Effect of concentration on hydrodynamic size of magnetite-based ferrofluid as a potential MRI contrast agent

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HIGHLIGHTS

► Stable magnetite-based ferrofluids were synthesized via co-precipitation method.
► Hydrodynamic size was evaluated via PCS technique.
► Effect of concentration was studied on hydrodynamic size.
► Stable ferrofluids were used as MRI contrast agents.

GRAPHICAL ABSTRACT

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In this work, ferrofluids containing dextran coated magnetite nanoparticles have been synthesized via co-precipitation method. FT-IR results verified presence of dextran molecules on the particles surface. TEM results showed that mean particle size is 7.23 nm, while mean hydrodynamic size determined via PCS technique varies between 39.8 and 125.8 nm depending on the ferrofluid concentration. The maximum hydrodynamic size was obtained in mid concentrations. To the best of our knowledge, effect of concentration on mean hydrodynamic size has not been systematically studied before. VSM results confirmed the superparamagnetic behavior of the synthesized nanoparticles with saturation magnetization of 5.82 emu/g. The stable ferrofluids were intravenously injected into mice and used as MRI contrast agent. Results showed that these ferrofluids can be considered as potential MRI contrast agents especially for imaging lymphatic system.

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1. Introduction

Ferrofluids containing magnetic nanoparticles have been widely used in various biological applications such as drug delivery [1–3], MRI contrast agent [4–8] and cancer therapy via hyperthermia [9,10]. Antibody conjugation is one of the conventional approaches of particle and drug delivery into target tissues [11,12]. Surface charge, size, shape and other physical and morphological properties of nanoparticles have a great role in determining the target tissue. For example, large and aggregated particles are mainly accumulated in tissues such as liver and spleen, however smaller ones (20–40 nm) are phagocytosed by macrophages of lymphatic system and mainly absorbed in lymph nodes [5,13]. So, hydrodynamic size of particles dispersing in liquid media including core size and surfactant layer thickness is an important parameter in drug delivery into target tissues and organs. This parameter is
usually determined via DLS (dynamic light scattering) or PCS (Photon correlation spectroscopy) techniques and has been widely used for evaluation of aggregate size [14–16]. One major parameter ignored in all of these investigations is effect of particles concentration on the hydrodynamic size. In other words, the concentration in which hydrodynamic size is measured is not mentioned in these papers. This subject is investigated in the present work and has not been systematically studied before in the best of our knowledge.

In this study, stable ferrofluids containing dextran coated magnetite nanoparticles were synthesized via co-precipitation approach using ferrous and ferric salts and a basic reducing agent according to the general reaction (1) [17]:

\[
\text{Fe}^{2+} + 2\text{Fe}^{3+} + \text{8OH}^- \rightarrow \text{Fe}_3\text{O}_4 + 4\text{H}_2\text{O}
\]  

(1)

The synthesized particles were characterized using XRD, TEM, VSM, FT-IR and PCS techniques. Finally, the stable ferrofluid was used as a MRI contrast agent for molecular imaging of mice lymphatic system. Results show that the synthesized ferrofluid can be considered as a potential MRI contrast agent.

2. Experimental

2.1. Materials and methods

All the chemical reagents used in this research were of analytical grade and used as received without further purification. The detailed synthesis process is described in the previous work [5]. Stable ferrofluids of dextran coated magnetite nanoparticles were synthesized via co-precipitation method using a conventional approach. Stoichiometric amounts of FeCl$_2$·4H$_2$O and FeCl$_3$·6H$_2$O for synthesizing 0.2 g Fe$_3$O$_4$ according to Eq. (1) were prepared and dissolved in distilled water in a three neck container using ultrasonic irradiation while Argon was blown into the solution. 0.6 g dextran 20000 kDa was added into solution too. After 5 min of ultrasonic irradiation, 0.4 molar NaOH was dropwise added into the solution until pH > 12. Ultrasonic irradiation was continued 30 min under Argon atmosphere. After that, HCl solution was added dropwise into the container under stirring until pH reached 7. The obtained stable ferrofluid was used for characterization and MRI tests in this work.

![Fig. 1](image1.png) TEM image of the synthesized sample at three concentrations: (a) 500 µg Fe/cc with mean particle size of 7.28 nm, (b) 2000 µg Fe/cc with mean particle size of 7.30 nm and (c) 3000 µg Fe/cc with mean particle size of 7.23 nm.

![Fig. 2](image2.png) VSM plot of the sample showing superparamagnetic behavior of synthesized nanoparticles.
2.2. Characterization

XRD patterns were taken by a Siemens D5000 X-ray diffractometer using graphite monochromatized high intensity Cu Kα radiation (λ = 1.5406 Å). A JEOL TEM JEM2010F was used to determine the average particle size and morphology of the powders on an accelerating voltage of 200 kV. The Beckman-Coulter N4-plus Submicron Particle Size Analyzer was employed for hydrodynamic diameter measurement in various Fe concentrations via PCS technique. The scattered light was measured at 90° from the incident beam while the sample temperature and pH was adjusted to 25 °C and 7.0, respectively. Also, a Lakeshore 7470 VSM was used for magnetic nanoparticles characterization. IR spectra were recorded on a Nicolet spectrometer (Magna 500). Powder samples were dried at 80 °C before fabrication of KBr pellet.

3. Results and discussion

TEM images of the synthesized sample are shown in three various concentrations in Fig. 1. As seen in these images, mean diameter of the synthesized particles is 7.28, 7.30 and 7.23 nm at sample concentrations of 500, 2000 and 3000 μg Fe/cc, respectively. As is predicted, the ferrofluid concentration has not a significant effect on mean particle size. H–M curve of the sample with saturation magnetization of 57.82 emu/gr obtained via VSM technique is presented superparamagnetic behavior of particles that is logic according to TEM size of particles (Fig. 2). Fig. 3 shows XRD plot of the sample in comparison with standard pattern of pure magnetite (JCPDS card no. 19-0629), confirming the inverse spinel structure of synthesized particles belonging to Fe₃O₄. Crystallite size of particles can be estimated from XRD pattern using the Scherrer equation \( d = \frac{0.9\lambda}{β\cosθ} \) where \( d \) is the crystallite size, \( λ \) is the X-ray wavelength.
and \( \beta \) is the full width at half maximum (FWHM) of the peak located at bragg angle \( (2\theta) \). Using \( \lambda = 1.5406 \AA, \beta = 1.106^\circ = 0.0193^\text{rad} \) and \( 2\theta = 54.8^\circ \), the average crystallite size of magnetite nanoparticles is about 7.54 nm which is close to the TEM results \((\sim 7.23 \text{ nm})\).

FT-IR spectra of the concentrated sample is presented in Fig. 4. The bands observed at 540, 1150 and 2910 cm\(^{-1}\) are related to stretching vibration mode of \( \text{Fe-O} \) in \( \text{Fe}_2\text{O}_4 \), asymmetrical stretching vibration mode of \( \text{C-O-C} \) in \( \alpha\)-glycoside bridge and asymmetrical stretching vibration mode of \( \text{C-H} \) of \(-\text{CH}_2\), respectively [18]. The band near to 1010 cm\(^{-1}\) is related to amorphous band [19]. The peaks located at 1340 and 1410 cm\(^{-1}\) are assigned to deformation vibration mode of \( \text{H-C-OH} \) band. Finally, the broad peak at 3550–3200 cm\(^{-1}\) is related to stretching vibration mode of \( \text{O-H} \). The above mentioned peaks confirmed presence of dextran coating on nanoparticles surface [18].

In Fig. 5, the hydrodynamic size histogram of particles in three various Fe concentrations has been displayed: 13, 200 and 3000 \( \mu \text{g Fe/cc} \). These data were obtained from PCS measurements for 14 various concentrations (Table 1). Each measurement was repeated 10 times in each concentration to obtain more validate results. The mean hydrodynamic size of the synthesized sample is plotted in Fig. 6 versus Fe concentration. As is seen from this figure and Table 1, mean hydrodynamic size is between 39.8 and 125.8 nm. In low Fe concentrations, it increases with increasing concentration, but in high Fe concentrations it has vice versa behavior. Hence, its maximum value is related to middle. Effect of concentration can be explained accordingly.

Attractive van der Waals and magnetic energies between particles dispersing in a liquid medium as a stable suspension can be written as [20]:

\[
U_{\text{vdw}} = -\frac{A}{6} \left[ \frac{2R^2}{r^2} - \frac{2R^2}{4r^2} + \ln \left( 1 - \frac{4R^2}{r^2} \right) \right]
\]

\[
U_{\text{mag}} = \frac{\mu_0 m_s^2 V^2 f(\theta)}{4\pi r^3}
\]

where, \( R \) and \( V \) are radius and volume of particle, respectively; \( A \) is Hamaker constant; \( r \) is inter-particulate distance; and \( m_s \) is saturation magnetization of sample. According to these equations, increasing nanoparticles concentration and decreasing \( r \) leads to increase attractive forces and formation of large agglomerates. Therefore, hydrodynamic size increases with concentration. On the other hand, increasing concentration from a definite value dramatically increases ferrofluid viscosity which causes hydrodynamic size reduction based on Stokes–Einstein equation:

\[
D_h = \frac{kT}{3\pi\mu D}
\]

where \( D_h \) is hydrodynamic diameter, \( k \) is Boltzmann constant, \( T \) is absolute temperature, \( \mu \) is viscosity and \( D \) is diffusion coefficient. It seems that in low concentrations, attractive forces are more effective in determining hydrodynamic size value, whereas viscosity increase is determinant in high concentrations. Hence, the maximum value of hydrodynamic size is related to mid concentrations.

The dextran coated magnetite nanoparticles prepared as stable ferrofluids were intravenously injected into mice via tail vein to investigate particles accumulation in targeted tissues (mainly lymph nodes in this study). As the mice weight was around 20 g, volume of mice blood was around 3 cc and maximal permissible dose is 2 mg Fe/body weight, 0.1 cc of ferrofluid were administrated with concentration of 0.4 mg/cc. Thus, injected Fe concentration is around 13 \( \mu \text{g (Fe)/cc (blood)} \) after several minutes of blood circulation. As shown in Figs. 5 and 6, mean hydrodynamic diameter of the particles is 44 nm in this concentration. As the ferrofluids pH was adjusted close to 7 and no antibody agent was conjugated into particles surface, the main targeting mechanism is based on particles size. As theory predicts [13], particles with mean

<table>
<thead>
<tr>
<th>Concentration (( \mu \text{g Fe/cc} ))</th>
<th>5</th>
<th>13</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>300</th>
<th>400</th>
<th>500</th>
<th>1000</th>
<th>2000</th>
<th>3000</th>
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</thead>
<tbody>
<tr>
<td>Hydrodynamic size (nm)</td>
<td>39.8</td>
<td>44.7</td>
<td>68.2</td>
<td>80</td>
<td>86.6</td>
<td>91.1</td>
<td>112</td>
<td>125.8</td>
<td>102.3</td>
<td>96.8</td>
<td>83.3</td>
<td>76.8</td>
<td>55</td>
<td>50.4</td>
</tr>
</tbody>
</table>

Table 1

Hydrodynamic size of sample A in various Fe concentrations.

![Fig. 6](image_url) Hydrodynamic size of the sample versus Fe concentration according to Table 1.

![Fig. 7](image_url) MR image of a mouse 24 h after IV injection of the sample showing nanoparticles accumulation in lymph nodes.
hydrodynamic size near the range 20–40 nm mainly accumulate in lymph nodes. This is true about the synthesized sample 24 h after IV injection (Fig. 7). In other words, the above mentioned amount of $D_0$, determined at 13 μg (Fe)/cc (blood) is more valid for determination of particles accumulation region.

4. Conclusion

Dextran coated magnetite nanoparticles were synthesized by coprecipitation method and stabilized in aqueous medium. In this study we demonstrate that hydrodynamic size measured via PCS technique is not a constant value and varies with particles concentration. This value varies between 39.8 and 125.8 nm for the studied samples. Although, we demonstrate that the synthesized nanoparticles are mainly accumulated in lymph nodes after administration in mice and significantly decrease signal intensity in these regions. Therefore, it can be considered and used as a potential negative MRI contrast agent especially for imaging lymphatic system.

References


