Research Article

Development of Stabilized Magnetite Nanoparticles for Medical Applications

Ioana Lavinia Ardelean, Luric Bogdan Nicolae Stoencea, Denisa Ficai, Anton Ficai, Roxana Trusca, Bogdan Stefan Vasile, Gheorghe Nechifor, and Ecaterina Andronesco

Faculty of Applied Chemistry and Material Science, Politehnica University of Bucharest, 1-7 Gh Polizu Street, 011061 Bucharest, Romania

Correspondence should be addressed to Denisa Ficai; denisaficai@yahoo.ro

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We report a facile method to synthesize magnetite nanoparticles with mesoporous structure by coprecipitation method using different stabilizing agents like salicylic acid, glutamic acid, and trichloroacetic acid. The stabilizing agents were used to prevent the aggregation of the magnetite nanocrystals and to obtain stable nanostructures even in the biological environment. The structure and morphology of magnetic nanocrystals were determined using X-ray diffraction (XRD), Fourier Transform Infrared (FTIR) spectroscopy, Brunauer-Emmett-Teller (BET) analysis, infrared (IR) spectra, scanning and transmission electron microscopy (SEM and TEM), high-resolution transmission electron microscopy (HRTEM), and selected area electron diffraction (SAED). The results reveal important differences between these magnetic nanoparticles (MNPs), which are mainly attributed to the stabilizing agents. The smallest nanoparticles were obtained in the presence of trichloroacetate ions. The mechanism of formation of these suprastructures is strongly correlated with the end functional groups of the stabilizing agent. Thus, the obtained nanoparticles are potential candidates for contrast agents as well as targeted carrier for specific diseases, especially cancer.

1. Introduction

Synthesis and characterization of porous materials have attracted considerable attention in recent years due to their industrial and biomedical applications: heavy metals removal, sensors, catalysis, magnetic recording and separation, photocatalysis, pigments, ferrofluids, magnetic resonance imaging, hyperthermia drug delivery, and so on [1–6]. Mesoporous iron oxides such as Fe_3O_4 and $\gamma\text{-Fe}_2\text{O}_3$ and CoFe_2O_4 are successfully used in the field of magnetic fluids, catalysis, biotechnology/biomedicine, magnetic resonance imaging, and magnetic recording devices because of their paramagnetism, biocompatibility, and safety [7–11]. Owing to their large surface area and well-defined pore structure and size, the lack of toxicity, and the possibility of targeted accumulation in the desired tissue, mesoporous Fe_3O_4 nanospheres are extensively used as targeted drug delivery systems [12].

Various techniques have been developed to synthesize some important transition metal oxides (such as Fe_3O_4 and

CoFe_2O_4) with various interior porous architecture (simple hollow spheres, core-shell and mesoporous structures [13–15]) using coprecipitation method [16], pyrolysis, hydrothermal reaction [17], microwave-assisted synthesis [18], and the thermal decomposition of organometallic compounds [19–22].

Liu et al. [23] prepared Palladium- (Pd-) loaded amino-group functionalized mesoporous Fe_3O_4 nanoparticles ($\text{Pd@Fe}_3\text{O}_4$) by a simple solvothermal method using ethanol as solvent. They show that the $\text{Pd@Fe}_3\text{O}_4$ modified the glassy carbon electrode (GCE) which then exhibited excellent electrochemical catalytic activities towards dopamine (DA) due to the synergetic effect between Pd and Fe_3O_4 .

Kumari et al. [24] synthesized magnetite nanospheres with hollow interiors using a simple, one-pot, and template-free solvothermal method with ferric chloride as the iron precursor, for the purpose of using this mesoporous nanosphere to remove heavy metals from waste water. The Langmuir adsorption capacities of the magnetic nanospheres were 9 and 19 mg/g for Cr^{6+} and Pb^{2+} , respectively.

Xia et al. [25] synthesized high-quality mesoporous Fe_3O_4 nanocages (MFONs) with a mesoporous structure and highly uniform dispersion by a facile complex-coprecipitation method at 100°C with addition of triethanolamine and ethylene glycol. They show that when used as an anode material for rechargeable lithium-ion batteries, MFONs anode presents high specific capacities and excellent cycling performance at high and low current rates thanks to the unique mesoporous cage-like structure and high specific surface area ($133\text{ m}^2/\text{g}$), which may offer large electrode/electrolyte contact area for the electron conduction and Li^+ storage.

The surface modification of the magnetic materials is essential for most of the chemical and biological applications and the performances of these materials can be designed by proper surface modification. Among the properties of interest, biocompatibility, biostability, and internalization are especially studied in the literature. It is well known that magnetite dispersibility and chemical stability in biological media are improved by functionalization with PEG (polyethylene glycol), dextran, albumin, chitosan, PVA (polyvinyl alcohol), PLGA (poly(lactic-co-glycolic acid)), and so on [26], but only a very limited number of studies present the functionalization of these nanoparticles with bifunctional small molecules like aminoacids, hydroxyacids, or haloacids [27, 28]. These bifunctional stabilizing small agents can be essential in allowing a better internalization of the nanoparticles inside the cells (especially when using aminoacids as functionalization agents), but they do not improve the dispersibility (because dispersability is ensured by the existence of highly charged groups such as citrate where three carboxylic groups are available for repulsive purposes). At this moment, a special attention is paid to folic acid (vitamin 12), a specific aminoacid, which proved to have a good internalization capacity for the magnetic nanoparticles, and it even allows a targeted delivery inside the tumoral cells [29–32].

The aminoacid or hydroxyacid based coating over the magnetic core offers the premises of high biocompatibility and an improvement in the capacity of internalization of these nanoparticles. In this study we report a facile method to synthesize magnetite nanoparticles with mesoporous structure by coprecipitation method using different stabilizing agents: salicylic acid, glutamic acid, and trichloroacetic acid. The very different structure and potential biological activity of the three stabilizing agents is important in designing new magnetic agents with desired properties. After the coating of the magnetic core, glutamic acid still has a free carboxyl group while salicylic acid has a hydroxyl group, each group being able to further bind molecules onto the surface [33]. Glutamic acid also acts as a powerful dispersing agent (like citrate) because of the net negative surface charge of the resulting nanostructures. The role of different stabilizing agents from the point of view of crystallinity, pore diameter, and surface area was analyzed. In order to determine the structure and morphology, the as-synthesized mesoporous Fe_3O_4 was characterized by powder X-ray diffraction (XRD), Fourier Transform Infrared (FTIR) spectroscopy, Brunauer-Emmett-Teller (BET), infrared (IR) spectra, scanning and

transmission electron microscopy (SEM and TEM), high-resolution transmission electron microscopy (HRTEM), and selected area electron diffraction (SAED).

2. Experimental Details

2.1. Materials and Methods. The main precursors used for the preparation of mesoporous magnetite are iron (III) chloride (FeCl_3) (Sigma-Aldrich), ammonium iron (II) sulfate hexahydrate ($\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$) (Silal Trading), sodium hydroxide (NaOH) (Silal Trading), salicylic acid ($\text{C}_6\text{H}_7\text{O}_3$) (Chimactiv), glutamic acid (Sigma-Aldrich), trichloroacetic acid (Carlo Erba RP ACS), and distilled water.

Mesoporous magnetite nanoparticles were prepared by precipitation method [34] with different stabilizing agents: salicylic acid, glutamic acid, and trichloroacetic acid. The precipitation bath was obtained as follows. First of all, the sodium hydroxide solution ($10\text{ g NaOH}/250\text{ mL}$ solution) was obtained and then mixed with further 2.5 g of the stabilizing agent, under vigorous mechanical stirring at room temperature. The pH of the precipitation bath was set at 10–11 with hydroxide solution. The precursors solution ($8.4520\text{ g Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ and 6.9920 g FeCl_3 in 100 mL distilled water) was sprayed into the obtained precipitation bath, under continuous mechanical stirring. The spraying rate was set at about $2\text{--}3\text{ mL}/\text{min}$. After precipitation, the mixture was placed in the ultrasonic bath for 30 minutes; then the mesoporous magnetite was filtered and washed, whenever needed, with distilled water until neutral pH and chloride-free filtrate were obtained. Finally, the synthesized mesoporous magnetite was dried in a vacuum oven at a temperature of 60°C (Figure 1) [34]. Based on the above presented procedure, magnetite (Fe_3O_4), magnetite with salicylic acid ($\text{Fe}_3\text{O}_4@\text{SAL}$), magnetite with glutamic acid ($\text{Fe}_3\text{O}_4@\text{GLU}$), and magnetite with trichloroacetic acid ($\text{Fe}_3\text{O}_4@\text{TCA}$) have been obtained.

The mesoporous magnetite nanoparticles were characterized by XRD, FTIR, SEM, TEM, and BET.

IR spectroscopy was performed on a Nicolet iS 50 FTIR spectrometer equipped with a broad range DTGS detector providing high-sensitivity infrared information from 4000 cm^{-1} to 100 cm^{-1} and an integral diamond ATR unit. The system is configured with the built-in iS50 ATR module and an iS50 ABX Automated Beamsplitter Exchanger. The spectra were recorded in ATR mode over the wave number range of $400\text{--}4000\text{ cm}^{-1}$, with a resolution of 4 cm^{-1} coadding 64 spectra for improving the quality of the spectra. For a better identification of the peaks, the obtained spectra were resolved using a Gaussian-Lorentzian peak resolve procedure assuming a linear baseline.

Morphological information was obtained by scanning electron microscopy of the gold-coated specimens. The analysis has been performed using a QUANTA INSPECT F SEM device equipped with a field emission gun (FEG) with a resolution of 1.2 nm and with an X-ray energy dispersive spectrometer (EDS).

X-ray diffraction analysis was carried out on a Panalytical X'Pert Pro MPD equipment, with a CuK_α radiation, over a scan range of 2θ of $10\text{--}90^\circ$.

FIGURE 1: Preparation flowchart of magnetic nanoparticles.

TEM analyses were performed on a Tecnai G2 F30 S-TWIN high-resolution transmission electron microscope (HR-TEM) equipped with energy dispersive spectroscopy (EDS) as well as a selected area electron diffraction detector (SAED). The microscope was operated in transmission mode at 300 kV; TEM point resolution was 2 Å and line resolution was 1 Å.

The Brunauer-Emmett-Teller (BET) analysis was performed on a Micrometrics Gemini V surface area and pore size analyzer.

3. Results and Discussion

3.1. Complex Thermal Analysis. The complex thermal analysis (Figure 2) was recorded in order to quantify the amount of stabilizing agent retained on the magnetic nanostructures. It can be seen that, among the three stabilizing agents, the lowest affinity to the magnetic core belongs to GLU (0.54%) and the highest belongs to TCA (7.28%) while SAL exhibits intermediary affinity (2.50%). Based on the TG data, the strong flattening of the XRD peaks of the $\text{Fe}_3\text{O}_4\text{@TCA}$ can be explained because of the high content of the stabilizing agent

(7.28%) while in the case of $\text{Fe}_3\text{O}_4\text{@SAL}$ (containing 2.50% stabilizing agent) the flattening is less significant (plane 422 disappears while plane 220 is strongly flattened). In the case of GLU stabilized magnetic nanostructures, the peaks are even stronger which can mean that the crystallization process was even better compared with the pure Fe_3O_4 sample. All these masses were determined at 600°C.

The differential thermal analysis reveals a stabilization effect of the salicylic acid, the most intense exothermic peak of pure magnetite (538.7 being shifted to 549.5°C) while in both the other cases this peak is shifted to lower temperature (498.0 and 525.2°C).

3.2. X-Ray Diffraction. X-ray diffraction was used in order to establish the formation of magnetite as a pure phase. The XRD patterns (Figure 3) of the synthesized mesoporous MNPs present only the characteristic peaks of magnetite [34] even if the crystallinity and the crystallite size depend on the used stabilizing agent. The main crystallization planes of inverse spinel magnetic crystals can be clearly identified by XRD ((111), (9220), (311), (400), (422), (511), and (440)), their relative intensity indicating a nonpreferential crystallization

FIGURE 2: Complex thermal analysis of Fe_3O_4 , $\text{Fe}_3\text{O}_4\text{@GLU}$, $\text{Fe}_3\text{O}_4\text{@SAL}$, and $\text{Fe}_3\text{O}_4\text{@TCA}$: (a) thermogravimetric analysis and (b) differential thermal analysis.

FIGURE 3: XRD patterns of the synthesized nanoparticles: (1) Fe_3O_4 , (2) $\text{Fe}_3\text{O}_4\text{@GLU}$, (3) $\text{Fe}_3\text{O}_4\text{@SAL}$, and (4) $\text{Fe}_3\text{O}_4\text{@TCA}$.

according to the literature [35] and ASTM 0075-1609. The average crystallite size is calculated using the Debye-Scherrer equation: $D = k\lambda/\beta \cos \theta$, where $k = 0.95$ is Scherrer's constant, λ is the X-ray wavelength, β is the full width at half-maximum, and θ is the Bragg diffraction angle. Based on this equation, the crystallite size (using the reflection plane (311), which can be measured for all the samples) was found to be 8.8, 11.3, 6.1, and 2.5 nm (Fe_3O_4 , $\text{Fe}_3\text{O}_4\text{@GLU}$, $\text{Fe}_3\text{O}_4\text{@SAL}$, and $\text{Fe}_3\text{O}_4\text{@TCA}$, resp.). The XRD patterns of Fe_3O_4 and $\text{Fe}_3\text{O}_4\text{@GLU}$ are similar, with almost the same shape and comparative peaks intensity which means that comparative crystallinity and crystallite size are obtained. In the case of $\text{Fe}_3\text{O}_4\text{@SAL}$, the characteristic diffraction peaks decrease as intensity, while, in the case of $\text{Fe}_3\text{O}_4\text{@TCA}$, even the main peak of magnetite could not be clearly identified.

3.3. FTIR. The FTIR data reveal some differences between the spectra of the samples, especially compared with pure magnetite (Figure 4). Based on the literature data [35], the main bands of magnetite corresponding to the vibration of $\text{Fe}^{3+}/\text{Fe}^{2+}-\text{O}^{2-}$ in tetrahedral and octahedral sites in the magnetite appear at ~ 400 and 568 cm^{-1} . It can be seen that both $\text{Fe}_3\text{O}_4\text{@SAL}$ and $\text{Fe}_3\text{O}_4\text{@GLU}$ exhibit high similitude with the pure magnetite, most probably because of the low content of the stabilizing agent (0.54 and 2.50 for GLU and SAL, resp.). In the case of the sample obtained in the presence of trichloroacetate, the strong band from $\sim 540 \text{ cm}^{-1}$ is only visible as a shoulder. The explanation is related to the presence of a high amount of trichloroacetate (7.28% based on the thermogravimetric data), as stabilizing agent covering the magnetite core. The presence of the trichloroacetate can be clearly identified after deconvolution between 400 and 1700 cm^{-1} , based on its main bands centered close to that of sodium trichloroacetate (based on the HR Aldrich FTIR Collection Edition II library): 685 (new peak), 710 (new peak), 723, 738 (new peak), and 845 cm^{-1} (Figure 4, insert). The shifting of these peaks is a proof of the strong interaction between magnetite core and trichloroacetate shell, along with the strong decrease of the strong peak of magnetite

TABLE 1: BET data of the synthesized MNPs samples.

Sample code	Samples name	Specific surface area S_{BET} (m^2/g)	Average pore size D_{BET} (nm)
Fe_3O_4 reference*	Fe_3O_4	156.0278 m^2/g	5.7466 nm
Fe_3O_4 @citrate*	Fe_3O_4 @citrate	257.15 m^2/g	2.8075 nm
Fe_3O_4 @SAL	Fe_3O_4 @salicylic acid	204.7198 m^2/g	5.2712 nm
Fe_3O_4 @GLU	Fe_3O_4 @glutamic acid	129.5527 m^2/g	8.3597 nm
Fe_3O_4 @TCA	Fe_3O_4 @trichloroacetic acid	287.7533 m^2/g	3.4475 nm

* Data published in [34].

FIGURE 4: FTIR spectra of the magnetic samples: Fe_3O_4 , Fe_3O_4 @TCA, Fe_3O_4 @SAL, and Fe_3O_4 @GLU; insert represents an overlay of Fe_3O_4 , Fe_3O_4 @TCA, and sodium trichloroacetate, over the region of 400–1800 cm^{-1} .

from $\sim 532 \text{ cm}^{-1}$ and the strong decrease of the Roentgen interferences characteristic for magnetite.

The most representative SEM images recorded at 50 (100) kx and 400 kx are presented in Figure 5. At high magnification, the nanostructured nature of these materials can be visualized. The morphology of Fe_3O_4 @GLU and Fe_3O_4 @SAL is similar to that of bare Fe_3O_4 while the Fe_3O_4 @TCA exhibits different morphology, perhaps due to the increasing content of the organic stabilizing agent and smaller particle size. These differences will be further resolved by (HR)TEM.

The TEM images were used to determine the size and morphology of the synthesized MNPs (Figure 6). The TEM images of the samples reveal some particularities. First of all, in all cases, the fine particles are strongly agglomerated because of the attractive forces between the magnetic nanoparticles. Based on the HRTEM images, the size and shapes of the nanoparticles can be evaluated. In the case of bare magnetite, spherical nanoparticles can be identified, the diameter of these nanoparticles being between 3 and 20 nm. In the case of Fe_3O_4 @GLU, two kinds of nanoparticles can be identified. The spherical nanoparticles have similar characteristics with those of pure magnetite but some rod-like nanoparticles can be also identified, their length reaching up to 50 nm, while the diameter is less than 10 nm. Based on the crystallite size determined by Debye-Scherrer equation, a good consistency between the TEM and XRD data can be concluded if considering also the capping effect induced

by the stabilizing agent [36] except for the case of the rod-like structures identified in Fe_3O_4 @GLU where these particles contain tens of crystallites. In the case of Fe_3O_4 @SAL and Fe_3O_4 @TCA, the size of the spherical nanoparticles is decreasing dramatically compared with the previous MNPs; the use of TCA as stabilizing agent induces the most important size reduction and, thus, has the best ability to stabilize the magnetite and can be correlated with the higher amount of stabilizing agent linked onto the magnetite core (7.28%). This conclusion is also supported by the SAED patterns of the four MNPs. In all cases, the selected area electron diffraction shapes confirm the formation of very fine nanoparticles, especially when TCA is used, the polycrystalline structure of the cubic spinel magnetic crystals being clearly highlighted, being in good accordance with the XRD data [37].

BET analysis was used to characterize the obtained mesoporous MNPs from the point of view of surface area. From the point of view of the average pore size and specific surface area, the stabilizing agents play an important role. According to Table 1, the pure magnetite has a specific surface area of $\sim 156 \text{ m}^2/\text{g}$ and an average pore size of 5.75 nm while the use of salicylic acid leads to an increased specific surface area and practically no change of the pore size; the use of glutamic acid leads to an important decrease of the specific surface area and an increase of the pore size while the use of trichloroacetic acid leads to a strong increase of the specific surface area (almost two times higher specific surface area

FIGURE 5: SEM images of the stabilized and nonstabilized magnetic nanoparticles obtained by coprecipitation.

compared with reference magnetite) and a corresponding decrease of the average pore size, down to 3.45 nm. The different behaviors are explained based on the different chemical structure of the three stabilizing agents and different amount of stabilizing agent adsorption onto the magnetic crystals. The strongest stabilization effect is ensured by TCA which conducts to a higher specific surface area being in good agreement with the TEM images (especially visible at low magnification, the first image of each sample). Compared with the well-established Fe_3O_4 @citrate superparamagnetic iron oxide nanoparticles, it can be concluded that TCA can ensure even better stabilization effect and the specific surface area can be improved by ~10% [34].

The mechanisms of the formation of the Fe_3O_4 @stabilizing agent is strongly dependent on some factors, the most important being the nature of the stabilizing agent and its concentration and pH. When the stabilizing agent is TCA, the single possibility of self-assembling is presented in Figure 7(a), carboxylate group being absorbed onto the magnetite core surface, while the trichloromethyl groups are oriented to the outer region of the core@shell structure. Between these core@shell individual structures hydrophobic interactions occur, thus leading to agglomerates. In the case of glutamic acid, as well as salicylic acid, the formation

FIGURE 6: TEM, HRTEM, and SAED patterns of the bare magnetite and stabilized MNPs.

FIGURE 7: Magnetite based suprastructures induced by stabilizing agents.

of $\text{Fe}_3\text{O}_4@\text{GLU}$ and $\text{Fe}_3\text{O}_4@\text{SAL}$ leads to the existence of multiple functional groups of the stabilizing agent, therefore allowing additional interactions between these moieties. So, along with the formation of the similar core@shell structures and agglomerates (assuming that COOH is in its anionic form and ammonium in its zero-valent form, namely, NH_2), as presented in the case of TCA, the additional interactions between different groups of the stabilizing agents also allow new stabilizing agent-stabilizing agent interactions (Figure 7(b), region delimited by green ellipse), leading to an increasing size of the core@shell structures and consequently lower specific surface area compared with $\text{Fe}_3\text{O}_4@\text{TCA}$. Even double layered shells can be imagined partly because the amino groups are in their cationic form and also water molecules can facilitate these interactions.

The as-obtained core@shell nanostructures can be imagined as potential carriers of a wide variety of biological active agents; the loading efficiency and the delivery behavior are controlled and induced due to the shell characteristics. The active agents can be attached to the shell, adsorbed into the magnetic core, or even distributed between the two components of the core@shell structures. These active agents will interact with the shell via hydrophobic or hydrophilic interactions. The use of salicylic acid as capping agent will allow the formation of strong hydrogen bonds with these nanostructures, while in the case of glutamic acid hydrogen bonds as well as electrostatic interactions will be possible, depending on the pH. In both cases covalent bonding of the active agent is possible via the reactive phenolic and carboxylic groups. In the case of trichloroacetic acid, especially hydrophobic and dipole based interactions will appear, but also more complex mechanisms of bonding are possible involving partial/full hydrolysis of the trichloro groups followed by covalent bonding. The main interactions between

the shell and the biological active agents are presented in Figure 8. The overall properties of the drug delivery systems are strongly dependent on many factors including the loading procedures, not only the nature and proportions of the components.

4. Conclusion

Three new magnetic nanoparticles were obtained starting from magnetite precursors and adequate stabilizing agents (salicylic acid, glutamic acid, and trichloroacetic acid) by spraying the magnetite precursors into the basic stabilizing agent solution. The results reveal important difference between these MNPs, these differences being induced by the used stabilizing agent. The smallest nanoparticles were obtained in the presence of trichloroacetate ions. The mechanism of formation of these suprastructures is strongly correlated with the end functional groups of the stabilizing agent. Comparing the glutamate and trichloroacetate, from both size and free functional groups, significant differences can be identified. In fact, the entire molecule excepting the carboxylate groups (highlighted with red) ensures the interaction of the stabilizing agent with the magnetic core as represented in Figure 7. It can be concluded that the agglomeration of the stabilized core@shell suprastructure is different because of the very different affinity of these end groups. In the case of glutamic acid, the ammonium and carboxylate groups can further interact and thus lead to larger particles and smaller core@shell structure as highlighted by TEM and HRTEM. The as-obtained nanoparticles are potential contrast agents as well as magnetic carriers of specific drugs for cancer diagnosis and treatments. Further works will be conducted in order to study the biocompatibility, cell internalization, and ability for targeted transport and delivery of various biological active agents including cytostatic drugs.

FIGURE 8: Potential interactions between the nanostructured core@shell structures and different biological active agents.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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