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SHORT COMMUNICATION

Synthesis and characterization of novel core-shell colloidal particles ZnO/poly(ethylcyanoacrylate)

Georgi G. Yordanov · Nina V. Kaneva · Ceco D. Dushkin

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Abstract Here we report the synthesis and physicochemical characterization of novel hybrid core/shell type ZnO/poly(ethylcyanoacrylate) colloidal particles. It is expected that coating ZnO colloidal particles with biocompatible and biodegradable poly(alkylcyanoacrylates) will pave the way toward the potential application of ZnO colloidal particles in biomedical research. Recent findings of cell selective toxicity indicate a potential utilization of ZnO colloidal particles in the treatment of cancer. For this purpose, ZnO colloidal particles have to be selectively delivered to the site of action by a suitable biocompatible and biodegradable carrier system. Toward this goal, poly(alkylcyanoacrylates) meet ideally the requirements for carrier systems in drug delivery due to their biocompatibility, biodegradability, low toxicity, and ability to overcome the multidrug resistance in cancer cells.

Keywords Poly(alkylcyanoacrylate) · Poly(ethylcyanoacrylate) · ZnO · Colloidal particles · Zinc oxide

Introduction

The integration of nanotechnology and biology provides the opportunity for the development of new inorganic–organic materials in the nanometer-size range that can be utilized in many practical applications in biological science and clinical medicine. Although our understanding of the functioning of the human body at the molecular scale has improved tremendously, advances in therapeutic options for treating severe diseases such as cancer and autoimmunity have lagged. Nanotechnology and nanomedicine can offer a targeted approach, which promises significant improvements in the treatment of cancer by increasing the cell-selective toxicity of drugs [1–9].

Recent work on cell-selective toxicity indicates the potential of ZnO colloidal particles in the treatment of cancer [10]. The key findings of this work support the view that ZnO colloidal particles induce toxicity in a cell-specific and proliferation-dependent manner with rapidly dividing cells being the most susceptible and quiescent cells being the least sensitive. The marked difference in cytotoxic response between cancer cells and their normal counterparts, as well as differences between activated and resting T lymphocytes, suggest an exciting potential for ZnO colloidal particles as novel means in cancer therapy [10]. However, one of the problems toward such application of ZnO colloidal particles is that they need to be selectively delivered to the site of action by a suitable biocompatible and biodegradable carrier system. On the other hand, the most perspective application of poly(alkylcyanoacrylates) in biology and medicine is their use as nanoparticle drug carriers [11–13]. This very exciting area of research has gained increasing interest in therapeutics, especially for cancer treatments. The poly(alkylcyanoacrylate) colloidal particles meet ideally the requirements for carrier systems due to a number of properties such as biocompatibility, biodegradability, low toxicity, and ability to overcome the multidrug resistance in cancer cells, which are the reasons for the intensive research in this area [14–24]. Today, poly(alkylcyanoacrylate) colloidal particles are considered the most promising colloidal drug delivery system and are already in clinical development for cancer therapy [25–29].

G. G. Yordanov · N. V. Kaneva · C. D. Dushkin (✉)
Department of General and Inorganic Chemistry,
Laboratory of Nanoparticle Science and Technology,
University of Sofia, Faculty of Chemistry,
1 James Boucher Blvd,
Sofia 1164, Bulgaria
e-mail: nhtd@wmail.chem.uni-sofia.bg

Here we report the preparation and physicochemical characterization of novel hybrid core-shell colloidal particles ZnO/poly(ethylcyanoacrylate). First, ZnO colloidal particles are synthesized by modified sol–gel procedure in 1-propanol using zinc(II) acetate as a Zn-precursor. Triethylamine (TEA) and ethyleneglycol (EG) are utilized as stabilizing additives in order to achieve good control over the size and morphology of the colloidal particles. Then, the as-prepared ZnO colloidal particles are coated for the first time with biocompatible and biodegradable polymer shell by interfacial polymerization of ethylcyanoacrylate monomer on the surface of ZnO colloidal particles. The initial ZnO, as well as the obtained core-shell colloidal particles ZnO/poly(ethylcyanoacrylate) are characterized by scanning electron microscopy, X-ray powder diffraction, Fourier transform infrared spectroscopy and dynamic light scattering in order to verify their performance.

Experimental procedures

Materials and reagents Ethyl-2-cyanoacrylate (ECA) monomer was purchased from Special Polymers (Bulgaria). Zinc acetate dihydrate (>99.5%), 1-propanol (>99.0%), and triethylamine (>99.5%) were from Fluka. Phosphate-buffered saline (PBS), Pluronic F68, and Polysorbate 80 were from Sigma. Triton X100 was from Merck. Absolute ethanol (99.7%) and acetone were from the Institute of Pure Compounds (University of Sofia, Bulgaria). Distilled water was used for all preparations. Acetone was distilled on a fractionation unit before utilization in nanoparticle preparations.

Synthesis of colloidal particles First, the ZnO colloidal particles were prepared by hydrolysis of zinc(II) acetate in 1-propanol as follows. Zinc acetate dihydrate (0.5 g), ethylene glycol (0.15 ml), and 1-propanol (20 ml) were mixed together in a round-bottomed flask with reversed condenser and stirred at room temperature for 15 min. Milky dispersion was obtained, which became clear after addition of triethylamine (0.35 ml). The resulting clear solution was stirred for 30 min at 80°C to obtain ZnO colloidal particles. The resulting ZnO colloidal particles were separated by centrifugation at 6,000 rpm for 30 min, twice washed with pure 1-propanol, and dispersed by ultrasonication in 1-propanol to obtain dispersion of ZnO colloidal particles (10 mg/ml).

Second, the polymer shells of poly(ethylcyanoacrylate) (PECA) were deposited on the ZnO cores. For this purpose, ethanol (10 ml) and ZnO colloidal particles (20 mg) were mixed in a 25-ml beaker at room temperature. The suspension was kept in an ultrasonic bath for 10 min to disperse the ZnO particles. The ECA monomer (10 µl), dissolved in dry acetone (0.5 ml), was dropwise added to the polymerization medium at room temperature upon magnetic stirring (600 rpm). After 3 h of stirring, the resulting suspension was centrifuged for 30 min at 6,000 rpm, and the obtained hybrid ZnO/PECA colloidal particles were resuspended in pure alcohol and centrifuged again. The samples were analyzed by dynamic light scattering (DLS) within 24 h after preparation.

To transfer the ZnO/PECA colloidal particles in water, we utilized the following protocol. Dispersion of ZnO/PECA colloidal particles in alcohol (5 ml) with desired concentration (usually 3 mg/ml) was centrifuged at 6,000 rpm for 30 min. The supernatant was decanted, and the sediment was washed twice with 0.01 M PBS buffer. Then, water solution (5 ml; 1% *w*/*w*) of a nonionic surfactant (Pluronic F68, Triton X100, or Polysorbate 80) was added. The sediment was suspended in the water solution of surfactant by ultrasonication for 10–20 min.

Characterization of colloidal particles The initial ZnO, as well the ZnO/PECA colloidal particles, was imaged by a scanning electron microscope (SEM) JSM-5510 (JEOL), operated at 10 kV of acceleration voltage. Samples for SEM were prepared by evaporation of dilute nanoparticle dispersion on a glass substrate and coating with gold thin film by JFC-1200 fine coater (JEOL).

DLS system Malvern 4700C (Malvern Instruments, UK) was used to measure the nanoparticle size and size distribution in 1-propanol at 25°C.

The X-ray powder diffraction (XRD) spectra were recorded at room temperature on a powder diffractometer (Siemens D500 with CuKα radiation within 2θ range 10–80° and step 0.05° 2θ and counting time 2 s/step).

Fourier transform infrared (FTIR) spectra were taken in the interval 400–4,400 cm^{-1} with Bruker Tensor 27 spectrometer, using KBr tablet technique.

Results and discussion

The preparation and properties of nanostructured ZnO has attracted a great attention, and a variety of methods have been employed to fabricate this material, including sol–gel, precipitation, microemulsion, solvothermal, and hydrothermal methods [30–34]. Zinc oxide colloidal particles can be synthesized by different methods as well. From these methods, the sol–gel synthesis is most popular because of its cheapness, reliability, repeatability, and simplicity. However, a good control of the size and morphology of colloidal particles is required in this process in order to prevent the growth of nuclei. One of the ways to do this is by providing a good adsorption of the surfactant onto the nanoparticle surface. For this purpose, surfactants species, such as monoethanolamine and diethanolamine are applied in the preparation of ZnO colloidal particles [35–37]. In our synthesis of ZnO colloidal particles, we use a combination of triethylamine (TEA) and ethyleneglycol (EG) as additives in order to achieve a good control over the nanoparticle growth. The TEA molecules may play an important role in the present work by serving as surface modifiers while their nitrogen donors bind to the Zn(II) on the surface of ZnO colloidal particles.

As the ligands (TEA molecules) are introduced into the Zn(II)-acetate solution, the Lewis bases of TEA most probably form complexes with Zn(II)-acetate, and the reaction system remains transparent. These complexes can act as a reservoir for Zn(II) by buffering its free concentration below the precipitation limit. The system becomes turbid within few minutes after the temperature is elevated to ~80°C. Raising the temperature promotes the dissociation of the zinc complex and leads to the controlled release of free Zn(II). We suppose that TEA reacts with the zinc(II) acetate to form triethylammonium acetate according to the following chemical equation:

$$\begin{aligned}&Zn(CH_3COO)_2 + H_2O + 2N(C_2H_5)_3\\&\quad\leftrightarrow ZnO + 2CH_3COONH(C_2H_5)_3\end{aligned} \tag{1}$$

As a result, polycrystalline ZnO colloidal particles are produced.

Typical SEM image of the as-prepared ZnO colloidal particles is shown in Fig. 1a. Colloidal particles with average diameter of about 50–70 nm are formed at the early stage of nanoparticle growth (within 10 min). As the reaction time is prolonged (up to 30 min), the particles become spherical-like with a mean diameter of about 230 nm. The histogram of their size distribution obtained by DLS is rather narrow (ca ±15%) (Fig. 2a). The experimental data, measured by DLS, are best fitted with a log-normal size distribution (Fig. 2) of specified average diameter and dispersion. The size-distribution, calculated by the theory of Lifshitz–Slyozov–Wagner (LSW), does not correspond to our experimental results, although it was previously used by other authors to explain the size distribution of ZnO nanoparticles [38].

Figure 3 shows typical XRD pattern of the as-obtained ZnO colloidal particles with the diffraction peaks well indexed to the hexagonal phase of wurtzite. The results indicate that pure phase of ZnO with no characteristic peaks for other crystalline impurities. The peaks prove that the products are crystalline with an average size of the ZnO crystallites of ~6.5 nm (calculated according to the Scherrer formula).

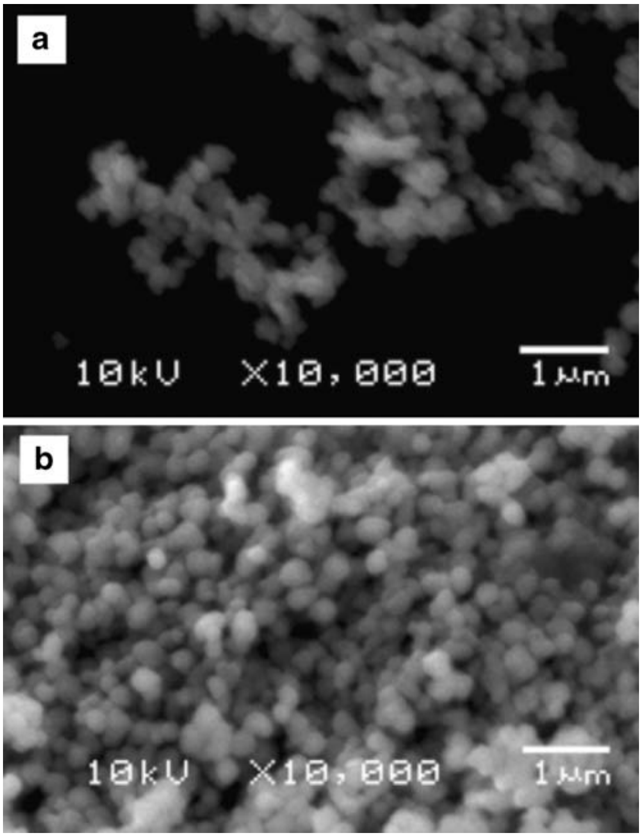

Fig. 1 SEM images of **a** initial ZnO colloidal particles, **b** ZnO/PECA colloidal particles

The FTIR spectrum of ZnO nanoparticle powder is shown in Fig. 4a. The vibration of ZnO particles only locates at about 450–470 cm^{-1}; however, other bands related to organic molecules appear in the spectrum. The FTIR spectrum shows three interesting regions. The first broad absorption band in the range of 3,000–3,750 cm^{-1} with a maximum at 3,474 cm^{-1} is attributed to water of hydration present (OH groups). The second region in the range 1,250–1,750 cm^{-1}, originates from the absorption of COO^{-} groups. The third region with peaks at 1,000–1,200 cm^{-1}, is assigned to the absorption of C–O stretching vibrations (1,021 cm^{-1}). Annealing of the as-prepared ZnO colloidal particles at 500°C for 1 h leads to increasing of the crystallite size to ~18 nm, keeping the same hexagonal crystal structure. Studies by FTIR spectroscopy shows that almost all organics and OH groups are removed after the annealing process, leading to the formation of pure ZnO, which is acompanied by drecrease of the sample weight with ~13 %. However, the non-annealed ZnO particles were utilized for the preparation of core-shell ZnO/poly (ethylcyanoacrylate) colloidal particles because the annealed sample contained some nondispersible aggregates.

The studies by SEM, DLS, and FTIR confirm the successful formation of poly(ethylcyanoacrylate) shell over

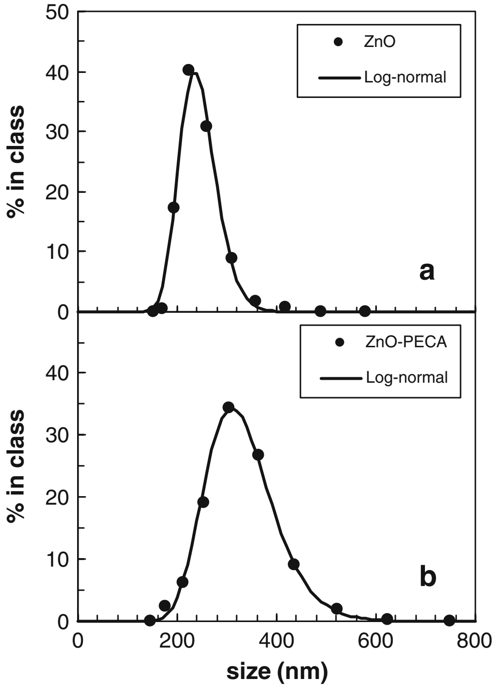

Fig. 2 Size distributions of the colloidal particles depicted in Fig. 1: **a** initial ZnO colloidal particles (average size 235 nm, dispersion 72 nm); **b** ZnO/PECA colloidal particles (average size 310 nm, dispersion 130 nm). The experimental data, measured by DLS (*dots*), are best fitted with a log-normal size distribution (*solid lines*) of specified average diameter and dispersion

the ZnO colloidal particles. A typical SEM image of the obtained core-shell colloidal particles ZnO/PECA is shown in Fig. 1b. Well-defined spheroid particles with a smooth surface and an average size about 320 nm are observed. DLS shows monomodal size distribution and confirms the larger size of ZnO/PECA colloidal particles (Fig. 2b) in comparison with the initial ZnO cores. The FTIR spectrum of pure PECA polymer (Fig. 4b) shows the characteristic stretches for C–H alkyl (2,990, 2,944, 2,909 cm^{-1}), CN (2,251 cm^{-1}), C=O (1,750 cm^{-1}), and C–O ester (1,256 and 1,015 cm^{-1}) bonds. The same stretches are observed also in the FTIR spectrum of the hybrid colloidal particles ZnO/PECA (Fig. 4c), which also confirms the successful formation of a PECA shell. The broad band centered at about 3,480 cm^{-1} in all FTIR spectra is attributed to O–H stretches.

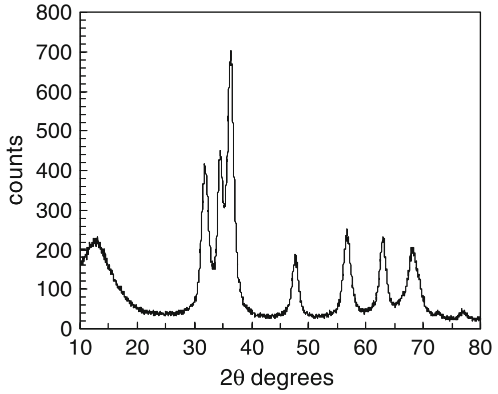

Fig. 3 XRD spectrum of ZnO colloidal particles with mean size of crystallites ~6.5 nm as calculated from the Scherrer formula

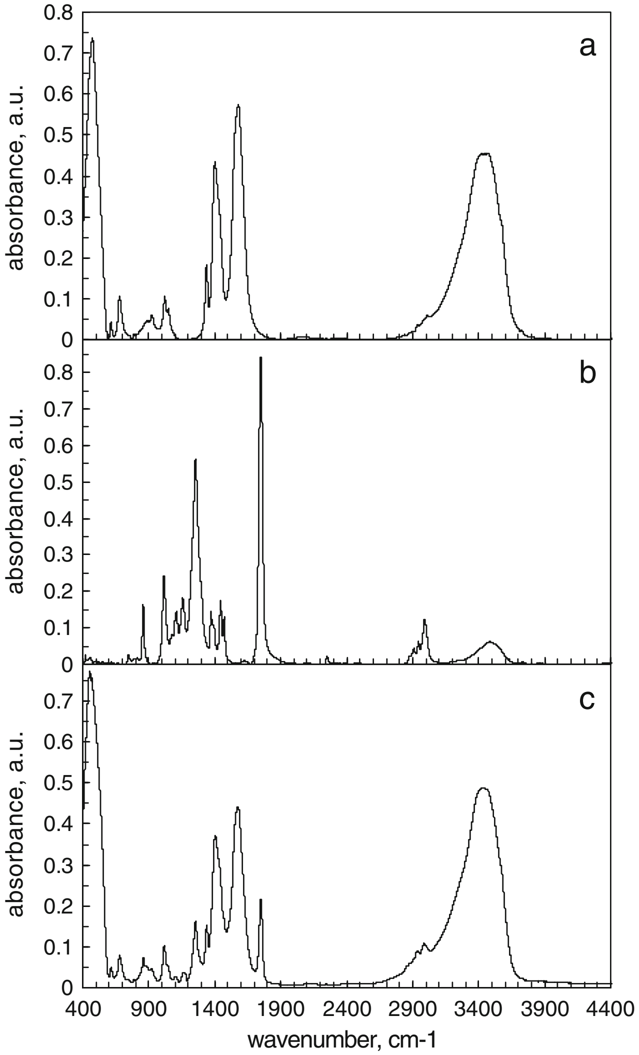

Fig. 4 FTIR spectra of **a** ZnO colloidal particles, **b** PECA, and **c** ZnO/PECA colloidal particles

Let us consider in more detail the possible mechanisms of polymer shell formation. The polymerization of alkylcyanoacrylates can generally occur according to three different routes: radical, anionic, and zwitterionic polymerization [13]. In our study, the polymerization of ECA is carried out in alcohol solution, where the hydroxyl groups (OH) could initiate zwitterionic polymerization at room temperature. The OH groups can also exist on the surface of ZnO colloidal particles. Therefore, there are two possible mechanisms that could explain the deposition of PECA shell over the ZnO colloidal particles. First, the OH groups from the molecules of alcohol could initiate zwitterionic polymerization, and thus, obtained PECA molecules become deposited on the surface of ZnO colloidal particles. Second, the OH groups from the surface of ZnO colloidal particles could also initiate the zwitterionic polymerization of ECA, leading to the formation of PECA polymer chain on the oxide surface (Fig. 5). Which one of these two mechanisms prevails in the PECA shell formation is difficult to be concluded at present.

Below, we give an approximate formula for calculation of the required amount of ECA monomer to form core-shell colloidal particles of desired shell thickness. We make the following assumptions: (1) all the monomer is consumed in the formation of polymer shell, (2) the shell thickness is the same in all colloidal particles, and (3) all the ZnO cores are of the same size. The total volume of ZnO cores (V_C) is given by the following equation:

$$V_C = \frac{m_{ZnO}}{\rho_{ZnO}} = \left(\frac{4\pi}{3} r^3\right) N \qquad (2)$$

Here m_{ZnO} is the mass of the ZnO colloidal particles, ρ_{ZnO} is the density of ZnO (5.606 g/cm^3), r is the average radius of ZnO colloidal particles, and N is the total number of colloidal particles. The total volume of PECA shell material (V_S) can be expressed as the difference between the total volume of core-shell colloidal particles (V_{CS}) and the total volume of cores (V_C) (Eq. 3):

$$V_S = V_{CS} - V_C = \frac{4}{3}\pi\left(R^3 - r^3\right)N \qquad (3)$$

Here R is the radius of core-shell ZnO/PECA colloidal particles. We assume that V_S is approximately equal to the volume of ECA monomer used (V_{ECA}), and by expressing N from Eq. 2 and making the respective substitution in Eq. 3, we obtain the following formula (Eq. 4):

$$V_{ECA} = \frac{m_{ZnO}}{\rho_{ZnO}} \frac{\left(R^3 - r^3\right)}{r^3} \qquad (4)$$

Equation 4 can be used to calculate the amount of ECA monomer required in a synthesis of core-shell ZnO/PACA colloidal particles with desired shell thickness. Our experiments show that Eq. 4 well correlates with the experimental results. For example, by using 20 mg ZnO cores with a size of 200 nm and 10 μl ECA, we obtained core-shell ZnO/PECA colloidal particles with size 310 nm (shell thickness ~55 nm). The respective theoretically calculated amount of ECA is 9.7 μl, which is in a very good agreement with the experiment.

The initial ZnO, as well as the hybrid core-shell ZnO/PECA colloidal particles are found unstable in distilled water and PBS buffer, rapidly flocculating into large aggregates. However, nonionic surfactants (in concentration of 1% *w*/*w*) could act as stabilizers for the colloidal dispersions in water. The ZnO/PECA colloidal particles are transferred in water solutions with the surfactants Triton X100, Pluronic F68 or Polysorbate 80, which are widely utilized in biomedical research. The obtained dispersions sediment slowly at room temperature, but could be redispersed upon ultrasonication.

Conclusions

The preparation and physicochemical characterization of novel core-shell colloidal particles ZnO/poly(ethylcyanoacrylate) is reported. The ZnO colloidal particles are synthesized

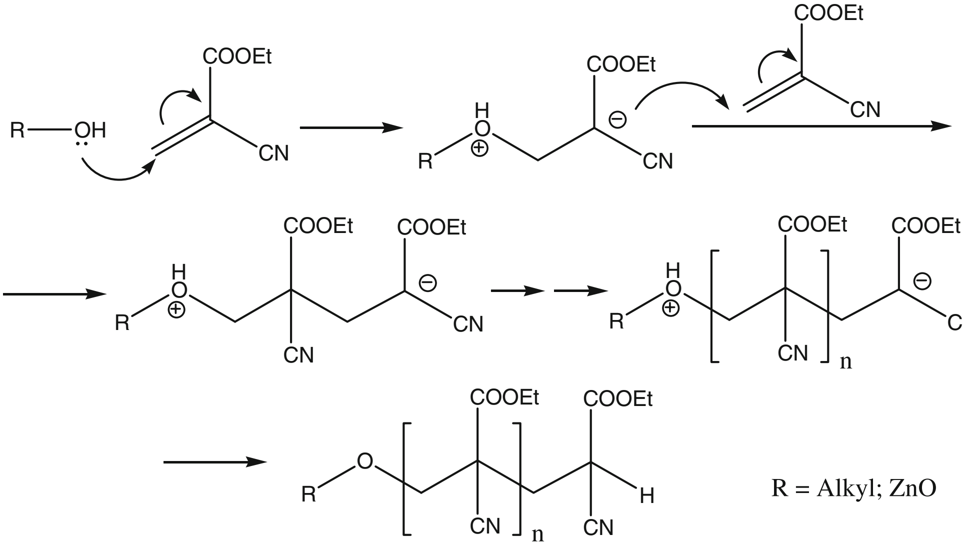

Fig. 5 Hypothetic mechanism of the zwitterionic polymerization of ethylcyanoacrylate. The OH groups, existing on the surface of ZnO, as well as the OH groups of the alcohol solvent may act as initiators of the polymerization reaction

by modified sol–gel method in 1-propanol using zinc(II) acetate as a Zn-precursor, as well as triethylamine and ethyleneglycol as stabilizing additives in order to achieve good control over the colloidal particles size and morphology. The obtained ZnO colloidal particles are polycrystalline (crystallite size of ~6.5 nm) and spherical-like with an average diameter of about 200 nm and a rather narrow size distribution (±15%). The ZnO colloidal particles are coated with polymer shell by polymerization of ethylcyanoacrylate monomer. The obtained core-shell colloidal particles ZnO/poly(ethylcyanoacrylate) could be successfully transferred into water solutions of nonionic surfactants, which is an important prerequisite for further biological tests.

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