GRAFTING OF MAGNETIC PARTICLES WITH POLY(2-ISOPROPENYL-2-OXAZOLINE)

CVEK Martin[1,2], MRLIK Miroslav[1], ILCIKOVA Marketa[3], MOSNACEK Jaroslav[3], KRONEK Juraj[3], SEDLACIK Michal[1]

[1]Centre of Polymer Systems, University Institute, Tomas Bata University in Zlin, Czech Republic, EU

[2]Polymer Centre, Faculty of Technology, Tomas Bata University in Zlin, Czech Republic, EU

[3]Polymer Institute, Slovak Academy of Sciences, Bratislava, Slovakia, EU

Abstract

Magnetic particles play an important role in modern biomedical applications including targeted drug delivery, local embolization of blood veins, hyperthermia etc. Therefore, the development of more effective systems with high biocompatibility is of interest for many researchers. Nevertheless, these magnetic systems have to meet certain criteria necessary for *in vivo* applications. We have considered key requirements desired from such materials, and we have prepared a promising system based on core-shell particles via surface-initiated atom transfer radical polymerization (ATRP). The finest grade of the carbonyl iron particles was used as a suitable core and the treatment of its surface in acidic environment ensured the presence of hydroxyl groups, which were further coupled with ethoxy groups of (3-Aminopropyl)triethoxysilane. After the functionalization, the immobilization of 2-Bromoisobutyryl bromide, which served as an initiator, was performed.

Finally, the initiator-treated particles were grafted with poly(2-isopropenyl-2-oxazoline) (PIPOx) under ATRP conditions as the PIPOx has recently shown a great potential in biomedical applications. The cleaning and washing procedures ensured high purity of the product. The reaction conversion, molar mass and dispersity of PIPOx grafts were investigated using nuclear magnetic resonance and gel permeation chromatography, respectively. The presence of grafted PIPOx was confirmed using Fourier-transform infrared, and energy-dispersive X-ray spectroscopies. The grafted PIPOx layer had negligible effect on particle magnetization as revealed via vibration-sample magnetometry. Synthesized core-shell structures may find utilization as a promising material for local embolization or may serve as a drug delivery system due to the presence of PIPOx bearing the active sites allowing the drug bonding.

Keywords: Carbonyl iron, surface modification, atom transfer radical polymerization, drug delivery, embolization, poly(2-isopropenyl-2-oxazoline)

1. INTRODUCTION

Magnetic particles with nm or μm dimensions have been recognized as promising materials in modern biomedical applications. Considerable research is being directed towards targeted drug delivery systems [1], hyperthermia [2, 3], local embolization of blood veins [4], or cell therapy (cell separation and labeling) [5]. For such systems the particles must satisfy several demands, including particle size and their distribution, which are the key criteria that determine the *in-vivo* distribution, biological fate or toxicity [6]. Magnetic nanoparticles have relatively high cell uptake, higher mobility due to their size when compared to microparticles. However, a limitation in particle size exists when approaching the nanometer range due to decreasing magnetization [7]. Therefore, the application of larger particles (diameter of 0.5-5 μm) with higher magnetization is necessary. As was presented elsewhere, these larger particles may be beneficial especially in drug delivery systems in order to target organs lying deep in the body cavity (8-12 cm from the body surface) [8].

Moreover, the particles used *in-vivo* have to be protected against reticuloendothelial system in order to prevent their degradation [8, 9]. As a suitable approach to enhance stability of the particles is the modification of their surface with various polymeric substances such as polyethylene glycol [10], poly(vinyl alcohol) [11],

dextran [12] etc. Conventional surface modifications are basically tools lacking a possibility to precisely control the thickness of modifying layer in nanometric scale, which can lead to inevitable decrease of particle magnetization. This drawback can be eliminated by the synthesis of core-shell particles via atom transfer radical polymerization (ATRP) that gives access to polymers with precisely controlled molecular weight, relatively low dispersities, defined molecular architecture and diverse functionalities [13]. Recently, we have shown that grafting of poly(glycidyl methacrylate) onto the carbonyl iron (CI) particles via surface-initiated ATRP had negligible effect on particle magnetization, but at the same time exceptionally improved their stability properties and cytotoxicity [14].

This study is focused on the synthesis of the CI particles ATRP-grafted with poly(2-isopropenyl-2-oxazoline) (PIPOx) as this polymer was found to be non-cytotoxic with no significant effect on the cellular immunological parameters [15]. Moreover, PIPOx is of special interest in drug and biomolecule bonding since it is bearing the functional groups, which represent an ideal site for coupling with pharmaceuticals. Thus, the design of multi-functional platform based on the CI particles grafted with PIPOx (CI-*g*-PIPOx) is presented and verified via spectroscopy methods, while the basic requirements such as particle magnetization is investigated, which are necessary first-stage testing characteristics from the application point of view.

2. EXPERIMENTAL

2.1. Materials

Carbonyl iron (CI, powder, HQ grade) particles were used as a suitable magnetic core for the preparation of intended magnetic carrier. Hydrochloric acid (HCl, 35%, p.a.) aqueous solution was employed as a chemical reagent to ensure reactivity of the CI particles. (3-Aminopropyl)triethoxysilane (APTES, ≥98%) provided a functionality, while 2-bromoisobutyryl bromide (BiBB, 98%) served as an initiator linked onto the APTES-treated particles. Initiator bonding was performed in the presence of proton scavenger, triethyleneamine (TEA, ≥99%). 2-isopropenyl-2-oxazoline (IPOx, 99%), ethyl 2-bromoisobutyrate (EBiB, 98%), *N,N,N´,N´´,N´´*-pentamethyldiethylenetriamine (PMDETA, ≥99%), copper bromide (CuBr, ≥99%) and anisole (99%) were used as a monomer, initiator, ligand, catalyst and solvent, respectively. All chemicals were purchased from Sigma Aldrich (USA) and were used without further purification (except for IPOx). IPOx was distilled prior its use in order to remove phenothiazine inhibitor. Tetrahydrofurane (THF, p.a.), acetone (p.a.), ethanol (absolute anhydrous, p.a.), toluene (p.a.), and hydrochloric acid (HCl, 35%, p.a.) were obtained from Penta Labs (Czech Republic). Deionized water (DW) was used during all experimental processes and washing routines.

2.2. The CI particle surface pre-conditioning and functionalization

The surface of the CI particles is under normal conditions covered with a thin oxide layer, which was removed in the first step in order to increase its reactivity. Therefore, the CI particles were treated in aqueous HCl solution similarly as in reference [16]. Subsequently, they underwent thorough cleaning procedures employing DW, ethanol, and acetone as washing agents. The washing process performed via decantation method was accelerated using a permanent magnet at the bottom of the beaker. The APTES was used as a modifier of the CI surface. The molecules of this organosilane are linked at the CI core by covalent Fe-O-Si bonds, while bearing the functionality provided by -NH_2 groups. The freshly-oxidized CI particles were dispersed in toluene and refluxed at elevated temperature, while the calculated amount of APTES was injected into the system [16]. Then, the mixture was let to cool down and thoroughly cleaned in toluene, ethanol and acetone using the accelerated decantation method as descried above.

2.3. Initiator immobilization and synthesis of CI-*g*-PIPOx entities

The presence of amine groups on the CI surface were preferably used to be linked with BiBB molecules. In a typical procedure [14], the functionalized CI particles (40 g), dried THF (100 mL) and TEA (16 mL) were mixed

under argon atmosphere at a temperature of ~5°C ensured by an ice/water bath, while BiBB (8 mL) was drop-wise added. The product was washed with THF (5x100 mL), and acetone (5x100 mL). The residual acetone from as-treated particles was removed by drying the product at mild temperature (40 °C) overnight.

The BIBB-treated particles (10 g) were transferred into Schlenk flask, which was evacuated and backfilled with argon several times. The argon-purged chemicals, namely IPOx (20.00 mL, 190.8 mmol), EBiB (0.2780 mL, 1.908 mmol), PMDETA (1.593 mL, 763.0 mmol), and anisole (XX mL) were gradually added. The presence of oxygen was minimized by degasing the system followed by several freeze-pump-thaw cycles. At a frozen state, the CuBr catalyst (273.9 mg, 1.908 mmol) was added as quickly as possible under gentle argon flow. The molar ratio of reactants [IPOx]:[EBiB]:[CuBr]:[PMDETA] was [100]:[1]:[1]:[4], while anisole served as a solvent in the amount of 50 vol.%. The polymerization process was initiated by immersing the flask into a silicone oil bath pre-heated to 60 °C. The reaction was stopped by opening the flask. The product was purified using anisole (3x50 mL), ethanol (5x100 mL) and finally with acetone (5x100 mL). The final product was dried overnight at 40 °C and stored in a desiccator.

2.4. Characterization

^{1}H nuclear magnetic resonance (NMR) spectra were recorded at 25 °C using an instrument (400 MHz VNMRS Varian, Japan) with deuterated chloroform ($CDCl_3$) as a solvent. The molar mass and polydispersity ($Đ$) of PIPOx chains were investigated using gel permeation chromatography (GPC) on the GPC instrument (PL-GPC220, Agilent, Japan) equipped with GPC columns (Waters 515 pump, two PPS SDV 5 μm columns (diameter of 8 mm, length of 300 mm, 500 Å + 105 Å)) and a Waters 410 differential refractive index detector tempered to 30 °C.

The samples for NMR spectroscopy and GPC analysis were prepared by their dilution with $CDCl_3$ and THF, respectively, followed by the purification process, in which they were passed through a neutral alumina column. The dimensions and morphology of bare CI particles as well as their CI-*g*-POx analogues was examined on the field-emission scanning electron microscope (SEM) (Nova NanoSEM 450, FEI, Japan). The instrument is equipped with an energy-dispersive (EDS) detector, which provided information about elemental composition of the samples. Fourier transform infrared (FTIR) spectra (64 scans, resolution of 4 cm^{-1}) were recorded on a Nicolet 6700 (Nicolet, USA) within a wavenumber range of 4000-600 cm^{-1}, while the attenuated total reflectance technique with a Germanium crystal was employed. The spectra were recorded at room temperature.

The magnetic properties of both particle variants (samples of approx. 150 mg) were investigated in the external magnetic fields in the range of ±15 kOe (±1160 $kA \cdot m^{-1}$) using a vibrating-sample magnetometer (VSM, Model 7404, Lake Shore, USA) at laboratory conditions. The amplitude of the vibration was set to 1.5 mm, while the vibration frequency was 82 Hz.

3. RESULTS AND DISCUSSION

Once the particles are intravenously administered into a living organism, they can be recognized by the host immune system and cleared by phagocytes from the circulation [6]. Therefore, the knowledge of modifying layer properties on molecular level has significant importance. The attention was paid to analyze the weight- (M_W) and number-average (M_n) molecular weights, and $Đ$ of PIPOx grafts. As revealed by GPC the aforementioned quantities were 2450 and 2600 $g \cdot mol^{-1}$, while $Đ$ equaled 1.07 implying the uniformity of grafted chains. The polymerization was carried out 2 hours, and its conversion was determined by NMR to be 21%.

The **Figure 1** presents the surface morphology of bare CI particles and their PIPOx-grafted analogues. The former exhibited quite smooth surfaces, while the latter exhibited rather rougher surface due to the presence of PIPOx. This distinctive difference in morphology indicates the successful grafting process. Further, both samples showed similar particle dimensions proving the theoretical assumption of PIPOx thickness in

nanometric scale. The EDS analysis clarified the surface chemical states of both materials. The bare CI particles contained mainly iron (~92.7 wt.%), while the small inclusion of carbon (~5.0 wt.%) and oxygen (~2.3 wt.%) can be attributed mainly to contaminants. Admittedly, the CI-*g*-PIPOx particles were also mainly consisted of iron (~90.5 wt.%), however increased amount of carbon (~5.4 wt.%) and oxygen (~2.5 wt.%), and the presence of expected PIPOx-related element such as and nitrogen (~1.6 wt.%) emphasized the hypotheses of ATRP-modified material.

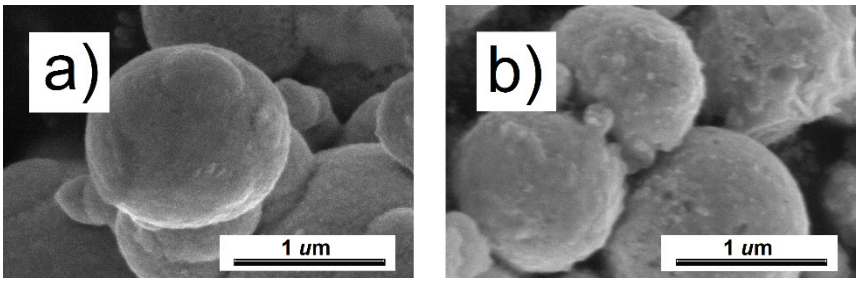

Figure 1 SEM micrographs of bate CI particles (*a*) and their PIPOx-grafted analogues (*b*)

The presence of the PIPOx grafts on the CI surface was verified also via FTIR spectroscopy. This method was used to monitor the individual steps in the particle synthesis including the initiator-treated CI. As seen in **Figure 2**, the differences in the spectra of studied materials were clearly distinguishable. In the spectrum of bare CI particles no obvious absorption peaks were detected. On the contrary, the initiator-treated particles exhibited increased absorption bands around 1219 and 1056 cm^{-1}, which were associated with the presence of O-Si bending and C-O stretching, respectively. An obvious peak at 741 cm^{-1} was a sign of C-Br group originating from the initiator. Finally, the spectrum of the CI-*g*-PIPOx entities was characterized by enhanced absorption levels around 3000 cm^{-1}, which reflected the presence of CH_3 and CH_2 groups. Additionally, the sharp peak at 1650 cm^{-1} was assigned to be a C=N group from oxazoline ring, while evident peaks at 1011, 984 cm^{-1} and finally 936 cm^{-1} represented typical absorption bands of oxazoline ring. To conclude, the FTIR was used as a complementary method to EDS in order to prove the presence of PIPOx grafts. Based on the results it can be asserted that the synthesis of CI-*g*-PIPOx particles can be considered as successful.

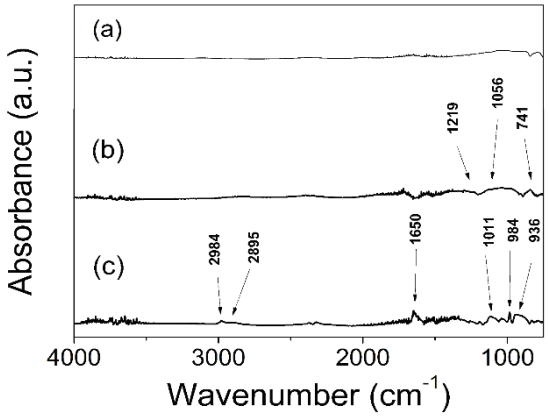

Figure 2 FTIR spectra of bare CI particles (*a*), initiator-treated particles (*b*) and their PIPOx-grafted analogues (*c*) with denoted characteristic wavenumbers

As seen in the **Figure 3**, both investigated particle types exhibited the typical characteristics of soft highly magnetic materials. Comparing the VSM spectra, it can be noticed that the magnetization of CI-*g*-PIPOx particles was slightly lower than in the case of original material. However, the decrease was quantified to be only ~2.5% at high fields (~1160 $kA \cdot m^{-1}$) when magnetization approaches the saturation state. The coercivity of the particles remained almost unchanged indicating fast demagnetization processes. In general, the

magnetic behavior of both materials was almost indistinguishable. Thus, the preserved magnetization of CI-*g*-PIPOx particles indicates that modified systems after other necessary tests may find utilization in the development of drug delivery systems with particular intention reaching the organs lying deep in the body cavity.

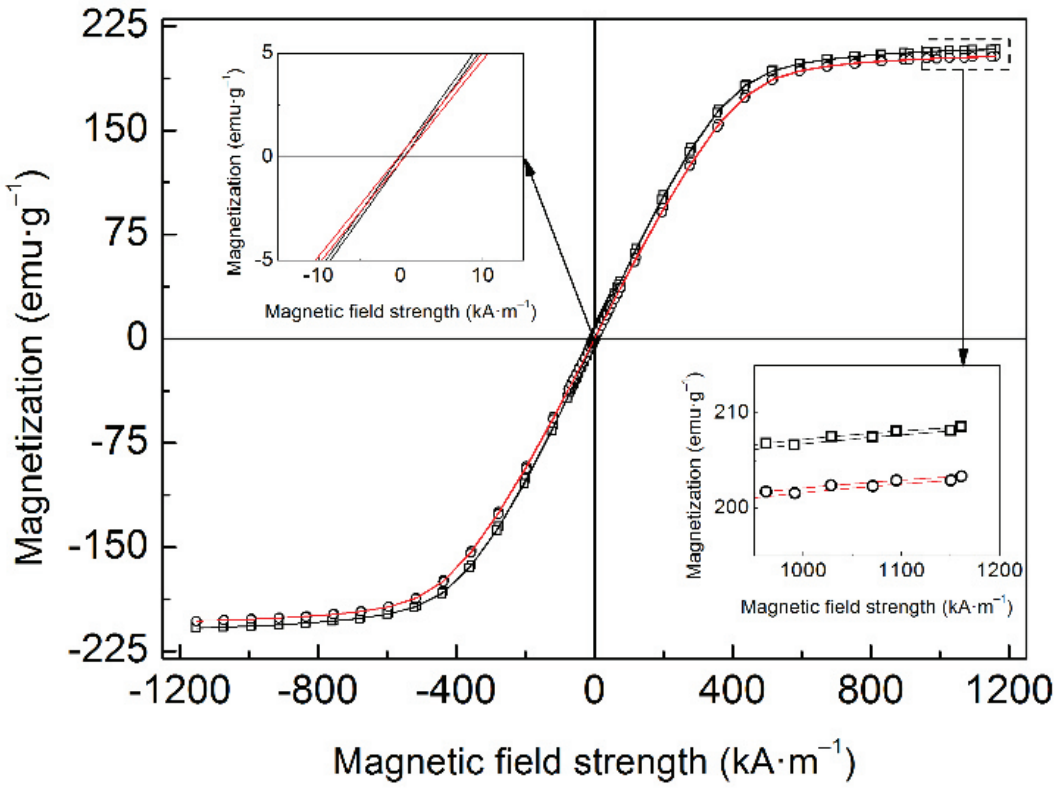

Figure 3 VSM spectra of bare CI particles (*squares*) and their PIPOx-grafted analogues (*circles*) with magnified coercivity (*upper-left*) and saturation magnetization (*lower-right*) regions

4. CONCLUSIONS

A design and preparation of the CI-*g*-PIPOx particles via surface-initiated ATRP is presented. Grafted PIPOx was characterized in term of its M_n and $Đ$ revealing the values of 2600 g·mol^{-1} and 1.07, respectively. The presence of PIPOx was verified via EDS and FTIR spectroscopies. The magnetization as a key factor for practical applications was negligibly decreased (only ~2.5% at magnetic field of ~1160 kA·m^{-1}). System described herein based on CI-*g*-PIPOx particles fulfill the fundamental criteria desired for such systems. Hence, after the necessary tests including, but not limited to, cytotoxicity, hemocompatibility etc. they are presumed to be promising candidates for the implementation to the next-stage *in-vitro* studies.

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