Core-shell fluorinated methacrylate nanoparticles with Rhodamine-B for
confocal microscopy and fluorescence correlation spectroscopy applications

Maciej Jarzebski, Yingze Zhang, Tomasz Sliwa, Jarosław Mazuryk, Tobiasz Deptula,
Malgorzata Kucinska, Marek Murias, Johan Buitenhuis, Jacek Gapiński, Adam Patkowski

Corresponding author

Address: Maciej Jarzebski, NanoBioMedical Centre, Adam Mickiewicz University, Umultowska 85, 61-614 Poznań, Poland. E-mail address: maciej@amu.edu.pl, phone no. +48 535 255 775, +48 618 296016

Formal publication: http://dx.doi.org/10.1016/j.jfluchem.2016.01.014

© 2016. This manuscript version is made available under the CC-BY-NC-ND 4.0 license
http://creativecommons.org/licenses/by-nc-nd/4.0/
Abstract

There is a need to develop nanosized and submicron sized stable and efficient fluorescent particles for confocal microscopy and fluorescence correlation spectroscopy, which are useful methods to study dynamics and interactions in complex colloidal systems and living cells. Fluorescent core-transparent shell fluorinated polymer nanoparticles exhibit low refractive index, close to that of water, high fluorescence efficiency, high stability and no cytotoxicity which makes them excellent probes for water based and living systems. Fluorescent core – transparent shell submicron particles were synthesized using 1H,1H-Heptafluoro-n-butyl methacrylate (HFBMA) as a monomer and Rhodamine-B-isothiocyanate as a dye. The fluorescent polymeric core was surrounded by a non-fluorescent shell obtained by the seeded growth synthesis. The spherical shape of the particles was confirmed by scanning electron microscopy and the particle size in suspension was characterized by dynamic light scattering (DLS) and fluorescence correlation spectroscopy (FCS). The effect of different reaction parameters, such as initiator concentration and polymerization time on the particle size and charge density as well as their toxicity were studied. The smallest nanoparticles, of diameter $d = 240$ nm for the core measured by DLS, were obtained with higher initiator concentration. Repeated confocal imaging, DLS and FCS measurements after a few months up to two years confirmed high fluorescence efficiency, stability and usefulness of prepared particles as versatile systems for confocal microscopy and fluorescence correlation spectroscopy studies in water-based colloidal suspensions. The cytotoxicity tests confirmed a possible use of these nanoparticles also in living cells.

Keywords: fluorinated fluorescent particles; core-shell polymers; laser scanning microscope; colloids interactions modeling; optically transparent polymers, toxicity tests.
Highlights:
- Fluorescent fluorinated polymer particles from 1H,1H-Heptafluoro-n-butyl methacrylate monomer with covalently bonded Rhodamine-B as a fluorescent dye were made.
- Emulsion polymerization resulted in particles with a hydrodynamic radius ranging from 120 to 291nm, determined by DLS and FCS.
- Spherical shape was confirmed by TEM, SEM and LSM images. The fluorescent behavior was proved by LSM and FCS.
- No toxic effects occurred on tested cell lines.

1. Introduction

New nanoparticles (NPs) with defined properties such as size [1,2], shape and electric charge [3] are needed in many fields of nanotechnology. These NPs may be pH-sensitive [4], biocompatible [5,6] and fluorescent and may be functionalized by specific reactive groups at the surface [7]. Among the reported work on complex systems based on functionalized nanoparticles [8,9] the most significant systems include NPs with a biocompatible shell. For example, toxic reagents or drugs are covered by a biocompatible shell to reduce the negative effects for living organism [10] in medical applications [11]. There are two major types of core-shell submicron nanoparticle systems: (i) NPs in which the core and the shell (or shells) are made from different materials, i.e. magnetic core (iron oxide) and biopolymer shell [12], and (ii) NPs in which the same material is used for the core and shell, i.e. fluorescent silica particles [1,2,4].

Modified polymers as well as quantum dots (QD) are the most frequently investigated types of fluorescent nanoparticles [14]. Polymeric materials are the most versatile systems due to the possibility of tuning their hydrophobic/hydrophilic properties and excellent fluorescence efficiency. Fluorescent nanoparticles often present reduced photobleaching and longer emission lifetimes than the free organic fluorophores. The seed growth is the mechanism of the size increase of polymer nanoparticles [13]. In the seed-growth process a drug or a fluorescent dye can be incorporated between the core and the shell [7]. Furthermore, lower
toxic effects are observed for labeled nanoparticles than for classical fluorophores. That makes the polymeric fluorescent nanoparticles a useful probe for studies of dynamics and interactions in colloidal and drug delivery systems [14,15] or in diagnostics applications [16-19]. Several techniques of dye incorporation into the polymer NPs were described in literature [15]. Two types of reaction can be distinguished: the pre-polymerization reaction with a fluorescent initiator or a fluorescent monomer and the post-polymerization reaction with a dye-functional reagent.

Fluorescent nanoparticles with defined properties can be used for imaging of cells and colloidal systems using laser confocal scanning microscope (LCSM). The confocal microscopes can recognize structures larger than about 300 nm. Using superresolution microscopy with stimulated emission depletion (STED) lateral resolution of about 40 to 50 nm can be obtained. For confocal microscopy imaging applications it is essential to incorporate a high number of dye molecules into the polymer structure. On the other hand, for the studies of particle interactions in complex systems it is crucial to observe and recognize each single particle. In this case it is preferable to use core-shell systems, where the dye is incorporated deeply into the particles core and shell is optically transparent. The advantages of such system are protection of the dye and low photobleaching effect even with higher laser beam power. Fluorinated polymeric materials are characterized by a low refractive index, close to that of water, which results in low scattering and reasonable optical transparency even for suspensions of relatively large NPs [20, 21]. Recently, some fluorinated NPs were reported with unique physical properties [22]. Fluorinated compounds also found some medical applications [A23,B24].

In this paper we present the results of the preparation and characterization of fluorinated fluorescent polymer nanoparticles and we show that these NPs are versatile systems for confocal laser scanning microscopy and fluorescence correlation spectroscopy studies of complex systems and living cells.
2. Materials and methods

2.1. Chemicals

1H,1H-Heptafluoro-n-butyl methacrylate (HFBMA) 97% was purchased from SynQuest Labs, a crosslinker ethylene glycol dimethacrylate (EGDMA) 98%) was purchased from Acros Organics. Sodium dodecyl sulfate (SDS) 99%, Rhodamine-B-isothiocyanate, allylamine 99.5% and methanol (99.8%) were purchased from Sigma-Aldrich and used without further purification. The initiator solution was prepared by dissolving 0.078 g of sodium bisulfite and 0.231 g potassium persulfate in 100 ml of deionized water (Milli-Q). The initiator concentration was given as the molar concentration of potassium persulfate and was adjusted by adding the proper amount of this initiator solution.

2.2. Particle preparation

2.2.1 Synthesis of fluorescent core

The polymerizable fluorescent dye was prepared by dissolving 12 µL of allylamine and 42.9 mg of Rhodamine-B-isothiocyanate in 1 mL of methanol for 1 h inside the oxygen and water free glovebox. The monomer HFBMA (30 ml) containing 10 wt.% of EGDMA was destabilized by washing it three times with 1M NaOH solution and two or three times with deionized water. For the core particle synthesis the 0.069 g SDS was dissolved in 300 mL of deionized water using a magnetic stirrer at 1200 rpm and maintaining a nitrogen flow over the liquid. Meanwhile the destabilized monomer was added with the crosslinker and the
fluorescent dye to the SDS solution in water at the concentration given above. The intensive stirring was continued while the reaction mixture was heated up to 73°C (slightly adjusted conditions from Ref. [20]). The Rhodamine-B caused an intensively dark pink color of the solution. To obtain a molarity of the persulfate ranging from $1.26 \cdot 10^{-4}$ to $1.97 \cdot 10^{-3}$ M between 5 and 100 mL of the initiator solution was added to the reaction mixture. A schematic diagram of a free-radical reaction is shown in Fig. 1. After a few minutes it was easy to follow the progress of a reaction, because the clear dark pink liquid turned to turbid pink (Fig. 2).

![Figure 2. Three steps in the synthesis of core-shell HFBMA. From left to right: monomer in the emulsion with RBITC (clear dark pink), after initiator addition (turbid pink), particles with shell (turbid light pink)](image)

The polymerization reaction was carried out for 12, 24 and 48 hours at 73°C. After each time period one third of the mixture was taken out from the flask and filtered through a filter paper to remove coagulum (by-product). Each time the by-product from the stirrer was removed. The reaction mixture of the core was filtrated through a double paper filter. The “dry” polymer concentration of the cleared filtrate was obtained by drying 0.5 mL of seed dispersion at 100°C for 2 hours, using aluminum dishes which were pre-dried for 1 hour at 300°C.

2.2.2 Synthesis of transparent shell

The second step of the particle preparation was to cover the fluorescent core by a transparent shell. The concentration of the seed dispersion (“dry” polymer) as determined above was always adjusted to 0.8wt.% for all seeded growth reactions. From this seed dispersion 300 mL was added to a three-necked round bottom flask and heated to 73°C under magnetic stirring at 1200 rpm and a nitrogen flow was maintained over the liquid. After
reaching 73°C, 2 mL of initiator solution was added, as described before. A few minutes later the addition of the monomer was started. For shell preparation 16 mL of destabilized monomer containing 10wt.% of the crosslinker was used. The monomer was added dropwise, using a syringe pump (with a speed of 4 mL per hour). The solution was stirred at 300 rpm. After addition of the whole amount of the monomer, the reaction mixture was stirred for 12 – 48 hours. Thereafter the whole solution was filtered through a paper filter.

Note that here we present results for shells prepared in one seeded growth step, but multiple seeded growth steps are also possible [20].

2.3. Characterization methods

The hydrodynamic radius of the particles was determined by dynamic light scattering (DLS), directly after the synthesis and for selected samples also after a couple of months. One droplet of the solution was diluted in 10 mL of deionized water (Millipore) prefiltered through a membrane filter with a pore size of 200 nm. DLS measurement were performed on a setup from ALV-Laservertriebgesellschaft (Langen, Germany) equipped with a Helium-Neon laser (632.8 nm) at 20°C (±0.1°C). The light scattering autocorrelation functions were obtained for scattering angles between 20° and 150° with an angular step of 5°. The diffusion coefficients were obtained directly from the autocorrelation functions by using CONTIN algorithm for multi-exponential decay analysis [25], built into the ALV-Correlator software (ver. 3.0). Due to the large size of studied NPs, their form factors were taken into account in the analysis and the solutions with the number average hydrodynamic radius were selected. The diffusion coefficient \( D \) is related to the particles hydrodynamic radius via the Stoke-Einstein relation:

\[
R_n = \frac{k_B T}{6\pi \eta D}, \quad R_n = \frac{\sum n_i R_i}{\sum n_i},
\]

where \( R_n \) is the number average hydrodynamic radius, \( n_i \) and \( D_i \) are the number concentration and radius of fraction \( i \), \( k_B T \) is the thermal energy at temperature \( T \) and \( \eta \) is the viscosity. More thorough details on DLS experiment are described by Berne and Pecora [26].
Imaging and structure analysis were performed using a scanning electron microscope (SEM) from Jeol, model 7001TTLS. The maximum acceleration voltage was 30 kV and the maximum resolution of the measuring system amounted to 1.5 nm.

Confocal images in water solutions were made, using an Olympus IX71 microscope with Fluoview-FV300 Laser Scanning Confocal System and a Zeiss Laser Scanning Microscope LSM780. For measurements slide channels (1 µ-Slide from Ibidi) were filled up. Dry samples were investigated using Olympus IX81, FV 1000 confocal microscope and a CCD camera. During preparation of dry samples, one droplet of solution was put into microscopic glass chamber (chambered coverglass 8 WELL from LAB-TEK) and dried for a few hours in air.

The electrophoretic mobility was measured using a Zetasizer 2000 from Malvern Instruments on highly diluted samples (0.002M TRIS solution). Estimated zeta potentials were calculated using the Smoluchowski approximation.

**Cytotoxicity assay**

Dulbecco's modified Eagle's medium with phenol red (DMEM), 0.25% Trypsin EDTA solution, phosphate buffered saline (PBS), Dimethyl sulfoxide (DMSO), methylthiazolydiphenyl-tetrazolium bromide (MTT) were obtained from Sigma Aldrich (St. Louis, Mi, USA). Penicillin-streptomycin and L-glutamine solution was obtained from Life Technologies (Grand Island, NY, USA). Dimethyl sulfoxide was obtained from POCh, S.A. (Avantor Performance Materials Poland S.A, Gliwice, Poland).

The two cell lines: human lung adenocarcinoma cancer cell line A549 and normal human fibroblast cell line CCD39Lu, were used for determining the cytotoxic activity of tested nanoparticles. Cell lines were obtained from European Collection of Cells Cultures (ECACC, Salisbury, UK) and cultured in DMEM medium containing 10% of FBS and supplemented with 1% of L-glutamine/penicillin/streptomycin solution. The cells were cultured at temperature 37°C, in a humidified atmosphere containing 5% CO₂.

The cytotoxic effect of selected samples was investigated using the MTT assay. In this assay, the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), a water soluble tetrazolium salt, is converted to an insoluble purple formazan by succinate
dehydrogenase in the mitochondria of viable cells. The formazan is dissolved in an appropriate solvent and analyzed spectrophotometrically (1).

Cells were seeded on 96 well plates at density 2x10^4 cells per well and incubated overnight under cell culture condition. Then, the tested nanoparticles were added at dilution 1:10; 1:20 and 1:40 in culture medium. The sterile water was used as a control. Cells were incubated 24 h and subsequently, the MTT assay was performed. Plates were washed twice with PBS and 170 µl MTT solution (5 mg/ml in PBS) was added to each well and incubated for 1.5 hours. After incubation the plates were centrifuged 3 minutes (1200 rpm, at room temperature) and formazan was extracted with 200 µL DMSO. The absorbance was measured at 570 nm with plate reader (Biotek Instruments, Elx-800). Cell viability was calculated as a percentage of the control.

The results are presented as the mean±SD from two experiments. The values were calculated using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA. Data were compared for statistical significance by the Dunnett’s Multiple Comparison Test, a probability value p< 0.05 was considered significant.

3. Results and discussion

We present results for shells prepared in one seeded growth step. In fact, during the synthesis, it was easy to see the increased turbidity as a first sign of the particle formation. Apart from that, also differences between seeds and core-shell particles dispersions can be observed easily by eye. We also note that after sedimentation of the particles during storage; the supernatant was almost colorless, which indicates that almost all Rhodamine B has been built into the polymer.
Figure 3: Typical SEM pictures of core-shell HFBMA particles at different magnifications. In all pictures the scale bar represents 1 µm.

The SEM pictures of different samples confirmed the spherical shape of core-shell fluorescent HFBMA particles (Fig.3). In Fig. 3D it can be seen that the core-shell particles are quite polydisperse. It may suggest that not all particles have a shell and it is possible that a second nucleation process occurred in some cases, as well.

Table 1: The values of the average particle hydrodynamic radius ($R_n$), initiator concentration, electrophoretic mobility ($\mu$) and zeta potential $\zeta$ for the core and core-shell nanoparticles

<table>
<thead>
<tr>
<th>Core (shell)</th>
<th>Synthesis time [h]</th>
<th>Initiator concentration [M·dm$^{-3}$]</th>
<th>$R_n$ [nm]</th>
<th>$\Delta R_n / R_n$</th>
<th>$\mu$ [µm·cm·s$^{-1}$·V$^{-1}$]</th>
<th>$\zeta$ [mV]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core (H10)</td>
<td>12</td>
<td>$1.97 \cdot 10^{-3}$</td>
<td>94</td>
<td>0.20</td>
<td>-5.59</td>
<td>-71.2</td>
</tr>
<tr>
<td>Core-shell (H23)</td>
<td>24</td>
<td>$5.36 \cdot 10^{-5}$</td>
<td>120</td>
<td>0.20</td>
<td>-5.85</td>
<td>-74.5</td>
</tr>
<tr>
<td>Core-shell (H25)</td>
<td>48</td>
<td>$5.36 \cdot 10^{-5}$</td>
<td>158</td>
<td>0.30</td>
<td>-5.48</td>
<td>-69.8</td>
</tr>
<tr>
<td>Core (H22)</td>
<td>48</td>
<td>$1.11 \cdot 10^{-3}$</td>
<td>160</td>
<td>0.1</td>
<td>-4.72</td>
<td>-60.0</td>
</tr>
<tr>
<td>Core (H14)</td>
<td>12</td>
<td>$1.26 \cdot 10^{-4}$</td>
<td>163</td>
<td>0.14</td>
<td>-5.01</td>
<td>-63.8</td>
</tr>
<tr>
<td>Core-shell (H26)</td>
<td>12</td>
<td>$5.36 \cdot 10^{-5}$</td>
<td>275</td>
<td>0.15</td>
<td>-5.33</td>
<td>-67.8</td>
</tr>
<tr>
<td>Core-shell (H27)</td>
<td>24</td>
<td>$5.36 \cdot 10^{-5}$</td>
<td>270</td>
<td>0.15</td>
<td>-5.68</td>
<td>-72.3</td>
</tr>
</tbody>
</table>
Table 1 shows the values of the hydrodynamic radius ($R_n$) and the polydispersity $\Delta R_n/R_n$ obtained from the CONTIN analysis of the DLS data, electrophoretic mobility ($\mu$) and zeta potential ($\zeta$) for uniformly labeled core and labeled core nonlabeled shell core–shell particles. As the naming convention for samples was rather trivial (letter “H” + synthesis number), the numbers in samples’ descriptions have no relation to their properties. In samples H23 and H22 a small amount (less than 20% by weight) of smaller NPs of size in the range of 20 – 30 nm was detected. The increase in the particle radii for the core-shell particles demonstrates a successful seeded growth reaction. In Table 1 we present the zeta potential and electrophoretic mobility for several core and core–shell particles. The zeta potential of the core and core–shell particles ranging from -60.0 to -74.5 mV indicates a relatively high charge of the NPs resulting in a high stability of the system. The $R_n$ values of the core are from 94 nm to about 160 nm, while the $R_n$ values of the core–shell particles are in the range of 120 – 275 nm. The dependence of the hydrodynamic radius of the fluorescent core particles on the initiator concentration and the polymerization time is shown in Fig. 4.

![Figure 4. Dependence of the hydrodynamic radius of the fluorescent core particles on the initiator concentration and the polymerization time.](image)

The particle size decreases with increasing initiator concentration. Slight changes in concentration (from $1.11\cdot10^{-3}\text{ mol}\cdot\text{dm}^{-3}$ to $1.97\cdot10^{-3}\text{ mol}\cdot\text{dm}^{-3}$) have a major impact on $R_n$ values (a decrease by about 60 nm). The smallest value of concentration of the initiator
which should be used for the fluorescent core synthesis was found to be $1.26 \cdot 10^{-4}$ mol·dm$^{-3}$. The same $R_n$ values (about 160 nm) were obtained for both $1.11 \cdot 10^{-3}$ mol·dm$^{-3}$ and for 10 times lower concentrations of the initiator ($1.26 \cdot 10^{-4}$ mol·dm$^{-3}$). At even lower initiator concentration $1.28 \cdot 10^{-5}$ mol·dm$^{-3}$ no nanoparticles were formed even after 48 hours, which confirms that there is a minimum initiator concentration needed for particle formation. The hydrodynamic radius obtained after 12, 24 and 48 hours with different concentrations of initiator, do not show any significant variation, therefore we conclude that particle formation finishes within 12 hours. The hydrodynamic radius of the NPs measured by means of DLS did not change over a long time up to two years. It is important to stress that all the NPs are quite monodisperse with $\Delta R_n/R_n$ in the range of $0.1 – 0.2$.

Examples of bright field and confocal microscopy images of the fluorescent fluorinated beads are given in Fig. 5. In order to obtain sharp images, only sedimented samples were measured. It may create an impression that the particles easily aggregate, which is not true. Even gentle mixing results in a well resuspended sample, which is corroborated by the DLS

Figure 5. Pictures of sedimented HFBMA particles: A (bright field), B (epi-fluorescence) microscopy (CCD camera, objective 100x/NA 1.4), C-D LSM 2-D scans.
and FCS results. In Fig. 5A and 5B images from a CCD camera in bright field and fluorescent mode, respectively, were presented. Figs. 5C-D present typical images resulting from a two-dimensional confocal LSM scan of HFBMA in water. Doing a z-scan (or three-dimensional imaging) some sedimentation process was observed, which is a result of the high density of the particles of about 1.6 g·mL⁻¹ [20]. The individual HFBMA particles can be easily distinguished even at high concentration. Therefore, the observation of the single particle behavior, possibly with core-shell structures, predisposes them as a model system for studying interactions in colloidal systems (e.g. living cells). An image of a fluorescent gelatine microgel droplet with incorporated HFBMA particles is presented in Fig. 6. The HFBMA beads can be easily distinguished despite the extremely low laser intensity of 0.002 % of the maximum value, while the values used in standard cell imaging are of the order of 1 %. The use of low power laser is recommended during studies conducted on living cells, as well as is in the system where fluorescent substances or dyes exhibit fast bleaching.

Figure 6. LSM image of HFBMA particles immersed in a gelatine microgel structure (microgel – yellow, HFBMA particles – pink)

The most meaningful way to characterize the fluorescence efficiency of the HFBMA NPs is to compare their counts per molecule (CPM) to that of a standard dye (Rhodamine B), possibly at the same incident laser beam intensity. The CPM value can be obtained from the
correlation functions (CFs) measured in fluorescence correlation spectroscopy (FCS) experiments. FCS measurements were performed for several HFBMA NPs of different structure (uniformly labeled and fluorescent core – non fluorescent shell NPs) and different radius in the range from 94 to 270 nm. Since the fluorescence efficiency of the HFBMA NPs is very high, we used the Zeiss ConfoCor3 instrument in a less efficient configuration with the long working distance objective Olympus LUCPLFL 40x/0.6 [27] and a very low power of the exciting diode laser ($\lambda = 561$ nm) of 0.01% of the total power (about 10 mW), in order to avoid the saturation of the detector. A laser power of 3.5% was used in the same setup for Rhodamine B. Typical CFs measured for the uniformly labeled (H22) and core-shell (H25, H27) HFBMA NPs are shown in Fig. 7.

The CFs were analyzed using the standard form:

$$G(t) = 1 + \frac{1}{N} \left( 1 + \frac{T}{1 - T} e^{-t/\tau} \right) \left( 1 + t/\tau \right)^{-1} \left( 1 + t/(\tau c^2) \right)^{-1/2},$$

(2)
where $T$ is the so called triplet fraction and $\tau_T$ is the characteristic time of the triplet contribution, $N$ – the number of particles in the confocal volume, $\tau$ - the diffusion time of NPs and $c = \sigma_z/\sigma_x$ is the aspect ratio of the Gaussian confocal volume. The value of CPM was obtained by dividing the total CR by N. The values of the correlation times, count rates (CR), count rates per molecule (CPM) and the laser power used to measure the CFs with the Olympus LUCPLFL 40x/0.6 objective are given in Table 2. The values of the hydrodynamic radius were calculated from the correlation times using the correction for particles of sizes comparable with the confocal volume radius $\sigma_x$ [28].

Table 2. The correlation times, count rates (CR), count rates per molecule (CPM) and the laser power used to measure the CFs with the Olympus LUCPLFL 40x/0.6 objective.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\tau$ [µs]</th>
<th>CR [kHz]</th>
<th>CPM [kHz]</th>
<th>Power [%]</th>
<th>$R_h$(core/shell) [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>RhB</td>
<td>103</td>
<td>7.87</td>
<td>1.90</td>
<td>3.5</td>
<td>0.537</td>
</tr>
<tr>
<td>H22</td>
<td>32600</td>
<td>25.0</td>
<td>158</td>
<td>0.01</td>
<td>150</td>
</tr>
<tr>
<td>H25</td>
<td>29960</td>
<td>34.6</td>
<td>155</td>
<td>0.01</td>
<td>95/150</td>
</tr>
<tr>
<td>H27</td>
<td>57350</td>
<td>30.2</td>
<td>155</td>
<td>0.01</td>
<td>153/270</td>
</tr>
</tbody>
</table>

The values of the CPM obtained in this way for the HFBMA NPs were about $2.8\times10^4$ times higher than the CPM for Rhodamine B, normalized to the same laser power. A similar comparison of the CPMs on a more efficient FCS setup equipped with the immersion objective Olympus UplanSApo 60x/1.3 Sil resulted in a ratio of $9\times10^4$. Thus, all the synthesized HFBMA NPs are characterized by a very high fluorescence efficiency which remained constant even after two years.

For selected particles Cytotoxicity assay were performed. The obtained data suggest that tested nanoparticles did not exert the cytotoxic activity (Table 3). The slight but statistical significant decrease of cancer cell A549 viability was observed after incubation with particles with diameter 456nm in all tested dilution. In the case of CCD39Lu the increase of
proliferation was observed for particle cores \( d = 340 \text{ nm} \) and \( d = 291 \text{ nm} \) in all tested dilution.

The cytotoxicity tests confirmed possible use of synthesized fluorescent particles for the investigations and modeling of interactions in colloidal or cells systems.

Table 3. The cell viability of A549 and CCD39Lu cell lines after treatment with core and core-shell HFBMA particles using MTT assay. The reference concentration was 5 %. Statistical significance is marked with an asterisk (*) \( p < 0.05 \), (**) \( p < 0.01 \); (***) \( p < 0.001 \). Statistical significance between groups was assessed by Dunnett’s Multiple Comparison Test.

<table>
<thead>
<tr>
<th>Nanoparticles type and diameter [nm]</th>
<th>Dilution factor</th>
<th>Cell viability [% of control]</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A549</td>
<td>CCD39Lu</td>
</tr>
<tr>
<td>Core</td>
<td>1:10</td>
<td>103.1±7.3</td>
<td>115.8±14.9*</td>
</tr>
<tr>
<td></td>
<td>1:20</td>
<td>102.1±8.2</td>
<td>103.9±10.7</td>
</tr>
<tr>
<td></td>
<td>1:40</td>
<td>97.9±7.5</td>
<td>108.3±17.3</td>
</tr>
<tr>
<td>Core</td>
<td>1:10</td>
<td>91.2±9.4</td>
<td>126.1±9.2***</td>
</tr>
<tr>
<td></td>
<td>1:20</td>
<td>94.1±12.5</td>
<td>125.5±7.5***</td>
</tr>
<tr>
<td></td>
<td>1:40</td>
<td>99.6±7.8</td>
<td>129.5±8.2***</td>
</tr>
<tr>
<td>Core-shell</td>
<td>1:10</td>
<td>104.6±6.4</td>
<td>123.9±8.9***</td>
</tr>
<tr>
<td></td>
<td>1:20</td>
<td>105.5±6.2</td>
<td>116.2±7***</td>
</tr>
<tr>
<td></td>
<td>1:40</td>
<td>103.8±65</td>
<td>110.4±5.8*</td>
</tr>
<tr>
<td>Core-shell</td>
<td>1:10</td>
<td>85.4±3.9***</td>
<td>107.7±5.5</td>
</tr>
<tr>
<td></td>
<td>1:20</td>
<td>90.8±5.3*</td>
<td>110.9±6.4*</td>
</tr>
<tr>
<td></td>
<td>1:40</td>
<td>91.7±4.6*</td>
<td>109.5±6.3</td>
</tr>
</tbody>
</table>
4. Conclusions

We synthesized fluorescent fluorinated polymer particles from 1H,1H-Heptafluoro-n-butyl methacrylate monomer with covalently bonded Rhodamine-B as a fluorescent dye. In addition, by using a seeded growth reaction these fluorescent core particles were coated with the non-fluorescent polymer, resulting in particles with a fluorescent core and a non-fluorescent shell. The polymer particles growth in the emulsion polymerization process is finished within 12 hours. The $R_n$ values of the number average hydrodynamic radius of the fluorescent core determined by the DLS varied between 94 and 163 nm and the polydispersity (PDI) was in the range of 0.1-0.2, decreasing with increasing size of the NPs. The core-shell particles are bigger by about 60 nm or more than the bare core. Spherical shapes of the NPs was demonstrated in the SEM images. Fluorescent behavior of the particles was confirmed in confocal laser scanning microscope imaging and FCS investigations. The stability of the particles was proved by repeating DLS and LSM tests after two months and after up to two years. The high fluorescent efficiency of the HFBMA particles makes them a very useful and versatile system for LSM imaging and FCS studies of the structure and dynamics in colloidal systems. The cytotoxicity tests confirmed a possible use of these nanoparticles also in living cells.

Acknowledgements

Authors thank prof. dr. Minne Paul Lettinga and dr. Peter Lang from Forschungszentrum Jülich, Institute of Complex Systems for help during synthesis and many fruitful discussions.

Maciej Jarzebski, Tobiasz Deptuła and Jarosław Mazuryk thank NanoBioMedical Centre in Poznan, and Faculty of Physics Adam Mickiewicz University for support by the International PhD Projects Programme of Foundation for Polish Science operated within the Innovative Economy Operational Programme (IE OP) 2007-2013 within European Regional Development Fund.

Authors gratefully acknowledge partial financial support from the National Centre for Research and Development under research grant "Nanomaterials and their application to biomedicine" PBS1/A9/13/2012.
References:


doi:10.1021/la010181y


dx.doi.org/10.1021/la5015708