



Magnetic silica hybrids modified with guanidine containing co-polymers for drug delivery applications



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ABSTRACT

Guanidine containing co-polymers grafted onto silica nanoparticles to form core-shell structure were prepared by sol-gel method in the presence of γ -Fe₂O₃ nanoparticles. The morphological features for uncoated and coated silica particles have been characterized with scanning electron microscopy. The results show that the polymer coated silicas exhibit spherical morphology with rough polymeric surface covered by γ -Fe₂O₃ nanoparticles. The grafting amount of guanidine containing co-polymers evaluated by thermogravimetric analysis was in the range from 17 to 30%. Then, the drug loading properties and cumulative release of silica hybrids modified with guanidine containing co-polymers were evaluated using molsidomine as a model drug. It was shown that after polymer grafting the loading content of molsidomine could reach up to 3.42 ± 0.21 and 2.34 ± 0.14 mg/g respectively. The maximum drug release of molsidomine is achieved at pH 1.6 (approximately 71–75% release at 37 °C), whereas at pH 7.4 drug release is lower (50.4–59.6% release at 37 °C). These results have an important implication that our magneto-controlled silica hybrids modified with guanidine containing co-polymers are promising as drug carriers with controlled behaviour under influence of magnetic field.

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1. Introduction

Inorganic nanoparticles are frequently engineered with organic components containing different functional groups in order to improve their physicochemical properties [1–3]. The numerous works have been published concerning modification of surface of nanoparticles by functional groups or polymers [4–6]. Such polymer coated nanoparticles have found a great interest in many branches of science and industrial areas as well. By varying the type of polymer the interaction of polymer coated nanoparticles with biological objects or biomolecules can be controlled [7]. Such valuable property has been successfully used for addressing some of the most significant biomolecules such as drugs at diseased tissues and cells.

It is well known that the surfaces of nanosized materials have a high free energy providing a high reactivity. Therefore, the surfaces of nanoparticles will adsorb biomolecules when it comes into contact with biological fluids leading to dysfunction of metabolism of cells [8–10]. Several methods have been suggested for reducing toxicity of nanoparticles. One of this is to manufacture protein corona nanoparticles based on surface modification of nanoparticles by proteins [11]. Although,

such topic is new and relevant, several papers about protein corona have already been published [12,13]. All authors in these papers announced about the problem of protein modification: structure of protein is very sensitive and gentle which may lead to protein denaturation and loss of its biological activity [13]. Another approach is based on polymer coating in order to form «core-shell» structure consisting of inorganic core (gold, silica, titania and zinc oxide etc.) and polymeric shell (Fig. 1a). The aim of polymeric shell is to protect from hazardous and toxic effect of nanoparticle. However, essential requirements have been involved for choosing polymers to modify surface of nanoparticles. They should be biocompatible, non-toxic, have its biological activity and high binding ability [14,15].

Compared with widely reported nanoparticles functionalized with organic compounds or biological species, silica coated with responsive polymers are of special interests due to the diversity, multifunctional capability [16]. Various polymers have been already synthesized and applied for surface modification of silica. The most commonly studies include application of poly(acrylamide) [17], polyethylene glycol [18], polyacrylic acid [19]. However, in an effort to further improve the physicochemical properties of polymer coated silica nanoparticles, new types of polymer hybrids should be considered. According to our recent studies concerning polymer coated silica, it has been suggested to consider guanidine containing polymers as the most promising candidates for surface modification due to their non-toxicity, biological activity and high binding ability [20,21]. It was demonstrated that polymethacryloyl

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guanidine hydrochloride (PMCGH) and polyacrylate guanidine (PAG) are pH-responsive. The drug loading and release behaviour of silica hybrids modified with PMCGH and PAG were carefully investigated by our research group. The obtained results emphasized that PMCGH and PAG are excellent candidates for surface functionalization and formation of pH sensitive nanoparticles which can be further recommended as drug carriers and effective hemoabsorbents for bilirubin removal [21, 22]. Here, we have continued our research focusing on application of new type of guanidine containing co-polymers: methacrylate guanidine with dialdehyde cellulose (MAG + DAC); diallyl dimethyl ammonium chloride with diallyl guanidine acetate (DDAC + DGA, 75:25) (Fig. 1b). Despite the fact that many researches deal with polymer coated silica using pH-responsive polymers, the application of guanidine containing co-polymers such as MAG + DAC and DDAC + DGA has not been reported so far to the best of our knowledge.

In recent years, application of magneto-controlled nanoparticles has become leading area in nanomedicine [23]. The high mobility of magnetic nanoparticles in solution under magnetic field allows using of nanoscale magnetic nanoparticles for highly selective and rapid separation of bacteria or delivery of anticancer drugs in blood plasma using a magnetic microfluidic device [24]. Many scientists have concentrated on synthesis and application of iron oxide nanocrystals in individual form (Fe_3O_4 and $\gamma\text{-Fe}_2\text{O}_3$) [25,26]. However, investigations in the field of multifunctional nanoparticles containing iron oxide nanocrystals as additional component are still relevant. In this contribution, we report sol-gel synthesis of polymer coated silica particles modified with guanidine containing co-polymers and $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles. There are three types of immobilization of $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles (Fig. 1c). Here, $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles were immobilized onto surface of silica particles with core-shell structure. Basically, it was developed new type of polymer coated $\gamma\text{-Fe}_2\text{O}_3$ /silica hybrids that allows the loading and transportation of drugs. To achieve that, the guanidine containing co-polymers were used. According to the works of E. V. Parfenyuk and A.V. Agafonov [27,28] molsidomine was chosen (Fig. 1d) as the most promising

candidate for the study of drug loading and cumulative release. There are only several papers are about molsidomine loading and study of its release. As it was reported in [27] that molsidomine is an active substance of a series of cardiovascular drugs which are widely used clinically. In accordance with work of A.V. Agafonov [27], the isoelectric point of molsidomine equals 4.4. It means the net charge on molsidomine can become positive or negative by changing the value of pH. Therefore, after preparation of our polymer coated silica particles, the drug loading and drug release of molsidomine were carefully investigated. The drug release of molsidomine was performed at pH 1.6 (stomach) and 7.4 (blood) with respect to suggestion to apply a novel form of molsidomine for oral and intravenous administration. The influence of guanidine containing co-polymers on drug loading and its release behaviour is discussed and recommendations concerning application of our prepared materials are suggested.

2. Experimental section

2.1. Chemicals

TEOS ($\text{Si}(\text{OC}_2\text{H}_5)_4$, $M_w = 208.3$ g/mol, 99%) and ammonia solution (4 wt%) were purchased from commercial chemical company "Ecos-1" (Russian Federation). NaOH, FeCl_3 ($M_w = 162.2$ g/mol, 99%) and molsidomine ($\text{C}_9\text{H}_{14}\text{N}_4\text{O}_4$, $M_w = 243.23$ g/mol) were purchased from Sigma-Aldrich (USA). The guanidine containing co-polymers: copolymer of methacrylate guanidine and dialdehyde cellulose (MAG + DAC); copolymer of diallyl dimethyl ammonium chloride with diallyl guanidine acetate (DDAC + DGA, 75:25) were provided by the department of macromolecular compounds of the Kabardino-Balkar State University and by the department of Chemistry of polyelectrolytes and biomedical polymers of A.V. Topchiev Institute of Petrochemical Synthesis, Russian Academy of Science. The full description of these guanidine containing co-polymers can be found in [29,30].

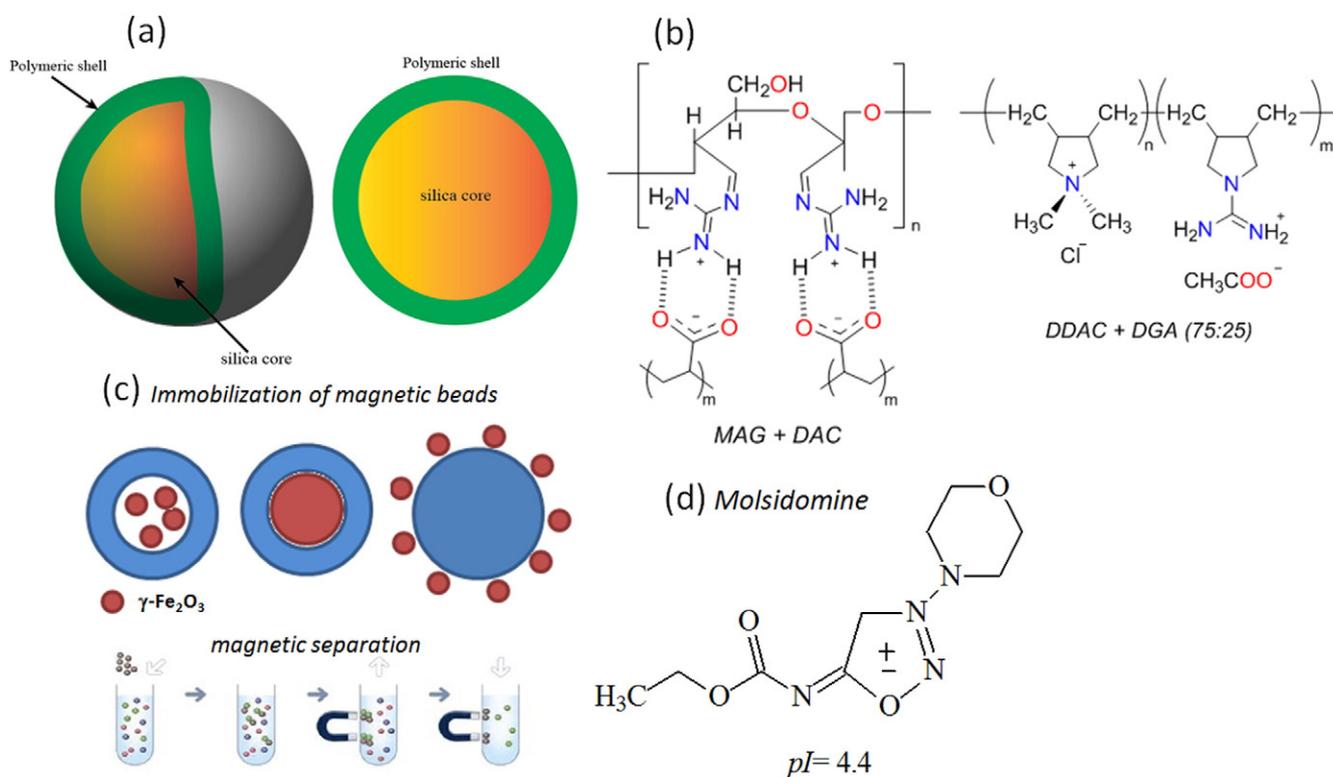


Fig. 1. (a) Schematic illustration of silica particle with «core-shell» structure; (b) structures of MAG + DAC and DDAC + DGA (75:25); (c) immobilization of magnetic beads and its use for magnetic separation; (d) molecular structure of molsidomine.

2.2. Synthesis of MAG + DAC-SNP- γ -Fe₂O₃ and DDAC + DGA-SNP- γ -Fe₂O₃

γ -Fe₂O₃/silica hybrids modified with guanidine containing co-polymers were prepared by sol-gel method. The sol-gel synthesis of γ -Fe₂O₃/silica magnetic nanocomposites was based on previously reported works [31,32,33] and adapted to our synthetic conditions. 0.92 mmol of FeCl₃ was dissolved in 10 mL of pure water (resistivity 18.2 M Ω cm) containing HCl (pH 2.20). Prepared solution stirred for 12 h at 80 °C. Then, the solution (reddish brown sols) was evaporated at 107 °C in open air in order to form solution 1. Ultrasonic treatment (Ultrasonic Cleaner GB-2500B, 42 kHz) was employed in order to improve the dispersibility and protect iron nanoparticles from aggregation. At the same time, 6 g of TEOS was mixed with 4.5 mL of H₂O containing from 0.1 to 0.3 g of co-polymer in order to form solution 2. After that, 4 mL of solution 1 was added to solution 2 and vigorously stirred until homogeneous mixture was being obtained. Then, the solution was stirred for 6 h. 0.0015 mL of aqueous ammonia solution (4 wt%) was added every 30 min as a catalyst. Finally, the obtained product was washed several times and dried under vacuum at 96 °C for 24 h. All synthetic procedures were performed in open air. Thus, the samples with the lowest and highest amount of grafted polymer were prepared, respectively. The same procedure was repeated for non-modified silica (SNP- γ -Fe₂O₃) but without adding of polymers. For polymer coated silica particles we have following abbreviation: MAG + DAC-SNP- γ -Fe₂O₃ and DDAC + DGA-SNP- γ -Fe₂O₃.

2.3. The effect of pH value on molsidomine loading

Typically, 50 mg of solid sample was added to 2 mL of 0.22 mg/mL of molsidomine. The suitable value of pH was adjusted by NaOH or HCl solution. The mixture was shaken during 20 h at room temperature and then centrifuged during 10 min at 132,000 rpm to remove the solid phase. The amount of molsidomine adsorbed was analyzed by measuring of its absorption in supernatant solution at the wavelength of 285 and 485 nm respectively. The molsidomine containing samples were carefully removed from solution and dried. Then they were tested for study of cumulative release.

2.4. Cumulative release of molsidomine

The study of drug release was performed according to our previous published work [22]. Briefly, 30 mg of drug adsorbed sample was dispersed within 10 mL of buffer (pH 1.6 and pH 7.4) under gentle stirring at temperature 37 °C. Regularly, the 1 mL of suspension was tested by UV-visible spectrometer at 285 nm (pH 1.6) and 310 nm (pH 7.4) and the required volume of fresh buffer was added to keep the conditions.

2.5. Characterization

2.5.1. Scanning electron microscopy

The images of uncoated and polymer coated silica particles were obtained using LEO 1550 Scanning Electron Microscope (SEM) which is available in Centre of MicroNano Technology (CMi) of Ecole Polytechnique Fédérale de Lausanne (EPFL). Before examining, all samples were mixed with ethanol to prepare suspension. The drop of the suspension was adhesive onto copper surface and dried. SEM imaging was performed under a working distance between 3 and 4 mm with acceleration voltages of 3–5 kV. The chamber vacuum was 10⁻⁷ mbar. We used SE2 signal (topography visualization) for SEM imaging.

2.5.2. Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectra of the samples were recorded on a Nicolet TM 4700 FTIR spectrometer ("Nicolet", USA) using KBr technique.

2.5.3. Thermal gravimetric (TG) analysis

TG analysis was used to determine the content of grafted polymer into silica core and conducted via STA 449 F3 Jupiter (Netzsch, Germany) in argon atmosphere from room temperature up to 900 °C with a ramp rate of 10 °C/min. An alumina crucible with a cover was used during thermal analysis.

2.5.4. X-ray diffraction (XRD) analysis

XRD patterns were recorded under a Bruker X-ray diffractometer D8 Advance using Cu-K α radiation over the range 10° ≤ 2 θ ≤ 70°.

2.5.5. Vibrating-sample magnetometer

A vibrating-sample magnetometer (VSM) (EG & G Princeton Applied Research vibrating sample magnetometer, model 155) was used at 300 K to measure the magnetic moment.

2.5.6. Zeta potential analyzer and dynamic light scattering (DLS) characterization

The zeta-potential of prepared samples was determined using Brookhaven ZetaPlus zeta potential analyzer. The samples were diluted with 10 mM NaCl solution in order to maintain a constant ionic strength. Electrophoretic mobility was measured and zeta potential (ζ) was calculated by the Smoluchowski equation: $\zeta = 4\pi \frac{\mu}{D}$, where n is the viscosity of the suspension at 25 °C, D is the dielectric constant of the solution at 25 °C and μ is the electrophoretic mobility of the particles ($\mu\text{m s}^{-1} \text{V}^{-1} \text{cm}^{-1}$). Measurements were performed at 0.4 g/L concentration of the suspension. All measurements were done in at least triplicate. The solution pH was adjusted using either dilute NaOH or HCl as required.

In this study, the hydrodynamic diameter and particle size distribution of the prepared materials were determined through dynamic light scattering (Brookhaven ZetaPlus particle size analyzer) with He-Ne laser ($\lambda = 663 \text{ nm}$) and scattering angle of 90° at 25 °C. All DLS measurements were carried out at 0.4 g/L concentration.

3. Results and discussion

3.1. Preparation of MAG + DAC-SNP- γ -Fe₂O₃ and DDAC + DGA-SNP- γ -Fe₂O₃

Polymer coated samples with core-shell structure and γ -Fe₂O₃ were synthesized by a modification of well-known Stöber method. The external surface of silica was coated with guanidine containing co-polymers which are able of pH-responsive properties. Then in the same time sol-gel modification was performed in aqueous solution in the presence of γ -Fe₂O₃ nanoparticles. Samples with three different contents of co-polymers were prepared. Thus, preparation of polymer coated silica particles with magnetic beads was the first task to be completed.

In order to remove weak unbonded polymers, the washing procedure was performed several times. Then we perform thermal gravimetric analysis to investigate the maximum available content of co-polymers onto silica matrix and their thermal stability (Fig. 2a). The TG curves of all samples with different amount of polymers show the mass loss of the organic fraction as it decomposes upon heating. The weight loss below 125 °C is assigned to adsorbed water and hydroxyl groups [34]. In case of polymer coated silicas, the TG curves show distinguishable few steps of mass loss associating with structure of guanidine containing copolymers. Around 200 °C the inclination was slightly changed that assume another process. In case of DDAC + DGA-SNP- γ -Fe₂O₃ we can observe a sharp weight loss from 200 °C to 400 °C associating with thermal decomposition of structure of copolymer. As for MAG + DAC-SNP- γ -Fe₂O₃ the process of thermal decomposition is longer (from 200 °C to 465 °C). We suppose that the process of thermal decomposition of MAG + DAC occurs at higher temperature compared to DDAC + DGA due to gain of effect of ionic bonds in stabilization of

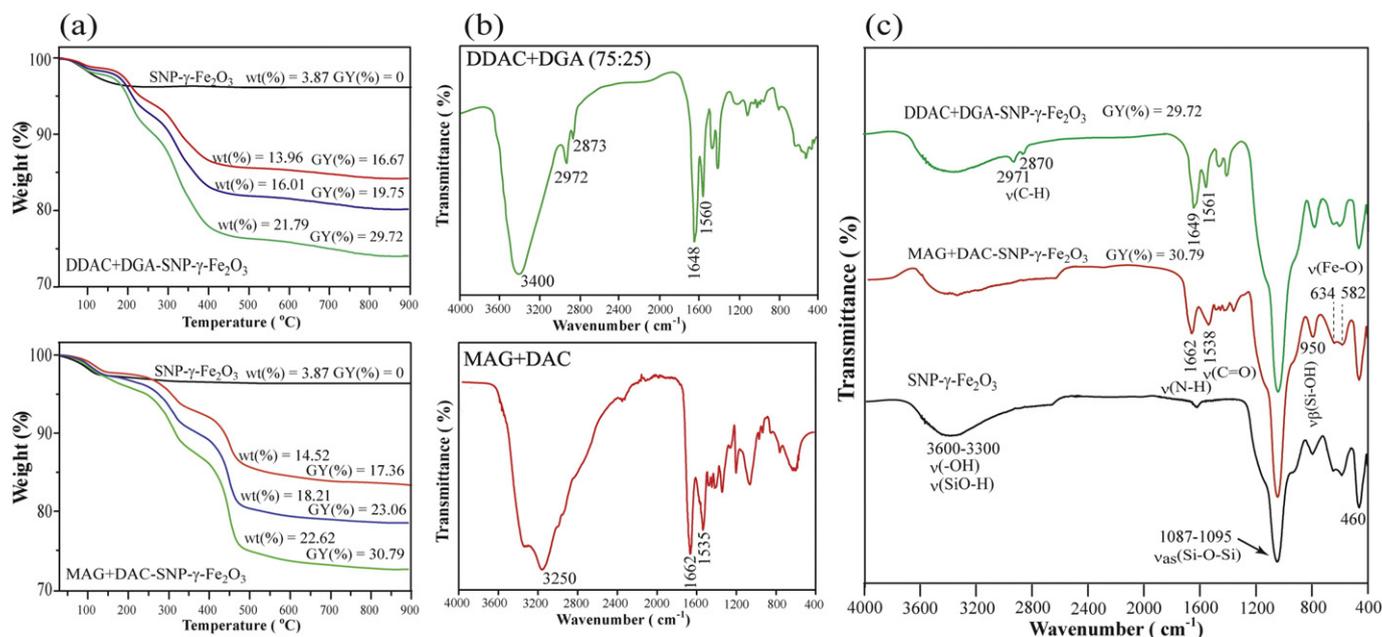


Fig. 2. (a) TG curves for uncoated and polymer coated silica particles; (b) FT-IR spectra of DDAC + DGA and MAG + DAC; (c) FT-IR spectra of uncoated and polymer coated silica particles.

MAG + DAC. Considering the structure of DDAC + DGA, it is clearly seen that the positive charge of this polymer is stabilized by anions (Cl^- and CH_3COO^-) whereas the positive charge of MAG + DAC is stabilized by less volatile anions (polyacrylic anions). Therefore, polyacrylic anions in MAG + DAC have impact on additional thermal stability of this polymer. The long tail after 465 °C was due to the carbonization of the decomposed products to ash.

The amount of grafted polymers has been calculated using following equation:

$$\text{Grafting yield (GY, \%)} = \frac{W_1 - W_0}{W_0} \times 100$$

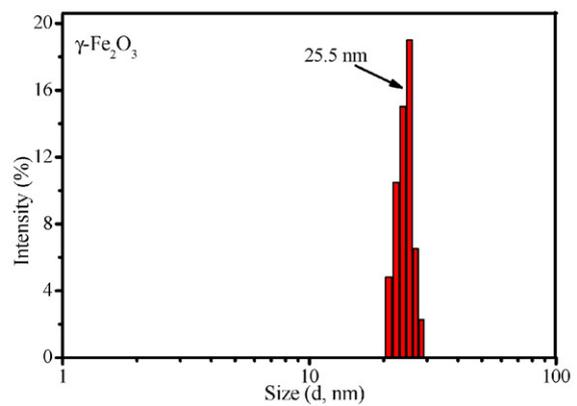
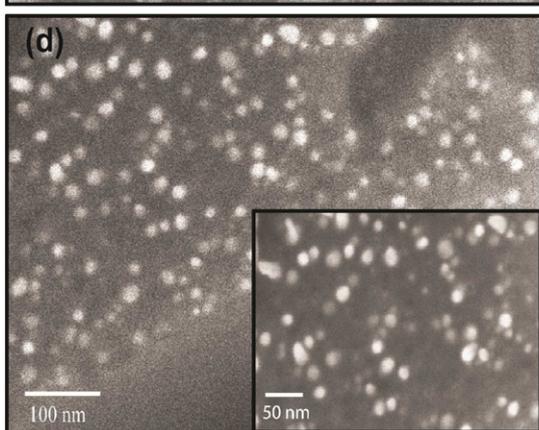
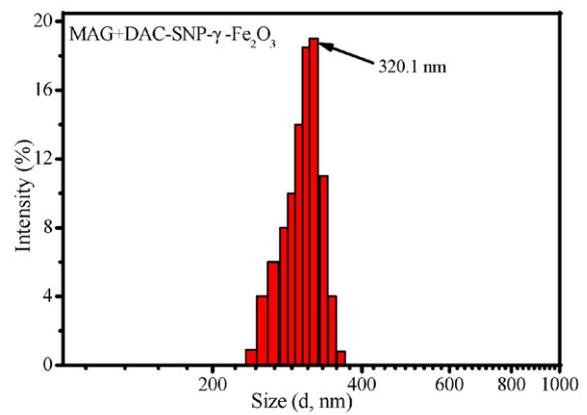
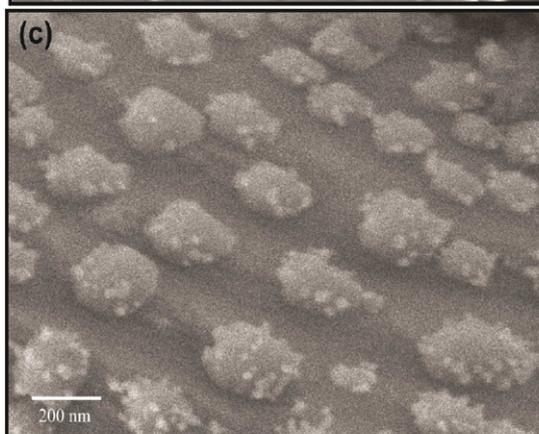
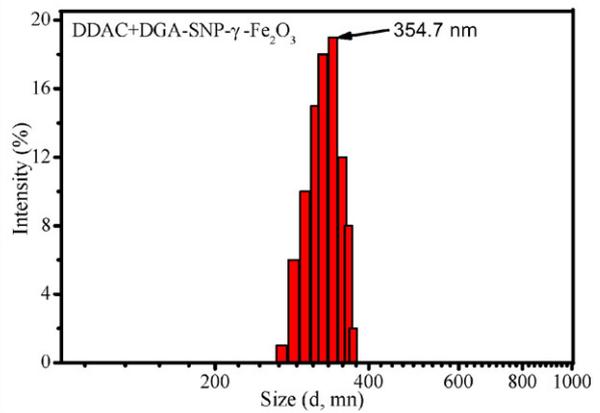
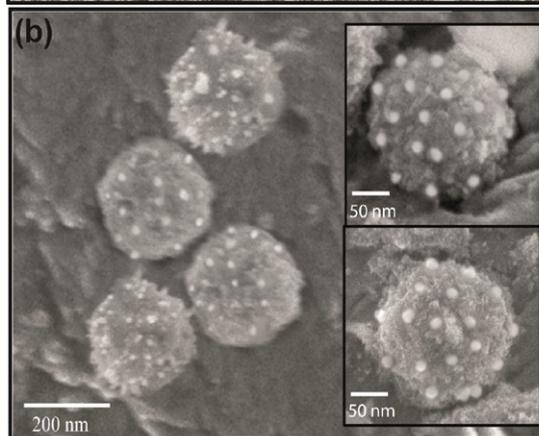
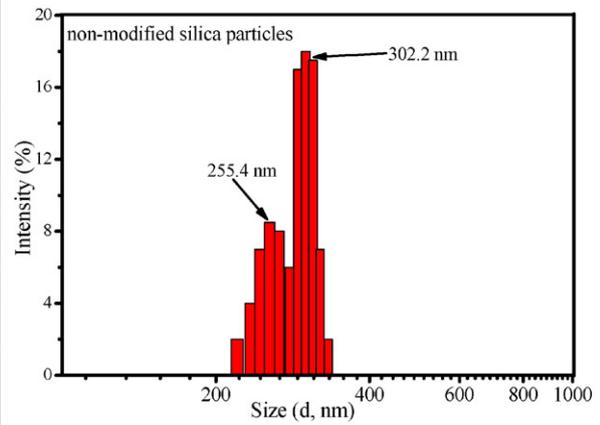
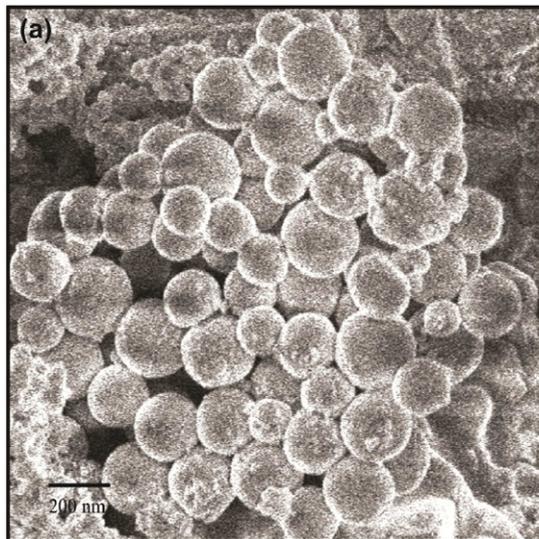
where W_1 and W_0 represent the weight of initial and grafted substrate, respectively.

In accordance with this equation, the grafting yield for MAG + DAC-SNP- γ -Fe₂O₃ and DDAC + DGA-SNP- γ -Fe₂O₃ ranges from ~17.36 (16.67)–30.79 (29.72) % (Fig. 2a). These experiments have shown the maximum amount of co-polymers which is managed to graft onto silica matrix. It should be noted that our obtained results concerning grafting capacity of guanidine containing co-polymers are in agreement with our previously published report where it has been shown that maximum grafting yield does not exceed 33–35% [22]. In addition, the quantitative data of elemental and thermo gravimetric analysis from this paper can be found in (Supporting information, Table 1S). Then, we focused only on investigation of polymer coated silicas with maximum content of guanidine containing co-polymers.

Next step was to conduct FT-IR spectroscopy in order to reveal the presence of functional groups of grafted co-polymers in our tested samples. FT-IR spectra of pure guanidine containing copolymers (MAG + DAC, DDAC + DGA) are shown in Fig. 2b in order to compare them with FT-IR spectra of polymer coated samples after sol-gel modification (Fig. 2c). According to analysis of FT-IR spectra of non-modified silica (SNP- γ -Fe₂O₃) and polymer coated silicas (MAG + DAC-SNP- γ -Fe₂O₃ and DDAC + DGA-SNP- γ -Fe₂O₃) we can observe a broad band in the range 3600–3300 cm^{-1} corresponding to the O–H stretching bands of hydrogen-bonded water molecules and SiO–H stretching vibrations [35]. The corresponding Si–OH bending mode is found around 950 cm^{-1} . The “bulk” vibrational modes corresponding to SiO₄ groups are observed at 1087–1095 cm^{-1} and

800 cm^{-1} (antisymmetric and symmetric Si–O–Si vibrations, respectively), with the bending vibrations near to 460 cm^{-1} [36]. It is clearly seen that after sol-gel modification the FT-IR spectra polymer coated silicas have shown new frequencies corresponding to the functional groups in guanidine containing copolymers. In case of DDAC + DGA-SNP- γ -Fe₂O₃ the band around 1649 cm^{-1} can be assigned to the combination of N=C, NH₂ and H–O vibrations. The intensive peak at 1561 cm^{-1} corresponds to the CH₃COO[−] vibrations in DDAC + DGA. The bands between 3000 and 2800 cm^{-1} correspond to intensive asymmetric and symmetric C–H stretching vibrations of methylene and methane groups in DDAC + DGA. As for MAG + DAC-SNP- γ -Fe₂O₃ it can be observed two most intensive peaks at 1538 and 1662 cm^{-1} respectively. The first one can be assigned to C=O groups in MAG + DAC and second one is associated with N–H vibrations. Perceptible differences were observed in 1500–1350 cm^{-1} regions which are responsible for carboxylic and amine bonds in guanidine containing copolymers. FT-IR spectra also give us the information about the presence of γ -Fe₂O₃ in our samples. From the spectra it can be seen that two broad peaks at 582 and 634 cm^{-1} were identified. These peaks correspond to Fe–O stretching and bending vibration mode of γ -Fe₂O₃ [37]. Thus, FT-IR spectroscopy has also shown the presence of guanidine containing copolymers and γ -Fe₂O₃ in our samples after sol-gel synthesis and consequently confirm silica modification by these chemical components.

Scanning electron microscopy was used in order to reveal any morphological changes of our samples after polymer coating and immobilization of γ -Fe₂O₃ (Fig. 3a–c). The SEM images displayed in Fig. 3a and Fig. 1S (Supporting information) showed that non-modified silica particles had the spherical shape with two nominal mean diameters of 210 and 150 nm respectively. Fig. 3b demonstrated that DDAC + DGA-SNP- γ -Fe₂O₃ preserved spherical morphological structure after modification. Although DDAC + DGA-SNP- γ -Fe₂O₃ shows a spherical morphology as individual silica particles but analysis of surface revealed some changes in surface structure. According to SEM analysis we can observe a rough surface which can be associated with formation of polymeric shell covering silica core. In other word, polymer coated silica particles with «core-shell» structure were obtained. As it can be seen from Fig. 3c, MAG + DAC-SNP- γ -Fe₂O₃ has slightly distorted spherical shape but the structure and morphology remain the same as it was mentioned above. A closer look at the fine structure of polymer coated silica particles exhibited the multiple white spots, i.e. γ -Fe₂O₃,



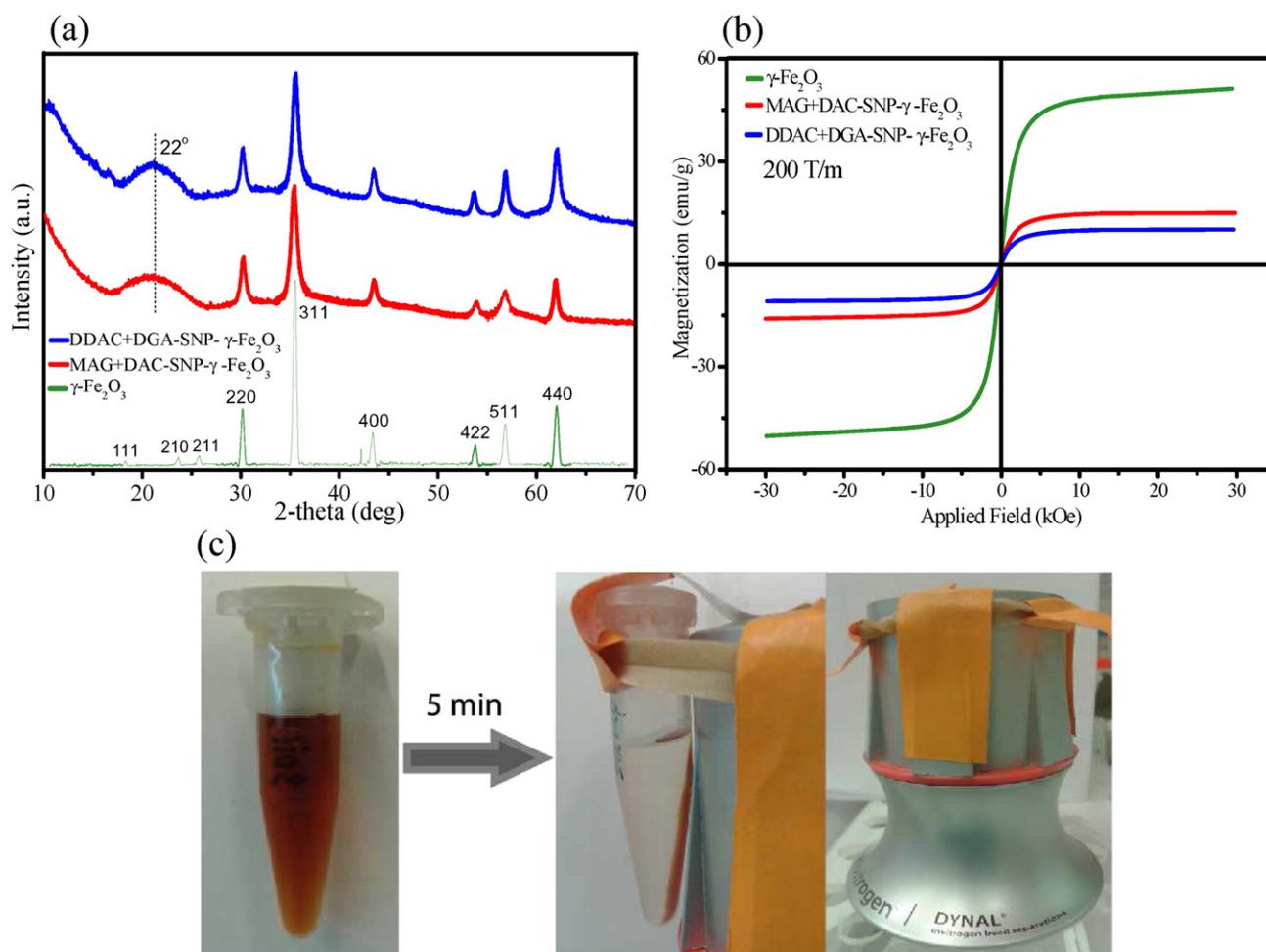


Fig. 4. (a) XRD pattern of synthesized materials; (b) Magnetization curves of prepared materials at room temperature; (c) photograph of the separation process of MAG + DAC-SNP- γ - Fe_2O_3 by a magnet.

were embedded around polymeric shell. According to SEM analysis (Fig. 3d), an average diameter of γ - Fe_2O_3 nanoparticles is around 17 nm.

In addition, the hydrodynamic diameter and size distribution were measured with DLS (Fig. 3). DLS histogram of non-modified silica particles showed bimodal particle size distribution with average hydrodynamic diameters of 255.4 and 302.2 nm. These results are in good agreement with SEM analysis but the diameter demonstrated in SEM is smaller than that detected using DLS method. This is because the diameter of particles obtained by dynamic light scattering reflected the hydrodynamic diameter of particles swelled in aqueous solution, while those observed by SEM were the diameters of dried particles [38]. DDAC + DGA-SNP- γ - Fe_2O_3 is characterized by narrow size distribution with average hydrodynamic diameter of 354.7 nm whereas MAG + DAC-SNP- γ - Fe_2O_3 shows wider size distribution with average hydrodynamic diameter of 320.1 nm. The average hydrodynamic diameter of γ - Fe_2O_3 was found to be 25.5 nm.

Then, XRD analysis was used for identification crystalline phase of iron nanoparticles in their individual form as well as in silica matrix of our prepared materials. Fig. 4a shows XRD patterns of pure γ - Fe_2O_3 and silica magnetic hybrids modified with guanidine containing copolymers (DDAC + DGA-SNP- γ - Fe_2O_3 and MAG + DAC-SNP- γ - Fe_2O_3). It is clearly seen that all diffraction peaks are in conformity

with standard XRD pattern γ - Fe_2O_3 (maghemite, JCPDS: 39-1346) [39]. For DDAC + DGA-SNP- γ - Fe_2O_3 and MAG + DAC-SNP- γ - Fe_2O_3 , the broad band centered at $2\theta = 22^\circ$ can be assigned to the amorphous silica (JCPDS: 29-0085). The other diffraction peaks correspond to γ - Fe_2O_3 (maghemite, JCPDS: 39-1346), which indicates that the material is composed of amorphous silica and crystalline magnetite. Since it is considered that our polymer coated silica particles should have magnetic properties, magnetic measurements on synthesized materials were performed. The magnetization gives the field-dependent magnetic curves (Fig. 4b). In case of SNP- γ - Fe_2O_3 the regime of saturation starts at about 2.5 kOe (0.25 T). Due to the magnetic field gradient value used in this study (200 T/m), the absolute value of the field within the experimental systems varies from 0 to 3 T. According to Fig. 4b we can clearly see that polymer coated silicas exhibit magnetic properties at room temperature with a saturation magnetization of 10.5 emu/g for DDAC + DGA-SNP- γ - Fe_2O_3 and 15.1 emu/g for MAG + DAC-SNP- γ - Fe_2O_3 . The decreased magnetization compared with free γ - Fe_2O_3 (51.3 emu/g) is due to non-magnetic components in each polymer coated sample [40]. MAG + DAC-SNP- γ - Fe_2O_3 can be separated from their aqueous dispersion in 5 min by simply using a hand-held magnet (Fig. 4d). Thus, such good magnetic response of polymer coated magnetic silica particles reveals the potential application for targeting and separation.

Fig. 3. (a) SEM images and dynamic light scattering (DLS) characterization of synthesized materials: (a) non-modified silica; (b) DDAC + DGA-SNP- γ - Fe_2O_3 ; (c) MAG + DAC-SNP- γ - Fe_2O_3 ; (d) γ - Fe_2O_3 nanoparticles.

3.2. pH-responsive effect of guanidine containing co-polymers on drug loading and drug release

As it is well known that ζ -potential is directly related to the net charges of the macromolecules or particles [41,42]. Therefore, we should characterize the surface charges of the samples using ζ -potential measurement in order to reveal electrostatic behaviour of surface charges of tested samples by varying pH value. Moreover, parallel with ζ -potential measurements it is necessary to detect the hydrodynamic diameter of synthesized materials at the same pH value. Fig. 5a exhibited the alteration tendency of ζ -potentials and hydrodynamic size of prepared materials as a function of pH values. In case of SNP- γ -Fe₂O₃, the ζ -potentials decreased from 7.1 mV to -41.4 mV when the pH values increased from 3.2 to 9.6. According to Fig. 4a, the *pI* of SNP- γ -Fe₂O₃ equals 3.4. Considering MAG + DAC-SNP- γ -Fe₂O₃ we can say that MAG + DAC-SNP- γ -Fe₂O₃ carries positive charges when the pH of solution is not higher than 6.7. It also shows zero ζ -potential at pH 6.7. The ζ -potential values of DDAC + DGA-SNP- γ -Fe₂O₃ are larger than the values of MAG + DAC-SNP- γ -Fe₂O₃ and zero ζ -potential was shifted (*pI* = 8.2). This result indicates that silica particles carry positive charges after co-polymer grafting at pH values up to 6.7 and 8.2 respectively. As it is clearly seen that the hydrodynamic diameter of synthesized materials dramatically increases close to the isoelectric point, which is in agreement with colloid science in general: ζ -

potential near zero leads to weak repulsion among particles, therefore, the aggregation of the particles is observed [43]. We should highlight that the functional groups of guanidine containing co-polymers play a key role to vary the charge densities and aggregation profile of nanoparticles. Thus, we believe that application of guanidine containing co-polymers would bring the superior drug loading and unique release behaviour. Therefore, the next step of our work is to study the loading of molsidomine. The drug loading capacity of non-modified and polymer coated silica particles at different pH values are presented in Fig. 5b. In all cases, we observe the gradual growth in drug loading capacity with further falling of its loading capacity when pH value is increased. As it was mentioned above, *pI* of molsidomine equals 4.4. At pH above 4.4, negatively charged molsidomine will bind with positively charged DDAC + DGA and MAG + DAC to form complex by the electrostatic interaction. However, in case of SNP- γ -Fe₂O₃, we also can observe some drug loading at pH around 4.2 even though the drug loading capacity of SNP- γ -Fe₂O₃ is very low (-0.31 ± 0.04 mg/g). The net charge of molsidomine is slightly positive at pH 4.2. Therefore, weak electrostatic forces between molsidomine and negatively charged surface of silica, non-specific van der Waals binding and hydrogen bonding can be involved in the process of drug loading. While we examine polymer coated silica particles, the increase in drug loading capacity is clearly seen. The molsidomine becomes negative charged when pH is higher 4.4 and there is a tendency in increase of drug loading capacity. However,

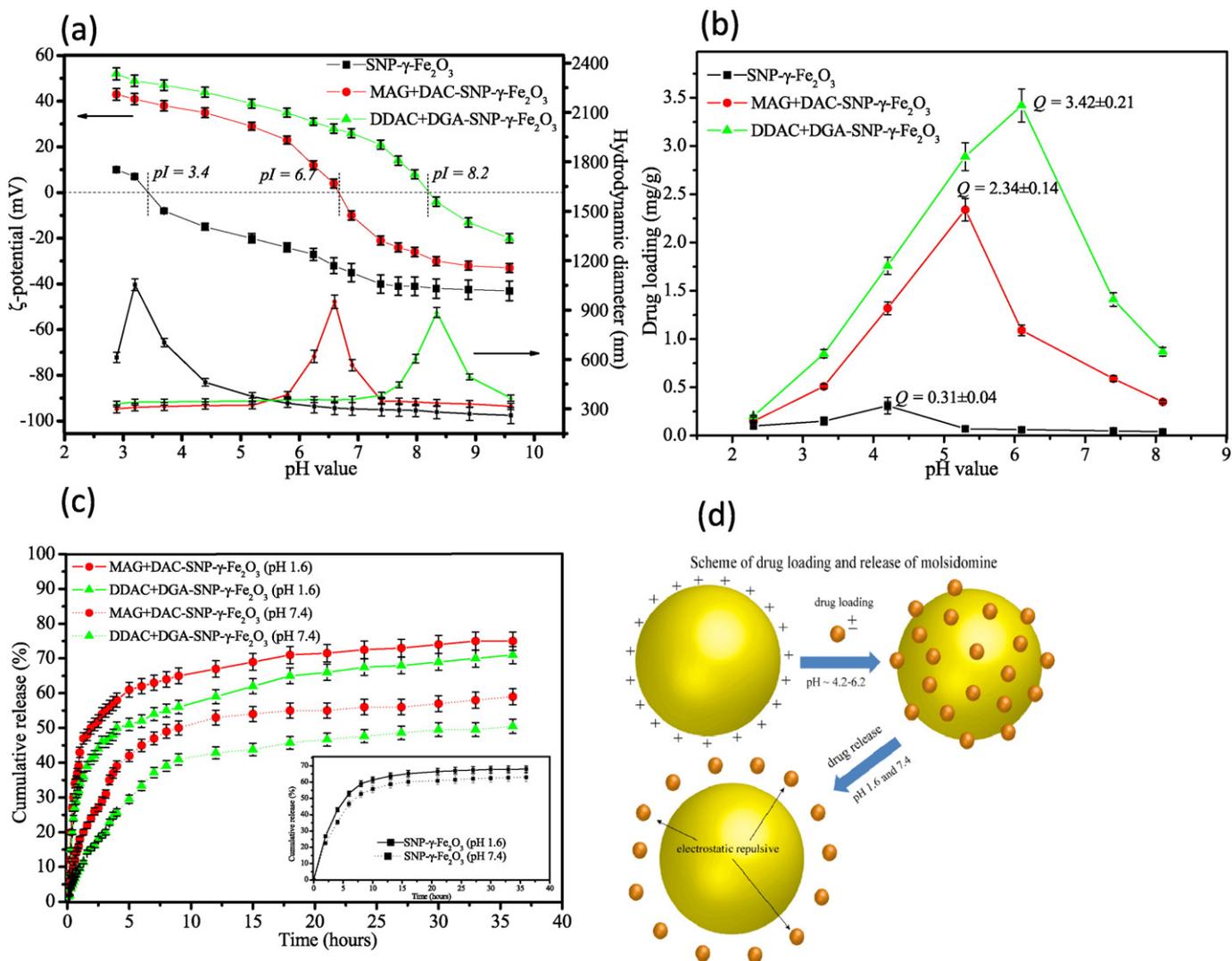


Fig. 5. (a) The zeta potential and hydrodynamic diameter vs. pH curves obtained for aqueous dispersion of our tested samples; (b) the effect of pH on drug loading of molsidomine; (c) the cumulative release of molsidomine at pH 1.6 and 7.4 from the surface of our tested materials; (d) the scheme of drug loading and release of molsidomine.

the drug loading capacity of polymer coated silicas is limited by electrostatic properties of used guanidine containing co-polymers. As for MAG + DAC-SNP- γ -Fe₂O₃ the maximum loading capacity equals 2.34 ± 0.14 mg/g at pH 5.3. Then, it decreased with increase in pH value which can be associated with growth of extent of deprotonated hydroxyl groups and functional groups of MAG + DAC onto silica surface. As a result, it might lead to electrostatic repulsion between silica surface and molsidomine. Only non-specific van der Waals binding and hydrogen bonding can influence on drug loading capacity. The maximum loading capacity of DDAC + DGA-SNP- γ -Fe₂O₃ was observed at pH 6.1 and equals 3.42 ± 0.21 mg/g. The shift in drug loading capacity of DDAC + DGA-SNP- γ -Fe₂O₃ can be due to the fact that DDAC + DGA remains positively charged up to pH 8.1 and can easily interact with negatively charged molsidomine. However, the rapid falling in drug loading capacity is observed after pH 6.1 due to deprotonation of hydroxyl groups of silica (—OH) and functional groups of DDAC + DGA. Comparing our results with other available materials, we can say that our polymer coated silicas are quite promising. For instance, in a recent report [28], E.V. Parfenyuk designed phenyl-modified composite materials based on silica and showed that maximum drug loading for molsidomine was 2.87 mg/g which is lower in comparison with our obtained results.

We further study cumulative release of molsidomine at pH 1.6 and 7.4 to evaluate the efficiency of our drug carriers for oral and intravenous administration. The cumulative release profiles of molsidomine were shown in Fig. 5c. It could be seen from Fig. 4c that cumulative release of molsidomine reached up to 75.1% at pH 1.6 in case of MAG + DAC-SNP- γ -Fe₂O₃. This is because of the fact that molecules molsidomine were protonized at pH 1.6, which would lead to electrostatic repulsion between molsidomine and positively charged surface of MAG + DAC-SNP- γ -Fe₂O₃. However, low solubility of molsidomine and hydrogen bonding have impact on deceleration of drug release. The cumulative release of molsidomine at pH 7.4 could reach only 59.6% when we study drug release in the presence of MAG + DAC-SNP- γ -Fe₂O₃. The existence of anionic form of molsidomine at pH 7.4 leads to the dissociation of electrostatic interactions between molsidomine and negatively charged of MAG + DAC-SNP- γ -Fe₂O₃. The similar situation can be observed in case of DDAC + DGA-SNP- γ -Fe₂O₃. However, compared to MAG + DAC-SNP- γ -Fe₂O₃ and SNP- γ -Fe₂O₃, the cumulative release of molsidomine from DDAC + DGA-SNP- γ -Fe₂O₃ is lower and equals 71.4% at pH 1.6 and 50.4% at pH 7.4 respectively. The poor solubility of molsidomine restricts the higher release amount of molsidomine from DDAC + DGA-SNP- γ -Fe₂O₃ containing 3.42 mg/g of molsidomine. The simple illustration of molsidomine loading and its release is presented in Fig. 5d.

To further investigate the effect of molsidomine loading on size distribution of polymer coated silica hybrids in aqueous solution, the hydrodynamic diameter of the particles were measured before and after adsorption with the molsidomine. The hydrodynamic diameter of the silica particles did not change significantly following after adsorption of molsidomine indicating that the drug does not extend beyond the Stern layer of the polymer coated silica hybrids (Supporting information, Fig. 2S).

Thus, we can conclude that our polymer coated silicas with magnetic properties are perspective as drug carriers of molsidomine with further release by changing pH values. In near future we are planning to test our materials for toxicity with LNCap and HeLa cells.

4. Conclusions

In this work, we have successfully designed a new magnetic polymer coated silica nanoparticles modified with guanidine containing co-polymers as effective carriers of molsidomine. γ -Fe₂O₃ nanoparticles were used in sol-gel process to functionalize the surface of polymer coated silica particles. The results reveal that novel materials exhibit core-shell structure with magnetic features. The combination of magnetic properties of polymer coated silica nanoparticles with pH

responsive co-polymers provides an efficient strategy for preparation of multifunctional materials for further development of effective drug carriers. Polymer coated silicas exhibited a high loading capacity, due to the strong electrostatic interactions of molsidomine and guanidine containing co-polymers. The drug loading and release behaviour were found to be dependent on the type of used guanidine containing co-polymer. Moreover, due to high binding ability of guanidine containing co-polymer we suggest to consider these co-polymers for modification of other inorganic materials such as TiO₂, Al₂O₃, carbon, Au etc.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.msec.2016.03.057>.

References

- [1] W.G. Kreyling, et al., In vivo integrity of polymer-coated gold nanoparticles, *Nat. Nanotechnol.* 10 (2015) 619–623.
- [2] G.P. Rivera, et al., The challenge to relate the physicochemical properties of colloidal nanoparticles to their cytotoxicity, *Acc. Chem. Res.* 46 (2013) 743–749.
- [3] H.W. Chen, P. Zou, J. Connarn, H. Pahalok, D.X. Sun, Intracellular dissociation of a polymer coating from nanoparticles, *Nano Res.* 5 (2012) 815–825.
- [4] K. Kobayashi, et al., Surface engineering of nanoparticles for therapeutic applications, *Polym. J.* 46 (2014) 460–468.
- [5] M.X. Xie, et al., Monofunctional polymer nanoparticles prepared through intramolecularly cross-linking the polymer chains sparsely grafted on the surface of sacrificial silica spheres, *Chem. Commun.* 51 (2015) 1842–1845.
- [6] Hsiang-Yu Chang, et al., Analyses of functional polymer-modified nanoparticles for protein sensing by surface-assisted laser desorption/ionization mass spectrometry coupled with HgTe nanomaterials, *Colloids Surf. B: Biointerfaces* 130 (2015) 157–163.
- [7] G. Marcelo, J.M.G. Martinho, J.P.S. Farinha, Polymer-coated nanoparticles by adsorption of hydrophobically modified poly(*N,N*-dimethylacrylamide), *J. Phys. Chem. B* 117 (2013) 3416–3427.
- [8] T. Cedervall, et al., Understanding the nanoparticle–protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles, *Proc. Natl. Acad. Sci.* 104 (2007) 2050–2055.
- [9] A.E. Nel, et al., Understanding biophysicochemical interactions at the nano–bio interface, *Nat. Mater.* 8 (2009) 543–557.
- [10] I. Lynch, K.A. Dawson, Protein–nanoparticle interactions, *Nano Today* 3 (2008) 40–47.
- [11] S. Ritz, et al., Protein corona of nanoparticles: distinct proteins regulate the cellular uptake, *Biomacromolecules* 16 (2015) 1311–1321.
- [12] Th. Winuprasith, et al., Alterations in nanoparticle protein corona by biological surfactants: impact of bile salts on β -lactoglobulin-coated gold nanoparticles, *J. Colloid Interface Sci.* 426 (2014) 333–340.
- [13] Sha Wan, et al., The «sweet» side of the protein corona: effects of glycosylation on nanoparticle–cell interactions, *ACS Nano* 9 (2015) 2157–2166.
- [14] S. Lukasiewicz, K. Szczepanowicz, Biocompatible polymeric nanoparticles as promising candidates for drug delivery, *Langmuir* 31 (2015) 6415–6425.
- [15] J. Sun, et al., Triboelectric nanogenerator based on biocompatible polymer materials, *J. Phys. Chem. C* 119 (2015) 9061–9068.
- [16] Y. Zhang, C.Y. Ang, Polymer-coated hollow mesoporous silica nanoparticles for triple-responsive drug delivery, *ACS Appl. Mater. Interfaces* 19 (2015) 18179–18187.
- [17] S. Baljit, G.S. Chauhan, S. Kumar, N. Chauhan, Synthesis, characterization and swelling responses of pH sensitive psyllium and polyacrylamide based hydrogels for the use in drug delivery, *Carbohydr. Polym.* 67 (2007) 190–200.
- [18] K. Knop, R. Hoogenboom, D. Fischer, U.S. Schubert, Poly(ethylene glycol) in drug delivery: pros and cons as well as potential alternatives, *Angew. Chem. Int. Ed.* 49 (2010) 6288–6308.
- [19] C. Müller, K. Leithner, S. Hauptstein, F. Hintzen, W. Salvenmoser, A. Bernkop-Schnürch, Preparation and characterization of mucus-penetrating papain/poly(acrylic acid) nanoparticles for oral drug delivery applications, *J. Nanoparticle Res.* 15 (2013) 1353.
- [20] A.S. Timin, A.V. Solomonov, E.V. Rummyantsev, Polyacrylate guanidine and polymethacrylate guanidine as novel cationic polymers for effective bilirubin binding, *J. Polym. Res.* 21 (2014) 400–409.

- [21] A.S. Timin, S.Yu. Khashirova, A. Zhansitov, E.V. Rumyantsev, Synthesis and application of silica hybrids grafted with new guanidine-containing polymers as highly effective adsorbents for bilirubin removal, *Colloid Polym. Sci.* 293 (6) (2015) 1667–1674.
- [22] A.S. Timin, S.Yu. Khashirova, A. Zhansitov, E.V. Rumyantsev, Application of guanidine-containing polymers for preparation of pH responsive silica-based particles for drug delivery systems, *Colloids and Surfaces A* 477 (2015) 26–34.
- [23] Ou Chen, et al., Magneto-fluorescent core-shell supernanoparticles, *Nat. Commun.* 5 (2014) 5093.
- [24] Juang-Jae Lee, et al., Synthetic ligand-coated magnetic nanoparticles for microfluidic bacterial separation from blood, *Nano Lett.* 14 (2014) 1–5.
- [25] Qi Wang, et al., Low toxicity and long circulation time of polyampholyte-coated magnetic nanoparticles for blood pool contrast agents, *Sci. Report.* 5 (2015) 7774.
- [26] Qin Yue, Jialuo Li, et al., An interface co-assembly in bi-liquid phase: toward core-shell magnetic mesoporous silica microspheres with tunable pore size, *J. Am. Chem. Soc.* 137 (2015) 13282–13289.
- [27] N.A. Alyoshina, A.V. Agafonov, E.V. Parfenyuk, Comparative study of adsorption capacity of mesoporous silica materials for molsidomine: effects of functionalizing and solution pH, *Mater. Sci. Eng. C Mater. Biol. Appl.* 40 (2014) 164–171.
- [28] E.V. Parfenyuk, E.S. Dolinina, Design of silica carrier for controlled release of molsidomine: effect of preparation methods of silica matrixes and their composites with molsidomine on the drug release kinetics in vitro, *Eur. J. Pharm. Biopharm.* 88 (2014) 1038–1045.
- [29] A. Hamrang, D. Balkose, *Applied Methodologies in Polymer Research and Technology*, Chapter 5: New biologically Active Composite Materials on the Basis of Dialdehyde Cellulose, 2015.
- [30] N.A. Sivov, et al., Biocide and Toxicological Properties of Synthesized Guanidine Containing Polymers and Their Structure, Chapter 27 of Book: *Modern Tendencies in Organic and Bioorganic Chemistry: Today and Tomorrow*, 2008.
- [31] E.M. Moreno, M. Zayat, M.P. Morales, C.J. Serna, A. Roig, D. Levy, Preparation of narrow size distribution superparamagnetic γ -Fe₂O₃ nanoparticles in a sol-gel transparent SiO₂ matrix, *Langmuir* 18 (2002) 4972–4978.
- [32] G. Ennas, A. Musinu, G. Piccaluga, D. Zedda, Characterization of iron oxide nanoparticles in Fe₂O₃-SiO₂ composite prepared by a sol-gel method, *Chem. Mater.* 10 (1998) 495–502.
- [33] J. Ge, Y. Hu, M. Biasini, W.P. Beyermann, Y. Yin, Supermagnetic magnetite colloidal nanocrystal clusters, *Angew. Chem. Int. Ed.* 46 (2007) 4342–4345.
- [34] L.T. Zhuravlev, The surface chemistry of amorphous silica, *Colloids Surf. A Physicochem. Eng. Asp.* 173 (2000) 1–38.
- [35] R. Mellaerts, J.A.G. Jammaer, M.V. Speybroeck, H. Chen, J.V. Humbeeck, P. Augustijns, G.V. Mooter, J.A. Martens, Silica carriers: a case study with itraconazole and ibuprofen, *Langmuir* 24 (2010) 8651–8659.
- [36] J. Zhou, L. Zhou, C. Dong, Y. Fend, S. Wei, J. Shen, X. Wang, Preparation and photodynamic properties of water-soluble hypocrellin A-silica nanospheres, *Mater. Lett.* 62 (2008) 2910–2913.
- [37] S.K. Sahoo, K. Agarwal, A.K. Singh, B.G. Polke, K.C. Raha, Characterization of γ - and α -Fe₂O₃ nanopowders synthesized by emulsion precipitation-calcination route and rheological behavior of α -Fe₂O₃, *Int. J. Eng. Sci. Technol.* 2 (2010) 118–126.
- [38] X. Liu, S. Su, F. Wei, X. Rong, Z. Yang, J. Liu, M. Li, Y. Wu, Construction of nanoparticles based on amphiphilic copolymers of poly(L-glutamic acid co-L-lactide)-1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine as a potential drug delivery carrier, *J. Colloid Interface Sci.* 413 (2014) 54–64.
- [39] Y. Shuming, Z. Zhou, G. Li, Structural evolution from mesoporous α -Fe₂O₃ to Fe₃O₄@C and γ -Fe₂O₃ nanospheres and their lithium storage performances, *Cryst. Eng. Comm.* 13 (2011) 4709–4713.
- [40] Ch. Baisong, Jia Guo, Congying Liu, Ji Qian, Wuli Yang, Surface functionalization of magnetic mesoporous silica nanoparticles for controlled drug release, *J. Mater. Chem.* 20 (2010) 9941–9947.
- [41] Jianing Qi, et al., Nanoparticles with dextran/chitosan shell and BSA/chitosan core-doxorubicin loading and delivery, *Int. J. Pharm.* 393 (2010) 176–184.
- [42] M.J. Murray, M.J. Snowden, The preparation, characterization and application of colloidal microgels, *Adv. Colloid Interf. Sci.* 54 (1995) 73–91.
- [43] H. Tang, J. Guo, Y. Sun, B. Chang, Q. Ren, W. Yang, Facile synthesis of pH sensitive polymer-coated mesoporous silica nanoparticles and their application in drug delivery, *Int. J. Pharm.* 421 (2011) 388–396.