In vitro MRI visualization of nanocomposite biodegradable microcapsules with tunable contrast†

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The microcapsules made of biodegradable polymers containing magnetite nanoparticles with tunable contrast in both T1 and T2 MRI modes were successfully prepared using layer-by-layer approach. MRI contrast of microcapsules was demonstrated to depend on distance between magnetite nanoparticles in the polymeric layers what is controlled by its concentration in the microcapsule shell. Five times increasing of average distance between nanoparticles in the microcapsule shell leads to changing intensity of MR signal by 100% for T1 and T2 both. Enzyme treatment of biodegradable shells resulted in changing of microcapsules MRI contrast. This evidence makes possible to follow on line capsule degradation while in vivo experiments are in place. It can be used for creation of new generation of drug delivery systems, including drug depot, with combined navigation, visualization and, remote activated release of bioactive substances in vivo.

1 Introduction

The modern medicine requires the new type of drug delivery carriers that will combine functions of in vivo navigation and visualization, carrier degradation, ability to deploy drug in controllable manner, including external triggering and sensing of important biological markers. This complex combination can be realized by fabrication of multifunctional carriers. Until now there are several types of drug delivery carriers developed. That includes nanoparticles, polymer micelles, liposomes, microcapsules. Nanoparticles, polymer micelles have a good cellular uptake and long circulation time, but their main downside is related with low amount of bioactive substance loaded that falls below demanded usual therapeutic dose. The liposomes are free of this deficiency of nanoparticles and polymer micelles but are not stable in long time and exhibit limited storage temperature dependent stability. The polymeric microcapsules fabrication approach is promising to create drug carriers with multiple functionality what could be a new generation of drug delivery systems.

There are several approaches for polymeric drug delivery carrier fabrication. There are miniemulsion polymerization, layer-by-layer assembly method, ultrasound assisted emulsion polymerization, electrospray. Advantage of layer-by-layer assembly for drug delivery systems in comparison with other methods is tunable structure and chemical composition at nanoscale level and as results it allow fabrication of microcapsules with sensitivity to external influences. The type of bioactive substance and external effect for release action depends on microcapsule biodistribution that can be controlled by type of in vivo administration (hypodermic, intravascular, intramuscular). The increasing of circulation time at intravascular injection can be achieved by PEG surface modification but the disadvantage of this approach is connected with growing allergy on the PEG. Other types of realization for targeted delivery based on surface modification are not working well in vivo due to the corona effect. Thus physical targeting of drug delivery is more promising approach. It can be realized by gradient of magnetic field, optical tweezers approach, energy cavitation bubble collapse. Anyway it is necessary to answer on the questions, where are nanocomposite capsules after in vivo injections and then how to navigate and activate them? The answer on this questions helps us make a decision related to selection of bioactive substances for encapsulation, selection of actions for remote release and navigation.

Nowadays, there are a number of methods for visualization of drug delivery systems which includes: MRI, ultrasound and photoacoustic imaging, X-ray tomography, bioluminescence imaging. Every method requires the application of contrast agents for this important task. There are Gd containing substances, manganese and iron oxide nanoparticles for MRI, plasmonic resonance nanoparticles for photoacoustic imaging, bubbles for ultrasound imaging, iodine or barium compounds for...
X-ray tomography, fluorescence dyes and quantum dots for bioluminescence imaging. Compared with other methods MRI has been demonstrated to have a very high resolution and penetration depth at high safety level. The main disadvantage of Gd containing contrast agent for MRI visualization of microcapsule shell is connected with higher toxicity of its compounds. Alternative contrast agent for capsules is magnetite nanoparticles, that usually demonstrated contrast in T2. It was shown before that MRI contrast depends on size, shape, aggregation level of nanoparticles. The magnetic properties of magnetite/polymer composite depend on the distance between nanoparticles. The advantage of layer-by-layer assembly is the control of the average distance between nanoparticles in the microcapsule shell. It can be obtained using variation of magnetite nanoparticle adsorption cycles or adsorbate concentration.

The LbL method can allow to prepare biodegradable and non biodegradable capsules by variation of polyelectrolytes. The ability of microcapsule shell biodegradation is important for biomedical applications of drug delivery systems. Release of bioactive substances depends on biodegradation rate of microcapsules shell, therefore existence of method for shell biodegradation is taken into account during calculation of therapeutic dose and time of medical treatment.

Therefore, the main goal of our work are control over MRI contrast of nanocomposite microcapsules by variation of distance between magnetite nanoparticles in the shell using variation of magnetite nanoparticle concentration during capsule shell preparation and study of in vitro biodegradation process of microcapsule shell induced by enzymes on the MR signal intensity contrast.

## 2 Experimental

### 2.1 Materials

Iron(III) chloride hexahydrate (99.8%, Sigma-Aldrich), iron(II) chloride tetrahydrate (99.8%, Sigma-Aldrich), sodium hydroxide (99.8%, Fluka), citric acid (99.8%, Sigma-Aldrich), sodium carbonate, calcium chloride, poly-L-arginine hydrochloride (Mw=15–70 kDa) (Sigma-Aldrich), dextran sulfate sodium salts (Mw=100 kDa)(Fluka), ethylene diamine tetraacetic acid disodium salt (Fluka), sodium chloride were used without additional purification. Trypsin solution was obtained from Biolot (Saint Petersburg).

### 2.2 Methods

#### 2.2.1 Synthesis of magnetite colloids

Magnetic nanoparticles were obtained by chemical precipitation from di- and trivalent salts of iron in presence of base. Initially, 0.65 g of FeCl₃·6H₂O and 0.24 g of FeCl₂·4H₂O were dissolved in water under room temperature with mixing. 100 mL of 0.1 M NaOH was added to reaction cell. For further colloids stabilization 25 mL of citric acid (16 mg/mL) solution was prepared. To remove oxygen from iron salts, stabilizing agent and sodium hydroxide solutions the nitrogen was bubbled across closed cells with mixtures during 10 minutes. Further, the iron salts solutions were injected to the sodium hydroxide solution during several seconds with active mixing, after that the solution was left under active mixing and nitrogen for 4 minutes resulting in formation of magnetic nanoparticles black sediment. 25 mL of citric acid (20 mg/mL) was added to suspension under constant mixing and nitrogen pressure. Dialysis of magnetic hydrosol was conducted during 4 days in 1.2 L vial under slow mixing. Mixing of reagents and washing steps were carried out under nitrogen. The average size of nanoparticles measured by DLS method was 13±3 nm (Fig. 1 a). This result has confirmed by TEM images (Fig. 1 b). Concentration of magnetite colloid was 7 mg/mL (from method of dry residue).

#### 2.2.2 Preparation of nanocomposite magnetic microcapsules

The layer-by-layer assembly method was used for the microcapsules preparation (Fig. 2 a). Microcapsules were obtained by adsorption of opposite charged poly-L-arginine hydrochloride (Parg) (concentration — 0.5 mg/mL in 0.15 M NaCl) and dextran sulfate sodium salts (Dex) (concentration — 1 mg/mL in 0.15 M NaCl), magnetic nanoparticles (MNPs) (1 mg/mL in water) on spherical surface of calcium carbonate cores with following extraction of cores under EDTA solution treatment (concentration 0.2 M in water). After each of adsorption steps as well as after dissolution of calcium carbonate cores suspension of the microparticles was centrifuged and washed two times by the pure water.

The calcium carbonate cores were obtained by mixing sodium carbonate and calcium chloride salts under constant mixing on the magnetic plate during 30 secs according to. The average size of calcium carbonate microparticles is 3.5±0.7 µm.

![Fig. 1](image1.png) a — Representative profile of magnetite nanoparticle size distribution obtained by dynamic light scattering; b — TEM image of magnetite nanoparticles.

![Fig. 2](image2.png) a — Scheme of microcapsule formation process: 1 — synthesized CaCO₃ particles (template); 2, 3, 4 — consequently adsorption of polyelectrolyte molecules and nanoparticles; 5 — dissolution of CaCO₃ template; b — shell structure of prepared microcapsules (containing one magnetite nanoparticle layer).
The structure of microcapsules shells was Parg/Dex/(Parg/MNPs)_3/Parg/Dex, and Parg/Dex/Parg/MNPs/Dex/Parg/Dex (Fig. 2 b). The average amount of magnetic nanoparticles in 1 mL of final suspension of polymer nanocomposite microcapsules with the three magnetite nanoparticle layers was 2.7 mg. The average concentration of microcapsule suspension was $5 \times 10^8$ mL$^{-1}$ of suspension from measurements by hemocytometer.

### 2.2.3 Enzyme treatment of microcapsule suspension.

To 1 mL of trypsin solution without any dissolution (25% of protein calculated by casein) 200 µL of initial microcapsule suspension was added. Tubes with final solution were placed to vials with water under +37°C for 30 min.

### 2.2.4 DLS, AFM, MRI, TEM.

The measurements of ζ-potential and size distribution of nanoparticles were performed using a Zetasizer Nano ZS instrument (Malvern Instruments Ltd., UK).

Atomic Force Microscopy (AFM) images were taken by using a NT-MDT Ntegra Spectra instrument operating in the tapping mode with NSG-10 tips from NT-MDT (Russia). TEM imaging was performed using a Zeiss EM 912 Omega (Zeiss, Germany) transmission electron microscope.

The thickness of the microcapsule shell is a half of double the shell thickness. The double shell thickness was measured by AFM method using the flat regions of the microcapsule shell profile by approach described in ref.47.

MRI was performed using a Philips Achieva 1.5T high field MRI scanner equipped with a phased array coil. T2- and T1-weighted quick “spin-echo” protocols (turbospinecho, TSE) were applied. Measurements were carried out with following parameters: the repetition time (TR) is 450 ms and the echo time (TE) is 15 ms for T1-weighted pulse sequence; the TR is 3000 ms, the TE is 47.7 ms for T2-weighted pulse sequence.

Decreasing of T1 relaxation time leads to increasing of MR signal in T1-weighted images. At the same time decreasing of T2 relaxation time falls to MR signal in T2-weighted images. 48

### 2.3 Results and discussion

The microcapsules obtained by layer-by-layer assembly method had shells containing one or three layers of magnetite nanoparticles. The average thickness of the microcapsules shell with the three magnetite nanoparticle layers was 54±5 nm. Data thickness obtained by AFM measurements (Fig. 3 a). Hence, the average thickness per one layer of magnetic nanoparticles was 16±2 nm that corresponding to the average size of magnetic nanoparticles 13±3 nm. The density of magnetite nanoparticle package was significantly higher in comparison with results obtained in.22 The thickness of the microcapsule shell with structure (PAH/Fe$_3$O$_4$)$_3$ was 45 nm. 22 As results the average thickness per magnetite nanoparticle layer was 5±1 nm and the average size of magnetic nanoparticles was 10±1 nm. The main forces of nanocomposite films formation during adsorption of polymers and nanoparticles are electrostatic interaction and entropy factor.49 Molecules of poly(allylamine hydrochloride) (PAH) has low surface charge density as weak polyelectrolyte comparing to poly-L-arginine hydrochloride (Parg) in water solution.50 In connection with the low surface charge PAH-shells with MNPs had low package density comparing to Parg-shells. The indirect proof of this theory is AFM images of microcapsules with Parg/Dex/(Parg/MNPs)$_3$/Parg/Dex structure (Fig. 3 b,d).

![AFM images of biodegradable microcapsules with three layers of magnetite NPs.](image)

The nanocomposite microcapsules containing one layer of magnetite nanoparticles with different concentration of magnetite nanoparticles in the microcapsule shell were prepared. As a result different volume fractions of magnetite nanoparticles for evaluating dependence of MR intensity on average distance between magnetite nanoparticles was obtained. The experiments were carried out for all samples with the same amount of magnetite nanoparticles. It has been obtained by variation of calcium carbonate microparticle number in water suspensions (S 1). This means that water suspension of microcapsules with highest concentration of magnetite nanoparticles in the shell has a lowest total number of microcapsules in the suspension. The microcapsule number was varied out by concentration changing of calcium carbonate microparticles that has been used as template for microcapsule shell forming. As a result, we have changing a total template surface area for adsorption at same magnetite nanoparticle concentration in the colloids that has been used during adsorption process (S 1).

The prepared samples were studied by MRI. T1- and T2-weighted MR images (bottom part of Fig. 4) of samples with different nanoparticle concentration in the microcapsule shell and as results different distance between nanoparticles (samples 1–5 at top part of Fig. 4) and respectively TEM images of nanocomposite microcapsule shell confirmed different average distance between nanoparticles depending on magnetite concentration (middle part of Fig. 4) were presented in the Figure 4. The Figure 4 demonstrated that MR contrast increases with decreasing of magnetite nanoparticle concentration in the nanocomposite micro-
capsule shell. Figure 5 represents a dependence of MR intensity on the average distance between nanoparticles in the microcapsule shell. The average distance between nanoparticles was calculated taking into account the average size and concentration of calcium carbonate and magnetite nanoparticles. For calculations the densities of vaterite and magnetite were taken as 1600 kg/m$^3$ and 5170 kg/m$^3$ respectively.$^{51,52}$

The MRI contrast of nanocomposite microcapsule was shown to depend on magnetite nanoparticle concentration in the microcapsule shell and as results distance between nanoparticles (Fig. 5 a, b). Normalized MR signal intensity change of microcapsule suspension for both T1 and T2 increases with increasing of magnetite nanoparticle distance in the microcapsule shell (Fig. 5). The obtained dependence can be explained by enhancing of magnetic interaction with decreasing of distance between nanoparticles. Qualitatively same behavior of polystyrene beads containing magnetite nanoparticles was demonstrated by laser tweezer method.$^{53}$ It was demonstrated that a magnetic interaction force and a magnetic force gradient were increased significantly if distance between beads is comparable with their size.$^{53}$

The dependence of MRI signal on microcapsule concentration was also studied. Figure 6 shows the MR images of samples containing suspension of microcapsules and magnetite colloids with information about magnetite concentration. The Figure 6 shows that MRI intensity dependence on microcapsule concentration is similar for colloids with the same amount of magnetite.

Both T1 and T2 dependences of MR signal intensity (SI) of magnetite hydrosol and microcapsule suspension as function of
magnetite concentration (Fig. 7 a, b) were obtained by analysis of data presented on Figure 6. Concentration dependencies for T1 weighted pulse sequence for both nanoparticles and LMC microcapsules have same shape (Fig. 7 a) as typical concentration dependence of MRI contrast agent.\textsuperscript{54} MRI signal intensity for magnetite nanoparticles is higher in comparison with LMC microcapsules at a same amount of magnetite (Fig. 7 a). It can be explained by higher magnetic interaction between magnetite nanoparticle in the microcapsule shell in comparison with one in magnetite hydrosol. The magnetite nanoparticles are fixed in the shell and average distance between nanoparticles is less than one for magnetite colloids. For HMC microcapsules the signal intensity change does not exceed 25%. It can be explained by the smaller distances between magnetite nanoparticles in the shell. Concentration dependencies for LMC microcapsules (Fig. 7 a, b) have not significant differences in comparison with nanoparticles hydrosol for both T1 and T2 modes.

In vitro study of MR contrast of microcapsule suspension with high concentration of magnetite nanoparticles in the shell at different concentration of microcapsules was carried out. Figure 8 (a, b) demonstrated the T1- and T2-weighted images of tubes containing magnetite water colloids (left column) and water suspension of magnetic polyelectrolyte microcapsules (right column) with magnetite concentration varied at interval 0.038–0.3 mg/ml. Figure 8 shows that microcapsule did not contrast in MRI images. The fermentative degradation of microcapsule shell induced by trypsin can destroy the peptide bonds of poly-L-arginine and as results the microcapsule shell.\textsuperscript{55} T1 and T2-weighted MR images of destroyed microcapsules demonstrated on the Figure 8 (a, b). Magnetite nanoparticles are released and exhibited the MR contrast after ferment induced capsule biodegradation. This fact was confirmed by TEM images (Fig. 9 a, b).

Figure 9 (a) demonstrated the nanocomposite shell before enzyme treatment. Insert of Figure 9 (a) shows that microcapsule shell contains high amount of magnetite nanoparticles and average distance between surfaces of nanoparticles is less than its size. After ferment treatment no unbroken microcapsule shells were found (Fig. 9 b). Figure 9 (b) exhibits fragments of nanocomposite microcapsule shell with different size and shape. Insert of Figure 9 (b) shows the average distance between nanoparticles in broken capsule shell is larger in comparison with unbroken one.

3 Conclusions

In this study we demonstrated fabrication of biodegradable microcapsules with magnetite nanoparticles in the shell with tunable MPI contrast and average size of microcapsule of 3.5±0.7 m. Variation of volume fraction of magnetite nanoparticle in the microcapsule shell and resulted average distance between nanoparticles can be obtained by changing of nanoparticle concentration in the colloidal suspension used for nanoparticle adsorptions. It was established that increasing of average distance between nanoparticles in the microcapsule shell leads to growth of contrast on the T1- and T2-weighted images. The maximum contrast for T1- and T2-weighted images was obtained at average distance between magnetite nanoparticles equal 54 nm that corresponds to 64 µg of magnetite per 1 ml of microcapsule suspension at concentration 5·10\textsuperscript{8} ml\textsuperscript{-1}. Enzymatically driven degradation of magnetic capsules was applied in vitro to mimic natural degradation. The biodegradation of microcapsule shell

![Figure 7](https://example.com/fig7.png)

Fig. 7 MR signal intensity change (%) as function of total amount of magnetite in the microcapsule suspension in comparison with one for magnetite colloids (square markers): (a) — T1 weighted and (b) — T2 weighted pulse sequence. The dependencies correspond to microcapsule suspensions with high (HMC, round markers) and low (LMC, triangle markers) concentration of magnetite nanoparticles in the shell.

![Figure 8](https://example.com/fig8.png)

Fig. 8 MR images of tubes with magnetite hydrosol and magnetic microcapsules (with three layers of magnetite) at varying concentrations before and after enzyme degradation. Concentration related to magnetite containment.
containing magnetite nanoparticles was demonstrated in vitro using MRI and TEM. The MRI contrast increase upon trypsin-induced degradation of microcapsules was demonstrated at initially highest concentration of magnetite nanoparticles in the shell. This finding makes possible to in-situ follow the degradation of polyelectrolyte capsules which are envisaged for in-vivo delivery. Combination of variable MRI visualization and magnetic properties of the capsules open an avenue for their use as drug carriers whose pathways in vivo can be well monitored in terms of visualization of delivery and degradation and external addressing.

4 Acknowledgements

The work was supported by Government of the Russian Federation (grant 14.Z50.31.0004 to support scientific research projects implemented under the supervision of leading scientists at Russian institutions and Russian institutions of higher education) and Saratov State University.

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