–1597
23. Pepys MB, Balz ML (1983) Acute phase proteins with special reference to C-Reactive protein and related proteins (pentraxins) and serum amyloid A protein. Adv Immunology 34:141–212
24. Aziz N, Fahey JL, Detels R, Butch AW (2003) Analytical performance of a highly sensitive C-Reactive protein-based immunoassay and the effects of laboratory variables on levels of protein in blood. Clin Diagn Lab Immunol 10:652–657
25. Otsuji S, Shibata H, Umeda M (1982) Turbidimetric immunoassay of serum C-Reactive protein. Clin Chem 28:2121–2124
26. Gasparyan VK (2002) Preparation and sensitization of polystyrene latex beads by some antigens and antibodies Factors affecting sensitivity and specificity of latex agglutination tests. J Immunoassay Immunochem 23:399–406
27. Gomes D, Algarra M, de los Rios MGD, Arrebola MM, Herrera-Gutiérrez ME, Seller-Pérez G, da Silva JCG (2012) CdSe and ZnSe quantum dots capped with PEA for screening C-reactive protein in human serum. Talanta 93:411–414
28. Luo Y, Zhang B, Chen M, Jiang T, Zhou D, Huang JFW (2012) Sensitive and rapid quantification of C-reactive protein using quantum dot-labeled microplate immunoassay. J Translational Med 10:1–9

29. Klayman DL, Griffin TS (1973) Reaction of selenium with sodium borohydride in protic solvents. A facile method for the introduction of selenium into organic molecules. J.Am Chem Soc 95: 197-199
30. Ganrot PO, Kindmark CO (1969) A simple two step procedure for isolation of CRP. Biochim Biophys Acta 194:443–449
31. Gasparyan VK (2005) Hen egg immunoglobulin Y in colloidal gold agglutination assay Comparison with rabbit antibodies. J Clin Lab Anal 19:124–127
32. Johnson U, Prellner K (1977) Purification of CRP on DEAE cellulose by a simple two step procedure utilizing the Ca dependency of the protein. Biochim Biophys Acta 495:349–353
33. Frimmel G (1987) Immunological Methods. Moscow, Medicine 392-394
34. Wuister SF, de Mello DS, Meijerink A (2004) Influence of thiol capping on the exciton luminescence and decay kinetics of CdTe and CdSe quantum dots. J Phys Chem B 107:17393–17397
35. Frens G (1973) Controlled nucleation for the regulation of the particle size in monodisperse gold suspension. Nature Physical Sci 241:20–24
36. Roh Y, Yi J, Kim Y (2010) Rapid, reversible preparation of size-controllable silver nanoplates by chemical redox. Langmuir 26: 11621–11623
37. Ridker PM, Hennekens CH, Burig JE, Rifai N (2000) CRP and other markers of inflammation in the prediction of cardiovascular diseases in woman. N Engl J Med 342:836–844
38. Gunter MJ, Stolzenberg-Solomon R, Cross AJ, Leitzmann MF, Weinstein S, Wood RJ, Virtamo J, Taylor PR, Albanes D, Sinha R (2006) A prospective study of serum C-reactive protein and colorectal cancer risk in men. Cancer Res 66:2483–2487