Polyelectrolyte Parg/DS submicrocapsules functionalized by magnetite nanoparticles as effective MR contrast agents

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Abstract. Polyelectrolyte microcapsules functionalized by Fe3O4 nanoparticles have proved to be perspective magnetic resonance (MR) contrast agents. Here we report about submicron-sized capsules with a shell made of poly-L-arginine and dextran sulfate. Such polymers are biodegradable, thus they are suitable to use as components of drug delivery carriers. To functionalize submicrocapsules by magnetite freezing and layer-by-layer method were used. MR study of obtained samples was carried out, and signal intensity change dependencies on submicrocapsules concentration by mass amount of iron oxide (III) nanoparticles were shown. The sample with optimal MR contrast characteristics was found as well, and an ability to use it as a bimodal MR agent was demonstrated.

1. Introduction
One of the main task of nanotechnology scientific investigations is to create carriers that can be effectively used for controlled drug delivery. Such carriers should provide not only a high drug loading, but also its transfer, local distribution and an ability to control carrier degradation and thus encapsulated substance release [1].

Different types of containers have been developed for the current moment [2-4]. Nevertheless, polymer capsules of micron and submicron size possess better characteristics in the view of loaded substance volume, time and temperature stability [5]. Layer-by-layer assembly [6] allows us to obtain polyelectrolyte shells functionalized by various inorganic, magnetic nanoparticles. Some substances can be additionally loaded into an inner volume of capsules as well.

Adding of magnetite nanoparticles to polymeric capsules can allow to control them by an external magnetic field and to visualize by magnetic resonance imaging (MRI) [7].

In order to obtain carriers described above polyelectrolyte Poly-L-arginin/Dextran sulfate (Parg/DS) submicrocapsules were prepared. Those ones of micron size were investigated earlier [8]. They contained Fe3O4 nanoparticles in a shell or/and an inner volume. To investigate the MR signal intensity dependence on magnetite content, carriers obtained were studied by MRI.
2. Methods and experiments
Magnetite nanoparticles were prepared by chemical precipitation. 20 mL of 1M NaOH and 150 mL of water were poured into the reaction cell and then heated until 40°C. For further colloid formation di- and trivalent iron salts solution was prepared. 1.3 g of FeCl₃ and 0.48 g of FeCl₂ were dissolved in 25 mL of pure water. For magnetite colloid stabilization 25 mL of 6M citric acid solution were prepared. After heating of NaOH, iron salts solution were injected there under nitrogen pressure. Then citric acid was added to stabilize magnetite nanoparticles had formed. TEM (transmission electron microscopy) of Fe₃O₄ nanoparticles are presented in figure 1, a. The size was 13±5 nm, measured by DLS (dynamic light scattering) method (figure 1, b).

Polyelectrolyte Parg/DS submicroparticles were formed by layer-by-layer assembly [6]. First, calcium carbonate cores were prepared by adding 0.4 mL of 0.5M sodium carbonate and 0.4 mL of 0.5M calcium chloride salts solutions to glycerin (4g) under constant mixing. After 1 hour submicron particles were washed three times by pure water, and then polymer shell were formed. Submicroparticles were functionalized by magnetite nanoparticles that were loaded into an inner volume by freezing and in a shell by Lbl method (according to the protocol from [8]). The loading method “freezing” was described in the manuscript [9], which is currently under revision. After the shell formation calcium carbonate cores were dissolved under EDTA (ethylenediaminetetraacetic acid disodium salt dehydrate) solution treatment (0.2M in water).

Two samples of magnetic polymeric submicrocapsules were synthesized: with low/high magnetite concentration in an inner volume and with 1 layer of magnetite nanoparticles in a shell, Lowcore+Lbl and Highcore+Lbl, respectively. Fe₃O₄ aqueous solution with 1.6 mg/mL concentration was used to freeze magnetite into calcium carbonate cores, and 0.56 mg/mL solution was used for Lbl assembly. SEM (scanning electron microscopy) images of obtained submicrocapsules are presented in figure 2. The presence of magnetite into the submicrocapsules was confirmed by EDXS analysis.

Figure 1. (a) TEM image of magnetite nanoparticles and (b) their size distribution, obtained from DLS.

Figure 2. SEM images of (a) Lowcore+Lbl and (b) Highcore+Lbl submicrocapsules, functionalized by magnetite nanoparticles.

Evaluation of a mass fraction of magnetite nanoparticles adsorbed on a shell and loaded by freezing into submicrocapsules was carried out by colorimetric titration in terms of the iron ions (III) qualitative reaction with ammonium thiocyanate. Obtained polymer capsules with magnetite nanoparticles were dissolved in 1M sulfuric acid. The reaction was carried out by direct mixing of 2 ml of the investigated
solution and 2 ml of 4M ammonium thiocyanate solution at rapid mixing, which results in formation of blood-red colour iron thiocyanate solution. Then a concentration of iron ions in the investigated solution was determined by colorimetric titration in terms of a standard iron (III) solution reaction with ammonium thiocyanate. The standard iron (III) solution was 1M sulfuric acid in which iron ions (III) were dissolved, its concentration was 0.1 mg/ml by iron amount.

The measurements of the magnetite nanoparticles size distribution were performed using a Zetasizer Nano ZS instrument (Malvern Instruments Ltd, UK).

TEM images of the magnetite nanoparticles were obtained with operating at 120 kV Libra-120 transmission electron microscope (Carl Zeiss, Germany). Investigated particles were deposited on a 300-mesh copper grid coated with formvar.

Scanning electron microscopy (SEM) was performed with MIRA II LMU (Tescan, Czech) microscope at operating voltage of 30 kV, in secondary and backscattering electron modes.

In vitro MRI study was carried out using a Philips Achieva 1.5 T high field MRI scanner equipped with a phased array coil. For obtaining T1-weighted images spin echo (SE) and gradient echo (FFE) sequences were applied, for T2-weighted images – turbo spin echo (TSE) sequence. The following parameters were used for conducting measurements: the repetition time (TR) was 488 ms and the echo time (TE) was 15 ms for the T1-weighted pulse SE sequence; the TR was 104 ms and the TE was 4.5 ms for the T1-weighted pulse FFE sequence; the TR was 3000 ms and the TE was 47.7 ms for the T2-weighted pulse TSE sequence. In order to increase MR signal in T1-weighted image the TR should be decreased, so tissues don’t manage to relax after an external influence. At the same time, the T2 relaxation time TE decrease leads to low MR contrast in T2-weighted image [10].

3. Results and discussion

Lowcore+Lbl and Highcore+Lbl submicrocapsules were obtained by varying the number of freezing loading cycle. As a result, each sample contained 40 mg of CaCO₃ cores, 3.03±0.11 mg and 13.26±0.55 mg of magnetite nanoparticles, respectively. In vitro MRI study was carried for different samples’ concentration to show the signal intensity dependence on the magnetite content in solution. For this T1 SE, T1 FFE and T2 TSE modes were used. MR images are performed in figure 3 and figure 4.

Dependences of MR signal intensity (SI) on submicrocapsules concentration by mass amount of magnetite are presented in figure 5 and figure 6 for Lowcore+Lbl sample; in figure 7 and figure 8 for Highcore+Lbl sample.

![Figure 3](image1.png)  
**Figure 3.** T1- and T2-weighted MR images of Lowcore+Lbl submicrocapsules, concentration by magnetite mass amount

![Figure 4](image2.png)  
**Figure 4.** T1- and T2-weighted MR images of Highcore+Lbl submicrocapsules, concentration by magnetite mass amount
Figure 5. Low core + Lbl normalized signal intensity (SI) dependence on concentration by magnetite mass amount for T1 mode

Figure 6. Low core + Lbl normalized signal intensity (SI) dependence on concentration by magnetite mass amount for T2 mode

Figure 7. High core + Lbl normalized signal intensity (SI) dependence on concentration by magnetite mass amount for T1 mode

Figure 8. High core + Lbl normalized signal intensity (SI) dependence on concentration by magnetite mass amount for T2 mode

The T1 SI change was about 300% for Low core + Lbl, which is 2 times lower than for magnetite aqueous solution. But this sample possess high contrast for T2 mode. It is nearly -100% change, which leads to almost full decay of the signal.

The High core + Lbl sample SI change from the data obtained is shown in figure 7 and figure 8. This sample possess high 300-600% T1 MR contrast for both SE and FFE sequences, and even the signal enhancement for the high concentration area where the signal usually decreases. The signal enhancement is near to that one for magnetite colloid. The curves observed are monotonous, do not have any additional peaks that provide the effective usage of the sample as T1 contrast agent. T2 mode was studied as well and provided 65% SI change. Having regard to the above, High core + Lbl submicrocapsules can be effectively used as bimodal MR contrast agent. Therefore, due to the high magnetite concentration such carriers can be controlled by an external magnetic field, which is necessary for controlled drug delivery.

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