A facile one-pot synthesis of poly(acrylic acid)-functionalized magnetic iron oxide nanoparticles for suppressing reactive oxygen species generation and adsorption of biocatalyst

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Abstract

Functionalized iron oxide nanoparticles (IONPs) have unique physical and chemical properties, which make them potential candidates for biomedical applications. In this study, a facile one-pot method is reported for the preparation of poly(acrylic acid) (PAA) functionalized IONPs through in situ free radical solution polymerization of AA and subsequent coprecipitation of Fe$^{3+}$ and Fe$^{2+}$ ions. The FTIR spectroscopic and TGA results indicated the successful formation and surface functionalization of IONPs with PAA. Electron micrographs showed that the prepared particles were of nano-sized and their shape is dependent on the concentration of PAA. pH-dependent variation of average hydrodynamic diameter confirmed the pH-responsivity of PAA-functionalized IONPs. Magnetic measurement suggested that the PAA functionalized IONPs were strongly paramagnetic (53.0 emu g$^{-1}$). Fenton-like catalytic generation is carried out to measure toxicity associated with the nanoparticles. The suppression ability for reactive oxygen species (ROS) generation associated with PAA-functionalized IONPs was studied via methylene blue degradation assay to address their toxicity profile. PAA-functionalized IONPs exhibited better suppression ability than that of the bare IONPs. The adsorption behavior of trypsin was also studied at different pH levels and a maximum adsorption is occurred on PAA-functionalized IONPs at pH 5.0. Catalytic behavior study confirmed higher activity of trypsin immobilized on PAA-functionalized IONPs than that of the reference IONPs. Therefore, the functionalized IONPs can be of high interest for magnetically recyclable biocatalyst carrier.

Introduction

Nowadays, the study of iron oxide nanoparticles (IONPs) is of great interest because of their potential biomedical applications including magnetic drug delivery, tumor-specific cell targeting, biosensor, magnetic resonance imaging, hyperthermia and bioseparation [1–8]. One of the most exciting properties of IONPs is magnetism, which allows them to be guided and stimulated to a desired site of the biological system by using an external magnet. It is revealed that the use of an external magnetic field to modulate magnetic property of IONPs is benign for cells [9]. In addition, different metabolic biochemical pathways including cytochrome P-450 functioning, mitochondrial oxidative phosphorylation, oxygen transportation, DNA and hemoglobin synthesis, and energy production are regulated by the ionic forms of iron, namely Fe$^{3+}$ and Fe$^{2+}$ ions [10]. However, the excessive availability of these ions may cause severe toxicity in cellular systems. For instance, the free iron ions released from the bare IONPs can be incorporated to the normal cellular iron pool resulting in iron overload to the iron carrier proteins, such as ferritin and transferin, and elevated intracellular and plasma iron concentration.
Magnetite particles have recently been reported as an adsorbent for basic dyes, and protein and gene carriers desirable features for pharmaceutical and bio-related applications. For example, PAA-functionalized magnetic anchor biomolecules and absorbing a large quantity of water, can be used as a superabsorbent. These all are existing onto the bare surface of IONPs. Moreover, PAA, having highly dense reactive functional groups to dissociation of carboxy groups of PAA makes a potential candidate to ligate metal cations, such as iron ions used without puriﬁcation. Loba, India, and was recrystallized from distilled water before use. Anhydrous FeCl₃, hydrated ferrous sulphate FeSO₄·7H₂O and trypsin were purchased from Merck, Germany. Trypsin (Pankreasprotease, Merck, Germany) was used for adsorption study without any puriﬁcation. Other chemicals were of analytical grade and used without puriﬁcation. Deionized water was distilled by using a glass (Pyrex) distillation apparatus and used throughout this work.

Experimental

Materials

AA was purchased from Fluka, Germany. Potassium persulphate (KPS) used as the initiator was purchased from Loba, India, and was recrystallized from distilled water before use. Anhydrous FeCl₃, hydrated ferrous sulphate FeSO₄·7H₂O and trypsin were purchased from Merck, Germany. Trypsin (Pankreasprotease, Merck, Germany) was used for adsorption study without any puriﬁcation. Other chemicals were of analytical grade and used without puriﬁcation. Deionized water was distilled by using a glass (Pyrex) distillation apparatus and used throughout this work.
Methods

Synthesis of PAA-functionalized magnetic Fe₃O₄ nanoparticles: PAA-functionalized Fe₃O₄ nanoparticles were prepared through in situ co-precipitation method. At first, various solutions of PAA were prepared by varying initial monomer contents (0.25, 0.50 and 1.0 g) in the recipe by free-radical solution polymerization of AA using KPS (1 wt% of monomer) as an initiator. The polymers synthesized with 0.25 g, 0.50 g and 1.00 g of AA are referred as PAA1, PAA2 and PAA3. The reaction mixture was mechanically stirred at 150 rpm and the polymerization reaction was carried out at 70 °C for 6 h under N₂ atmosphere. Then, the temperature of the polymerization reaction mixture was cooled down to 50 °C, and the aqueous solutions of FeCl₃ (0.98 g in 50.0 ml) and FeSO₄·7H₂O (0.83 g in 70.0 ml), which were prepared under N₂ purging, were poured into the medium containing PAA.

Aqueous NH₄OH (25%, 50.0 ml) was added immediately to the reaction mixture to increase the pH at 10.0, and the reaction was further continued more 16 h at a stirring speed of 1400 rpm. At the end of the reaction, the brown dispersion was turned into black. The obtained dispersion was cooled down to room temperature and washed 5 times with deionized distilled water using an external magnet.

Characterization

Scanning electron microscopy (SEM) images were obtained on a JEOL (Tokyo, Japan) JSM-6510 scanning electron microscope. The dilute dispersion was dropped onto a mica sheet and the sample-sheet was dried in a vacuum desiccator overnight. The dried particles were coated with Au by sputtering before SEM observation. The size and morphology of the designed particles were observed using a transmission electron microscope (TEM) (JEM-1230, JEOL, Japan). The sample was diluted down to around 0.01% solid content and a small drop of nanoparticle dispersion was placed on a carbon-coated copper grid. The sample grid was dried at a reduced temperature under vacuum. The images were taken at an accelerating voltage of 100 kV. Fourier transform infrared (FTIR) spectro-photometer (PerkinElmer, FTIR-100, UK) was used to get an insight about functionalization of the particles. The spectra were taken using KBr pellets and were scanned within the range of 200–4000 cm⁻¹. Hydrodynamic average diameter of the particles was determined by using a dynamic light scattering (DLS) NICOMP 380 particle sizer (Santa Barbara, California, USA). The particle dispersion was diluted down to around 0.01% solid content. At respective pH, the intensity weighted average hydrodynamic diameters of the magnetic particles were measured. Each measurement was carried out in duplicate and the reproducibility of the size measurement was within ±5%. Thermal degradation study of the samples was carried out on a thermogravimetry analyzer (TGA) (EXSTAR-6000) from Seiko Instruments Inc. Japan. Weight loss of the dry powder samples was measured from ambient temperature to 800 °C at a heating rate of 20 °C min⁻¹ under N₂ atmosphere and the data was recorded. The x-ray diffraction (XRD) patterns of the powder samples were taken with a scanning x-ray diffractometer (Bruker D8 Advance, Germany) using CuKα radiation (λ = 1.54 Å), AQ6, a tube voltage of 33 kV and a tube current of 45 mA. The intensities were measured at 2θ values from 5° to 80° at a continuous scan rate of 10° min⁻¹ with a position-sensitive detector aperture at 3° (equivalent to 0.5° min⁻¹ with a scintillation counter). Vibrating sample magnetometer (VSM) (Micro Sense, EV9, USA) was used for magnetic property analysis.
Suppressing ability for reactive oxygen species (ROS) generation by functionalized IONPs
The suppressing capability of ROS generation by the reactive surface of bare IONPs and PAA- functionalized IONPs was assessed via methylene blue (MB) degradation assay at ambient conditions. The degradation experiments were carried in an aqueous MB solution (10 ml, 5 μg/ml) in presence of different amounts (1.0 and 1.5 mg) of functionalized and bare IONPs. The degradation of MB was initiated by spiking the samples with an aqueous H2O2 solution (30%, 245 mM). After incubation, the bare- and functionalized nanoparticles were magnetically decanted, and the absorbance of the supernatants was measured at 660 nm using a UV-visible spectrophotometer.

Adsorption of trypsin as a biocatalyst
Five mixtures (15.0 ml) were prepared from the dispersion of washed PAA-functionalized IONPs (0.01 g) and an aqueous solution of trypsin (200 mgg⁻¹ of functionalized IONPs) using 5 vials (20.0 ml). The pHs of mixtures were immediately adjusted at 5.0, 7.0 and 10.0 and then, the sample vials were transferred into a water bath maintained at 25 °C temperature with occasional shaking. After 45 min incubation, the IONPs were separated magnetically followed by centrifugation at 10,000 rpm for 5 min in order to remove any particle left in the supernatant. The concentration of trypsin in the supernatant was measured by a UV-visible spectroscopy monitoring the absorbance at the wavelength (λmax) of 280 nm. The amount of adsorbed trypsin onto the IONPs surface was calculated by subtracting the concentration of free trypsin from the initial concentration.

Measurement of enzyme activity
The activity of the free and immobilized trypsin was determined by the degradation assay of N- benzoyl-DL-arginin-p-nitroanilid hydrochlorid (BAPNA) as substrate, monitoring the increase of absorbance at 410 nm for p-nitro aniline using UV-visible spectrophotometer. The activity (per U) is defined as μmol BAPNA hydrolyzed within 1 min applying an absorption coefficient of ε₄₁₀ 8800 M⁻¹ cm⁻¹. For the activity of free trypsin 0.835 ml solution of 220 mM BAPNA was prepared in DMSO and then diluted with 20.0 ml of water and 0.5 ml of phosphate buffer solution (pH; 8.0). Finally, 10.0 ml of trypsin solution (0.1 mg mL⁻¹) was added to the mixture to measure the catalytic activity. The activity of immobilized trypsin on Fe₃O₄ and Fe₃O₄/PAA1 were also analyzed with same procedure; where trypsin was first immobilized on the nanocomposites at pH; 5.0.

Results and discussion
The surface morphologies of bare and PAA- functionalized IONPs nanoparticles were studied by SEM images (figure 1). SEM images reveal the formation of nanosized bare IONPs (15 ~ 25 nm) and PAA1 functionalized IONPs nanoparticles (5.0 ~ 7.5 nm). The shapes of both bare and functionalized IONPs are almost spherical. The average size of IONPs is reduced, when they were prepared in the presence of PAA.

Here PAA is functioning as a surface stabilizer by both steric and electrostatic repulsions and controlling particle growth process during IONPs formation, whereas in the absence of PAA the particle nuclei formed initially and preceded the growth unhindered resulting relatively larger particles. Relatively large-sized bare-IONPs is more prone to aggregation due to magnetic attraction forces. PAA1-functionalized IONPs showed smoother surface, while bare IONPs showed rough surface. A difference in contrast is also visible in the SEM images between bare- and PAA- functionalized IONPs. This difference is possibly due to the formation of PAA
soft hairy structure, which has been collapsed on the surface of IONPs and serving as glue, during drying of the sample.

The morphologies and size distributions of bare IONPs and IONPs in PAA1- and PAA2- functionalized IONPs were further observed by TEM as illustrated in figure 2. The TEM image of bare IONPs shows no clear boundary among individual particles and average diameter is approximately 25 nm, which is in good agreement with the SEM result. PAA1-functionalized IONPs exhibited almost regular size and distribution having an average diameter of 7 nm. The very thin layer coating of PAA1 onto the surface of IONPs was not observable due to absence of any heavy atoms under high energy electron beam. Therefore, the TEM result is also in agreement with those of the SEM images. In the presence of PAA2, interestingly produced IONPs with larger sizes and deviation from the regular shape. Some bridge molecules onto the surface of IONPs

The chemical structure of bare IONPs and PAA-functionalized IONPs were studied by FTIR spectroscopy. Figure 3 shows a comparison of the FTIR spectrum of bare IONPs with that of various PAA-functionalized IONPs. In the spectrum of bare IONPs, the characteristic stretching vibration bands appeared at 424 and 578 cm\(^{-1}\), which are assignable to the Fe–O bond vibrations. These bands were shifted to 429 and 583 cm\(^{-1}\) in the FTIR spectra of PAA functionalized IONPs due to the polyelectrolytic interaction of the carboxylate moieties with the surface of IONPs. A stretching broad band occurred in the region of 3200–3500 cm\(^{-1}\) and a bending band was also observed at 1625 cm\(^{-1}\) indicating to –O–H moieties of the adsorbed and hydrogen bonded water molecules onto the surface of IONPs [32, 34, 35]. In the spectrum of pure PAA, a strong band at 1718 cm\(^{-1}\) assignable to the carbonyl group stretch of the PAA and the band at 1410–1450 cm\(^{-1}\) are corresponded to the stretch of C–O and the deformation vibration of OH, and this band was shifted to 1400 cm\(^{-1}\) in the spectra of PAA functionalized IONPs [36]. In addition, the presence of two new signals at 1454 and 1552 cm\(^{-1}\) indicates the chemical interaction of magnetite with PAA, while the absence of the peak at around 1700 cm\(^{-1}\) is indicating bidentate chelation of the carboxylate moieties to Fe atoms of the IONPs surface [37–40]. The peak intensity at 1645 cm\(^{-1}\) was increased (figures 3(b)–(d)) than that of the peak of bare IONPs due to C=O stretching vibrations, which indicated that some of the carboxyl moieties of PAA were bonded to nanoparticles either in monodentate form or existing as a free acid [40]. All these results confirm the surface functionalization of IONPs with PAA.

The crystal structure of bare IONPs and the effect of PAA functionalization on the crystal structure of IONPs were analyzed by XRD technique. Figure 4 shows the XRD patterns of bare IONPs and, PAA1 and PAA3-functionalized IONPs. The XRD profile (figure 4(a)) of bare IONPs showed characteristic six diffraction peaks at 2\(\theta\) values of 30.08°, 35.45°, 43.50°, 53.54°, 57.11° and 62.78°, which are assignable to (220), (311), (400), (422), (511), and (440) Brava is planes of fcc Fe\(_3\)O\(_4\), respectively (ICPD\(_\)S card no. 19–0629) [32]. These reflections also appeared in the XRD patterns of PAA functionalized IONPs (figures 4(b) and (c)) and no any additional sharp peak of other iron compounds is observable, which imply the phase purity, in \textit{in situ} formation of Fe\(_3\)O\(_4\) and its crystal structure was not hindered by PAA functionalization. Further, the intensity of diffraction signals remains

Figure 2. TEM images of (a) bare IONPs, (b) PAA1 functionalized IONPs (c) PAA2 functionalized IONPs.
almost identical after surface modification. In addition, a broad reflection also observed at 2θ region of 17 to 24° in the XRD profile of PAA1 functionalized IONPs due to the presence of amorphous PAA on the surface of the particles that demonstrates the successful functionalization of the nanocomposite particles [41, 42]. However, in figure 4(c) for PAA3-functionalized IONPs, some intense peaks for IONPs have become weak which might be due to the surface coverage of IONPs with PAA3.

The thermal behaviors of the functionalized IONPs and PAA contents were evaluated by TGA. Figure 5 shows the thermograms of bare IONPs and PAA-functionalized IONPs. Until 150 °C, the bare IONPs showed only 8.15% mass loss due to the elimination of bound water molecule from the surface of IONPs and the weight retained almost constant up to 800 °C. Comparatively, two significant mass loss regions were observed in the TGA thermogram of PAA-functionalized IONPs. The initial stage of the weight loss is due to the dehydration of loosely adsorbed and hydrogen-bonded water molecules on the surface. A sharp decrease occurred (figure 5(b) and (c)) at 300 to 500 °C originated from the decarboxylation and degradation of PAA chain and the residual weigh was remained unchanged to 800 °C [43–45]. On the other hand, IONPs modified with PAA2 showed higher weight loss, 28.5 wt.%, than that of IONPs modified with PAA1 (10.5 wt.%).

The magnetic properties of the dried powder samples of bare IONPs and PAA-functionalized IONPs were studied by VSM technique, where their magnetization was measured as a function of the applied magnetic field.
at ambient conditions (Figure 6). The saturation of magnetization ($M_s$) 51.6 emug$^{-1}$ for PAA1-functionalized IONPs is slightly higher than that of the bare IONPs (49 emug$^{-1}$). This enhancement in $M_s$ is due to the improvement in the uniformity of surface property and colloidal stability of the IONPs via chemical modification with carboxylate moieties of PAA [46]. Besides, the bare surface is very prone to air oxidation during synthesis, purification and storage resulting so called magnetic dead-layer on the outer surface of IONPs, which reduces the $M_s$ value. The negligible remanence and coercivity of the functionalized IONPs indicates that the prepared nanoparticles are superparamagnetic and suitable for magnetic guiding applications.

The pH dependent hydrodynamic average diameter ($D_h$) of PAA functionalized IONPs were analyzed by DLS method at 25 °C (Figure 7). For PAA2-functionalized IONPs, the $D_h$ value was decreased from 665 to 374 nm with increasing the pH value of the dispersion. The increment of pH of the dispersion promotes the formation of $-\text{CO}_2^-$ moieties and enhanced electrostatic repulsions among the polymer segments resulted larger hydrodynamic spheres. In the case of PAA1-functionalized IONPs monotonic gradual decrease of $D_h$ is not observed with increasing the dispersion pH, where the $D_h$ is decreased from 500 to 396 nm up to pH 7.0 and then slightly increased to 433.2 nm at pH 11. Additionally, a rapid sedimentation of the IONPs was observed at less than pH 6 and the $D_h$ was not measurable. This is due to the protonation of ionic carboxylate ($-\text{COO}^-$) ions to form non-ionized carboxy ($-\text{COOH}$) groups that reduced the inter chain electrostatic repulsions promoting rapid sedimentation of the dispersed particles. As described, the higher amount of PAA2 improved the dispersity of IONPs even under acidic conditions, while the lower amount of PAA1 could not stabilize the dispersibility under acidic conditions.

In a Fenton-like system, Fe$^{2+}$ ion reacts with H$_2$O$_2$ to produce highly reactive hydroxy radical (HO') and Fe$^{3+}$ ion that reacts with another molecule of H$_2$O$_2$ to generate reactive hydroperoxy (HOO') radical. These radicals can easily attack positively charged MB molecules to convert them into colorless species. The active surface of bare IONPs (Fe$_3$O$_4$) is capable to catalyze the formation of ROS through the leaching out of the iron...
ions to increase the Fenton and Haber-Weiss reactions resulted in adverse oxidative stress to cells inside the body [47]. Therefore, the degree of ROS formation can be suppressed by coverage the active surface of IONPs ($\text{Fe}_3\text{O}_4$) with suitable polymeric moieties [48]. It is reported that polymer-modified IONPs containing the $\text{–COO}^-$ groups interacted very strongly with MB by the electrostatic interactions enhancing its adsorption onto the surface of IONPs [19]. Therefore, the synthesized bare and functionalized IONPs were tested via MB degradation assay to investigate their toxicity profile in vitro.

First, the degradation experiments were examined using bare IONPs to confirm the mechanism. Figure 8(a) shows the UV-visible spectra of MB without any treatment, MB treated with $\text{H}_2\text{O}_2$, MB treated with bare $\text{Fe}_3\text{O}_4$, and MB treated with the mixture of bare $\text{Fe}_3\text{O}_4$ and $\text{H}_2\text{O}_2$. The UV-visible absorbance maxima for MB were found at 660 nm regardless of the conditions of the MB degradation. Only the treatment of MB with bare $\text{Fe}_3\text{O}_4$ decreased the absorbance, while other treatments did not. This result indicates that ROS generation is enhanced by releasing iron ions from the bare surface of $\text{Fe}_3\text{O}_4$ induced by $\text{H}_2\text{O}_2$ and this result is a good agreement with earlier reports [48].

Figure 8(b) shows the concentration dependent degradation of MB by the bare $\text{Fe}_3\text{O}_4$ and PAA functionalized IONPs. Here, $A_1/A_0$ denotes the ratio of the concentration of MB at a given time after treating with nanoparticles in presence of $\text{H}_2\text{O}_2$ with respect to the initial one. The bare IONPs showed the highest degree of the MB degradation due to easy release of Fe ions from the bare surface to participate in the Fenton chemistry via generating ROS while the PAA.functionalized IONPs exhibited significantly lower degrees of the MB degradation. This lower degree of the MB degradation indicates not only very minute leaching of Fe ions from the functionalized surface but also insufficient coverage of the IONPs surface with PAA. Higher amount of PAA in the PAA2-functionalized IONPs resulted in the lowest degree (0.01 mg g$^{-1}$) of MB degradation due to the efficient suppression of leakage of Fe ions by the robust coverage of the IONPs surface. Therefore, PAA-functionalization on IONPs effectively inhibit the Fenton-like reaction and suppressed the generation of ROS [49, 50]. This result demonstrates that PAA-functionalized IONPs may resist to endogenous hydrogen peroxide of cells and useful for biomedical applications.
Trypsin adsorption assay was carried out to measure the ability of functionalized IONPs to carry biocatalyst at different pH values. The pI value of trypsin is 10, and trypsin electrostatically interacts with carboxylate moieties under pH below the pI. pH-dependent adsorption behavior of trypsin onto the bare surface of IONPs and PAA1-functionalized IONPs are shown in Figure 9. For PAA1-functionalized IONPs, the maximum amount of adsorbed trypsin was found to be 145.0 mg g$^{-1}$ at pH 5.0 and almost 54% adsorption was dropped with changing pH to 6.0. This adsorption behavior depends on the balance of the ionization of both the adsorbent (basically PAA and IONP for bare IONP) and trypsin. The pI value of trypsin is 10, and the cationic character of trypsin becomes stronger as the decrease of pH from 10.0. Of course, trypsin becomes anionic above pH 10.0. The pKa of PAA is 4.2, and the anionic character of PAA becomes stronger as the increase of pH. The electrostatic interaction between trypsin and PAA becomes strongest at a moderate pH resulting in formations of sufficient amounts of the cationic groups in trypsin and the carboxylate groups in PAA. Under higher pH, trypsin weakens its cationic character and the ability to interact with PAA becomes weaker. Under lower pH, the amounts of the carboxylate groups in PAA are reduced and the ability to interact with cationic trypsin becomes weaker. The adsorption behavior can be explained well with the aforementioned ionization behaviors.

However, same type of adsorption curve observed for bare Fe$_3$O$_4$ particles over the same pH range. First adsorption increased from 111.0 to 228.5 mg g$^{-1}$ due to the change of pH from 5.0 to This large adsorption at pH 6.0 is due to the conversion of doubly protonated hydroxyl (–FeOH$_2^+$) into singly protonated hydroxy (–FeOH) moieties, which reduced the repulsion between particles and trypsin [51, 52]. After pH 6.0, the amount of total adsorption starts to decrease gradually up to pH value 9.0 due to the partial conversion of –FeOH group into –FeO$^-$. This generated negative charge repels the biomolecule trypsin. At pH higher than 9.0 total adsorption drops sharply and becomes 0 mg g$^{-1}$ at pH value 10.0, due to almost 100% conversion of –FeOH groups to –FeO$^-$. Compared to PAA-functionalized IONPs, bare-IONPs exhibited higher amount of trypsin adsorption except pH 5. Nonetheless, the lower colloidal stability of bare Fe$_3$O$_4$ particles limits their application for this adsorption study.

The activity of free and adsorbed trypsin are measured by same conditions and given in table 1. The highest activity (5970.0 Ug$^{-1}$) was obtained for free trypsin, which confirmed that the trypsin solution relatively retained its native structure with prolonging time and did not undergo intense self-digestion. The activity of immobilized trypsin on IONPs (2270.0 Ug$^{-1}$) and PAA1 functionalized IONPs (3210.0 Ug$^{-1}$) are lower compared to free trypsin. The plausible reason of lower activity might be for strong interaction with nanoparticles as well as partially blocking of the active sites of biocatalyst trypsin [3]. Whereas, trypsin immobilized PAA1

![Figure 9. Adsorption behavior of trypsin on bare IONPs and PPA1-functionalized IONPs at different pH.](image)

| Condition                      | Relative activity (%) | Enzyme activity (Ug$^{-1}$) |
|--------------------------------|-----------------------|-----------------------------|
| Free trypsin                   | —                     | 5970.0                      |
| Immobilized trypsin on bare IONPs | 38.02                | 2270.0                      |
| Immobilized trypsin on PAA1-functionalized IONPs | 53.77                | 3210.0                      |

Table 1. Catalytic activity of trypsin at free and immobilized state.
functionalized IONPs exhibited higher amount of activity than that of the reference IONPs. The higher amount of trypsin adsorption on PAA1 functionalized nanoparticles at certain pH; 5.0 are the possible reason higher activity of trypsin immobilized on PAA1-functionalized IONPs.

Conclusion

A facile one-pot method was used for the successful preparation of PAA-functionalized IONPs. The successful synthesis of nanosized particles were confirmed by SEM and TEM images, while the surface functionalization of IONPs with PAA was proved by FTIR spectroscopy and TGA. The amount of AA presents in the reaction system of iron oxide synthesis significantly improved the efficacy of functionalization and stabilization of the dispersion of IONPs. The PAA-functionalized IONPs stably dispersed under a wider pH range than that for bare IONPs, and the hydrodynamic diameter was decreased as the increase of the pH. The magnetization curve of PAA-functionalized IONPs indicated the superparamagnetic nature. ROS generation study revealed that the PAA-functionalization of IONPs minimized the production of hydroperoxy or hydroxy radicals occurring with bare IONPs. In addition, the synthesized PAA-functionalized IONPs effectively adsorbed a significantly improved the efficiency of functionalization and stabilization of the dispersion of IONPs. The PAA-functionalized IONPs exhibited higher amount of activity than that of the reference IONPs. The higher amount of trypsin adsorption on PAA1 functionalized nanoparticles at certain pH; 5.0 are the possible reason higher activity of trypsin immobilized on PAA1-functionalized IONPs.

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