BioNanoSci.
DOI 10.1007/s12668-017-0400-8

A Facile Method of Preparation of Polymer-Stabilized Perfluorocarbon Nanoparticles with Enhanced Contrast for Molecular Magnetic Resonance Imaging

Lev L. Gervits[1] · Andrey V. Shibaev[2] · Mikhail V. Gulyaev[2] · Vyacheslav S. Molchanov[2] · Nikolai V. Anisimov[2] · Yury A. Pirogov[2] · Alexei R. Khokhlov[2] · Olga E. Philippova[2]

Abstract We present a new efficient method of incorporation of gadolinium chelates into polymeric shell surrounding liquid nanoparticles of perfluorotributylamine, which does not degrade the particles stability. The payload reaches ca. 10^5 Gd ions per nanoparticle giving large local concentration of contrast agent. The relaxivity of Gd-loaded nanoparticles estimated by magnetic resonance imaging at 7 T magnetic field is as high as 10^5–10^6 mM^{-1} s^{-1}. These nanoparticles are promising for dual-mode imaging simultaneously on two nuclei ^1H and ^{19}F, thus enhancing the quality of visualization.

Keywords Emulsion · Proxanol block copolymer · Perfluorotributylamine · Gadolinium chelates · Contrast agents · Magnetic resonance imaging

1 Introduction

Water-soluble polymers are widely used for the stabilization of different nanoparticles, in particular, for biomedical application [1–4]. Biocompatible nonionic amphiphilic macromolecules, e.g., poly(ethylene oxide) (PEO) derivatives adsorbed on nanoparticles permit not only to avoid particle aggregation but also to prevent the rapid uptake of intravenously injected particles by the cells of the reticuloendothelial system [5, 6]. In addition, the polymeric shell of nanoparticles provides the possibility to include on their surface different agents and functional groups [7].

Among various nanoparticles for biomedical applications, perfluorocarbon nanoparticles are of special interest, because they are nontoxic, biologically inert, and not metabolized in the body [8–10]. In particular, they are quite prospective for in vivo magnetic resonance imaging (MRI) [8–13] taking into account that ^{19}F nuclei are absent in most biological tissues, which eliminates the problem of interfering background signals. The introduction of proton contrast agents on the surface of such particles allows one to perform simultaneous ^1H and ^{19}F imaging, thus improving significantly the quality of visualization [10, 14–16]. For this aim, gadolinium chelates are usually used because they are among the most effective clinically approved proton contrast agents [17–19]. To the best of our knowledge, by now, gadolinium chelates were incorporated mainly on the surface of perfluorocarbon particles stabilized by phospholipids [14, 16, 18]. For this purpose, gadolinium chelates with lipophilic chains like 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) or C6-DSPE were used, but they are quite expensive. In this paper, we propose to incorporate gadolinium chelates on the surface of perfluorocarbon nanoparticles stabilized by polymer. For these studies, the particles of perfluorotributylamine (PFTBA) were chosen. This fluorocarbon has nine chemically and magnetically equivalent fluorine atoms of trifluoromethyl groups per molecule, which give a strong peak in NMR spectrum [20]. Due to its highly hydrophobic and lipophobic properties, PFTBA is known to form very stable emulsions [21], which can be stored for a long time at room temperature. In this study, the PFTBA particles were covered by poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer Proxanol-268,

✉ Olga E. Philippova
phil@polly.phys.msu.ru

[1] A.N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, Moscow, Russia 119991

[2] M.V. Lomonosov Moscow State University, Moscow, Russia 119991

Published online: 23 February 2017

which plays a crucial role in the system: it stabilizes the particles and accommodates the added proton contrast agent, gadolinium chelate. Also, Proxanol-268 is expected to prevent opsonization and prolong circulation half-life of the particles [5, 6]. The elaborated method of incorporation of gadolinium chelates in the polymeric shell of the perfluorocarbon nanoparticles gives an easy way to get bimodal contrast agent for molecular imaging simultaneously on two kinds of nuclei: ^{1}H and ^{19}F.

2 Experimental Section

2.1 Materials

Perfluorotributylamine $N(CF_2CF_2CF_2CF_3)_3$ (State Institute of Applied Chemistry, Russia) and poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer Proxanol-268 (Perftoran, Russia) were used without further purification. Proxanol-268 with molar mass (MM) of ca. 13,000 g/mol has two terminal hydrophilic blocks of 110 ethylene oxide units each and a central hydrophobic block with 45 propylene oxide units (EO_{110}-PO_{45}-EO_{110}). Its composition is rather similar to that of Pluronic F-68: EO_{78}-PO_{30}-EO_{78} [22].

Three different commercial contrast agents based on gadolinium chelates were chosen for the studies: gadodiamide (trademark Omniscan®), gadopentetate dimeglumine (Magnevist®), and gadobutrol (Gadovist®) (Fig. 1). They are formulated at a gadolinium concentration of 0.5 mol/L except Gadovist®, which has a gadolinium concentration of 1.0 mol/L.

2.2 Preparation of Particles

The introduction of gadolinium chelates on the surface of nanoparticles was performed as follows. First, 15 ml of the commercial solution of contrast agent containing 0.5 mol/L of gadolinium ions was poured into 105 ml of 8 wt% aqueous solution of Proxanol-268 and rigorously stirred with high-pressure homogenizer Donor-1 during 3 min at 200 Atm. Then, 30 ml of PFTBA was added dropwise to the resulting solution under continuous stirring (3 min, 200 Atm). Finally, the emulsion thus obtained was strongly homogenized during 15 min at 600 Atm. To some samples, 150 ml of 1.8 wt% aqueous NaCl solution was added at this final stage.

In order to determine the amount of gadolinium ions adsorbed on the particles, the PFTBA particles were removed by centrifugation (1 h, 40,000 g), and the residual amount of gadolinium ions in the external aqueous solution was analyzed by X-ray fluorescence method.

2.3 X-Ray Fluorescence

X-ray fluorescence measurements were performed on Carl Zeiss VRA-30 X-ray spectrometer using X-ray tube with Mo anode (40 kV, 20 mA).

2.4 Dynamic Light Scattering

Dynamic light scattering (DLS) measurements were made with an ALV/DLS/SLS-5000 compact goniometer system at a wavelength of 632.8 nm at 25.0 ± 0.1 °C. For these studies, the initial emulsions were tenfold diluted with distilled water. The autocorrelation functions of the scattered intensity were analyzed by using CONTIN method [23] to obtain the distribution of decay times. Under the assumption that the scattering particles behave as hard spheres in dilute solution, the relaxation time distribution obtained was converted into an apparent hydrodynamic size distribution using the Stokes-Einstein relationship. The details of the DLS data treatment are described elsewhere [24–26].

2.5 Transmission Electron Microscopy

Transmission electron microscopy (TEM) measurements were performed on a LEO912 AB OMEGA microscope at an accelerating voltage of 100 kV. To prepare the samples for these measurements, a drop of emulsion was deposited on a 140-mesh Formvar-coated copper grid and dried on the air.

2.6 Magnetic Resonance and Nuclear Magnetic Resonance Spectroscopy

Magnetic resonance measurements (high-resolution spectra and MRI registrations) were carried out at 7 T Bruker Scanner BioSpec 70/30 USR equipped with Topspin® 2.0 and ParaVision® 5.0 software. The volume resonator (transceiver coil) was modified to register not only ^{1}H NMR (300 MHz) but also ^{19}F NMR (283 MHz) signal. T1 and T2 relaxation time measurements were performed using the MRI pulse sequences RAREVTR (saturation recovery with variable TR) and MSME (multi spin multi echo), respectively. The relaxivity values were determined from the concentration dependences of 1/T1 and 1/T2.

3 Results and Discussion

Three different clinically approved gadolinium-containing contrast agents currently used in MRI were chosen for the introduction on the surface of particles (Table 1). They differ: (1) by the molecular structure of their gadolinium-chelate complex (cyclic or linear) and (2) by the presence of higher

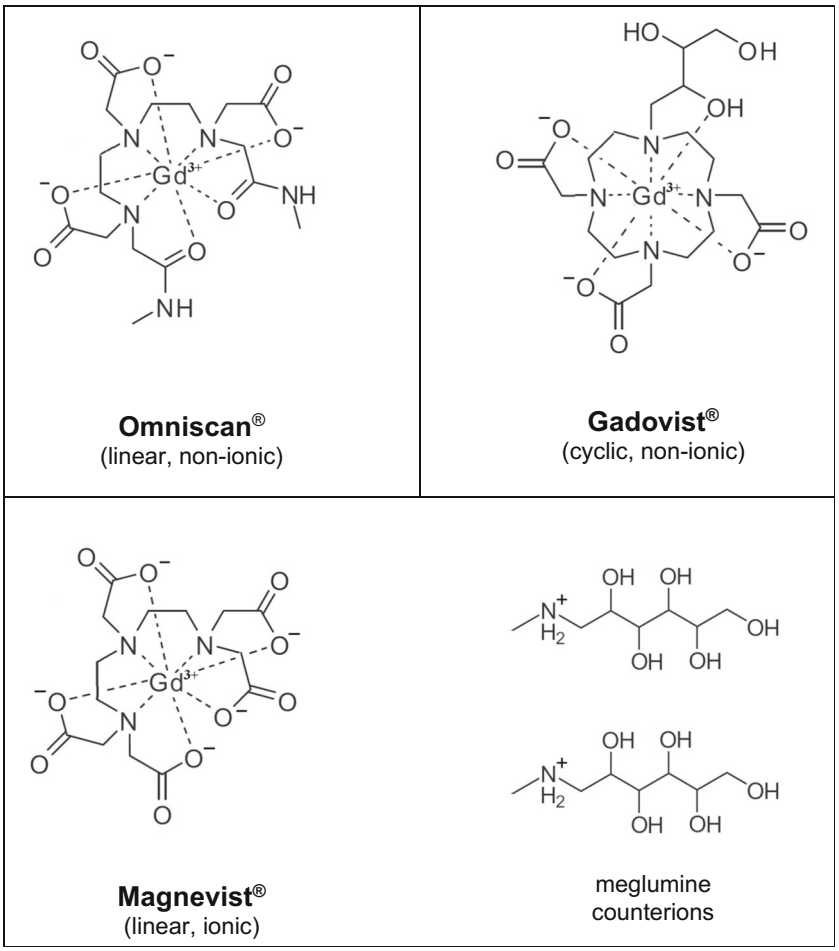

Fig. 1 Chemical structure of contrast agents under study

amount of charged groups than necessary to compensate the charge of Gd^{3+} ion (ionic or nonionic) (Fig. 1). In the case of ionic contrast agent (Magnevist®), a protonated amino saccharide N-methyl-D-glucamine (meglumine) acts as a counterion (Fig. 1).

All these gadolinium chelates are highly soluble in water. However, to provide better contrast, they should be located in close proximity of perfluorocarbon particles and not in the bulk of water. In other words, the chelates should be incorporated in the polymer layer adsorbed on the surface of particles. In this paper, the following technique of incorporation of gadolinium chelates into polymer (Proxanol) shell of perfluorocarbon particles is proposed. First, the gadolinium chelates were mixed with Proxanol in order to obtain polymer-coated chelates. Then, the polymer-coated chelates (instead of "pure" polymer) were used to stabilize the perfluorocarbon particles in the emulsion. In this way, three different systems were prepared. Their compositions are listed in Table 1. Also,

Table 1 Composition of polymer-stabilized PFTBA emulsions with incorporated gadolinium ions

Contrast agent	PFTBA concentration (*v*/*w*%)	Gd ions concentration (g/L) in the solution before and after removal of particles		Amount of Gd ions (mmol) in the solution before and after removal of particles		Fraction of Gd ions localized on the particles	Radius of particles (nm)	Amount of Gd ions per particle
		Before	After	Before	After			
Omniscan®	20	9.81	1.09	7.5	0.83	0.89	80	2.8×10^5
Magnevist®[a]	10	4.36	0.57	7.5	0.98	0.87	80	5.6×10^5
Gadovist®[a]	10	8.72	0.75	15	1.29	0.91	55; 135	–

[a] In physiological salt solution

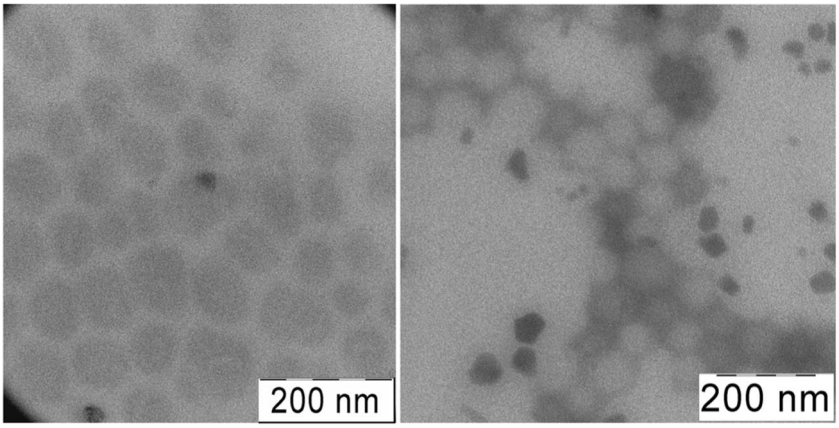

Fig. 2 TEM images of PFTBA (*left*) and PFTBA-Omniscan® (*right*) emulsions

Table 1 presents the results of the determination of the amount of gadolinium ions remaining in the external solution after the separation of the particles by centrifugation. It is seen that most of the ions (87–91%) are not released to the solution, i.e., they are associated with the particles. So, polymer provides a very effective binding of gadolinium to the surface of the particles. Note that a twofold dilution of the emulsions with salt solution did not deteriorate the association of gadolinium ions with the particles (Table 1). Transmission electron microscopy data (Fig. 2) demonstrate that gadolinium ions are located in the shell of perfluorocarbon particles. One can see that in the absence of Gd chelates, the shell of perfluorocarbon particles is lighter than their core. Such picture was observed for many perfluorocarbon emulsions [16]. On the other hand, in the presence of Gd chelates, the shell becomes darker than the core. This may indicate that electron-dense heavy gadolinium ions are accumulated in the shell of particles. These ions strongly scatter electrons forming a dark zone of intense contrast.

The effect of incorporated gadolinium chelates on the size of emulsion particles was investigated by DLS. It was found that all the samples under study demonstrate q^2-dependent translational diffusion of the particles. The particle size distribution functions obtained by CONTIN method are shown in Fig. 3. It is seen that in the presence of linear chelates Omniscan® and Magnevist®, the distribution is monomodal with the average hydrodynamic radius of particles equal to ca. 80 nm, which is close to that of nonloaded PFTBA particles. At the same time, in the presence of cyclic chelate (Gadovist®), the particle size distribution function is bimodal (Fig. 3). This can reflect the process of destabilization of the emulsion with the formation of larger particles, which will be discussed later.

To estimate the average number of gadolinium ions adsorbed on a single particle, the amount of gadolinium ions adsorbed on all particles was divided by the number of the particles NP, which can be determined by formula $NP = V_{FC}/v_p$, where V_{FC} is the whole volume of perfluorocarbon in the emulsion, and v_p is the volume of a single particle. The volume of one particle was estimated from the value of its hydrodynamic radius measured by DLS (Table 1) under assumption that it has a spherical shape. The calculations were performed only for PFTBA-Omniscan® and PFTBA-Magnevist® emulsions with monomodal distribution of particles. They show that ca. 10^5 gadolinium ions are located on the surface of one nanoparticle (Table 1). This is a rather large amount of gadolinium similar to that entrapped in 200–300-nm polymeric nanocapsules [7].

What can be the reason of such an effective binding? Since the gadolinium chelates are not soluble in perfluorocarbons, they are expected to locate in the polymeric shell of the particles. PEO representing one of the blocks of Proxanol is known to interact with different cations [27, 28] surrounding them like a crown ether. However, very often nonionic polymer cannot expel negatively charged ligand bound to trivalent gadolinium cation [29–32], and a triple complex Gd(III)-ligand-polymer is formed [31, 32]. The complexes of gadolinium chelates with different nonionic polymers (dextran, hydroxypropylstarch, inulin, polyvinylpyrrolidone, PEO) were described in the literature [31, 32]. In particular, they were developed in order to decrease the toxicity of the chelates without significant deterioration of their relaxivity [32]. These studies were performed with analogs of Magnevist® (gadopentetate disodium or dipiperasinium salts) and different nonionic polymers: polyvinylpyrrolidone (MM 12000 g/mol), dextran (MM 60000 g/mol), PEO (MM 1500 g/mol) [32]. It was shown that polymer decreases the toxicity of chelates by 15%. Most probably, a similar triple complex Gd(III)-ligand-Proxanol is formed in the present system. In this complex, the polymer can interact with the chelates surrounding gadolinium, as they possess some groups (–OH, –NH) capable of forming H bonds with ether oxygens of PEO [33].

One can suggest that the incorporation of a rather large amount of gadolinium chelates into the polymeric shells stabilizing perfluorocarbon nanoparticles could deteriorate the emulsion stability. To explore this problem, the size distribution of particles loaded with contrast agent was characterized by DLS 8 months after the preparation of the emulsions. The results are presented in Fig. 3. It is seen that in the emulsions

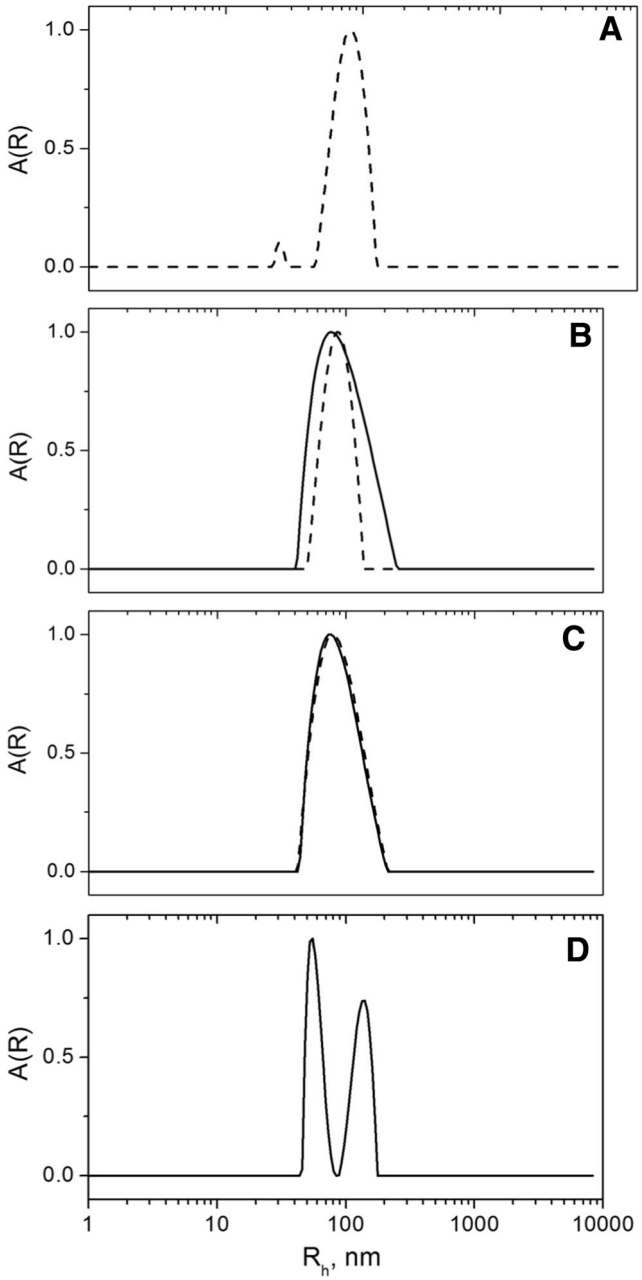

Fig. 3 Hydrodynamic radius distribution of particles in the PFTBA, PFTBA-Omniscan®, PFTBA-Magnevist®, and PFTBA-Gadovist® emulsions at scattering angle $\theta = 90°$ stored for 1 week (*solid line*) and 8 months (*dotted line*) after preparation

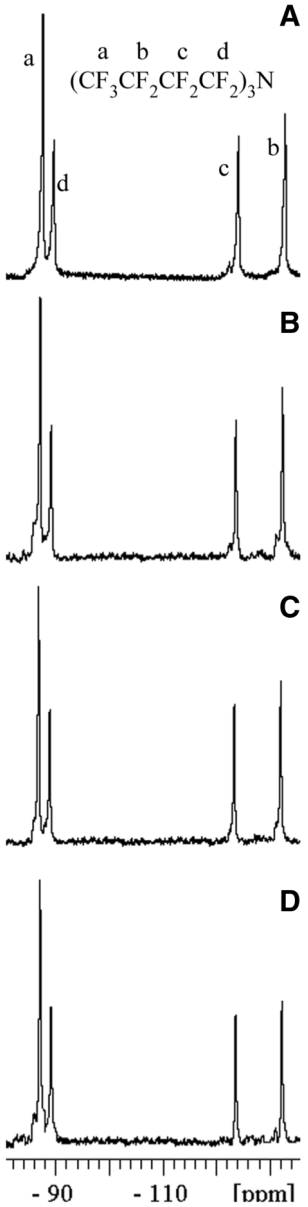

Fig. 4 ^{19}F NMR spectra of the initial PFTBA emulsion **A** and PFTBA emulsion with different contrast agents: **B** Omniscan®, **C** Magnevist®, **D** Gadovist®

with linear chelates (nonionic Omniscan® and ionic Magnevist®), the size distribution of particles does not change appreciably during 8 months of their storage at 4 °C. Therefore, the linear chelates do not induce the destabilization of the emulsion independently of their charge. By contrast, in PFTBA emulsion containing cyclic chelate, Gadovist®, a phase separation occurred. Probably, cyclic chelates induce more significant perturbation of the polymer layer stabilizing the particles, because they are more bulky and more rigid than the linear ones. Therefore, the proposed method of incorporation of gadolinium ions into the shell of PFTBA particles is efficient mainly for linear ligands (both ionic and nonionic).

Fig. 4 allows us to compare ^{19}F NMR spectra of the PFTBA emulsion before and after the addition of gadolinium ions. It is seen that the spectra of all emulsions contain four major peaks (their assignment is given in Fig. 4). Introduction of Gd chelates induces a frequency shift and a broadening of the peaks indicating the interaction of the PFTBA particles with paramagnetic Gd ions.

The efficiency of gadolinium chelates to change the relaxation time of PFTBA emulsions was estimated by MRI. The gadolinium-based contrast agents are known to enhance MRI

Table 2 Relaxation time T1 and relaxivity r_1 of protons in PFTBA emulsions and aqueous solutions of the corresponding gadolinium chelates

System	T1 (ms)[a]	r_1 (mM^{-1} s^{-1})	
		Per Gd ion	Per particle
PFTBA	1800	–	
Magnevist®	55	2.8	
Omniscan®	57	3.0	
Gadovist®	48	3.4	
PFTBA-Magnevist®	40	4.6	2.6×10^6
PFTBA-Omniscan®	63	1.8	5.0×10^5
PFTBA-Gadovist®	52	3.8	–

[a] Concentration of Gd ions 10 mmol/L

signal by reducing the longitudinal (T1) or transverse (T2) relaxation time of spins [29]. As PFTBA emulsion contains both hydrogen and fluorine atoms, the relaxation time values were determined both for ^{1}H and ^{19}F nuclei.

The results of the measurements of T1 and T2 relaxation times of protons are presented in Tables 2 and 3. It is seen that the addition of paramagnetic ions at the concentration of 10 mmol/L to PFTBA emulsion leads to a very significant (by several dozen times) reduction of T1 and T2.

When comparing the observed relaxivity for the emulsion of gadolinium-loaded nanoparticles with that of aqueous solution of the corresponding contrast agent, one can see that it is almost the same only for PFTBA-Gadovist® (Tables 2 and 3). For PFTBA-Omniscan®, it is somewhat lower, and for PFTBA-Magnevist®, it is somewhat larger than for the free contrast agents. The relaxivity defined as the relaxation enhancement normalized to a 1 mM concentration of the paramagnetic ion depends on many parameters including the exchange rate between water molecules freely moving in the solution and those bound to Gd chelate as well as the rotational correlation time of the Gd ions [34, 35]. One can suggest that in the system under study, the polymeric shell can (1) slow down the water exchange with bulk solution and (2) hinder the rotation of Gd ions. The first of these effects lowers relaxivity, whereas the second one increases it. The rotation of Gd ions should be especially sensitive to the strength of interaction of Gd chelate with Proxanol, which is expected to increase in the following sequence: Omniscan® < Gadovist® < Magnevist® taking into account the amount of functional groups (–OH and –NH) in the chelate (including counterions) capable to form hydrogen bonds with the polymer (Fig. 1). A stronger effect of polymer on ionic chelate Magnevist® may be also due to the fact that this chelate should be entrapped in polymeric shell together with its counterions. The presence of counterions in close vicinity of the charged chelate can restrict appreciably the rotation of Gd ion. Thus, the increase of relaxivity in the order PFTBA-Omniscan® < PFTBA-Gadovist® < PFTBA-Magnevist® seems to be primarily due to the slowing down of the rotation of Gd ions. In these systems, the maximum enhancement of the relaxivity per single Gd ion reaches 60% (Tables 2 and 3).

Table 3 Relaxation time T2 and relaxivity r_2 of protons in PFTBA emulsions and aqueous solutions of the corresponding gadolinium chelates

System	T2 (ms)[a]	r_2 (mM^{-1} s^{-1})	
		Per Gd ion	Per particle
PFTBA	650	–	
Magnevist®	50	3.4	
Omniscan®	53	3.3	
Gadovist®	44	4.1	
PFTBA-Magnevist®	35	4.8	2.7×10^6
PFTBA-Omniscan®	60	1.9	5.3×10^5
PFTBA-Gadovist®	48	4.3	–

[a] Concentration of Gd ions 10 mmol/L

However, the most important effect is provided by the accumulation of many gadolinium ions in the polymer shell of the particle, which allows to deliver a large number of contrast agents to the site of interest and, therefore, to get a pronounced enhancement of sensitivity [32] as the relaxivity of protons per particle is tremendously high—up to ca. 10^6 mM^{-1} s^{-1} (Tables 2 and 3).

As to fluorine nuclei, the data obtained show that their T1 relaxation time does not change in the presence of gadolinium. For example, for the initial PFTBA emulsion, $T1_{19F} \approx 250 \pm 15$ ms, whereas for the PFTBA-Omniskan®, $T1_{19F} \approx 230 \pm 10$ ms. This could indicate that the gadolinium ions are not located in close contact with perfluorocarbon atoms and therefore are unable to alter local magnetic fields in the vicinity of ^{19}F spins. But this suggestion contradicts to the observed frequency shift in ^{19}F spectrum induced by Gd ions indicating the close proximity of these ions to perfluorocarbon. Another explanation of the low effect of gadolinium on T1 relaxation time of the fluorine nuclei can be connected with high strength of magnetic field in our experiments (7 T). As was evidenced earlier [15], the strongest impact of gadolinium on T1 of perfluorocarbon particles is observed at 1.5 T (clinical field strength), and then it tremendously decreases with increasing field strength becoming just marginal (ca. 20%) at 11.7 T.

Therefore, gadolinium ions located in polymer shell surrounding liquid perfluorocarbon particles do not affect the T1 relaxation time of fluorine atoms, but induce a very large decrease of T1 relaxation time of protons in PFTBA emulsion.

4 Conclusions

Thus, in this paper, we propose a facile method of accumulation of clinically approved contrast agents based on gadolinium chelates on the surface of perfluorocarbon particles. It requires just the addition of gadolinium chelates to polymer solution before a "standard" procedure of the preparation of perfluorocarbon emulsion. This method permits to concentrate up to 5.6×10^5 gadolinium ions on one perfluorocarbon particle and get as high relaxivity per particle as 10^6 mM^{-1} s^{-1}. This approach seems to be quite promising for the creation of new dual-mode MRI contrast agents.

Acknowledgments The financial support of the Russian Ministry of Education and Science under agreement no. 14.604.21.0060 (identifier RFMEFI60414X0060) is gratefully acknowledged.

References

1. Sakai, T., & Alexandridis, P. (2004). Single-step synthesis and stabilization of metal nanoparticles in aqueous Pluronic block copolymer solutions at ambient temperature. *Langmuir, 20*, 8426–8430. doi:10.1021/la049514s.
2. Santander-Ortega, M. J., Jódar-Reyes, A. B., Csaba, N., Bastos-González, D., & Ortega-Vinuesa, J. L. (2006). Colloidal stability of Pluronic F68-coated PLGA nanoparticles: a variety of stabilization mechanisms. *Journal of Colloid and Interface Science, 302*, 522–529. doi:10.1016/j.jcis.2006.07.031.
3. Jain, T. K., Foy, S. P., Erokwu, B., Dimitrijevic, S., Flask, C. A., & Labhasetwar, V. (2009). Magnetic resonance imaging of multifunctional Pluronic stabilized iron-oxide nanoparticles in tumor-bearing mice. *Biomaterials, 30*, 6748–6756. doi:10.1016/j.biomaterials.2009.08.042.
4. Wulff-Pérez, M., Torcello-Gomez, A., Galvez-Ruiz, M. J., & Martin-Rodriguez, A. (2009). Stability of emulsions for parenteral feeding: preparation and characterization of o/w nanoemulsions with natural oils and Pluronic f68 as surfactant. *Food Hydrocolloids, 23*, 1096–1102. doi:10.1016/j.foodhyd.2008.09.017.
5. Batrakova, E. V., & Kabanov, A. V. (2008). Pluronic block copolymers: evolution of drug delivery concept from inert nanocarriers to biological response modifiers. *Journal of Controlled Release, 130*, 98–106. doi:10.1016/j.jconrel.2008.04.013.
6. Owens, D. E., & Peppas, N. A. (2006). Opsonization, biodistribution, and pharmacokinetics of polymer nanoparticles. *International Journal of Pharmaceutics, 307*, 93–102. doi:10.1016/j.ijpharm.2005.10.010.
7. Sharma, S., Paiphansiri, U., Hombach, V., Mailänder, V., Zimmermann, O., Landfester, K., & Rasche, V. (2010). Characterization of MRI contrast agent-loaded polymeric nanocapsules as versatile vehicle for targeted imaging. *Contrast Media & Molecular Imaging, 5*, 59–69. doi:10.1002/cmmi.364.
8. Kraft, M. P., & Riess, J. G. (2007). Perfluorocarbons: life sciences and biomedical uses. *J Polym Sci A: Polym Chem, 45*, 1185–1198. doi:10.1002/pola.21937.
9. Kaneda, M. M., Caruthers, S., Lanza, G. M., & Wickline, S. A. (2009). Perfluorocarbon nanoemulsions for quantitative molecular imaging and targeted therapeutics. *Annals of Biomedical Engineering, 37*, 1922–1933. doi:10.1007/s10439-009-9643-z.
10. Winter, P. M. (2014). Perfluorocarbon nanoparticles: evolution of a multimodality and multifunctional imaging agent. *Scientifica, 2014*, 1–10. doi:10.1155/2014/746574.
11. Mason, R. P., Antich, P. P., Babcock, E. E., Gerberich, J. L., & Nunnally, R. L. (1989). Perfluorocarbon imaging in vivo: a ^{19}F MRI study in tumor-bearing mice. *Magnetic Resonance Imaging, 7*, 475–485. doi:10.1016/0730-725X(89)90402-5.
12. Srinivas, M., Heerschap, A., Ahrens, E. T., Figdor, C. G., & de Vries, I. J. M. (2010). ^{19}F MRI for quantitative *in vivo* cell tracking. *Trends in Biotechnology, 28*, 363–370. doi:10.1016/j.tibtech.2010.04.002.
13. Ebner, B., Behm, P., Jacoby, C., Burghoff, S., French, B. A., Schrader, J., & Flögel, U. (2010). Early assessment of pulmonary inflammation by ^{19}F MRI in vivo. *Circulation. Cardiovascular Imaging, 3*, 202–210. doi:10.1161/CIRCIMAGING.109.902312.
14. Morawski, A. M., Winter, P. M., Crowder, K. C., Caruthers, S. D., Fuhrhop, R. W., Scott, M. J., Robertson, J. D., Abendschein, D. R., Lanza, G. M., & Wickline, S. A. (2004). Targeted nanoparticles for quantitative imaging of sparse molecular epitopes with MRI. *Magnetic Resonance in Medicine, 51*, 480–486. doi:10.1002/mrm.20010.
15. Neubauer, A. M., Myerson, J., Caruthers, S. D., Hockett, F. D., Winter, P. M., Chen, J., Gaffney, P. J., Robertson, J. D., Lanza, G. M., & Wickline, S. A. (2008). Gadolinium-modulated ^{19}F signals from perfluorocarbon nanoparticles as a new strategy for molecular imaging. *Magnetic Resonance in Medicine, 60*, 1066–1072. doi:10.1002/mrm.21750.
16. De Vries, A., Moonen, R., Yildirim, M., Langereis, S., Lamerichs, R., Pikkemaat, J. A., Baroni, S., Terreno, E., Nicolay, K., Strijkers, G. J., & Grüll, H. (2014). Relaxometric studies of gadolinium-functionalized perfluorocarbon nanoparticles for MR imaging. *Contrast Media & Molecular Imaging, 9*, 83–91. doi:10.1002/cmmi.1541.
17. Caravan, P., Ellison, J. J., McMurry, T. J., & Lauffer, R. B. (1999). Gadolinium (III) chelates as MRI contrast agents: structure, dynamics, and applications. *Chemical Reviews, 99*, 2293–2352. doi:10.1021/cr980440x.
18. Sherry, A. D., Caravan, P., & Lenkinski, R. E. (2009). A primer on gadolinium chemistry. *Journal of Magnetic Resonance Imaging, 30*, 1240–1248. doi:10.1002/jmri.21966.
19. Amoroso, A. J., & Pope, S. J. A. (2015). Using lanthanide ions in molecular bioimaging. *Chemical Society Reviews, 44*, 4723–4742. doi:10.1039/C4CS00293H.
20. Balinov, B., & Söderman, O. (2001). Emulsions—the NMR perspective. In J. Sjoblom (Ed.), *Encyclopedic handbook of emulsion technology* (p. 295). New York, Basel: Marcel Dekker Inc..
21. Krafft, M. P., Riess, J. G., & Weers, J. G. (1998). The design and engineering of oxygen-delivering fluorocarbon emulsions. In S. Benita (Ed.), *Submicron emulsions in drug targeting and delivery* (pp. 235–334). Amsterdam: Harwood Academic Publishers.
22. Nakashima, K., Anzai, T., & Fujimoto, Y. (1994). Fluorescence studies on the properties of a Pluronic F68 micelle. *Langmuir, 10*, 658–661. doi:10.1021/la00015a012.
23. Provencher, S. W. (1982). A constrained regularization method for inverting data represented by linear algebraic or integral equations. *Computer Physics Communications, 27*, 213–227. doi:10.1016/0010-4655(82)90173-4.
24. Korchagina, E. V., & Philippova, O. E. (2010). Multichain aggregates in dilute solutions of associating polyelectrolyte keeping a constant size at the increase in the chain length of individual macromolecules. *Biomacromolecules, 11*, 3457–3466. doi:10.1021/bm100990u.
25. Korchagina, E. V., & Philippova, O. E. (2012). Effects of hydrophobic substituents and salt on core-shell aggregates of hydrophobically modified chitosan: light scattering study. *Langmuir, 28*, 7880–7888. doi:10.1021/la3013409.

26. Korchagina, E. V., & Philippova, O. E. (2015). Ion-specific self-assembly of hydrophobically modified polycation of natural origin. *Macromolecules, 48*, 8622–8628. doi:10.1021/acs.macromol.5b02213.
27. Fenton, D. E., Parker, J. M., & Wright, P. V. (1973). Complexes of alkali metal ions with poly(ethylene oxide). *Polymer, 14*, 589. doi:10.1016/0032-3861(73)90146-8.
28. Harris, M. J. (1992). Introduction to biotechnical and biomedical applications of poly(ethylene glycol). In M. J. Harris (Ed.), *Poly(ethylene glycol) chemistry: biotechnical and biomedical applications* (pp. 1–14). New York: Plenum Press.
29. Juang, R. S., & Chen, M. N. (1996). Measurement of binding constants of poly(ethyleneimine) with metal ions and metal chelates in aqueous media by ultrafiltration. *Industrial and Engineering Chemistry Research, 35*, 1935–1943. doi:10.1021/ie950551b.
30. Maketon, W., Zenner, C., & Ogden, K. L. (2008). Removal efficiency and binding mechanisms of copper and copper-EDTA complexes using polyethyleneimine. *Environmental Science & Technology, 42*, 2124–2129. doi:10.1021/es702420h.
31. Rongved, P., & Klaveness, J. (1991). Water-soluble polysaccharides as carriers of paramagnetic contrast agents for magnetic resonance imaging: synthesis and relaxation properties. *Carbohydrate Res, 214*, 315–323. doi:10.1016/0008-6215(91)80038-O.
32. Kulakov VN, Khokhlov VF, Goltyapin YV, Nikitin SM, Sergeev PV, Shimanovsky NL, Panov VO, Bolotova EN, Klimova TP (2000) Magnetic resonance contrast composition. Patent RU 2150961.
33. Philippova, O. E., Kuchanov, S. I., Topchieva, I. N., & Kabanov, V. A. (1985). Hydrogen bonds in dilute solutions of poly(ethylene glycol). *Macromolecules, 18*, 1628–1633. doi:10.1021/ma00150a018.
34. Merbach, A. E., & Tóth, È. (2001). *The chemistry of contrast agents in medical magnetic resonance imaging*. Chichester: John Wiley & Sons.
35. Botta, M., & Tei, L. (2012). Relaxivity enhancement in macromolecular and nanosized Gd^{III}-based MRI contrast agents. *European Journal of Inorganic Chemistry, 2012*, 1945–1960. doi:10.1002/ejic.201101305.