Supporting information

Effect of Cationic Brush-Type Copolymers on Colloidal Stability of GdPO₄ Particles with Different Morphology in Biological Aqueous Media

Vaidas Klimkevicius¹,‡, Matas Janulevicius¹,‡, Aleksandra Babiceva¹, Audrius Drabavicius² and Arturas Katelnikovas¹,*

¹ Institute of Chemistry, Vilnius University, Naugarduko 24, LT-03225 Vilnius, Lithuania.
² Centre of Physical Science and Technology, Sauletekis av. 3, LT-10257 Vilnius, Lithuania.

Email: arturas.katelnikovas@chf.vu.lt

Number of pages: 8
Number of figures: 5

Content

S1. Instrumental analysis methods
   S1.1. Size exclusion chromatography (SEC) measurements
   S1.2. Nuclear magnetic resonance (NMR) measurements
   S1.3. X-Ray diffraction (XRD) measurements
   S1.4. Scanning electron microscope (SEM) measurement
   S1.5. TEM measurements

S2. Controlled hydrothermal synthesis of GdPO₄ particles with different morphology
   S2.1. Synthesis procedure
   S2.2. Crystallographic data

S3. Investigation of synthesized cationic brush-type polyelectrolytes
   S3.1. SEC data
   S3.2. NMR data

S4. Stability of GdPO₄ particles in biological aqueous media.
S1. Instrumental analysis methods

S1.1. Size exclusion chromatography (SEC) measurements. The macromolecular parameters of the synthesized p(METAC-stat-PEO₁₉-MEMA) copolymers such as number average and weight average molecular weights (M_n and M_w), and dispersity (D = M_w/M_n) were determined by size exclusion chromatography (SEC). Viscotek TDAmx (Malvern) system equipped with a triple detection array (TDA305) consisting of a differential refractive index detector (RI), light scattering detector (LS) simultaneously measuring the scattered light (laser 3 mW, 670 nm) at two angles – right-angle (90°) and low-angle (7°), and four-capillary bridge viscosity detector (IV) was used. Viscotek (Malvern) columns AGuard (50 × 8.0 mm) and A6000M General Mixed Aq (300 × 8.0 mm) filled with porous polyhydroxymethacrylate, particle size 13 µm, nominal pore size 1.5 × 10⁴ Å, exclusion limit M_w (for Pollulan) < 2·10⁷ g/mol, were employed for the separation of hydrophilic polymeric samples. A 250 mM sodium acetate buffer (pH 4.0) was used as an eluent, flow rate was 0.5 mL/min. The temperature of the column oven and of the detectors was maintained at 30.0 °C. The prepared diluted polymer samples with concentration of 2-4 mg/mL were injected into SEC equipment (constant injection volume 100 µL). SEC measurements of the diluted aliquots were triplicated. SEC data were collected and processed using OmniSEC software (Malvern, v. 5.12).

Normalization of the constant values of the SEC detectors (RI, LS, IV) was performed using PEO standards for triple calibration PolyCAL™ TDS-PEO-N (M_w 24 kDa, Malvern) at a concentration of 2.468 mg/mL.

S1.2. Nuclear magnetic resonance (NMR) measurements. The NMR experiments were conducted at a temperature of 22 °C using a Bruker Ascend™ 400 MHz spectrometer (Bruker). \(^1\)H NMR measurements were performed employing a 90° single-pulse sequence for 128 scans with a 5 s recycle delay. \(^1\)H NMR spectra of p(METAC-stat-PEO₁₉-MEMA) copolymers with three different compositions were recorded in D₂O. The concentration of the samples was 20 mg/mL.

S1.3. X-Ray diffraction (XRD) measurements. Crystalline phase of the particles was identified by performing powder XRD measurements on a Bruker D8 Advance da Vinci design diffractometer working using parallel beam geometry.
S1.4. Scanning electron microscope (SEM) measurement. The morphology and size of sintered GdPO₄ particles was investigated using scanning electron microscopy (SEM). SEM images were taken with high-resolution scanning electron microscope (FE SEM) Hitachi SU-70, with accelerating voltage up to 10 kV. Samples for SEM were prepared by dispersing particles in distilled water and adding 20 μL of aqueous dispersion on a Si plate.

S1.5. TEM measurements. For morphology, size, shape and coating evaluation, FEI Tecnai F20 X-TWIN transmission electron microscope (TEM) was used. Measurements were carried out using 200 kV accelerating voltage, images obtained by using Gatan Orius CCD camera.

S2. Controlled hydrothermal synthesis of GdPO₄ particles with different morphology.

S2.1. Synthesis procedure. Gd(NO₃)₃ (0.5 mL, 0.4 mmol) was dissolved in 20 mL of deionized water and stirred for 15 min. Later, tartaric acid (1.20 g, 8 mmol) dissolved in 20 mL of deionized water was dropwise added to Gd(NO₃)₃ solution and allowed to stir for additional 30 min at room temperature to form Gd-tartaric acid complex. After complex formation the pH value of aqueous mixture was set to 10 by using ammonium hydroxide. Then, required amount of NH₄H₂PO₄ (depending on desired morphology of the particles to be obtained) was dissolved in 20 mL of deionized water and added dropwise into solution of Gd-tartaric acid complex, under vigorous stirring. The volume of reaction mixture was adjusted to 80 mL by adding distilled water. Afterwards, the mixture was poured into Teflon bottle autoclave, sealed and placed into hydrothermal reactor (Berghof) equipped with BTC-3000 Temperature Controller and Data Logger (Berghof) for 12 h at 160 °C temperature. Finally, the reaction product was separated by centrifugation at 7500 rpm (centrifuge model Eppendorf 5804), and washed four times with deionized water, dried to constant weight under reduced pressure at 40 °C.
S2.2. Crystallographic data. After performing X-ray diffraction analysis synthesized GdPO₄ particles were identified to possess rhabdophane crystalline phase. Reference card No. PDF ICDD 00-039-0232.

Figure S1. XRD patterns of synthesized GdPO₄ particles with different morphology: nanorods (b), hexagonal nanoprisms (c), submicrospheres (d); XRD reference pattern of GdPO₄ (a).
S3. Investigation of synthesized cationic brush-type polyelectrolytes

S3.1. SEC data. Molecular weight distribution (MWD) curves of p(METAC-stat-PEO<sub>19</sub>MEMA) samples with different composition is presented in Fig. S2. The unimodal distribution of molecular weight and low dispersity proves a well-controlled character of the RAFT polymerization process.

![Molecular weight distribution curves](image)

**Figure S2.** Molecular weight distribution curves of synthesized p(METAC-stat-PEO<sub>19</sub>MEMA) containing different charge density (different amount of METAC monomeric groups in composition): 65 mol % (high, (a)), 47 mol % (medium) (b)) and 27 mol % (low (c)).

S3.2. NMR data. The structure of synthesized p(METAC-stat-PEO<sub>19</sub>MEMA) copolymers were proved from <sup>1</sup>H NMR spectra (Fig. S3). The exact composition was calculated by comparing integrals of typical peaks of each monomer (see magnified area in Fig.S3) using Eq. S1:
where $F_1$ – the positive charge containing ammonium groups (of METAC monomer) in copolymer composition (mol %), $\int 2.26$ and $\int 3.35$ represent the integrals of chemical shift of $-N(CH_3)_3 (9H)$ groups (in METAC) and $-OCH_3 (3H)$ groups in PEO$_{19}$MEMA, respectively.

**Figure S3.** $^1$H NMR spectra of $p$(METAC-stat-PEO$_{19}$MEMA) with high (a), medium (b), and low (c) charge density.
S4. Stability of GdPO$_4$ particles in biological aqueous media.

Figure S4. Visual evaluation of GdPO$_4$ particles ((nanorods (1 bare, 2 modified), hexagonal nanoprisms (3 bare, 4 modified), submicrospheres (5 bare, 6 modified)) stability in biological aqueous media (consisting 10 vol. % human blood plasma, pH 6.5 (Ref.)) over time: (a) 0 h; (b) 1 h; (c) 2 h; (d) 3 h.
Figure S5. Particle size distribution in aqueous dispersions (pH 6.5) of GdPO₄ particles with different morphologies (nanorods (a), hexagonal nanoprisms (b) and submicrospheres (c)) after incubation in protein-rich aqueous media (consisting 10 vol. % human blood plasma, pH 6.5). 1 – the initial PSD; 2 – bare particles; 3 – modified particles.