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Impact of Amino-Acid Coating on the Synthesis and Characteristics of Iron-Oxide Nanoparticles (IONs)

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Iron-oxide nanoparticles (IONs) with biocompatible coatings are the only nanostructural materials which have been approved by the FDA for clinical use. Common biocompatible coatings such as hydrocarbons, polymers, and silica have profound influences on critical characteristics of IONs. Recently, amino acids were introduced as a novel biocompatible coating. In the present study, the effects of amino acids on IONs synthesis and characteristics have been evaluated. Magnetite nanoparticles with L-arginine and L-lysine coatings were synthesised by a coprecipitation reaction in aqueous solvent and their characteristics were compared with naked magnetite nanoparticles. The results showed that amino acids can be a perfect coating for IONs and would increase particle stability without any significant effects on the critical properties of nanoparticles such as particle size and magnetization saturation value.

Key Words : Amino acid, Arginine, Lysine, Magnetic nanoparticles, Synthesis

Introduction

Magnetite (Fe_3O_4) is one of the major iron ores found in the earth's crust and magnetite nanoparticles are the most common iron oxide nanoparticles (IONs) in nanotechnology. It is indeed a binary ionic compound, made of a metal (Fe) and a non-metal (O) *via* chemical combination of iron(II) oxide with iron(III) oxide. It is known as iron(II, III) oxide or ferrous-ferric oxide, but its IUPAC name is iron(II) di-iron(III) oxide with $FeO{\cdot}Fe_2O_3$ formula (see inset in Scheme 1). Magnetite crystalline structure has a cubic inverse spinel structure which consists of a closed-packed oxygen arrangement with the Fe(II) ions and half of the Fe(III) ions in octahedral coordination and the other half of the Fe(III) ions in tetrahedral coordination with oxygens.[1] In aqueous solutions, unoccupied atomic orbitals of co-ordinatively unsaturated Fe atoms on the surface of IONs would co-ordinate with hydroxyl ions or water molecules which share their lone electron pair with Fe. This co-ordination resulted in water molecule dissociation and IONs surface covered by hydroxyl groups (Scheme 1).[2]

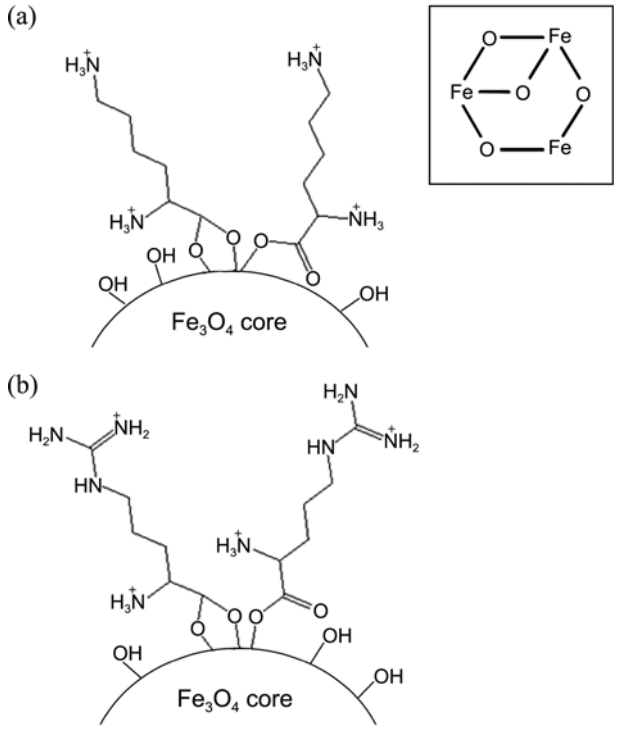
IONs have applications in medicine as a new contrast agent in magnetic resonance imaging (MRI), targeted drug delivery and nano heat sources in hyperthermia techniques. Also, IONs have been used in the biological sciences for cell labeling, cell separation, RNA and DNA purification, enzyme and protein immobilization,[3-6] and even for pathogen and non-pathogen bacteria separation.[7-10] IONs with biocompatible coatings are the only nano-sized structures which were approved by FDA for clinical use.[11,12] Naked IONs have significant toxic effects in all tested cell lines and can disturb cell membrane functions leading to leakage of lactate dehydrogenase and large aberrations in the cell membrane, with bubble-shaped protrusions extending from the cell body.[13,14] There is evidence that bare IONs can reduce microfilament formation and disrupt actin distribution within the main bodies of the cells, resulting in smaller cells with smaller structures and a spherical shape.[14-16] Naked IONs produce reactive oxygen species (ROS) in the Fenton's reaction (Eqs. (1) and (2)[17]) and disturb the balance between oxidative pressure and antioxidant defence of cells, causing membrane lipid peroxidation, DNA breakage, protein oxidation and giving rise to necrosis and apoptosis.[12,17,18] All these toxic effects could be significantly eliminated by use of biocompatible coatings, such as dextran,[13,14,17,19] polyvinyl alcohol,[19,20] poly(ethylene-glycol),[21] pullulan,[15] dimercaptosuccinic acid[22] and proteins, like albumin[13] and transferrin.[14]

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-} \quad (1)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OOH^{\bullet} + H^{+} \quad (2)$$

In recent years, there has been increasing interest concerning amino acid coatings of IONs. Amino acids are suitable because they play a very important role in the body. Therapy with amino acid imbalance (TAAI) or with amino acid dosages have been widely used to treat cancer sufferers because some amino acids have been shown to reduce tumours. Excess amino acids, such as arginine and leucine, lead to tumour shrinkage or even tumour cells dying out.[23,24] Amino acids interact with the nanoparticle's surface through the carboxyl groups and side chains which are exposed to the exterior.[24-26] The schematic possible structures for amino-acid coating on Fe_3O_4 nanoparticles have previously been suggested based on attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) (Scheme 1).[25]

Those with side-chain functional groups also provide an active group for interaction with an enormous variety of biological molecules and ligands. Synthesis of IONs with an amino-acid coating could be done in a one pot aqueous reaction. This simple synthesis pathway and lack of organic solvents have increased popularity of amino-acid coatings. All coating materials have undesirable effects on ION characteristics. Common biocompatible coatings such as silica,[27-29] hydrocarbons and polymers[30,31] significantly increase the particles size and reduce their magnetic saturation value. The aim of the current survey is to evaluate the impact of amino-acid coating on the IONs synthesis and characteristics. Magnetite nanoparticles were synthesised and coated by L-lysine and L-arginine amino acids. Prepared nanoparticles were characterized and compared with naked magnetite nanoparticles.



Scheme 1. Possible structures for L-lysine (a) and L-arginine (b) coating on Fe_3O_4 nanoparticles, the inset is showing the molecular structure of Fe_3O_4.

Experimental

Synthesis of Amino Acid Coated IONs. Amino-acid coated magnetite nanoparticles were synthesised by the one pot synthesis method. 1.17 g of ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) and 0.6 g of ferrous sulphate tetrahydrate ($FeSO_4{\cdot}4H_2O$) (molar ratio 1.75:1, respectively) were dissolved in 50 mL deionized water and the mixture was stirred vigorously under N_2 atmosphere at 70 °C. After 30 min, 1.6 g of L-lysine or 1.5 g of L-arginine were dissolved in 6 mL distilled water and rapidly added to the mixture and the reaction was stirred for another 30 minutes. Then 5 mL NH_4OH (32%) was rapidly added and stirring continued for 1.5 h. Prepared particles were separated magnetically, washed with distilled water three times, and dried in an oven at 50 °C overnight.[23,25,26,32]

Synthesis of Naked IONs. Naked nanoparticles were synthesised as described above, but without adding any amino acid. Briefly, 1.17 g of ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) and 0.6 g of ferrous sulphate tetrahydrate ($FeSO_4{\cdot}4H_2O$) were dissolved in 50 mL deionized water and the mixture was stirred vigorously under N_2 atmosphere at 70 °C. After 1 h, 5 mL ammonium hydroxide (32%) was rapidly injected into the mixture and stirred for another 1 h. The black precipitate was separated by magnet, the particles were washed three times with distilled water, and dried in an oven at 50 °C overnight.[3]

Characterization of IONs. Prepared particles were characterized by a Fourier transformed infrared (FTIR) spectrometer (Bruker, Vertex 70, FTIR Spectrometer) using KBr pellets containing 1.5 mg sample and 15 mg KBr. Differential scanning calorimetry (DSC) analysis was done in aluminium pans containing 7 mg sample and a pan containing Al_2O_3 was used as reference (BAHR Thermoanalyser DSC 302).[30,33] Measurements were performed from 25 °C to 499 °C with a scanning rate of 10 °C min^{-1} under ambient atmosphere and nitrogen was used for sweeping (5 °C min^{-1}). Saturation magnetisation (M_S) values of IONs were evaluated by a vibrating sample magnetometer (VSM) at room temperature (Meghnatis Daghigh Kavir Co., Iran). A drop of nanoparticle dispersion (100 μg mL^{-1} distilled water) was dripped on a carbon-coated copper grid and transmission electron microscopy (TEM) micrographs obtained with Philips CM 10, TEM, operated at HT 100 Kv. Particle size distribution and mean particle size were determined by measuring diameters of one hundred nanoparticles randomly selected on the TEM images.[26,34] The crystallinity of synthesised Fe_3O_4 nanoparticles was studied with an x-ray diffractometer (Siemens D5000).

Results and Discussion

The coprecipitation reaction of Fe^{+2} and Fe^{+3} in aqueous medium is the most common and environmental friendly process for the synthesis of IONs. In this method (bottom-up) magnetite nanoparticles are obtained through Eq. (3).[35]

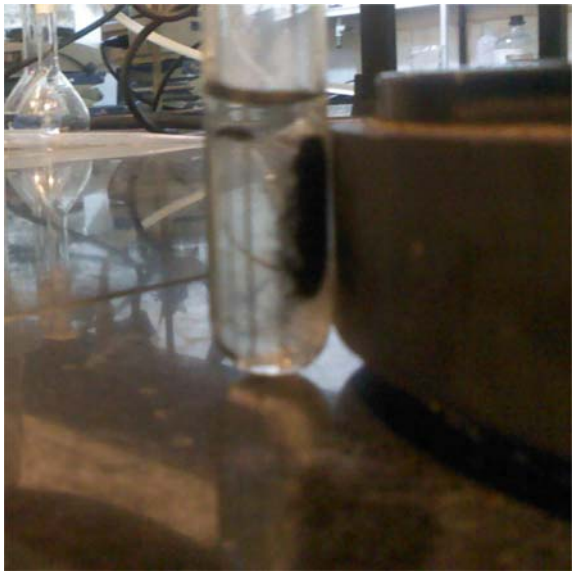


Figure 1. Immediate response of prepared magnetite nanoparticles to the permanent magnet.

$$Fe^{+2} + 2Fe^{+3} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O \quad (3)$$

In this study, as ammonium hydroxide was added to the iron salts solution a sudden colour change to dark black was observed and Fe_3O_4 cores were formed. By following the reaction, magnetite cores were grown and nanoparticles became larger.[2] All prepared particles, amino-acid coated and naked, were sensitive to a magnetic field and responded quickly to the permanent magnet (Figure 1).

The presence of amino acids in the iron salts mixture has an inhibitory effect on the growth of magnetite nanoparticles and reduces particle size.[26] To alleviate this impact, the reaction time of synthesis in the presence of amino acids has been increased to 1.5 h in contrast to 1 h in the absence of amino acids. More time is therefore provided for the growth of nanocrystals and prepared amino-acid coated nanoparticles will be more similar to naked nanoparticles in size. Figure 2 shows the appearance of prepared magnetite nanoparticles and the insets correspond to particle size distribution. The diameter of naked nanoparticles was 7-20 nm with mean particle size of 11 nm. L-arginine coated nanoparticles were from 5 to 12 nm with mean particle size of 8 nm. Nanoparticles with L-lysine coating were measured 4-10 nm and mean particles size was 7 nm. Despite more synthesis time, amino-acid coated particles were smaller than naked particles. Various amino acids have different effects on synthesised particles' size. According to the results of current study and Patel *et al.*,[23] presence of L-lysine in the reaction mixture has a greater size reducing effect than either L-arginine or L-glutamine. Additionally, the presence L-arginine or L-lysine in the iron salts mixture during ION synthesis resulted in the production of nanoparticles with a narrower particle size distribution.

The FTIR spectra of Fe_3O_4 nanoparticles are presented in Figure 3. The Fe-O characteristic peaks of magnetite nano-

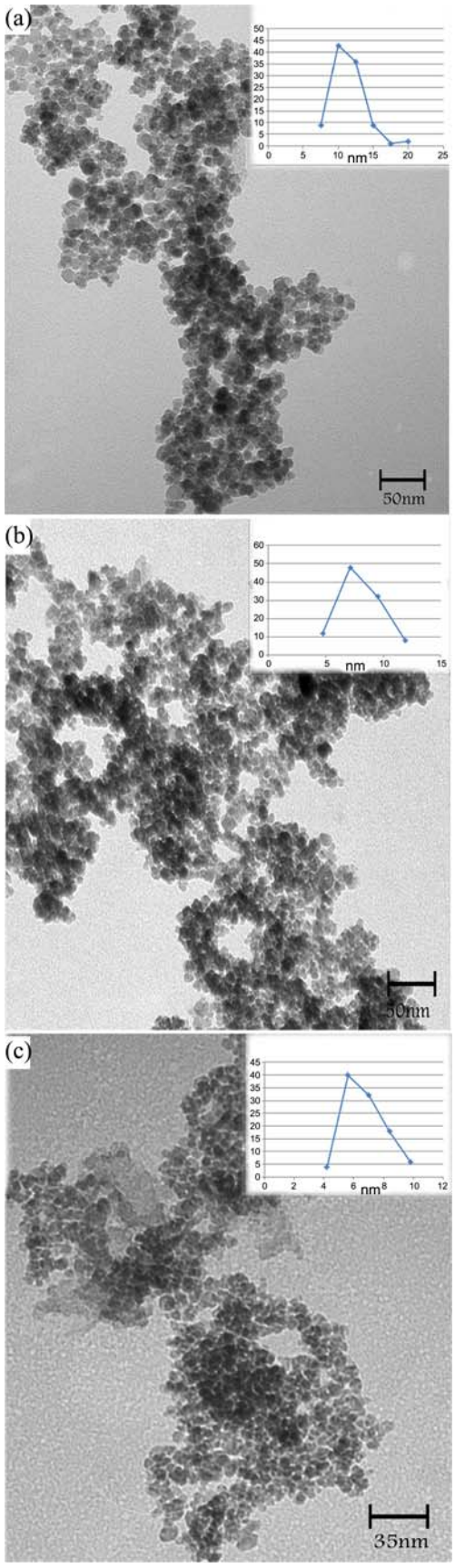


Figure 2. Transmission electron micrographs of naked (a), L-arginine coated (b) and L-lysine coated (c) magnetite nanoparticles, the insets are corresponding particle size distribution.

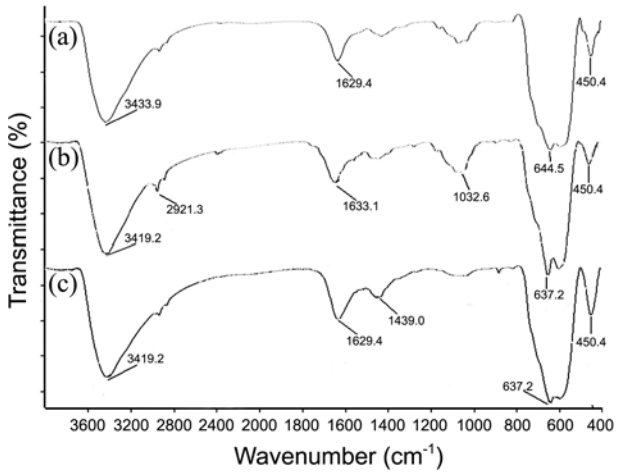


Figure 3. FTIR spectra of naked (a), L-arginine coated (b), and L-lysine coated (c) magnetite nanoparticles.

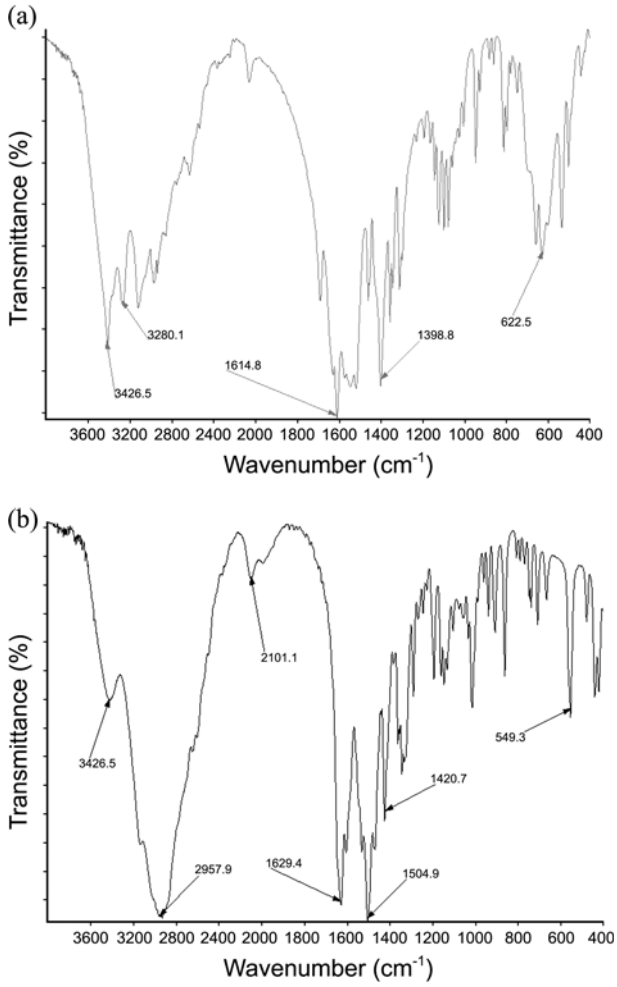


Figure 4. FTIR spectra of pure L-arginine (a) and L-lysine (b).

particles appear at about 640 cm^{-1}, split into two peaks, and 450 cm^{-1}. In aqueous medium, the surface of the magnetite nanoparticles has been modified by OH groups, due to co-ordination of unsaturated surface Fe atoms with hydroxyl ions or water molecules. These OH groups absorb IR waves at about 1630 cm^{-1} (deforming) and 3400 cm^{-1} (stretching).[2-4,6,35-37] In amino-acid coated nanoparticles the C=O and C-O stretching vibrations can be seen at ~1630 cm^{-1} and ~1439 cm^{-1} respectively. The peak at 2921 cm^{-1} is due to C-H stretching vibration and N-H stretching vibration overlaps with OH stretching at 3419 cm^{-1}. FTIR diagrams of pure L-arginine and L-lysine are shown in Figure 4. Compared to pure amino acids, shortening of the carboxyl group's peak in Figure 3(b and c) is due to interaction with OH groups at the surface of the nanoparticles.[24-26]

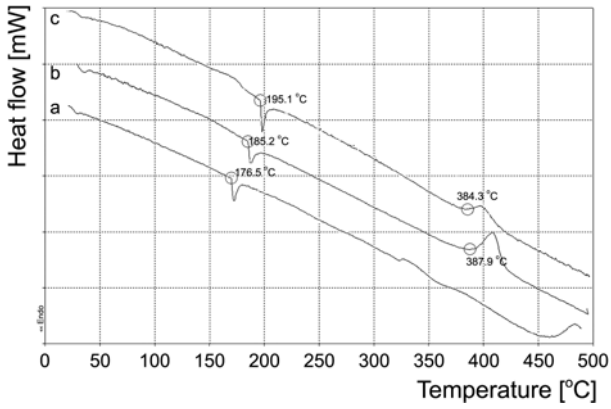


Figure 5. DSC curves of naked (a), L-arginine coated (b), and L-lysine coated (c) magnetite nanoparticles.

DSC curves of magnetite nanoparticles are shown in Figure 5. An endothermic peak, due to oxidation and change in crystallinity of Fe_3O_4 crystals, can be seen between 150 °C and 200 °C.[33] In naked nanoparticles, this change appears at 176.5 °C; however oxidation of amino-acid coated particles occurs at higher temperatures, 185.2 °C and 195.1 °C in L-arginine and L-lysine coated particles, respectively. Decomposition of amino-acid coating occurred at about 380 °C and produced an exothermic peak.[26] Depending on the amino acid, the coating could increase the oxidation stability of IONs. DSC analyses show that L-lysine coated particles are more stable than those with L-arginine coating. Although the oxidation reaction of L-arginine coated nanoparticles occurred in lower temperature than L-lysine coated particles, the former reaction occurred more slowly and produced a broader endothermic peak. It is known that features of transformation from magnetite to maghemite, and hence hematite by oxidation, are influenced by magnetite crystal size.[33] In small crystals, the surface to volume ratio is high and reaction rates are fast, so oxidation is achieved rapidly at lower temperatures.[33] The smaller nanoparticles, which have been synthesised in the presence of amino acids, are more stable and oxidised in higher temperatures.

Results of saturation magnetisation analysis can be seen in Figure 6. No hysteresis is seen and magnetization curves are completely reversible exhibiting the superparamagnetic behaviour of the prepared particles. The saturation magnetization (Ms) values read from the magnetization plots[34] were found to be 60 emu/g for naked, 52 emu/g for L-Arg coated Fe_3O_4 and 42 emu/g for L-Lys coated Fe_3O_4. Coating of magnetite nanoparticles with paramagnetic amino acids would reduce the saturation magnetization (Ms) values. This

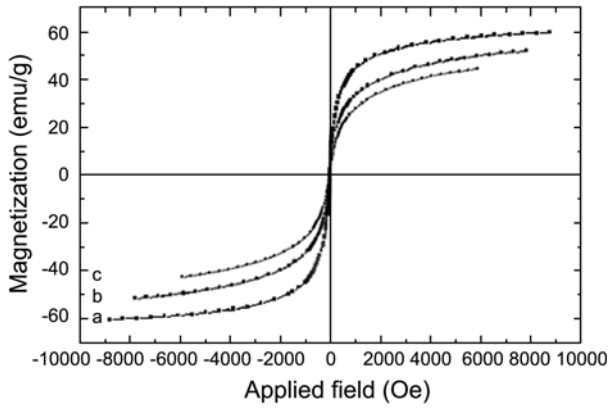


Figure 6. Vibrating sample magnetometer (VSM) diagrams of naked (a), L-Arg coated (b) and L-Lys coated (c) Fe_3O_4.

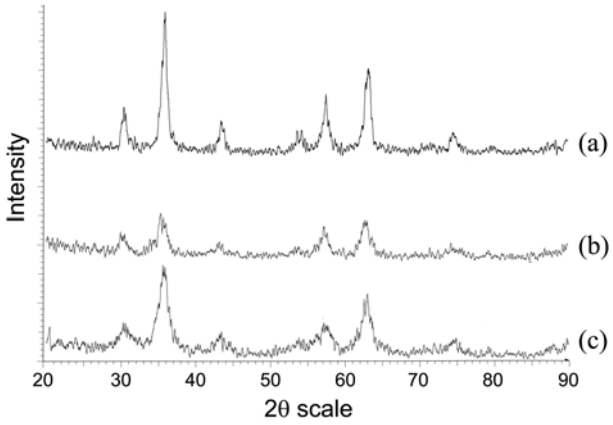


Figure 7. X-ray power diffraction patterns of naked (a), L-arginine coated (b) and L-lysine coated (c) magnetite nanoparticles.

reduction is in direct relation to amino-acid coating density. Nanoparticles with a denser coating, which have been synthesised in higher amino acid concentrations, are less sensitive to a magnetic field.[26] This is considerable that by synthesis of magnetite nanoparticles in higher concentrations of amino acids, smaller particles would be obtained and to some extend the reduction in Ms value is due to reduction in size of produced nanoparticles.[38] In contrast to the large reduction in magnetisation by other biocompatible coatings such as hydrocarbons, polymers and silica,[29,30] the reduction due to amino-acid coating is negligible.

X-ray power diffraction patterns of naked and amino-acid coated nanoparticles are demonstrated by the characteristic features of magnetite nanoparticles having intensity peaks at values expressed in 2θ degrees of 30, 35.5, 43, 57, and 63 (Figure 7). The smaller size of the amino-acid coated magnetite particles led to the XRD peak broadening in (b) and (c) spectra.[39-42]

Conclusion

In the current study, naked and amino-acid coated magnetite nanoparticles were synthesised and the effects of amino acids on synthesis and characteristics of prepared particles were evaluated. Amino-acid coatings increase ION stability and modify the surface of nanoparticles by addition of functional groups for interaction with a wide variety of molecules and ligands. This biocompatible coating has no undesirable effect on the main characteristics of IONs, however, it is worth mentioning that according to our laboratory experiments, amino-acid coated IONs are less colloidally stable in an aqueous matrix than polymer coated IONs.

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