Synthesis and characterization of new functionalized polymer-Fe₃O₄ nanocomposite particles

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Abstract. In this study, Fe₃O₄ nanoparticles (NPs) were functionalized with copolymer or terpolymer bearing glycidyl methacrylate (GMA) moieties making them suitable for potential applications as drug delivery systems (DDS). For this purpose, the surface of magnetic nanoparticles was first coated with 3-(trimethoxysilyl) propyl methacrylate (MPS) by a silanization reaction to introduce reactive methacrylate groups onto the surface. Subsequently, monomers were grafted onto the surface of modified-MPS particles via two polymerization methods: seed emulsion (GMA, divinylbenzene, DVB, and styrene, S) and distillation – precipitation (GMA and DVB). The obtained nanocomposite particles were characterized by FTIR (Fourier transform infrared spectroscopy), DR UV-Vis (diffuse reflectance ultraviolet – visible spectroscopy), TEM (transmission electron microscopy) combined with EDS (energy dispersive X-ray spectroscopy) analysis and DLS (dynamic light scattering). FTIR spectroscopy showed that indeed a polymer – Fe₃O₄@MPS composite was obtained. TEM and EDS analysis showed that the seed emulsion method resulted in nanosized, 100 nm Fe₃O₄@MPS core/polymer shell NPs, forming long chains. On the contrary, the distillation – precipitation method caused the formation of an inverted structure, i.e. polymer core coated by a Fe₃O₄@MPS shell, which exhibited a very coarse size distribution varying from several hundreds to over 2 μm.

Keywords: nanocomposites, modified Fe₃O₄ nanocomposite particles, drug delivery system, spectroscopy, TEM

1. Introduction

Targeting drug-delivery systems (DDS) in the form of functionalized nanoparticles has been intensively explored especially for tumor diagnostics and therapeutics [1–4]. This drug delivery method is attractive, because the substances are easily administered and transported to the target under certain conditions, allowing a safer and more effective tissue-specific release of drugs [5]. However, not every substance can be used as DDS. There are several conditions that must be satisfied so that the nanoparticles could be used to deliver drugs. These comprise among others: small size, the ability to carry a wide variety of chemotherapeutic agents with sufficient drug space to meet the desired amount of biologically active drugs, the ability to release the drug at a predictable rate,

biocompatibility, minimal antigenic properties, biodegradability, minimized toxicity of the breakdown products [6]. Liposomes, polysaccharides, nanocrystals, dendrimers, and polymeric, inorganic and modified nanoparticles meet all of these conditions [7–16]. Magnetic nanoparticles could be potentially used for drug delivery, as they can be easily handled by an external magnetic field and are preferentially taken up by the target tissue. They also offer the possibility of being used in magnetic resonance imaging (MRI) [17, 18]. However, in most of the cases where magnetic nanocarriers have been used, difficulties in achieving the above mentioned objectives appeared. However, it seems that the main factor determining the possibility of using nanoparticles as DDS is the strength of their magnetic force, which must be sufficient to

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overcome the force of blood flow and to accumulate drugs only at target site [19]. Depending on their magnetic properties, nanoparticles can be divided into pure metals, their alloys and oxides [20–23].

Iron oxide nanoparticles, due to the favorable features they exhibit, are the only type of magnetic nanoparticles approved for clinical use by Food and Drug Administration. Unique properties of Fe_3O_4 nanoparticles, e.g. superparamagnetism, a large surface-to-volume ratio, high surface area, low toxicity, easiness of modification, offer enormous potential in the fields of immobilization of biomaterials [24–27], bioseparation [26–31] and bioengineering usage [32–36]. Moreover, the most important fact is the natural occurrence of iron oxides in human heart, spleen and liver [37], what implies their biocompatibility and non-toxicity at a physiological concentration.

Since the pure Fe_3O_4 nanoparticles often have poor stability and dispersion, various modification methods have been exploited to obtain soluble and biocompatible Fe_3O_4 nanoparticles. The resulting modified Fe_3O_4 nanoparticles have been extensively used for various applications. However, it is of crucial importance that the materials used for modification are non-toxic. The aim of this study was to verify, which synthesis procedure allows obtaining surface modified Fe_3O_4 nanoparticles with a size below 100 nm. This biocompatible nanocomposite (NC) could find potential application as DDS. For this purpose, the surface of the synthesized Fe_3O_4 nanoparticles was modified by two functionalization methods: seed – emulsion and distillation – precipitation polymerization. The global information on the composites was obtained by DLS as well as FTIR and UV-Vis spectroscopy. The combination of TEM imaging with EDS chemical mapping allowed to detect the core-shell structure of the synthesized composites and geometrically locate the respective elements present in the material. A variety of potential applications as well as limitations of the functionalized Fe_3O_4 nanocomposite particles were discussed.

2. Experimental

2.1. Materials and chemicals

Divinylbenzene (DVB, 80%, Aldrich, Germany), glycidyl methacrylate (GMA, >97%, Aldrich, Germany) and styrene (S, >99%, Fluka, Switzerland) were used as monomers. They were purified from inhibitors by

extraction with an aqueous solution of 5% NaOH and 20% NaCl. Initiators (2,2'-azobis(2-methylpropanitrile), AIBN, 98%, China and potassium persulfate, KPS, >99%, Germany) were purchased from Sigma-Aldrich and used as purchased. Other chemicals, sodium citrate bihydrate (POCh, Poland), 3-(trimethoxysilyl)propyl methacrylate (98%, Aldrich, China), iron(II) sulfate tetrahydrate (Sigma-Aldrich, India), iron(III) chloride hexahydrate (CHEMPUR, Poland), ammonia solution (28 wt%, POCh, Poland), and organic solvents (acetonitrile, ethanol, POCh, Poland) were also used as purchased.

2.2. Synthesis of citrate stabilized Fe_3O_4 particles

The synthesis of the Fe_3O_4 nanoparticles was performed in a glass reactor (100 ml) equipped with a heating coat, a mechanical stirrer, a condenser, a dropping funnel and a source of argon. The reactor was connected to a thermostat and immersed additionally in an ultrasonic bath. $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (5 mmol) and $\text{FeSO}_4 \cdot 4\text{H}_2\text{O}$ (2.5 mmol) were dissolved in deionized water (15 mL). The resulting solution was heated to 70 °C and ammonia solution (4 mL, 28 wt% was added dropwise. After 30 minutes, a solution of sodium citrate (2 mmol, 0.5 g/mL) was added to stabilize the precipitated black colored nanoparticles of Fe_3O_4 . Next, the reaction temperature was raised to 90 °C and the reaction was continued with simultaneous sonication of the mixture constantly for 60 minutes. The resulting magnetite particles were separated from the solution by using a strong magnet. They were then rinsed many times with deionized water. The magnetic properties were also used to isolate the particles at this stage. The final product was kept dispersed in water (10 mL).

2.3. Modification of magnetite particles

The modification was performed in the same glass reactor (100 mL) equipped as described above. Ethanol (30 mL), 3-(trimethoxysilyl)propyl methacrylate (0.95 mL, 4 mmol), and ammonia (2 mL of 25 wt% solution) were added to the water dispersion of the Fe_3O_4 particles obtained in the previous step. The mixture was heated in the reactor to 70 °C and mixed for 24 hours. The resulting product of a silanization reaction, $\text{Fe}_3\text{O}_4@\text{MPS}$, being in the form of bright brown particles, was rinsed with water and

Figure 1. Synthesis and modification of Fe₃O₄ NPs to obtain Fe₃O₄@MPS

ethanol, by decantation using a strong magnet, and dried under reduced pressure. This methodology applied previously by Xu *et al.* [38] allowed obtaining nanoparticles capable to copolymerize with other unsaturated monomers. The scheme of synthesis and modification of Fe₃O₄ nanoparticles is presented in Figure 1.

2.4. Synthesis of functionalized composite particles

2.4.1. Method I – seed emulsion polymerization

For a typical polymerization procedure, Fe₃O₄@MPS particles (40 mg) were placed in a 100 mL glass reactor, equipped with a mechanical stirrer and a source of argon, and dispersed in demineralized water (50 mL). The dispersion was sonicated for 30 minutes. After that time, styrene (0.208 g, 2 mmol) was added to the mixture and the sonication was continued for further 30 minutes. Next, the reaction mixture was heated to 80 °C and a water solution of potassium persulfate (0.6 mL, 10 mg/g) was added. After next 1 hour, divinylbenzene (0.029 g, 0.22 mmol) and glycidyl methacrylate (0.079 g, 0.55 mmol) were added to the mixture. The polymerization was continued for 16 hours at 90 °C. The final product, Fe₃O₄@MPS@GMA-S-DVB, was rinsed with water and ethanol, by decantation using a strong magnet, and dried under reduced pressure.

2.4.2. Method II – distillation – precipitation polymerization

Distillation – precipitation polymerization was performed in three-neck flask equipped with a mechanical stirrer, a Dean Stark apparatus and a source of argon. The conditions of the polymerization were similar to those applied in [39]. For a typical polymerization procedure, Fe₃O₄@MPS dispersion in acetonitrile (40 mg/80 mL) was sonicated for 30 minutes using an ultrasonic bath and a mixture of glycidyl

Figure 2. Simplified scheme of syntheses of two types of Fe₃O₄-polymer composite particles: Fe₃O₄@MPS@GMA-S-DVB (method I, right side); Fe₃O₄@MPS@DVB-GMA (method II, left side)

methacrylate (1.92 g, 13.5 mmol), divinylbenzene (0.19 g, 1.5 mmol) and AIBN (40 mg) was added. The polymerization was carried out in the boiling state of the reaction mixture for 2 hours, until distilling about 50% of acetonitrile. The resulting particles (Fe₃O₄@MPS@DVB-GMA) were rinsed with a 1:1 v/v mixture of ethanol and acetonitrile, by decantation using a strong magnet, and dried under reduced pressure.

The simplified scheme of syntheses of Fe₃O₄-polymer composite particles using method I and method II is presented in Figure 2.

2.5. Characterization of Fe₃O₄ and modified magnetic particles

2.5.1. Particle size measurements

Dynamic Light Scattering (DLS) technique was used to assess particle size distribution. The measurements were performed at 20 °C using a Zetasizer Nano ZS instrument from Malvern with a red laser (633 nm, He-Ne, 4.0 mW). The measurement angle was set at 173° (Backscatter NIBS default).

2.5.2. TEM

The structure of the synthesized Fe₃O₄ modified particles (Fe₃O₄@MPS), the composite particles obtained using seed emulsion polymerization (Fe₃O₄@MPS@GMA-S-DVB) and distillation – precipitation polymerization methods (Fe₃O₄@MPS@DVB-GMA) were analyzed by transmission electron microscopy (TEM). A drop of each suspension was deposited on a TEM Cu grid coated with a carbon foil, and subsequently dried. The sample was observed in the scanning transmission electron microscopy (STEM) mode using the high angular annular dark field (HAADF) detector on a FEI Tecnai Osiris operating at 200 kV with resolution of 0.15 nm in STEM. The chemical analysis of the samples was

performed by energy dispersive spectroscopy using Super EDX.

2.5.3 Infrared spectroscopy measurements

FTIR spectra were recorded using a Thermo Scientific Nicolet 8700 spectrometer and the KBr pellet method or an FTIR Nicolet iN10 MX microscope in the reflectance mode. All the analyses were performed in the mid IR range, more precisely between 400–4000 cm^{-1} wavelength. 64 or 128 scans were used to obtain the spectral resolution of 0.1 cm^{-1} .

2.5.4. UV-Vis

Diffuse reflectance UV-Vis spectra were recorded using a Jasco V-670 spectrophotometer equipped with a Jasco ISN-723 UV-Vis NIR 60 mm integrating sphere. BaSO_4 was used as a standard. The resolution was chosen to be 1 nm and the scan speed was 1000 nm/min. The spectral range was from 190 nm to 800 nm.

2.5.5. TGA analysis

TGA analyses were performed using a METTLER TOLEDO TGA/DSC 1 analyzer. Samples (2–2.5 mg) were heated from 25 to 800 $^{\circ}\text{C}$ (10 $^{\circ}\text{C}/\text{min}$) under nitrogen atmosphere (50 mL N_2/min).

3. Results

3.1. Particle size distribution

The first information about the size distribution of the synthesized particle particles was obtained from

Figure 3. DLS spectrum of size distribution observed for $\text{Fe}_3\text{O}_4@\text{MPS}$ (·····), $\text{Fe}_3\text{O}_4@\text{MPS}@GMA-DVB$ (—), $\text{Fe}_3\text{O}_4@\text{MPS}@GMA-S-DVB$ (---) nanoparticles

the DLS measurements. Their results are shown in Figure 3. The $\text{Fe}_3\text{O}_4@\text{MPS}$ particles had an average size of 40 nm and relatively low size dispersion. This made them interesting as potential seeds for emulsion and distillation-precipitation polymerization functionalization. The peaks with maxima at approximately 100 nm size were observed for composite particles functionalized by seed emulsion polymerization ($\text{Fe}_3\text{O}_4@\text{MPS}@GMA-S-DVB$) and distillation – precipitation polymerization ($\text{Fe}_3\text{O}_4@\text{MPS}@GMA-DVB$) methods. However, in these cases the dispersion of the particle size was clearly larger.

3.2. Structural characterization

The general morphology of the seed emulsion polymerization produced $\text{Fe}_3\text{O}_4@\text{MPS}@GMA-S-DVB$ NPs is presented in the HAADF STEM image Figure 4a. The aim of the synthesis was to obtain nanocomposite particles consisting of a Fe_3O_4 core surrounded by a polymer shell. From Figure 4a can be clearly seen, that the synthesis of the nanocomposite particles was successful, when seed emulsion polymerization was applied. The particles are interconnected by their shells, forming long chains. However, the agglomeration effect observed in STEM HAADF image (Figure 4a) could result from the magnetic interactions between nanoparticles and not from any covalent chemical connection between the polymer shells. The majority of $\text{Fe}_3\text{O}_4@\text{MPS}@GMA-S-DVB$ NPs has a core-shell structure as shown in Figure 4b. Some of the NC particles contain more than one $\text{Fe}_3\text{O}_4@\text{MPS}$ nanoparticles. Nevertheless, most of the particles have a size around 100 nm, which is consistent with the result obtained by DLS. However, smaller, agglomerated $\text{Fe}_3\text{O}_4@\text{MPS}$ NPs, Figure 4c, and larger NC nanoparticles, which are surrounded by numerous $\text{Fe}_3\text{O}_4@\text{MPS}$ NPs, Figure 4d, can be found in the sample.

The STEM HAADF imaging, Figure 5a, revealed that the sample of the nanocomposite particles obtained using distillation-precipitation polymerization consists of large, perfectly spherical particles with diameters varying from about 500 nm to over 2 μm .

The white contrasted shell on the spherical particles in Figure 5a, stems from the presence of smaller particles agglomerated on their surface. This observation is indeed confirmed by the EDS chemical mapping, Figure 5. Indeed, in the EDS elemental map

Figure 4. STEM HAADF images of the $\text{Fe}_3\text{O}_4@\text{MPS}@\text{GMA-S-DVB}$ nanoparticles synthesized by seed emulsion polymerization: (a) overview image; (b) individual nanoparticle with a clearly visible $\text{Fe}_3\text{O}_4@\text{MPS}$ core surrounded by a polymer shell; (c) magnified view of agglomerated $\text{Fe}_3\text{O}_4@\text{MPS}$ NPs; (d) polymer nanoparticle surrounded by $\text{Fe}_3\text{O}_4@\text{MPS}$ NPs

Figure 5. Chemical mapping by EDS of the $\text{Fe}_3\text{O}_4@\text{MPS}@\text{GMA-DVB}$ particles: (a) STEM HAADF image with the corresponding maps: (b) Fe; (c) O; (d) Si; (e) collective map of O, Fe and Si

Figure 6. Detailed chemical mapping of the $\text{Fe}_3\text{O}_4@\text{MPS}@\text{GMA-DVB}$ nanoparticle shell: (a) STEM HAADF image; (b) magnified view of the Fe_3O_4 particles forming the shell; the corresponding EDS maps: (c) Fe; (d) Si; (e) collective map of O, Fe and Si

the stronger iron signal is detected on the polymer spheres, Figure 5b. Also the oxygen signal is stronger on the circumference of the spheres, Figure 5c. According to the EDS maps the distribution of Si is uniform in the polymer spheres, Figure 5d.

The agglomeration of the $\text{Fe}_3\text{O}_4@\text{MPS}$ NPs on the surface of the GMA-DVB polymer spheres is even better visible on the detailed view of the sphere surface in the STEM HAADF image with the corresponding EDS maps, Figure 6. Individual $\text{Fe}_3\text{O}_4@\text{MPS}$ NPs are visible on the magnified HAADF image in Figure 6b. The Fe signal is clearly stronger on the surface of the polymer spheres, Figure 6c. The Si signal is detected not only in the polymer core of the spheres but also at the same location as the Fe signal.

3.3. FTIR and DR UV-Vis analysis

Figure 7 shows a comparison of the FTIR spectra collected for the four types of samples bearing Fe_3O_4 NPs. The absorption bands present in the FTIR spectrum of the unmodified particles at 570 cm^{-1} corre-

spond to Fe–O–Fe skeletal vibrations in Fe_3O_4 , and 3420 cm^{-1} can be attributed to the symmetric stretching vibrations of OH groups (the first of the bands). The presence of symmetrical and asymmetric stretching vibrations of COO^- groups at 1615 and 1400 cm^{-1} strongly suggests that indeed citrate coated magnetite particles were obtained (Figure 7a) [40]. The synthesized Fe_3O_4 particles were subsequently treated with 3-(trimethoxysilyl)propyl methacrylate to attach methacrylate groups on the surface of magnetite nanoparticles. The success of magnetite modification under the applied conditions was proved by a FTIR spectrum presented in Figure 7b. The strong absorption bands found in the spectrum of the modified particles at 1750 cm^{-1} are attributed to ester carbonyl groups. The bands at 1263 and 1128 cm^{-1} correspond to the stretching vibrations of C–O–C and/or Si–O–Si skeletal vibrations. Thus it seems that the attachment of methacrylate moieties on the surface of Fe_3O_4 particles was successful [41]. The FTIR spectrum of the particles synthesized using the emulsion poly-

merization method is shown in Figure 7c. The bands attributed to styrene, divinylbenzene and glycidyl methacrylate were found as dominant peaks in the collected spectrum. The bands observed in the range of $2850\text{--}3050\text{ cm}^{-1}$ confirm the presence of aliphatic and aromatic hydrocarbon groups, respectively. The absorption band at $\sim 1720\text{ cm}^{-1}$ corresponds to the ester carbonyl group stretching vibrations. The bands at 1050 and 1250 cm^{-1} can be assigned to the asymmetric and symmetric C–O–C skeletal vibrations and those at $750\text{--}800\text{ cm}^{-1}$, observed in the range characteristic of the epoxy ring stretchings, indicate the presence of glycidyl methacrylate mers in the synthesized polymer particles. The FTIR spectrum (Figure 7d), which is registered for the product of distillation – precipitation polymerization, is dominated by bands related to the presence of repeating units of GMA and DVB. Absorption bands corresponding to the vibrations of the hydrocarbyl groups ($2850\text{--}3050$, 1600 , 1490 and 1450 cm^{-1}), ester carbonyl groups (1720 cm^{-1}), C–O–C moieties (approx. 1100 cm^{-1}) and epoxy groups ($900\text{--}840\text{ cm}^{-1}$) are observed in the spectrum. Due to the low percentage of the modified magnetite particles, a smaller absorption in the range

Figure 7. Typical FTIR spectra of: (a) Fe_3O_4 nanoparticles (KBr), (b) $\text{Fe}_3\text{O}_4\text{@MPS}$ nanoparticles, (c) $\text{Fe}_3\text{O}_4\text{@MPS@GMA-S-DVB}$, (d) $\text{Fe}_3\text{O}_4\text{@MPS@GMA-DVB}$

Figure 8. DR UV-Vis spectra recorded for the synthesized particles: $\text{Fe}_3\text{O}_4\text{@MPS}$ ($\cdots$), $\text{Fe}_3\text{O}_4\text{@MPS@GMA-S-DVB}$ (—), $\text{Fe}_3\text{O}_4\text{@MPS@GMA-DVB}$ (---)

typical for magnetite (from approx. 570 cm^{-1}) is observed [42, 43].

To determine the optical properties of the modified and functionalized Fe_3O_4 nanoparticles the DR UV-Vis spectra were collected. They are shown in Figure 8. For the $\text{Fe}_3\text{O}_4\text{@MPS}$ NPs the maximum absorption at about 320 nm was found, which corresponds to previously published data [44]. It was shifted towards higher wavelength values in the cases of functionalized Fe_3O_4 nanoparticles, i.e. the red shifts were observed.

3.4. TGA analysis

Figure 9 presents TGA curves obtained for the particles provided by seed emulsion and those prepared by distillation-precipitation polymerization. The samples of the particles were heated slowly up to 800°C . In both cases the relatively slow weight loss was ob-

Figure 9. TGA curves of $\text{Fe}_3\text{O}_4\text{@MPS@GMA-S-DVB}$ (—), $\text{Fe}_3\text{O}_4\text{@MPS@GMA-DVB}$ ($\cdots$)

served up to about 300 °C. It results from removal of absorbed water and/or other solvents used during preparation of the particles. Above this temperature the rate of decomposition clearly increased and reached the maxima at 415 °C for Fe₃O₄@MPS@GMA-S-DVB and at 390 °C for Fe₃O₄@MPS@GMA-DVB particles. Additionally, in the case of Fe₃O₄@MPS@GMA-DVB the second, local increase in decomposition rate was observed with the maximum at about 740 °C.

4. Discussion

Modified and functionalized Fe₃O₄ nanoparticles are increasingly used as DDS [45–49]. The aim of this study was to functionalize Fe₃O₄@MPS NPs and obtain core-shell nanocomposites with a Fe₃O₄@MPS core surrounded by the DVB-(S)-GMA polymer shell to make these materials useful for medical applications.

Materials, which are dedicated as DDS, must meet several conditions, which were mentioned in the introduction. Among others, materials introduced into the human body, must not be toxic [6]. The modification and functionalization of the studied nanocomposites required a selection of the suitable monomers. 3-(Trimethoxysilyl)propyl methacrylate – MPS, divinylbenzene – DVB, glycidyl methacrylate – GMA, and styrene – S seem to meet the requirements of lack of toxicity for human body after their polymerization.

Another requirement for particles applied as DDS is their minimal size. Thus, their diameter must be between 2 and 100 nm, as smaller nanoparticles would pass through the blood-brain barrier [6]. However, drug release is affected by particle size. Smaller particles have larger surface area, therefore, most of the drug would be at or near the particle surface, leading to fast drug release. While, larger particles have large cores, which allow more drug to be encapsulated and slowly diffuse out. Smaller particles also have tendency to aggregate during storage and transportation of nanoparticle dispersion.

The results obtained by DLS indicate that the diameter of the Fe₃O₄@MPS and Fe₃O₄@MPS@GMA-S-DVB (particles obtained using seed emulsion polymerization) is within the range from 2–100 nm. The presence of the nano-sized particles was also detected by the DLS measurements in the sample of the

particles obtained by distillation-precipitation polymerization. However, this technique of heterogeneous polymerization provided larger particles as well, reaching about 2 µm in diameter.

The micro-size of the synthesized particles does not disqualify them, however, as potential materials for drug delivery. Such micro-sized particles were studied, for example, by Tao and Desai, who developed microfabrication as controlled delivery devices [50]. These devices provide the capacity to target cells, promote unidirectional controlled release, and enhance permeation across the intestinal epithelial barrier [50–52]. Another application of microparticles are biosensors and for size exclusion chromatography [52, 53].

Much attention has been devoted to optimize surface coating of magnetic NPs formulations for their use as DDS [54]. Without an inert coating, Fe₃O₄ NPs are subject to opsonization and rapid clearance from the blood by the reticuloendothelial system (RES). In our case, the formation of polymer – Fe₃O₄ nanocomposites was confirmed by FTIR measurements. This is evidenced by the absorbance maxima at wavelengths of the wave numbers of ~1720, 1050, 1250, and 750–800 cm⁻¹ corresponding to the vibrations from the mers of glycidyl methacrylate in the synthesized polymer particles [42, 43]. However, FTIR spectroscopy provided only information about the chemistry of nanocomposites. To verify their morphology, STEM analysis, including EDS was performed. Scanning transmission electron microscopy imaging allowed observing the shape and size of the synthesized composite particles. However, STEM enables only visualizing the core and the shell of the particles, while it is impossible to determine their chemical composition. Thus EDS mapping was applied, as it is the only technique that allows visualizing the geometric position of the respective chemical elements present in the individual particles. Consequently, information on the location of the elements used for functionalization of the composites was obtained.

STEM analysis of nanocomposites with Fe₃O₄ synthesized using the seed emulsion polymerization functionalization method confirmed that the core-shell structure was indeed achieved. The nanocomposites were composed of a Fe₃O₄@MPS core surrounded by a polymer shell. The functionalization prevented the

100 nm polymer nanocomposites from agglomeration.

On the contrary, the distillation – precipitation polymerization method resulted in nanocomposite particles consisting of Fe_3O_4 @MPS NPs immersed entirely in a bulk polymer core. Additionally, the particles are surrounded by a Fe_3O_4 @MPS shell, as was clearly shown by STEM and EDS analysis. Indeed, the Si signal is not only detected in the polymer core of the composite spheres but also in the same location as the Fe signal, confirming the presence of the MPS shell around the Fe_3O_4 cores. The presence of the MPS shell around the Fe_3O_4 NPs was also confirmed by FTIR. After subtracting the spectrum collected for pure Fe_3O_4 NPs using the OPUS software from the spectrum of Fe_3O_4 @MPS particles no peak corresponding to pure Fe_3O_4 was visible.

STEM imaging of the Fe_3O_4 @MPS nanocomposites functionalized by the seed emulsion polymerization method showed a relatively uniform size of the NC particles, around 100 nm, confirmed by DLS measurements. In the case of the Fe_3O_4 @MPS nanocomposites functionalized by the distillation – precipitation polymerization method, the detailed STEM analysis showed a very large particle size distribution, both nano- and micro-sized particles. The micro-sized particles are perfectly spherical and reach sizes up to around 2 μm . The large size distribution makes them rather unsuitable as DDS.

An important prerequisite is that nanoparticles for DDS applications should not be agglomerated. This is one of the principal problems for researchers synthesizing nanoparticles. The STEM images of the Fe_3O_4 @MPS@GMA-S-DVB nanocomposites clearly show that the functionalization by the seed emulsion polymerization method prevented the 100 nm nanocomposite particles from agglomeration. Conversely, the surface of Fe_3O_4 @MPS@DVB-GMA spheres was coated by Fe_3O_4 @MPS NPs, what shows that MPS modification only, does not prevent the Fe_3O_4 NPs from agglomeration.

Fe_3O_4 introduced into the structure of the polymer particles maintains magnetic properties, useful, for example for separation of the catalyst or sorption applications, controlled delivery of drugs or medical imaging [53, 55–57]. The size of iron oxide cores is an important parameter that affects the magnetic properties of NPs [49, 58]. The magnetic properties

of the synthesized nanoparticles can be applied in magnetic resonance imaging (MRI) [17]. Moreover, they can be used as DDS for antibiotics [59], anti-cancer drugs [60], neurotransmitters [61]. Furthermore, the presence of modifiable functional groups facilitates the attachment of different therapeutic agents.

Considering the obtained results, as well as the literature data, it is clearly visible that the modified and functionalized Fe_3O_4 nanoparticles could be potentially used like DDS. The spectroscopic measurements allowed for identifying the introduced chemical changes into Fe_3O_4 @MPS nanoparticles functionalized by two different methods. However, the TEM imaging has shown that only the seed emulsion polymerization functionalization method of Fe_3O_4 @MPS nanoparticles was successful, resulting in the aimed nanocomposite structure consisting of a Fe_3O_4 @MPS core and a polymer shell.

TGA analyses of the particles showed that the total weight loss of Fe_3O_4 @MPS@GMA-S-DVB particles was only about 50%. This finding can be additional proof of the core-shell nature of the particles provided by seed emulsion polymerization (Method I). In the case of Fe_3O_4 @MPS@GMA-DVB particles the total weight loss of the analyzed sample amounted to about 90%. It proves that the particles synthesized by method II (precipitation – distillation polymerization) has mainly of organic nature, i.e. the inorganic particles are immersed in the polymer bulk.

5. Conclusions

The present study describes the synthesis, modification and functionalization of Fe_3O_4 nanoparticles. Two different functionalization methods of the Fe_3O_4 @MPS nanoparticles were investigated: seed - emulsion and distillation – precipitation polymerization. FTIR spectroscopy showed that in both cases a polymer – Fe_3O_4 nanocomposite was obtained and the polymer chains had been effectively grafted onto the surface of Fe_3O_4 @MPS nanoparticles. As biocompatible polymers were used for the functionalization of the Fe_3O_4 @MPS nanoparticles, the resulting product could be applied potentially as DDS. However, morphological analysis by TEM showed that only the seed emulsion polymerization method resulted in a Fe_3O_4 @MPS core/polymer shell structure of the nanoparticles. DLS and TEM measurements con-

firmed that their size was mainly in the range of 100 nm. The polymer shell prevented the NPs from agglomeration. In the case of the distillation – precipitation polymerization method the large, perfectly spherical nanocomposite particles coated by Fe₃O₄@MPS NPs were mainly obtained. However, the TEM investigation, supported by DLS measurements, showed that these polymer core composite spheres had size varying from nanometers to over 2 μm.

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