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Techniques of controlling hydrodynamic size of ferrofluid of gelatin-coated magnetic iron oxide nanoparticles

**Babita Gaihre · Myung S. Khil · Jung A. Ko ·
Hak Y. Kim**

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Abstract In this study, magnetic iron oxide (IOPs) nanoparticles were coated with gelatin B, and various parameters were investigated to find out effects of those parameters in the overall hydrodynamic size of the colloidal dispersions. Two different types of coating techniques, viz., in situ and separately precipitation/coating technique were investigated. In in situ precipitation/coating technique, precipitation, and coating of the IOPs were done simultaneously, while in separately precipitation/coating technique, the IOPs particles were separately precipitated and purified before surface coating was done. The colloidal dispersion obtained from these two methods showed drastically different viscosities as well as hydrodynamic size. It was found that in situ precipitation/coating technique gave smaller-sized monodispersed particles compare to separately precipitation/coating technique. In addition to the above two techniques, the desolvation/cross-linking technique was also investigated and found to, further, reduce the size of the ferrofluid prepared by the in situ and separately precipitation/coating techniques. The ferrofluids prepared using in situ as well as separately precipitation techniques were highly stable and did not sediment for more than 1 month. However, the desolvation/cross-linking technique gave dispersion with reduced stability. Nevertheless, by adjusting suitable combination of acetone and glyceraldehydes, ferrofluid with better stability could be produced by this technique.

Introduction

The critical emerging issues in the use of magnetic iron oxide (IOPs) nanoparticles concern surface modification of the nanoparticles to impart biocompatibility, targeting specificity, and assimilation of biological properties to the nanoparticles. These issues are essential if the fabrication is for the various biomedical applications as without surface modification the IOPs tend to agglomerate, which lead to rapid elimination by macrophages, loss in magnetic characterization, and decrease in surface activity [1].

The long-ranged attractive magnetic force between the IOPs particles is the main cause of rapid aggregation and sedimentation of the IOPs. This behavior can be removed or reduced by introducing long-ranged repulsive forces. One effective way to induce the repulsive force to the particles is to coat the particles with hydrophilic polymers whose long loops and tails extent out from the IOPs surface into solution leading to steric stabilization. So far different types of synthetic and natural polymers have been investigated in order to stabilize the ferrofluids [1, 2]. However, very few works have been done using gelatin as a coating material [3].

Gelatin, a protein derived from collagen, is a commonly used plasma expander. The properties such as water solubility, biodegradability, biocompatibility, pH-induced surface charge [4], and presence of multifunctional groups like –NH$_2$, –COOH in the gelatin chain make gelatin a

B. Gaihre · J. A. Ko
Department of Bionanosystem Engineering, Chonbuk National University, Chonju 561-756, Republic of Korea

M. S. Khil · H. Y. Kim
Center for Healthcare Technology Development, Chonbuk National University, Chonju 561-756, Republic of Korea

M. S. Khil · H. Y. Kim (✉)
Department of Textile Engineering, Chonbuk National University, Chonju 561-756, Republic of Korea
e-mail: khy@chonbuk.ac.kr

suitable polymer for the surface modification of the IOPs to stabilize the ferrofluid and to impart the useful properties of gelatin to the IOPs. Moreover, gelatin can be fabricated as microspheres and nanospheres depending on the technique used for the fabrication process and found to enhance tumoral cell phagocytosis [5].

In our earlier works, we have investigated effect of different parameters in the adsorption of gelatin to the IOPs surface [6] and stability of colloidal dispersion of the ferrofluids [6, 7] and bioactivity of the gelatin coated IOPs [8]. Although gelatin coating could impart biological functionality to the IOPs [8] and increase the colloidal stability due to steric stabilization [6, 7], the long loops and tails of the steric groups, i.e., polymers affected the overall hydrodynamic size of the dispersion [7]. It has been shown that reduction of size increases the intracellular uptake of the particle leading to effective biomedical application of the materials [9]. So, the aim of the current study is to investigate different techniques in order to reduce hydrodynamic size of the ferrofluid dispersion of gelatin-coated IOPs without affecting the stability of the colloidal dispersion. Normally, the surface modification of the IOPs is done by dispersing the particles in the solution of the coating polymers in presence [3, 10] or absence of cross-linking agent [11]. Furthermore, coating of IOPs as soon as they are produced gives monodispersed particles with reduced size [1]. We investigate all these techniques to control the hydrodynamic size of the ferrofluid of gelatin-coated IOPs.

Experimental

Materials

The starting materials were gelatin type B from bovine skin (~225 bloom, Sigma–Aldrich, USA), iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, Fisher Scientific, UK), iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, Fisher Scientific, UK), sodium hydroxide (NaOH, Fisher Scientific, UK), acetone (CH_3COCH_3, Samchun Chemical, Korea), 25% glutaraldehyde ($OHC(CH_2)_3CHO$, Sigma–Aldrich, USA).

Deionized water purged with nitrogen gas was used in all the steps involved. The glutaraldehyde solution was diluted to 4% by adding deionized water and used for the cross-linking process. A stock solution of the ferrous and ferric salts (with Fe(II)/Fe(III) = 0.5 molar ratio) was prepared by dissolving 5.4 g $FeCl_3 \cdot 6H_2O$ and 2 g $FeCl_2 \cdot 4H_2O$ in 60 mL 0.16 M aq. HCl and same stock solution were used in all the experiments for the precipitation of the IOPs [7].

Controlling hydrodynamic size of the ferrofluid dispersion

In order to control the hydrodynamic size of the ferrofluid dispersion, two different techniques viz coating of separately precipitated IOPs (defined as separately precipitation/coating technique) and simultaneous precipitation and coating (defined as in situ precipitation/coating technique) were investigated. In addition to the aforementioned techniques, a well-known technique, i.e., desolvation/cross-linking technique was also investigated.

In situ versus separately precipitation/coating technique and characterization

In order to see the effects of the aforementioned techniques on the size of the ferrofluid, the two types of dispersions were prepared in identical condition. For the preparation of ferrofluid dispersion using in situ precipitation/coating technique, 3 mL of 1 M NaOH and 15 mL gelatin (5% w/v, pH 5) solution were added to 2 mL iron chloride stock solution, simultaneously. The precipitates were, then, redispersed by brief sonication followed by dialysis at 37 °C for 24 h.

Similarly, for the preparation of ferrofluid dispersion using separately precipitation/coating technique, the IOPs were precipitated separately from 3 mL of 1 M NaOH and 2 mL iron chloride stock solution. After purification through dialysis, 15 mL gelatin (5% w/v, pH 5) solution was added to the IOPs dispersion followed by incubation at 37 °C for 24 h. Finally, the homogenous dispersions of the surface-coated IOPs were collected by removing the aggregates by centrifugation at 3000 rpm for 2 min. The dispersion would be referred to as GIOIBPs and GIOBPs for in situ and separately precipitation/coating techniques, respectively.

The hydrodynamic size of the dispersion was investigated after 24 h to allow dispersion to reach steady state. The samples for this analysis were prepared by diluting 30 μL of the ferrofluids to 3 mL by adding deionized water and measurements were done using dynamic light scattering technique (DLS, Malvern System 4700, Malvern, UK) at room temperature 25 °C. X-ray diffractometer (XRD, Lad-X 600, Shimadzu, Japan) utilizing CuKα radiation (l = 1.54053 A), operating at 30 mA and 40 kV was used to investigate the crystalline phases. The scan rate and scan angle were fixed at 4°/min and 20–70, 2θ degree, respectively. The gelatin-coated IOPs nanoparticles were collected from the ferrofluid dispersion, using magnet and air-dried before the analysis.

Desolvation/cross-linking technique

The desolvation/cross-linking technique has also been used to reduce the size and stabilize the ferrofluids. In this technique, suitable amount of desolvating (acetone) and cross-linking (glutaraldehyde) agents are added to the dispersion [3, 7]. In the present work, the desolvation/cross-linking technique was also investigated to reduce the size of the ferrofluids obtained by both in situ as well as separately precipitation/coating techniques.

In situ precipitation/coating technique In order to see the effect of desolvation/crosslinking technique on the hydrodynamic size of the ferrofluid prepared by the in situ precipitation technique, the GIOIBPs-2 dispersion was prepared at first by adding 3 mL 1 M NaOH and 12 mL gelatin (5% w/v, pH 5) solution to the 2 mL iron chloride stock solution, simultaneously. The resulting black precipitates were stirrer for further 30 min before magnetic collection. The collected precipitates were, then, redispersed in 12 mL of deionized water with brief sonication and dialyzed against deionized water overnight. After removing the aggregates by centrifugation at 3000 rpm for 2 min, the homogenous dispersion was treated with various amounts of acetone and glutaraldehyde for desolvation and cross-linking, respectively, incubating the dispersion for 2 h after each step. Lastly, acetone and unreacted glutaraldehyde were removed by dialysis against deionized water for 2 h.

Separately precipitation/coating technique Similarly, in order to see the effect of desolvation/crosslinking technique on the hydrodynamic size of the ferrofluid prepared separately by the precipitation technique, the GIOBPs-5 dispersion was prepared by incubating 5 mL of the dialyzed IOPs dispersion (1 mg/mL) and 1 mL gelatin (5% w/v, pH 5) solution for 24 h at 37 °C to allow for extensive adsorption to take place. The homogeneous dispersion was, then, treated with required amount of acetone and glutaraldehyde solution for desolvation and cross-linking, respectively, incubating the dispersion for 2 h after each step. Acetone and unreacted glutaraldehyde were removed by dialysis for 2 h. The hydrodynamic size of the dispersions was measured after 24 h using DLS as discussed in the above sections.

Viscosity affecting the hydrodynamic size the ferrofluid

In order to see the effect of viscosity on the hydrodynamic size of the ferrofluids, the ferrofluids with three different viscosities were prepared using both in situ and separately precipitation/coating techniques.

Using in situ precipitation/coating technique, the ferrofluid dispersions were prepared as discussed in the "Desolvation/cross-linking technique" section by varying volumes of iron chloride stock solution to 0.5, 1, and 2 mL. The dispersion would be referred to as GIOIBPs-0.5, GIOIBPs-1, and GIOIBPs-2, respectively. Similarly, using separately precipitation/coating technique, the ferrofluid dispersions were prepared as discussed in the "Desolvation/cross-linking technique" section by varying volumes of dialyzed IOPs dispersion (1 mg/mL) to 0.1, 2, and 5 mL. The dispersions would be referred to as GIOBPs-0.1, GIOBPs-2, and GIOBPs-5, respectively. The dispersions were not treated with the desolvating and cross-linking agent.

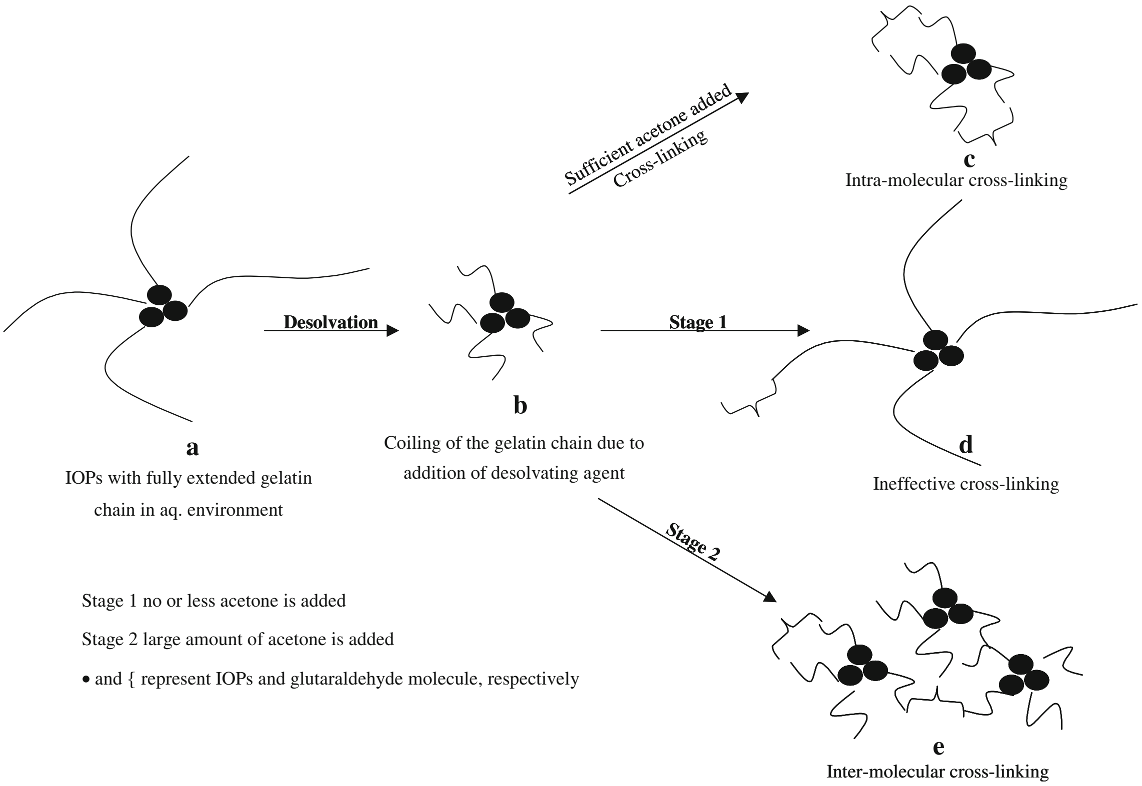
The viscosity measurement of the dispersions was done using viscometer (DV-III Ultra Programmable Rheometer, Brookfield, USA) with spindle no. 61 at 25 °C. The torques of the dispersions were in between 30 and 60. The hydrodynamic size of the dispersions was measured after 24 h using DLS as discussed in the "In situ versus separately precipitation/coating technique and characterization" section.

Dispersibility and stability of the ferrofluid

The morphology and dispersibility of the gelatin-coated IOPs ferrofluids prepared by in situ as well as separately precipitation/coating techniques were analyzed using transmission electron microscopy (BIO-TEM, H-7650, Hitachi, Japan), operating at 100 kV and atomic force microscopy (AFM, Nanoscope IV multimode, Veeco, USA) in taping mode. The 30 μL of GIOIBPs and GIOBPs dispersions discussed in the "In situ versus separately precipitation/coating technique and characterization" section were diluted to 3 mL by adding deionized water. A drop of the diluted dispersions was placed on the TEM girds and silicon plates and air-dried before analysis by TEM and AFM analyzer, respectively. The stability of the ferrofluid dispersions was investigated visually by observing the dispersion for various intervals of time. The more detail study on the stability of the ferrofluid can be found in our previous works [6, 7].

Results and discussion

Several types of hydrophilic polymers have been investigated in order to stabilize IOPs dispersions [1, 2]. These polymers, especially those having certain functional groups and water-loving chains, are believed to adsorb to the surface of the IOPs and long loops and tails extend into solution, which leads to steric stabilization of the dispersions [12, 13] as shown by Scheme 1a. Although, the



Scheme 1 Theory of desolvation and cross-linking process leading to formation of particles with various sizes

polymer coating could increase the colloidal stability due to steric stabilization [2, 7], the long loops and tails of the steric groups would affect the overall hydrodynamic size of the dispersion [7]. So, in order to reduce hydrodynamic size of the ferrofluid dispersions of the gelatin-coated IOPs, without affecting the stability of the colloidal dispersion, different techniques of surface modifications were investigated. The surface coating of the IOPs with gelatin was done by two different methods, viz., in situ precipitation/coating technique and separately precipitation/coating technique. In addition, desolvation/cross-linking technique was also investigated to see the effect of this technique on the hydrodynamic size of the ferrofluids prepared by above-mentioned in situ and separately precipitation techniques.

In situ versus separately precipitation/coating technique

Table 1 presents the physicochemical properties of the ferrofluid dispersions produced by the two different techniques. It can be observed that under identical condition, the ferrofluid obtained by the in situ precipitation/coating technique had very low viscosity compared to the dispersion obtained by the separately precipitation/coating technique. The ferrofluid obtained by the in situ precipitation/coating technique was free-flowing liquid in room temperature with dramatically reduced size, while the ferrofluid obtained by the separately precipitation/coating technique turned into gel within 1 h and had large size.

Table 1 Effect of the in situ and separately precipitation/coating techniques on the hydrodynamic size of the ferrofluids

Sample	Liquid property	Viscosity (cps)	Size (nm)
GIOBPs	Turns gel within 1 h	14	3,839.9 ± 500.9
GIOIBPs	Free-flowing liquid	14	360.2 ± 10.0

According to the hypothesis given by Loeb [14] and Kunitz [15], gelatin solution has micellar structures that consist of water-soluble constituent held in a network of insoluble fibers. Osmotic pressure of the soluble gelatin inside the micelles causes swelling of the micelles, which leads to the higher viscosity of the solution. When any salt or any particle that does not penetrate the micelle is added to the solution, it causes decrease in viscosity due to shrinkage of the micelles [16]. In our present work, during in situ precipitation/coating process, the gelatin and basic solution were added, simultaneously, to the iron chloride stock solution. The resulting black residues were redispersed in water with brief sonication and un-dispersed aggregates were removed by centrifugation. However, for coating of separately precipitated IOPs, the IOPs were dispersed in gelatin solution followed by incubation, i.e., the condition was less drastic than in situ precipitation/

coating. Furthermore, in contrast to in situ precipitation/coating technique, very insignificant amount of black residues were found to precipitate. It seems that the insoluble fibrous portion of gelatin were separated as aggregates leaving only water-soluble gelatin in the dispersion obtained by in situ precipitation/coating technique, which gave the liquid with less viscosity. Since the condition was less severe, separation of fibrous portion of gelatin was not possible when coating was done by separately precipitation/coating technique leading to dispersion with very high viscosity as shown by Table 1. Furthermore, on decreasing gelatin concentration entanglement of gelatin layer between coated IOPs became less (or less crowding due to adsorbed chain), which might be the cause of decreased size of the ferrofluid obtained by the in situ precipitation/coating technique as seen in Table 1.

Desolvation/cross-linking technique

In order to see the effect of desolvation/crosslinking technique, the GIOIBPs-2 and GIOBPs-5 dispersions obtained using in situ and separately precipitation/coating techniques, respectively, were treated with different amounts of acetone and glutaraldehyde and the results are presented as Table 2. The table indicates decrease in size of the ferrofluids on addition of suitable amount of desolvating and cross-linking agents. The table, further, shows the marked difference between the volume of acetone consumed by ferrofluids prepared by in situ and separately precipitation/coating. For example, the ferrofluid obtained by in situ precipitation/coating technique consumed only 25 to 50 µL acetone/mL of dispersion, while the ferrofluid obtained by the separately precipitation/coating technique consumed ten times larger amount of acetone.

Table 2 Effect of desolvation/cross-linking technique on the hydrodynamic size of the dispersions obtained by in situ (a) and separately (b) precipitation/coating technique

Acetone (µL/mL)	GTA (µL/mL)	Size (nm)
(a)		
0	0	176.0 ± 5.0
0	6	130.4 ± 1.5
0	10	134.3 ± 1.0
25	6	125.5 ± 1.7
25	10	125.9 ± 1.1
50	6	122.4 ± 0.5
50	10	125.8 ± 1.0
(b)		
0	0	1,190 ± 114.0
250	6	978.6 ± 50.0
500	6	332.4 ± 12.5
750	6	511.63 ± 50.0
1000	6	Precipitation

It has been shown that gelatin can undergo irreversible adsorption to the metal oxide surface [17–19]. Furthermore, it has been shown that polymers having carboxylic and amine functional group have high affinity with the IOPs [12, 13]. The adsorption of gelatin to the IOPs can be visualized as in Scheme 1a, in which the gelatin layers extent from the surface of the IOPs into water. When desolvating agent such as acetone or ethanol is added to the aqueous solution of gelatin, some of the water molecules surrounding the gelatin chains are replace by acetone resulting in the blockage of intermolecular hydrogen bonding between the water molecules and the gelatin chains [20, 21]. The result is change in conformation of the gelatin chains to form coil-like structures (Scheme 1b). Addition of water reversed the process, hence, resolvating the chains again to water. However, on addition of glutaraldehyde, at this time, the hydrogen bond forming groups in the gelatin chain could bind with the functional groups of the glutaraldehyde, thus, preventing the intermolecular hydrogen bonding between the gelatin chain and water molecules [22], hence, blocking the full extension of the chains (Scheme 1c). The overall result is the decrease in hydrodynamic size as shown in Table 2. Furthermore, as discussed in the "In situ versus separately precipitation/coating technique" section, due to separation of insoluble fibrous portion as aggregates, the ferrofluid prepared by in situ precipitation/coating technique contained less amount of gelatin. Due to the presence of this less amount of gelatin, less amount of acetone was consumed by the dispersion prepared by in situ precipitation/cross-linking technique compared to that prepared by separately precipitation/coating technique.

Intermolecular/intermolecular cross-linking affecting size of the ferrofluid

In present work, the role of the glutaraldehyde was to bind the functional groups of gelatin (cross-link) so that the gelatin chains are not fully extended, thereby, decreasing the hydrodynamic size of the ferrofluids [22]. The cross-linking might occur between functional groups of two different gelatin chains (intermolecular cross-linking) or between functional groups of same gelatin chain (intramolecular cross-linking). Depending upon the extent of coiling and the concentration of the coiled structures, two stages might occur. Stage 1, when no and/or insufficient acetone is added as shown in Scheme 1d. The cross-linking at this stage might give ineffective cross-linking because the chains are extended far away for the glutaraldehyde molecule to make intramolecular cross-linking, which leads to resolvation of gelatin chain,

Table 3 Hydrodynamic size of the colloidal dispersion of GIOBPs-5 at each steps of preparation for dispersion obtained by separately precipitation/coating technique

	Hydrodynamic size (nm)	
	250 μL of acetone[a]	500 μL of acetone[a]
After acetone addition	376.1 ± 2.0	263.5 ± 55.0
After GTA addition (6 μL)	345.9 ± 15.5	256.2 ± 63.7
After dialysis	978.6 ± 50.0	332.4 ± 12.5

[a] Added per milliliter of dispersion

when acetone is removed, leading to formation of the large sized particles. Stage 2, when an extra amount of desolvating agent is added as shown in Scheme 1e. The cross-linking at this stage also gives larger-size colloidal dispersion. However, the cause of the large-sized colloidal dispersion might be due to over crowding of the coiled structure, which facilitates the intermolecular bonding between gelatin chains.

To support this theory, the hydrodynamic size of the dispersion was measured after each step of preparation and the result is presented in Table 3. It was found that the hydrodynamic size of the colloidal dispersion was more or less similar after addition of acetone (250, 500 μL) and glutaraldehyde (6 μL). However, after dialysis the hydrodynamic size was found to increase for the dispersion in which less amount of acetone (250 μL) was added (Table 3). But, such marked increase in size did not occur when 500 μL of the acetone was added to the dispersion. It seems that 250 μL of the acetone was insufficient for the gelatin chain to have sufficient coiling so that glutaraldehyde could cross-link the chains, intramolecularly. So, after removal of acetone the chains are extended back leading to the large hydrodynamic size as shown in Scheme 1d. On the other hand, when 1000 μL of acetone was added to the dispersion, the nanoparticles turned into aggregates and settled down to the bottom within two hours. This might be due to formation of a larger amount of coiled structure, which leads to increased hydrophobic interaction between them, hence, destabilizing the dispersion. On addition of 6 μL of glutaraldehyde to the dispersion, hardening of the particles occurred leading to formation of aggregates, irreversibly, as suggested in Scheme 1e. In this experiment, it was found that 500 μL acetone/mL of GIOBPs-5 dispersion was sufficient to make the intramolecular cross-linking leading to formation of colloidal dispersion with nanometer hydrodynamic size.

Viscosity affecting the hydrodynamic size of the ferrofluids

In the "In situ versus separately precipitation/coating technique" section, it was observed that the ferrofluids obtained by the two different techniques in the identical condition had drastically different size and viscosity. The GIOIBPs ferrofluid was free-flowing liquid with reduced size, while the GIOBPs ferrofluid was large sized, which turned into gel in room temperature with in 1 h. So, in order to find out the correlation between viscosity and size, the dispersions with three different viscosities were prepared using both in situ and separately precipitation/coating techniques. Table 4 represents the effect of viscosities on the hydrodynamic size of the dispersions prepared by in situ as well as separately precipitation/coating techniques. By observing the table it can be concluded that the size of the ferrofluids can be reduced to nanometer size by decreasing the viscosity of the dispersion.

Table 4 Effect of viscosity on the hydrodynamic size of the dispersions obtained by in situ (a) and separately (b) precipitation/coating technique

Sample	Liquid property	Viscosity (cps)	Size (nm)
(a)			
GIOIBPs-0.5	Turns gel after 24 h	6	356.4 ± 25.5
GIOIBPs-1	Free-flowing liquid	4	288.0 ± 10.0
GIOIBPs-2	Free-flowing liquid	3	176.0 ± 5.0
(b)			
GIOBPs-0.1	Turns gel within 1 h	14	3,839.9 ± 500.9
GIOBPs-2	Turns gel within 12 h	10	1,740.9 ± 100.5
GIOBPs-5	Turns gel after 24 h	6	1,190 ± 114.0

Dispersibility and stability of the ferrofluids

As discussed in the "In situ versus separately precipitation/coating technique" section, the hydrodynamic size of the GIOIBPs decreased drastically compared to GIOBPs. Not only the hydrodynamic size, but also the dispersibility of the colloidal dispersion was affected (Figs. 1 and 2). The dispersion prepared by in situ precipitation/coating technique gave very well-dispersed morphology of size around 26 nm (Fig. 1a) compared to second method, which gave larger clusters of size around 80 nm (Fig. 1b) with each of the clusters carrying further small grain-like structures. Similarly, the TEM images in Fig. 2 also showed monodispersed particles for the ferrofluid prepared by the in situ precipitation/coating technique, while clusters with each cluster carrying spherical nanoparticles of size around 9 nm could be seen for ferrofluid prepared by the separately precipitation/coating technique. Because gelatin molecules cannot absorb the electric beam and become invisible, the dark spots seen in Fig. 2 could be the IOPs. The selected area diffraction pattern (SADP) in Fig. 2c

Fig. 1 AFM images of GIOBPs (**a**) and GIOIBPs (**b**)

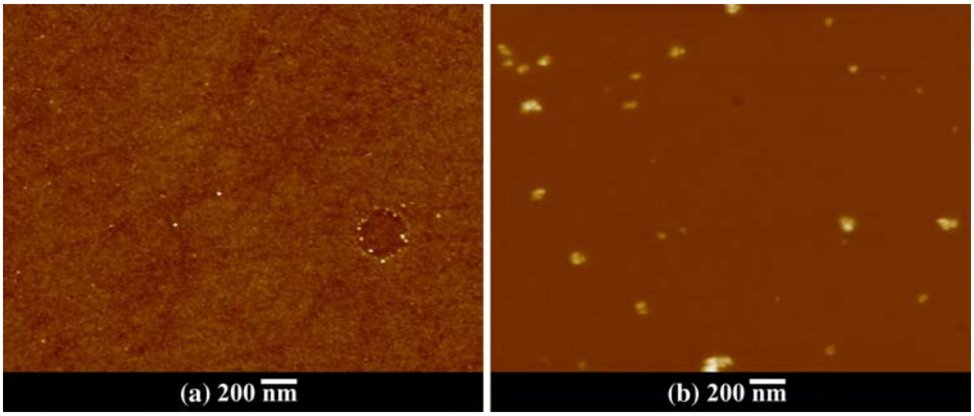
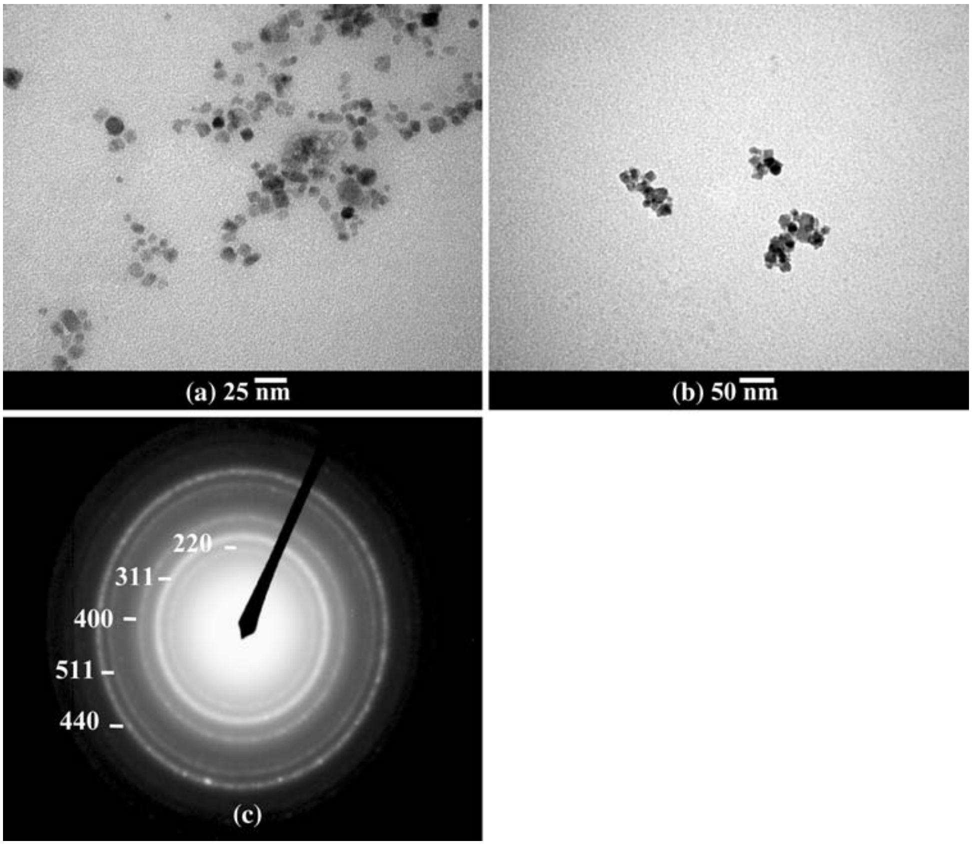


Fig. 2 BIO-TEM images of GIOBPs (**a**), GIOIBPs (**b**), and SADP (**c**)



supports the idea. Furthermore, the positions and relative intensities of the peaks at 2θ angles of 30.1, 35.5, 43.3, 53.4, 56.4, and 62.6 in XRD spectrum in Fig. 3 indicated formation of the magnetite or maghemite [23]. The SADP along with the XRD peaks conformed the crystalline structures of IOPs.

In aqueous system, IOPs are coordinated with water molecules, which share their electron pairs with iron atom. Upon adsorption, the water molecules usually dissociate resulting in a surface covered by hydroxyl groups coordinated to the underlying iron atoms [24]. The process can be continuous leading to adsorption of more water molecules, which hydrogen bond to the surface hydroxyl groups. Such adsorbed water molecules promote self-adhesion of the IOPs, which can lead to the formation of clusters [18]. In this work, since preparation of the GIOBPs involved use of separately prepared IOPs, we could expect aggregation of the tiny particles of the IOPs during purification step. When these particles were surface coated chances of coating "in-group" were higher compared to individual particles as confirmed by Figs. 1b and 2b. However, in the preparation of the GIOIBPs, since the IOPs were coated as soon as they

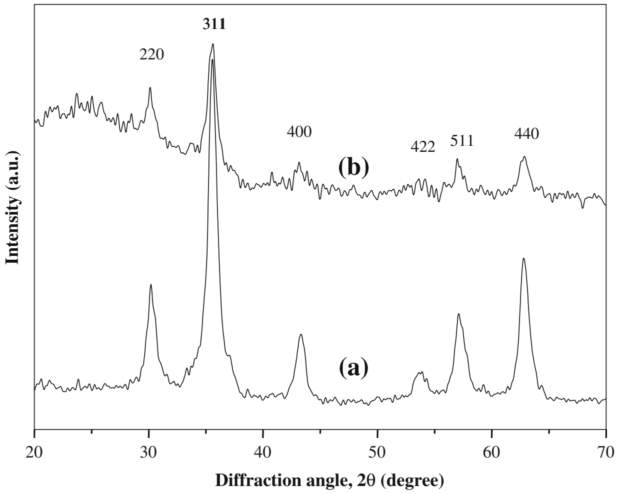


Fig. 3 XRD spectrum of IOPs (a) and GIOBPs (b)

were prepared, such aggregation was prevented as shown by Figs. 1a and 2a.

Theoretically, the biomedical applications involve the injection of the ferrofluids intravenously or directly into the area where treatment is desired [9]. For this purpose, the ferrofluid should be free-flowing, stable liquid. In the present work, all most all ferrofluids prepared using in situ as well as separately precipitation/coating techniques, discussed in the sections “In situ versus separately precipitation/coating technique” and “Viscosity affecting the hydrodynamic size of the ferrofluids”, were highly stable and did not sediment for more than 1 month, which indicates gelatin as an effective stabilizer. Figure 4b shows the representative picture of the dispersions stabilized by gelatin, which was free-flowing and stable for at least 1 month in the physiological pH. However, the IOPs dispersion prepared in identical condition but without gelatin started to aggregate and sediment within 5 min (Fig. 4a). The detail study concerning the stability test in different pH and ionic concentration can be found in our previous work [6].

However, on addition of desolvating and cross-linking agents, the stability of ferrofluid dispersion prepared by in situ as well as separately precipitation/coating techniques decreased. The stability of the dispersions decreased from 1 month to 1 day depending upon the volume of the agents used. For example, in Table 2b, sedimentation of the dispersion occurred when volume of acetone increased above 1000 μL/mL, probably, due to hydrophobic interaction between large numbers of coiled structure (Scheme 1e) as discussed in the “Intermolecular/intermolecular cross-linking affecting size of the ferrofluid” section. When glutaraldehyde was added it bonded with the amine groups of gelatin [22], which resulted in the decrease in water-loving functional groups, leading to decrease in water solubility, hence, stability of the ferrofluid dispersions. Nevertheless, by adjusting suitable combination of acetone and glyceraldehydes, ferrofluid with better stability and size could be produce. The detail study concerning the effect of acetone and glutaraldehyde on stability of the dispersion can be found in our previous work [7].

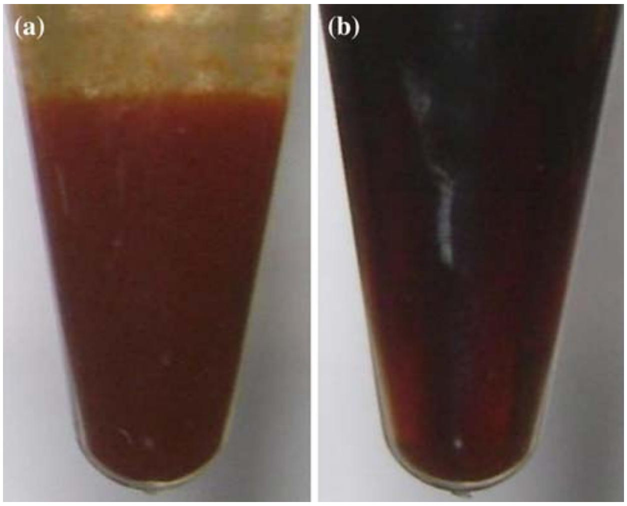


Fig. 4 Picture of uncoated (**a**) and gelatin coated (GIOIBPs-2) (**b**) IOPs dispersions

Conclusions

This study investigated various coating techniques in order to control the hydrodynamic size and stability of the gelatin-coated IOPs ferrofluids. It was observed that the hydrodynamic size of the gelatin-coated IOPs dispersion could be reduced to nanometer size if surface modification of IOPs is by in situ precipitation/coating technique and by reducing viscosity of the ferrofluids. In addition, the size could also be reduced from micro to nanometer size by adding sufficient amount of desolvating and cross-linking agent. Furthermore, the TEM and AFM images indicated that the in situ precipitation/coating technique gave monodispersed ferrofluid compared to the separately precipitation/coating technique, which resulted in dispersion with clustered IOPs. The ferrofluids prepared using in situ as well as separately precipitation techniques were highly stable and did not sediment for more than 1 month. However, the desolvation/cross-linking technique gave dispersion with reduced stability. Nevertheless, by adjusting suitable combination of acetone and glyceraldehydes, ferrofluid with better stability could be produce by this technique.

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