

Research Article

Superparamagnetic Iron Oxide (Fe_3O_4) Nanoparticles Coated with PEG/PEI for Biomedical Applications: A Facile and Scalable Preparation Route Based on the Cathodic Electrochemical Deposition Method

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Cathodic electrochemical deposition (CED) is introduced as an efficient and effective method for synthesis and surface coating of superparamagnetic iron oxide nanoparticles (SPIONs). In this way, bare Fe_3O_4 nanoparticles were electrosynthesized through CED method from aqueous solution $\text{Fe}^{3+} : \text{Fe}^{2+}$ chloride (molar ratio of 2 : 1). In the next step, the surface of NPs was coated with polyethyleneimine (PEI) and polyethylene glycol (PEG) during the CED procedure, and PEG/PEI coated SPIONs were obtained. The prepared NPs were evaluated by powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), dynamic light scattering (DLS), vibrating sample magnetometer (VSM), and field-emission scanning electron microscopy (FE-SEM). The pure magnetite phase and nanosize (about 15 nm) of the prepared NPs were confirmed by XRD and FE-SEM. The presence of two coats (i.e., PEG and PEI) on the surface of electrosynthesized NPs was proved via FTIR results. The percentage of polymer coat (37.5%) on the NPs surface was provided by TGA analysis. The high magnetization value, negligible coercivity, and remanence measured by VSM indicate the superparamagnetic nature of both prepared NPs. The obtained results confirmed that the prepared Fe_3O_4 nanoparticles have suitable physicochemical and magnetic properties for biomedical applications.

1. Introduction

In recent years, the application of magnetic nanoparticles (MNPs) in areas of bionanotechnology and biomedicine has been highly interesting, which is mainly due to the multiple properties offered by this type of material [1–3]. MNPs properties are essentially originated from their size and composition, when compared to the bulk material. Applications in the mentioned areas necessitate MNPs forming of stable colloidal suspensions, in addition to being compatible, nontoxic, and nonimmunogenic. Iron oxide MNPs are constructed by

a magnetite (Fe_3O_4) or maghemite ($\gamma\text{-Fe}_2\text{O}_3$) core coated with biocompatible surface layer like a polymer. The biocompatibility and nontoxicity of the surface coating are very interesting features of nanoparticles that have direct effects on cellular uptake of nanoparticles, blood circulation, metabolism, and biodistribution [4, 5].

Up to now, there are many reports on the preparation of MNPs through various chemical methods like as sol-gel, coprecipitation, hydrothermal method, thermal decomposition, microemulsion, and colloidal chemistry method. In addition to these methods, electrochemical synthesis can be

applied for the preparation of MNPs. But, this method has been rarely studied and only a few reports are available in literature [6–9]. As an electrochemical procedure, cathodic electrochemical deposition (CED) has advantages of facility and ability of controlling the purity, crystallinity, and size of synthesized NPs by manipulating the applied conditions like current, potential, electrolyte composition, pH, and concentration [10–13]. It was reported that cathodic deposition of Fe_3O_4 nanoparticles (NPs) can be easily achievable *via* base (OH^-) electrogeneration on the cathode surface [6–9]. For the first time, basic work on the CED preparation of Fe_3O_4 nanoparticles has been reported by Marques et al. [6, 7] where they observed that the CED method results in Fe_3O_4 nanoparticles with controlled size and dispersion. Recently, we report the preparation of Fe_3O_4 nanoparticles from aqueous medium through CED method and their in situ surface coating with polyethylene glycol, polyvinyl chloride, and poly(vinylpyrrolidone) polymers [8, 9]. It was reported that conducting polymers have outstanding mechanical, electrical, and thermal properties. This is the reason that these materials have been targeted for many potential applications [10–17]. Here, we applied, for the first time, this method for preparation of double coated iron oxide nanoparticles with desired size and magnetic behavior from aqueous medium. The polyethylene glycol (PEG) and polyethylene imine (PEI) were used as the coating agents. In the electrodeposition experiments, the electrochemical conditions were first optimized to obtain the bare or uncoated Fe_3O_4 NPs with proper characteristics. The purity, well dispersion, nanosize, and superparamagnetic property of the prepared nanoparticles were identified *via* XRD, FE-SEM, TEM, and VSM analyses. PEG and PEI polymers were then coated on the surface of Fe_3O_4 NPs during their CED synthesis. The double coat on the surface of the deposited NPs was confirmed by FTIR, DSC-TGA, and DLS analyses. In spite of the most used chemical routes which have multistep procedure and require long time (8–12 h) and high temperature (40–80°C) for coating process, our developed method was in situ, simple, one-pot, and time conserving. Furthermore, this one-pot, in situ, and simultaneous double coating of surface of Fe_3O_4 NPs has not been reported until now.

2. Experimental

2.1. Materials. Ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), ferrous chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), polyethylene glycol (PEG, $M_w = 4000$), polyethyleneimine (PEI, $M_w \sim 25000$), and ethanol ($\text{C}_2\text{H}_5\text{OH}$) were purchased from Merck. Graphite plate and stainless steel (316L) were purchased from local companies. All the chemicals used were of reagent grade.

2.2. Preparation Procedure of Nanoparticles. Bare nanoparticles of Fe_3O_4 were prepared through cathodic electrochemical deposition (CED) method. Galvanostat regime was applied in the electrodeposition experiments. In this way, a two-electrode set-up was designed based on the stainless-steel cathode centered between two graphite anodes, as shown in Figure 1. A mixed solution of 0.005 M [FeCl_2

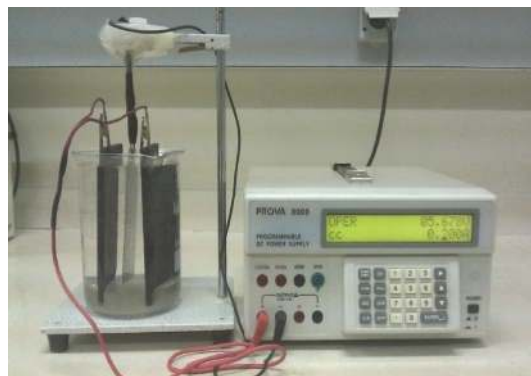


FIGURE 1: Electrochemical set-up used for electrosynthesis of nanoparticles.

and FeCl_3 (2.4 g/l)] with molar ratio of 1:2 was prepared and as the bath composition. The deposition experiments were performed using a DC power supply (PROVA 8000). The current density of 10 mA cm^{-2} was applied for 30 min to the electrochemical cell and black deposit was formed on the steel cathode surface. After the deposition step, the steel substrate was removed from the electrolyte and rinsed with distilled water several times to remove free anions. Then, the black deposit was scraped from the electrode surface and dissolved in the ethanol solution to be centrifuged at 6000 rpm for 30 min. After this step, the deposits were washed several times with ethanol, separated by magnet, dried in a vacuum oven, and subjected to characterization analyses.

2.3. Preparation Procedure of Coated Nanoparticles. The similar electrochemical set-up and conditions were used for the preparation of polymer coated nanoparticles. A mixed solution of 0.005 M [$\text{FeCl}_2/\text{FeCl}_3$: with molar ratio of 1:2] and 2 gr/L [PEG (1 gr) + PEI (1 gr)] was prepared as deposition bath. The bare Fe_3O_4 nanoparticles were synthesized from the deposition bath of 0.01 M [$\text{FeCl}_2/\text{FeCl}_3$] with molar ratio of 1:2. The electrosynthesis process was then carried out in the presence of PEG and PEI polymers with applying current density of 10 mA cm^{-2} for 30 min. The black deposit was performed at this step on the cathode surface. After the deposition step, the purification steps were done as follows: (i) the steel substrate was removed from the electrochemical cell and washed several times with deionized water. (ii) The deposits were scraped from the surface of steel sheet and dissolved in ethanol solution. (iii) Washing several times with ethanol was done to remove the free and uncoated polymers to the surface of deposit. (iv) Then, the deposits were dispersed in ethanol and centrifuged at 6000 rpm for 30 min. (v) After this step, the black powder was separated from ethanol solution by magnet, and the obtained wet powder was dried in a vacuum oven. Finally, the prepared powder was characterized by various analyses.

2.4. Characterization Procedure. The XRD patterns of the prepared samples were recorded on a Philips powder diffractometer PW-1800 with $\text{Co K}\alpha$ radiation ($\lambda = 1.789 \text{ \AA}$). The

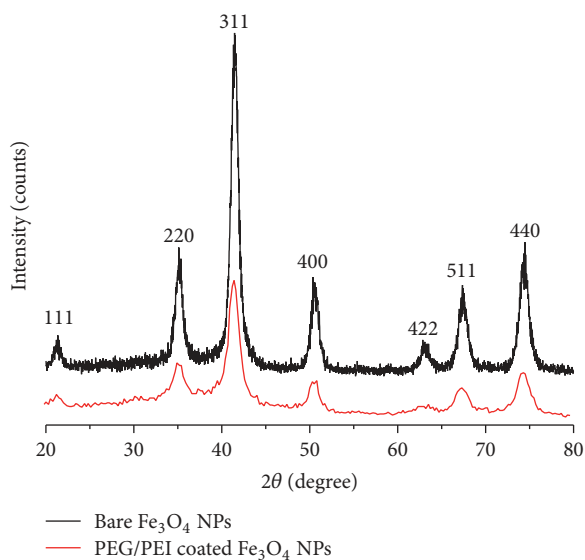


FIGURE 2: XRD patterns of the electrosynthesized (a) bare and (b) PEG/PEI coated Fe_3O_4 NPs.

crystallite sizes are estimated from the X-ray line broadening using Scherrer formula. The infrared spectra were recorded in the range $4000\text{--}400\text{ cm}^{-1}$ on a Fourier transform infrared spectrometer (FTIR, Bruker Vector 22 spectroscope). The morphological micrographs were taken by field-emission scanning electron microscope (FE-SEM, TE-SCAN Model MIRA3, operating voltage 30 kV) and Zeiss transmission electron microscope (TEM, Zeiss EM 900 with accelerating voltage of 80 kV) for particle size determination. Thermal behavior analysis (DTA-TG) was done in N_2 between room temperature and 600°C at a heating rate of 5°C min^{-1} using a STA-1500 thermoanalyzer. Dynamic light scattering (DLS) measurement was performed using Malvern 4700 Autosizer employing a 7132-digital correlator for the determination of hydrodynamic diameter. Vibrating sample magnetometer (VSM) (Model: Lake shore 7400, United States) was employed to study the hysteresis loops and the magnetic properties of the magnetite nanoparticles.

3. Results and Discussions

The XRD patterns of both prepared samples are shown in Figure 2. From these patterns, it can be easily observed that the prepared samples are composed of crystalline single phase cubic inverse spinel Fe_3O_4 structure, where the position and relative intensity of all observed diffraction peaks are matched well with those of the JCPDS Card number 88-0315 for magnetite. No peak is observed from any impurities. Notably, the characteristic peaks of the coated nanoparticles have no shift in the position but have some broadening, indicating that the coated sample has small crystalline size as compared with uncoated one. Furthermore, the peak intensity of the coated sample is lower than that of uncoated one, which is related to the existence of PEG/PEI coat on the surface of Fe_3O_4 nanoparticles. The crystallite size was calculated by measuring the half-height width of the strongest reflection

plane (i.e., 311), using the well-known Scherrer formula ($D = 0.9\lambda/\beta \cos(\theta)$), where β is the full width at half maxima (FWHM) of the (311) peak. Based on the calculations, crystallite sizes of the bare and coated Fe_3O_4 NPs were estimated to be 10.1 nm and 8.3 nm, respectively. These findings confirmed that the pure magnetite phase of iron oxide can be easily prepared by direct current CED method.

To better characterize the phase and composition of the prepared nanoparticles, FTIR analysis was performed. Figure 3 exhibits the IR spectra of the prepared nanoparticles. The FTIR spectrum of the uncoated NPs (in Figure 3(a)) has the characteristic absorbance bands of nanosized magnetite, that is, two strong bands around 565 and 621 cm^{-1} , resulting from a split of the ν_1 band of the Fe-O bond and a weaker band at 421 cm^{-1} , corresponding to the ν_2 band of the Fe-O bond [17, 18]. The absorption bands around 1633 and 3448 cm^{-1} originate from stretching and deformation vibrations of hydroxyl groups connected to the surface of nanoparticles [8]. For the coated NPs, the three mentioned peaks are located around 571 , 625 , and 441 cm^{-1} , which exhibit a little blue shift. This shift implicates the change in environment of the NPs after coating with PEG/PEI, where the new bands between iron oxide and OH^- groups of PEG and amine groups of PEI have been formed [18, 19].

In the spectrum of the coated nanoparticles, there are some new bands, which can be assigned to the following vibrations: the bands at 3435 and 1646 cm^{-1} due to the $-\text{NH}_2$ groups in PEI structure [13, 19, 20]. Furthermore, the CH_2 bending vibration at 2882 cm^{-1} , the stretching of N-C-H at 2812 cm^{-1} , the scissoring of CH_2 at 1475 cm^{-1} , the wagging and twisting of CH_2 at 1354 and 1297 cm^{-1} , and the stretching of C-N at 1129 cm^{-1} are all the characteristic IR peaks of PEI [13, 19, 20]. Also, a wide peak at $3200\text{--}3700\text{ cm}^{-1}$ and sharp one at 1005 cm^{-1} are related to the vibrations of O-H and C-O-C bonds of PEG, respectively. The bands of O-H vibration modes of PEG are also seen at 864 and 1156 cm^{-1} [17, 19]. All of these findings completely confirmed the presence of both PEI and PEG on the surface of electrosynthesized nanoparticles and hence the prepared NPs are double coated. So, it can be stated that in situ, on-pot, and simultaneous double coating of iron oxide surface during the electrodeposition process is achievable by our used strategy.

FE-SEM observations were used to provide the morphological properties of the prepared samples which are shown in Figure 4. It is evident that both prepared Fe_3O_4 samples are nearly spherical in shape at nanoscale. In fact, both uncoated and coated samples have particle morphology with spherical shape and no obvious aggregation. The mean diameter of both uncoated and coated particles was measured to be around 20 nm. Notably, the PEG/PEI coated NPs have relative better dispersion as compared with bare NPs. For better observation of morphology of the prepared NPs, the TEM observations of NPs are shown in Figure 5. From TEM images in Figure 5, it is clearly observable that both prepared NPs have spherical shape. The PEG/PEI coated NPs exhibit better dispersibility, as seen in Figure 5(b), where the bare NPs are rather agglomerated. The less agglomerated texture of the PEG/PEI coated NPs can be related to the effect of polymer

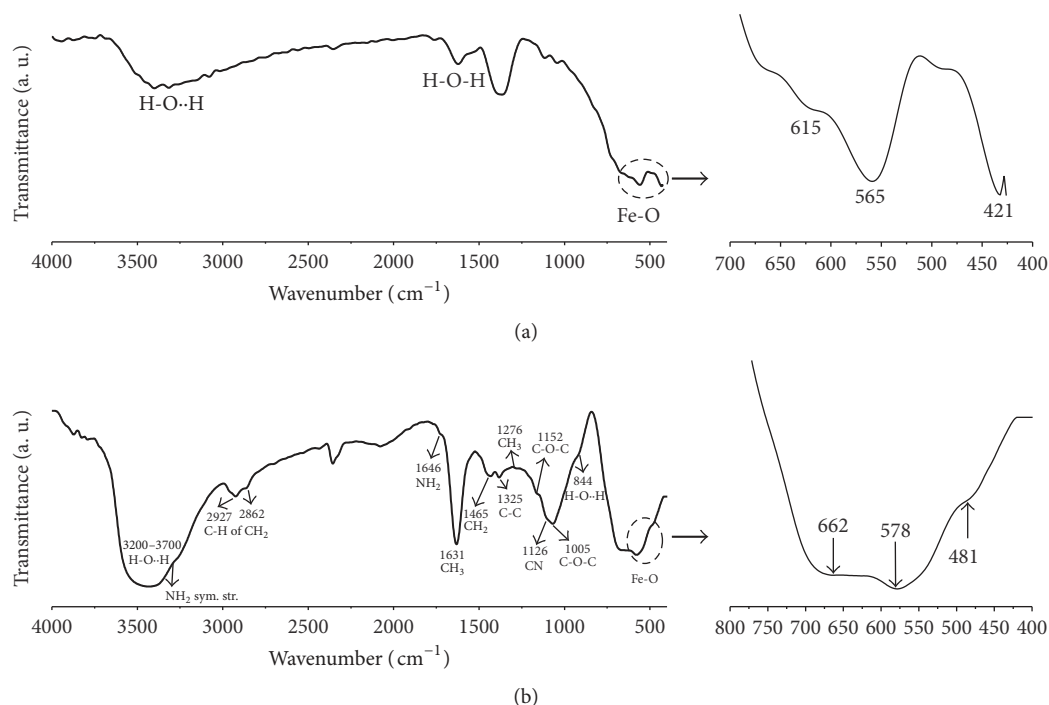


FIGURE 3: IR spectra of the prepared (a) bare and (b) PEG/PEI coated Fe_3O_4 NPs.

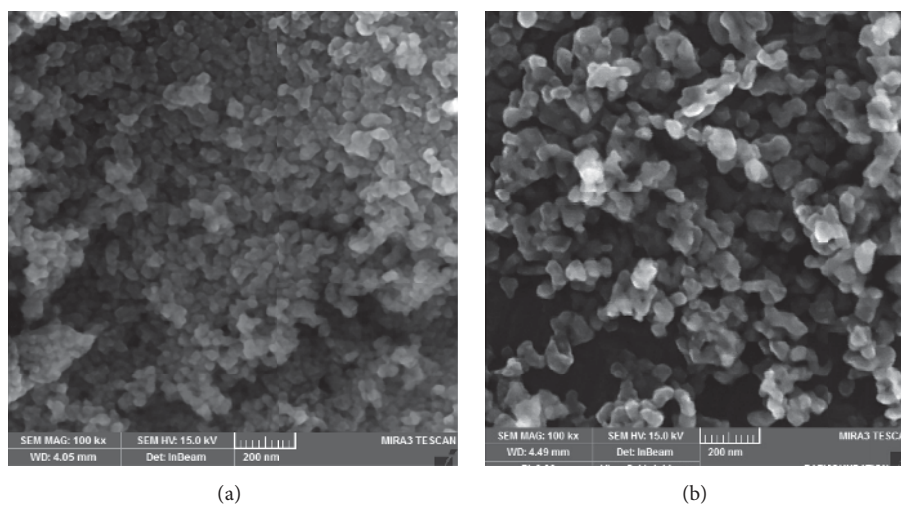


FIGURE 4: FE-SEM images of the prepared (a) bare and (b) PEG/PEI coated Fe_3O_4 NPs.

layer during the particle formation. It seems that coating of the Fe_3O_4 NPs with polymer leads to decreasing the magnetic interaction among the particles due to their reduced magnetism (as confirmed by VSM in Figure 8) and prevents their agglomeration. From TEM images, the mean diameters of the bare and PEG/PEI coated particles were estimated to be 10 nm and 15 nm, respectively. This size increase confirmed the presence of the polymer layer on the Fe_3O_4 particles.

TGA and DSC curves of the prepared nanoparticles are shown in Figure 6. It can be seen that coated NPs have completely different thermal behavior as compared with bare NPs.

This difference may be originated from the presence of polymer coat on the surface of NPs. The uncoated nanoparticles were thermally stable and no special endo and/or exothermic peak was observed in the DSC curve (Figure 6(a)), and correspondingly there was no essential weight loss over the entire temperature range in TG curve (Figure 6(b)). For the bare NPs, TG curve exhibited only 1.6% weight loss, where the largest portion of these weight losses occurred at the temperatures of 25–200°C. Hence, this weight loss is related to removal of the physically adsorbed water and/or hydroxyl groups on the surface of Fe_3O_4 nanoparticles. For the coated

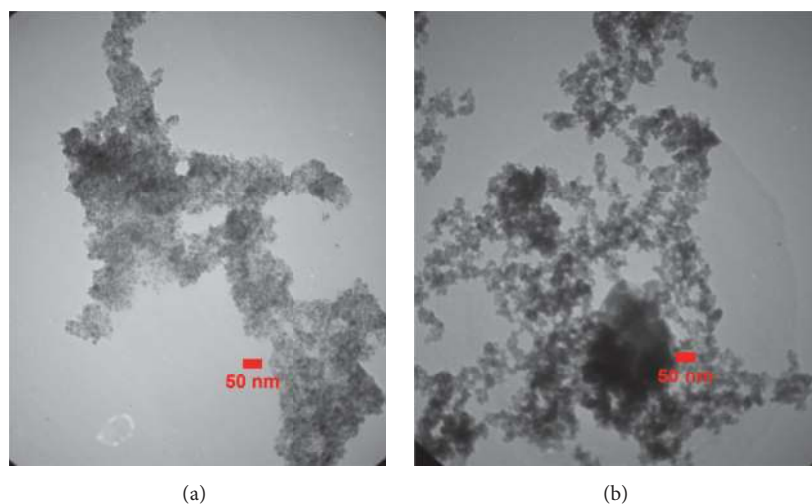


FIGURE 5: TEM images of the prepared (a) bare and (b) PEG/PEI coated Fe_3O_4 NPs.

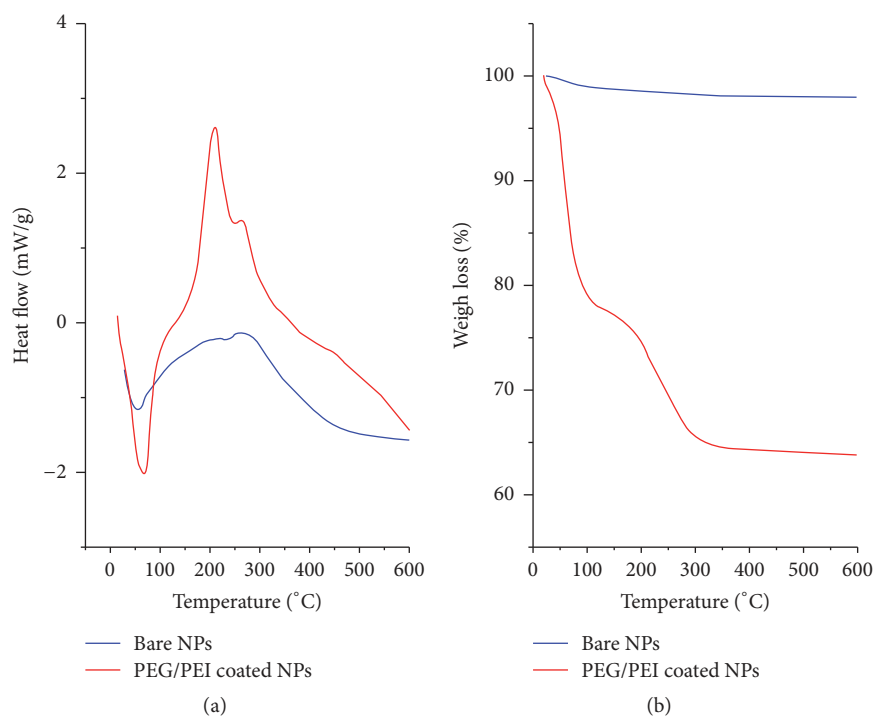


FIGURE 6: DSC-TG curves for the prepared (a) bare and (b) PEG/PEI coated Fe_3O_4 nanoparticles.

nanoparticles, DSC curve in Figure 6(a) exhibits a multistep exothermic peak at the temperature range of 100–600°C. Respectively, TG curve shows three sharp weight losses with total losses of about 37.5% (Figure 6(b)). It was reported that the decomposition of pure PEG started at around 350°C and ended at 425°C [24]. Furthermore, it was also observed that the decomposition of PEG coat of NPs is started around 177°C and ended at around 352°C [25–28]. It has been observed that PEI coat on the surface of NPs is decomposed at the temperatures of 200–400°C with a relative sharp peak at 250°C [21, 29–31]. Hence, the weight loss of 37.5% results from

the decomposition of PEG and PEI polymers from the surface of NPs. After 500°C, there is no obvious change in weight throughout the temperature range up to 600°C (Figure 6), indicating the complete decomposition of coating agents. These results confirmed the in situ double coating of Fe_3O_4 nanoparticles during their electrosynthesis procedure.

DLS analysis was used for determination of the hydrodynamic diameter of both uncoated and coated nanoparticles. Figure 7 indicates the measured particle size of the bare and PEG/PEI coated nanoparticles. For the bare NPs (Figure 7(a)), the mean hydrodynamic diameter was measured

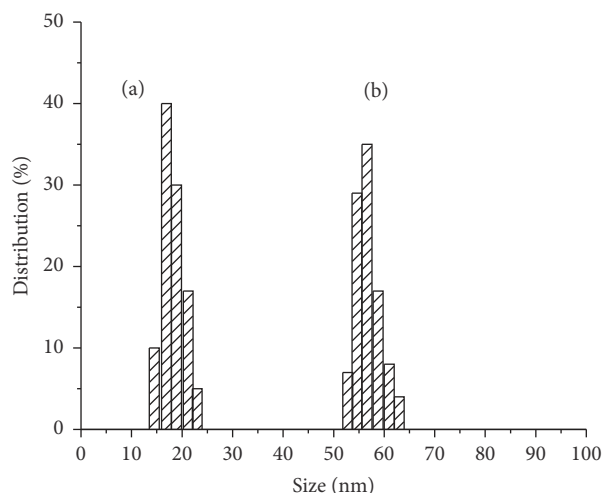


FIGURE 7: Particle size distributions of (a) bare and (b) PEG/PEI coated Fe_3O_4 NPs.

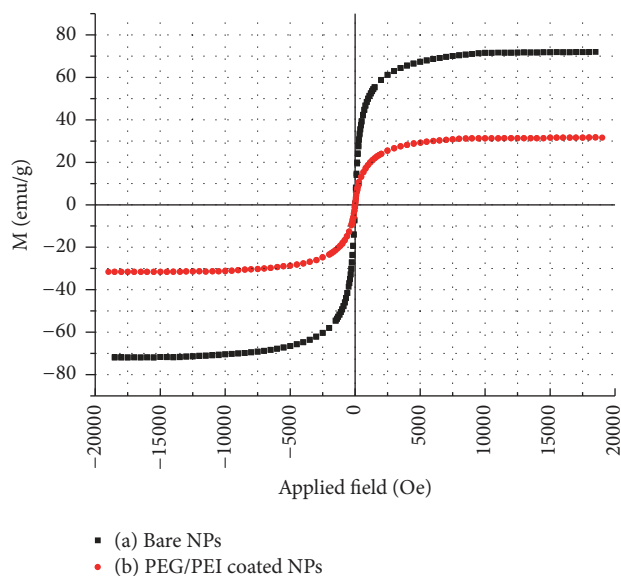


FIGURE 8: VSM curves for the prepared (a) bare and (b) PEG/PEI coated Fe_3O_4 NPs.

to be ~ 20 nm. For the coated NPs, this value is observed to be ~ 58 , which is larger than that of bare NPs. These results clearly prove the coat layer on the surface of electrodeposited NPs.

The magnetic characteristics of the prepared nanoparticles in the presence of magnetic field were measured using a vibrating sample magnetometer (VSM). Figure 8 shows the hysteresis loops of the bare and PEG/PEI coated NPs at RT. For both prepared NPs, the magnetization reduces from plateau state to zero on removal of the magnetic field. This behavior proved that our particles are superparamagnetic. The uncoated NPs exhibit saturation magnetization (M_s) of 71.5 emu/g and have small remanent magnetization ($M_r \approx 0.81$ emu/g) and coercivity ($C_e \sim 2.3$ Oe) indicating their suitable magnetic behavior. The polymer coated nanoparticles exhibit $M_s \approx 32.5$ emu/g, $M_r \approx 0.54$ emu/g, and $C_e \approx 1.25$ Oe.

These findings indicate that our prepared particles have proper behavior and can be used in biomedical applications.

4. Conclusion

Bare and polymer coated Fe_3O_4 nanoparticles were prepared via cathodic electrochemical deposition method. The prepared bare and PEG/PEI coated nanoparticles were characterized by FTIR, DLS, TGA, FE-SEM, TEM, and VSM. The results confirmed the prepared nanoparticles have proper physicochemical and magnetic properties for biomedical applications.

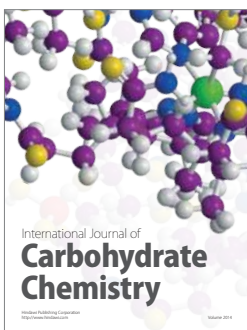
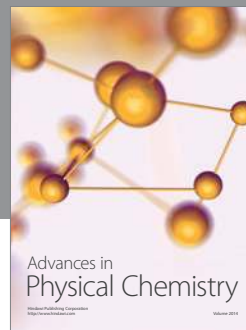
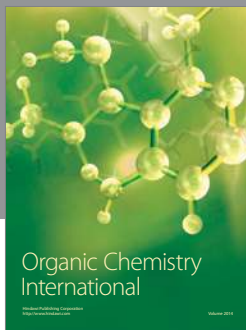
Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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