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An investigation on the optimum conditions of synthesizing a magnetite based ferrofluid as MRI contrast agent using Taguchi method

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In this study, some stabilized magnetite based ferrofluids were synthesized using Dextran as a stabilizing agent. In order to achieve optimum experimental conditions for synthesizing ferrofluids as MRI contrast agents, the Taguchi method was used. This approach was employed to design and minimize the number of required experiments. By using the Taguchi orthogonal (L16) array, four parameters including solution temperature and alkalinity, reaction temperature and stirring rate were selected at four predetermined levels for 16 experiments. Synthesizing processes established based on this set of experimental conditions were carried out and the obtained ferrofluids were characterized using PCS, VSM, TEM and FT-IR techniques. The obtained results were used and analyzed through the Qualitek-4 software and the proposed optimum experimental conditions were used for synthesizing the desired sample. Finally, this sample was used as a potential MRI contrast agent for imaging lymph nodes.

Keywords: *magnetite; Dextran; Taguchi method*

1. Introduction

Magnetite nanoparticles have been widely used in biological applications especially as Magnetic Resonance Imaging (MRI) contrast enhancement agent [1, 2] and also in cancer therapy [3, 4] and drug delivery [5]. Among various types of conventional surfactants such as Dextran [1, 5], PEG [6] and PVP [7], Dextran has frequently been used for stabilizing the magnetite based ferrofluids. This surfactant is very popular because of its good biocompatibility and excellent stabilizing property, so that ferrofluids synthesized in the presence of this surfactant usually show long-term physical and chemical stability.

Regardless of other factors, the target tissue is determined by the mean hydrodynamic size of magnetite nanoparticles. For example, nanoparticles with the mean hydrodynamic size between 20 and 40 nanometers are mainly accumulated in lymph nodes, while large and aggregated particles (the mean hydrodynamic size between 80 and 150 nanometers) are quickly absorbed in a liver and spleen and the largest ones (about 300 nanometers) are used for MR imaging or drug delivery into gastrointestinal system [1, 5].

Among various approaches for synthesizing magnetite based ferrofluids such as co-precipitation [8, 9], hydrothermal [10–12] and thermal decomposition [13], co-precipitation is rather a simple and conventional method. Unfortunately, the co-precipitation method usually leads to formation of ferrofluids with broad particle size and hydrodynamic size distribution. It is mainly due to the non-uniform nature of precursors mixing and reacting in the reaction container. For example, stirring conditions, including material velocity and agitation intensity, are completely different in the points located near and far from the magnetic or mechanical stirrer. In a conventional co-precipitation process of magnetite formation, from chloride salts as the iron precursors and ammonia solution as the reducing agent, Fe_3O_4 particles are formed via a simple one-stage reaction that can be written accordingly [1, 9]:

$$FeCl_2 + 2FeCl_3 + 8NH_4OH \rightarrow Fe_3O_4 + 8NH_4Cl + +4H_2O \quad (1)$$

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As the entire reaction occurs in a few seconds, any inhomogeneity of material flux at various points of reaction container leads to formation of particles with a broad size distribution histogram. Besides, it seems that the results obtained by this method are not as repeatable as a thermal approach such as hydrothermal process. Various parameters such as solution temperature, pH, etc. strongly affect hydrodynamic size distribution and investigation of the effect of all these parameters on the suspension properties requires too many experiments. It seems that "design of experiments" (DOE) methods can be useful for evaluating the optimum conditions for the synthesis process.

In this study, Taguchi orthogonal (L16) array was used for design of experiments and four parameters including solution temperature and alkalinity, reaction temperature and stirring rate were selected at 4 levels for 16 experiments. Taguchi method has been used by some material science researchers for designing experiments and optimizing experimental conditions [14–16]. Hydrodynamic size and saturation magnetization of these 16 samples were measured using Photon Correlation Spectroscopy (PCS) and Vibrating Sample Magnetometery (VSM) techniques. As the lymph nodes in this study were target tissues, samples with the particle size ranging between 20 and 40 nanometers seemed to be proper [5]. The optimum experimental conditions assessed with Qualiteck-4 software were considered for synthesizing the desired ferrofluid. This sample was injected intravenously into a rat and used as a potential MRI contrast agent.

2. Experimental

2.1. Materials and methods

All chemicals were of analytical grade and were used as received without further purification. Sixteen magnetite based ferrofluids were synthesized via conventional co-precipitation approach using $FeCl_2{\cdot}4H_2O$ and $FeCl_3{\cdot}6H_2O$ as iron precursors, NH_4OH as reducing agent and Dextran 15 kDa as the stabilizing surfactant. Stoichiometric amounts of the iron salts were dissolved in distilled water under N_2 atmosphere in a three neck container using a magnetic stirrer. After that, Dextran was added into the container and stirring was continued for 10 minutes. Predetermined amount of ammonia solution was suddenly added into the container while magnetic stirring and N_2 blowing were still in progress. The solution color changed immediately into black and stirring was continued for 30 minutes in a fixed temperature. After that, the synthesized ferrofluids were cooled down to room temperature under N_2 atmosphere and prepared for VSM, PCS, Transmission Electron Microscopy (TEM) and Fourier Transform Infrared Spectroscopy (FT-IR) were used for characterizations. The detailed amounts of reactants and experimental conditions are listed in Table 1.

2.2. Characterization instruments

JEOL TEM, JEM-2010F was used to determine the average particle size and morphology of the powders at an accelerating voltage of 200 kV. Sample preparation was performed through the conventional method in which one trickle of ferrofluid was located on the surface of a carbon coated copper grid and used as TEM sample after the trickle drying. The trickle concentration was of the order of 1 μgFe/ml. Malvern instrument was employed for hydrodynamic diameter measurement via PCS technique. Concentration of all PCS samples was adjusted to 13.3 μgFe/ml. This concentration was selected regarding to the particle concentration in rat's bloodstream after ferrofluid injection. Magnetization saturation of the samples was measured using Lakeshore 7470 VSM. As the synthesized ferrofluids were very stable and preparation of solid sample was difficult, magnetic characterization was performed directly on the suspension samples instead of the solid ones. VSM samples concentration was adjusted to 2 mgFe/ml. FT-IR spectra were recorded on a Nicolet spectrometer (Magna 500). Powder samples were dried at 80 °C before fabrication of KBr pellet. MR imaging was performed with a 1.5 T (GE medical system) by

Table 1. Experimental conditions for synthesizing samples T_1 to T_{16}. These sets of experimental conditions have been assessed by Qualiteck-4 software according to Taguchi method.

Sample	T (°C)	Stirring Rate (rpm)	Dextran Concentration (gr/cc)	NH_3 Molarity	*D_{PCS} (nm)	M_s (emu/gr)
T_1	25	250	0.00459	0.39	120±5	27.05
T_2	25	500	0.00612	0.52	88.4±3	26.10
T_3	25	750	0.00765	0.65	78.3±7	35.82
T_4	25	1000	0.00918	0.78	86.4±2	25.13
T_5	35	250	0.00612	0.65	115.6±5	57.94
T_6	35	500	0.00459	0.78	111.1±8	28.21
T_7	35	750	0.00918	0.39	70.6±9	40.16
T_8	35	1000	0.00765	0.52	91±4	37.77
T_9	45	250	0.00765	0.78	253.7±5	41.12
T_{10}	45	500	0.00918	0.65	71.2±5	41.13
T_{11}	45	750	0.00459	0.52	128.6±6	31.36
T_{12}	45	1000	0.00612	0.39	95±9	34.58
T_{13}	55	250	0.00918	0.52	66.5±6	40.38
T_{14}	55	500	0.00765	0.39	86.4±3	45.02
T_{15}	55	750	0.00612	0.78	99±4	28.15
T_{16}	55	1000	0.00459	0.65	221±8	47.73

*Each value is result of at least 3 measurements.

using a knee coil for transmission and reception of the signals.

3. Results and discussion

3.1. VSM

The values of magnetization saturation of the samples are listed in Table 1. These data have been extracted from VSM diagrams (Fig. 1). As seen in this figure, all samples show superparamagnetic behavior revealing small size of particles. This phenomenon is due to the large amount of the heat energy, kT, in comparison with the magnetic energy, KV, for the small particles, where k is the Boltzmann constant, T is the absolute temperature, K is the magnetic anisotropy constant of magnetite nanoparticles and V is the nanoparticle volume. When the particle size decreases from a definite value, the superparamagnetic critical size, V, diminishes to such an extent that $KV \ll kT$. So, no permanent magnetic moment remains inside the particle due to the thermal vibrations. Although the bulk magnetite shows ferromagnetic behavior, magnetite nanoparticles with the small sizes have no coercivity and no hysteresis loss region in their M-H diagrams. These so-called "superparamagnetic particles" are suitable for biomedical applications such as MRI contrast agents in which magnetic attraction and aggregation of magnetite nanoparticles is not desirable in the absence of an external magnetic field, but is required in the presence of such a field.

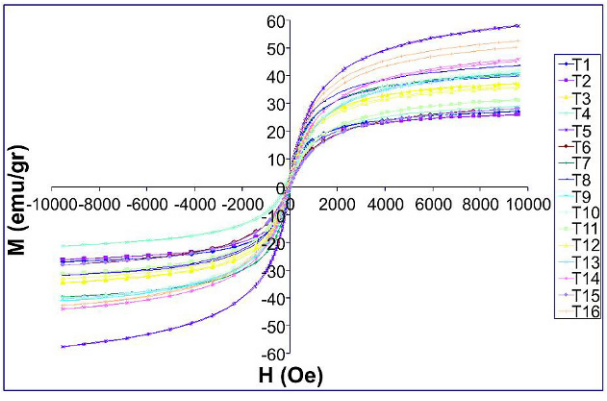


Fig. 1. VSM digram of samples T_1 to T_{16}.

Table 2. Optimum conditions proposed by Qualitek-4 software for synthesizing a ferrofluid with mean hydrodynamic size of 23.2 nm.

Factor	Level Description	Level	Contribution
T (°C)	35	2	−25.846
*Stirring Rate	500	2	−27.579
**Dextran Concentration	0.00918	4	−23.566
NH_3 Molarity	0.52	2	−22.796
Total contribution from all factors			−99.788
Current grand average of all performance			123.003
Expected result at optimum condition			23.216

*rpm
**g/cc

3.2. PCS

The values of hydrodynamic size of samples T_1 to T_{16} are listed in Table 1. These data have been obtained via PCS technique. As seen in this Table, the mean hydrodynamic size ranges between 66.5 and 253.7 nanometers for these samples. All of these values are out of the range 20 – 40 nanometers, which is the proper range for drug delivery into lymph nodes [5]. Regarding a minimum value of the mean hydrodynamic size (mode "smaller is better"), we have assessed with the Qualitek-4 software, using the obtained data, a set of optimum experimental conditions which is shown in Table 2. According to this table, the synthesis process at 35 °C and 500 rpm in the presence of 0.52 mole NH_4OH and Dextran concentration of 9.18 mg/cc leads to formation of a ferrofluid with the mean hydrodynamic size of 23.2 nm. This size is suitable for drug delivery into lymph nodes. Synthesis experiment established according to Table 2 led to formation of a stable ferrofluid with the mean hydrodynamic size of 26.8 nm which is close to the predicted value ($T_{opt.}$ sample). Hydrodynamic size histogram of the $T_{opt.}$ sample is shown in Fig. 2. This sample was injected into the rats at the concentration of 2 mgFe/cc and used as a potential MRI contrast agent.

3.3. FT-IR

FT-IR spectrum of the $T_{opt.}$sample is shown in Fig. 3. The presence of the observed peaks

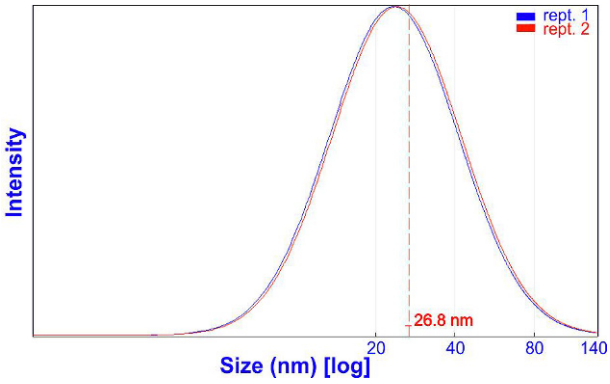
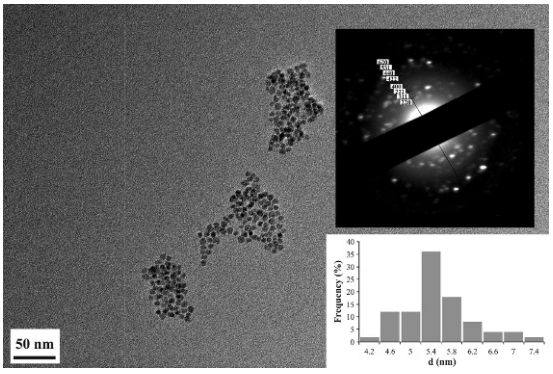


Fig. 2. Hydrodynamic size histogram of sample $T_{opt.}$ obtained from PCS measurement with mean hydrodynamic size of 26.8 nm. This sample has been synthesized at 35 °C and 500 rpm in the presence of 0.52 mole NH_4OH and Dextran concentration of 9.18 mg/cc.

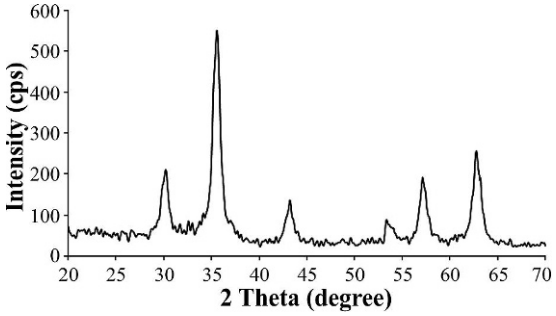
can be explained as follows: the band observed at 540 cm^{-1} is related to stretching vibration mode of Fe–O in Fe_3O_4 [17]; the bands at 850 and 900 are characteristic of (1→3)-α-D-glucan [18]; the peak observed at 1000 cm^{-1} is due to the great chain flexibility present in Dextran around the glycosidic bonds [19]; the bands at 1120 and 2900 are related to asymmetrical stretching vibration mode of C–O–C in α-glycoside bridge and asymmetrical stretching vibration mode of C–H of $–CH_2$, respectively [18]; the peaks located at 1320 and 1400 $cm^{-1}$are assigned to deformation vibration mode of H–C–OH band; the band at the region of 1620 cm^{-1} is due to carboxyl group [20]; finally, the broad peak at 3400 $cm^{-1}$is related to hydroxyl stretching vibration of the polysaccharide [21]. The above mentioned peaks confirm the presence of Dextran coating on the nanoparticles surface.

3.4. TEM and XRD

TEM image of the $T_{opt.}$ sample is presented in Fig. 4a. As seen in the size distribution histogram of this sample, some semi-spherical particles with a mean size of 5.5 nm are synthesized with a rather narrow size distribution. The mean particle size was calculated by measuring 50 particles. Diffraction pattern of this sample reveals reverse spinel structure of the nanoparticles. The relevant



(a)



(b)

Fig. 3. (a):TEM image, size distribution and diffraction pattern of sample $T_{opt.}$ with mean particle size of 5.49 nm. The rings related to crystallographic planes of magnetite structure including planes (220), (311), (222), (400), (422), (440), (531) and (620) are illustrated in diffraction pattern, revealing formation of Fe_3O_4 nanoparticles; (b): XRD pattern of the dried sample $T_{opt.}$.

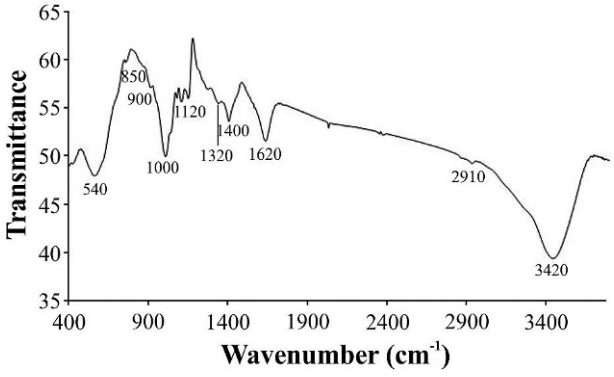
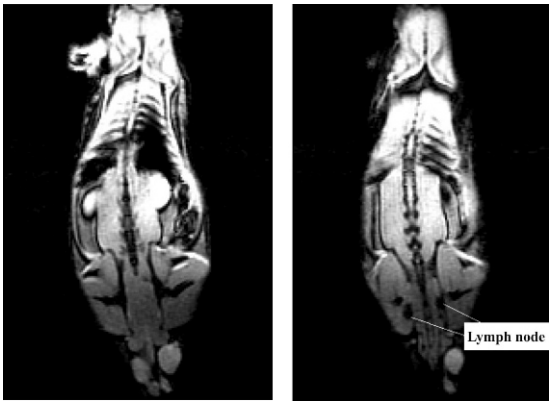


Fig. 4. FT-IR plot of sample $T_{opt.}$.



(a) (b)

Fig. 5. MR image of rat (a) before and (b) 24 hours after IV injection of sample $T_{opt.}$ revealing particles accumulation in lymphatic nodes.

rings are illustrated in diffraction pattern. XRD pattern of the dried powder $T_{opt.}$ is shown in Fig. 4b, confirming the reverse spinel structure of this sample. Crystallite size of synthesized particles can be calculated from the XRD pattern with the Scherrer equation:

$$d = \frac{0.9\lambda}{\beta \cos\Theta} \qquad (2)$$

where, d is the calculated size, λ is the X-ray wavelength, β is the full width at half maximum (FWHM) of the peak and Θ is the diffraction angle. The average crystallite size of magnetite nanoparticles estimated from equation 2 is about 6.1 nm, which is in good coincidence with the TEM result (5.5 nm).

3.5. MRI

MR images of the rat before and 24 hours after intravenous injection of the $T_{opt.}$ sample are shown in Fig. 5. The detailed protocol of animal study and MR imaging has been described in a previous work [1]. According to theory, particles with mean hydrodynamic size between 20 and 40 nanometers have a long circulation time in bloodstream and finally are absorbed in lymph nodes [5]. As seen in Fig. 5b, signal decrease is evident in the marked regions in comparison with Fig. 5a, confirming accumulation of the nanoparticles in these lymphatic zones. So, this sample can be considered as a potential MRI contrast agant for further investigations.

4. Conclusions

In this study, optimized conditions for synthesizing magnetite/Dextran ferrofluids as potential MRI contrast agents were determined based on the particles mean hydrodynamic size. Using the Taguchi method through orthogonal L16 array and considering solution temperature and alkalinity, stirring rate and Dextran concentration as variable parameters, the optimized conditions for synthesizing ferrofluids, suitable for MR imaging of rats' lymphatic system were found: T = 35 °C, stirring rate = 500 rpm, NH_4OH amount = 0.52 mole and Dextran concentration = 9.18 mg/cc. These conditions led to formation of a stable ferrofluid with a mean hydrodynamic size and a mean particle size of 26.8 and 5.5 nanometers, respectively. The optimized ferrofluid was administrated intravenously into a rat and used as MRI contrast agent. MR image verified accumulation of the particles in the lymph nodes and due to signal decrease in these regions a suitable contrast was obtained in the image.

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