Journal of Applied Chemistry   JAC
                                        http://chemistry.journals.semnan.ac.ir

Using the design experiment Response surface methodology for the preparation of three-component nanoparticles

Fariba Tadayon[1], reza Jamshidi[2,*], parisa Jamshidpoor[1]

[1] Department of Chemistry, North Tehran Branch, Islamic Azad University, Tehran, Iran
[2] Department of biochemistry, faculty of veterinary, semnan university, Semnan, Iran

Abstract

In this study, three-component nanoparticles of iron, nickel and lithium were synthesized with two methods of combined technique (co-precipitation, hydrothermal) and pechini sol-gel. The optimum conditions for producing MNPs by co-precipitation method were found to be at pH=13 with constant stirring rate of 1000 rpm and at temperature of 72 °C under nitrogen atmosphere. The situation for preparing nanoparticles by pechini sol-gel method including dissolving nitrate salt of three metals in water then adding citric acid and ethylene glycol respectively in temperatures 60 and 80 ^{0} C with pH=7 and placing sample in temperatures 190 and 500 ^{0} C for 720 and 60 minutes respectively. In the comparison of two methods, morphology and size of produced magnetic nanoparticles with combined method of co-precipitation- hydrothermal was more suitable. Response surface methodology was used to verify the best synthesized nanoparticles in size. Silica and amine groups in the coated nanoparticles were loaded to target drugs.

Keywords: Magnetic Nanoparticles; Response surface methodology; Pechini sol-gel; Hydrothermal.

1. Introduction

Advances in nanotechnology and molecular biology are rapidly enabling the development of nanoparticles (NPs) with specific functional properties that address the shortcomings of traditional disease diagnostic and therapeutic agents [1-3]. Biocompatible magnetic nanoparticles based on iron oxide NPs are key components for biomedical applications such as drug delivery systems [4-6], hyperthermia [7], MRI contrast enhancement [8-10] and magnetic particle imaging (MPI) [11].

An important point in development of nanoparticles to be used in biomedical applications is that the particle size and distribution should be controllable [12]. MNPs based on nickel composition are highly interesting materials due to their superparamagnetic behavior at room temperature. For biomedical applications, important results are directly obtained by conjugation of Ni–MNPs with an enzyme [13]. Thus, they may be used for diagnostic and therapeutic applications [14-17].

* .**Corresponding author:** Assistant Professor of Biochemistry, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran. E-mail: r_jamshidi@semnan.ac.ir

MNPs are being developed as drug carriers thanks to careful nanostructure construction (tailored drug release characteristics, low immunogenicity, etc.), yielding improved treatment efficacy and reduction of unwanted side effects [18, 19]. As a drug delivery, the particle should be biocompatible to the body's immune system. In order to be most effective, carrier particles must have a long retention time while circulating in the blood [20]. For this purpose, silica MNPs must be coated. Silica has been known to be one of the most ideal coating layers for MNPs due to its reliable chemical stability, biocompatibility and reactivity with various coupling agents, making them suitable for conjugation with drugs for in vivo applications [21].An experimental design was developed to decrease the number of experimental runs and consider the interaction of parameters to recognize true optimum points with reasonable runs. Response surface methodology (RSM) has been reported to be an effective tool for optimization of a process in which the independent parameters have a combined effect on the desired response. RSM is a method that combines statistics and mathematics to analyse the relative significant influential factors in a system [22].

The preparation of MNPs will be examined in this study by the sol-gel method and combined technique, which consists of the controlled precipitation and hydrothermal methods. Another aim of this paper is applying RSMtooptimize the effect of various experimental factors on the size of silica coated magnetic nanoparticles (SiO_2-MNPs).

2. Experimental

2.1 Reagents and apparatus

All chemicals and reagents used in this work were of analytical grade and were obtained from Merck Company. Deionized distilled water (DDW) was used to prepare all the solutions.

X-ray diffraction (XRD) (X'Pert Pro MPD, PANalytical, 2009) was used in order to examine the PS and the crystal structure. The PS and morphology of the MNPs were investigated by employing scanning electron microscopy (SEM) (KYKY-EM3200, Madell Technology, USA) at 15 kV. Furthermore, the Fourier transform infrared spectroscopy (FT-IR) was employed to explore the absorption spectra of the SiO2-MNPs from 450 to 400 cm_1 (Nicolet Magna 550 series II FTIR, USA). The NP powder was mixed with KBr and the spectra were obtained through the analyzer. The magnetic properties of the samples were then studied using vibrating sample magnetometer (VSM). Ultrasonic (Bandelin SONOREX RK 514H, capacity 18.7 L, 600 W) was employed for mixed phase synthesis.

2.2 Optimization of MNPs

Central composite design has been studied by many statisticians in response surface analysis and is perhaps the most popular class of second order designs. Central composite design (CCD), the most popular RSM and Minitab 17.1 statistical software were used to determine the optimum conditions in co-precipitation method and produce the best nanoparticles in size. Three experimental effective factors, stirration rate (rpm), temperature (˚C) and pH were selected to run the optimization method. Particle size was considered as the response. Three levels of the factors were used in optimization by RSM (table 1). Experimental factors were selected based on having the most significant effect on the efficiency of the controlled precipitation method. Previous experimental trials were used to select Factor levels.

Table 1. Factors used in the factorial design

Factors/Level	-1	0	1
Rate (rate/min) (X1)	500	1000	1500
pH (X2)	9	11	13
Temperature (X3)	60	72	85

A set of 20 experiments were designed by Minitab (Table 2). The XRD spectra were utilized to determine the crystallographic identity of the produced material, and phase purity was considered for calculating the mean particle size; it must be explained that this was done according to the broadening of the most prominent peak observed in the XRD profile.The

diffraction peaks of the XRD patterns of the prepared MNP samples can be seen in Fig. 1, as determined by Mini tab software and the Scherrer equation. The SiO2-MNPs showed minor strong reflection peaks at low 2θ values that originated from the amorphous silica matrix, due to the thin silica coating layer in SiO2-MNPs.

Table 2. Particle size obtained with RSM design.

Std order	Run order	Block	X1:rate	X2:pH	X3:Temperature	YExp: MNP size(nm)
1	1	1	500	9	60	54.73
15	2	1	1000	11	72	45.62
8	3	1	1500	13	85	55.89
13	4	1	500	11	72	58.12
12	5	1	1000	11	85	53.09
11	6	1	1000	11	60	49.51
20	7	1	1000	11	72	45.02
3	8	1	500	9	85	50.95
5	9	1	1500	9	60	70.16
2	10	1	500	13	60	64.43
17	11	1	1000	11	72	45.35
9	12	1	1000	9	72	47.29
6	13	1	1500	13	60	44.73
16	14	1	1000	11	72	45.82
10	15	1	1000	13	72	43.05
19	16	1	1000	11	72	45.07
14	17	1	1500	11	72	58.39
4	18	1	500	13	85	69.80
7	19	1	1500	9	85	72.00
18	20	1	1000	11	72	47.15

2.3 Synthesis of Li$_{0.25}$Ni$_{0.5}$Fe$_2$O$_4$ NPs by combined technique

Magnetic nanoparticles of Li$_{0.25}$Ni$_{0.5}$Fe$_2$O$_4$ were prepared by a combined technique, which consists of the controlled precipitation and hydrothermal methods. First in co-precipitation method, sufficient amounts

of FeN$_3$O$_9$.9H$_2$O, LiNo$_3$, and NiN$_2$O$_6$.6H$_2$O (Li: Ni: 2Fe) were mixed in distilled water at room temperature and stirred approximately at 1000 rpm for 15 min. Next, based on optimum conditions in table 2 for preparing MNPs of the least size, the mixture was placed in an oil bath heated to 72˚C and subjected to

nitrogen atmosphere with constant stirring at 1000rpm for 40 min.Afterwards, 1.2 M NaOH solution was added drop-by-drop to the solution under nitrogen atmosphere. Following this in hydrothermal method, the mixture was transferred to a Teflon-lined autoclave and maintained at 190 ˚C for 12 h.The products were washed three times with distilled water and then dried at 80˚C for 15 h in a vacuum oven. The MNPs were obtained heated at 800 ˚C for 2 h.

The Stöber method was used for coating MNPs with silica [23]. SiO_2-MNP was prepared by mixing certain amounts of ethanol, distilled water and tetraethyl orthosilicate (TEOS) in a 250 mL two neck flask and stirred magnetically at 1500 rpm, in a 40 ˚C water bath for about 30 min. Sufficient amount of 25% NH_4OH was added to the solution and reacted under mechanical stirring for 30 min. Subsequently, Magnetic multicomponent nanoparticles were added to the solution and stirred for another 6 h. The SiO_2-MNPs were separated by magnetic field, washed with absolute ethanol three times and dried at 80 ˚C in a vacuum oven. SiO2-MNPs were functionalized by (3-Aminopropyl) triethoxysilane (APTES) as an amine agent. Enough amounts of SiO_2-MNPs were washed in ethanol and water for 30 min with an ultrasonic processor. APTES and dimethylformamide were added to the solution and shaken in a shaker at 350 rpm for 2 h. The SiO_2-MNPs were isolated with a magnet and washed with phosphate-buffered saline (PBS) three times. Finally, glutaraldehyde (GA) was added to the SiO_2-MNP suspension in PBS and shaken for 1 h. The activated SiO_2- MNPs were prepared by washing suspension three times with PBS.

2.4 Synthesis of LiNiFe₂O₄ NPs by pechini sol-gel method

Magnetic nanoparticles of nickel Lithium ferrite were synthesized by pechini sol-gel method [25].

Stoichiometric amounts of $FeN_3O_9.9H_2O$, $LiNo_3$, $NiN_2O_6.6H_2O$, Citric acid and ethylene glycol were used as precursor materials. For the hydrolysis reaction, sufficient amount of metal nitrates was mixed in distilled water at room temperature under magnetic stirring at 1500 rpm for 15 min. Controlled amounts of Citric acid as chelating agent were added to the solution at 60 ˚C and mixed for 1 h. A little amount of NH_4OH was added to the solution to reach the pH at about 7. The solution was heated to 80 ˚C then ethylene glycol as a polymerization agent was added to the mixture and stirred magnetically for another 1 h. The wet gel was dried in an oven at 190 ˚C for 12 h. For the calcination process, dry gel was heated at 500 ˚C for 1 h. finally MNPs were formed.

3. Results and Discussion

3.1Preparation and properties of MNPs

To determine the optimal parameters required for the preparation of MNPs, CCD was employed as a reliable methodology to evaluate the interactions between the independent variables. Accordingly, the influence of pH, temperature and rate of reaction was studied. The MNP PS obtained under each synthesis condition and the relationship between the reaction conditions and PS can be seen in Table 2. By comparing the data obtained in this study with those already found in the previous studies, it was clear that the PS obtained here was smaller; in fact, it was the smallest, with the run 15 MNP PS of 43.05 nm.

3.2Studying three-dimensional response surface plot

Results of the twenty designed experimental runs were investigated by using three-dimensional response surface to study the effects of factors and their interactions on synthesis of MNPs. (fig.1)
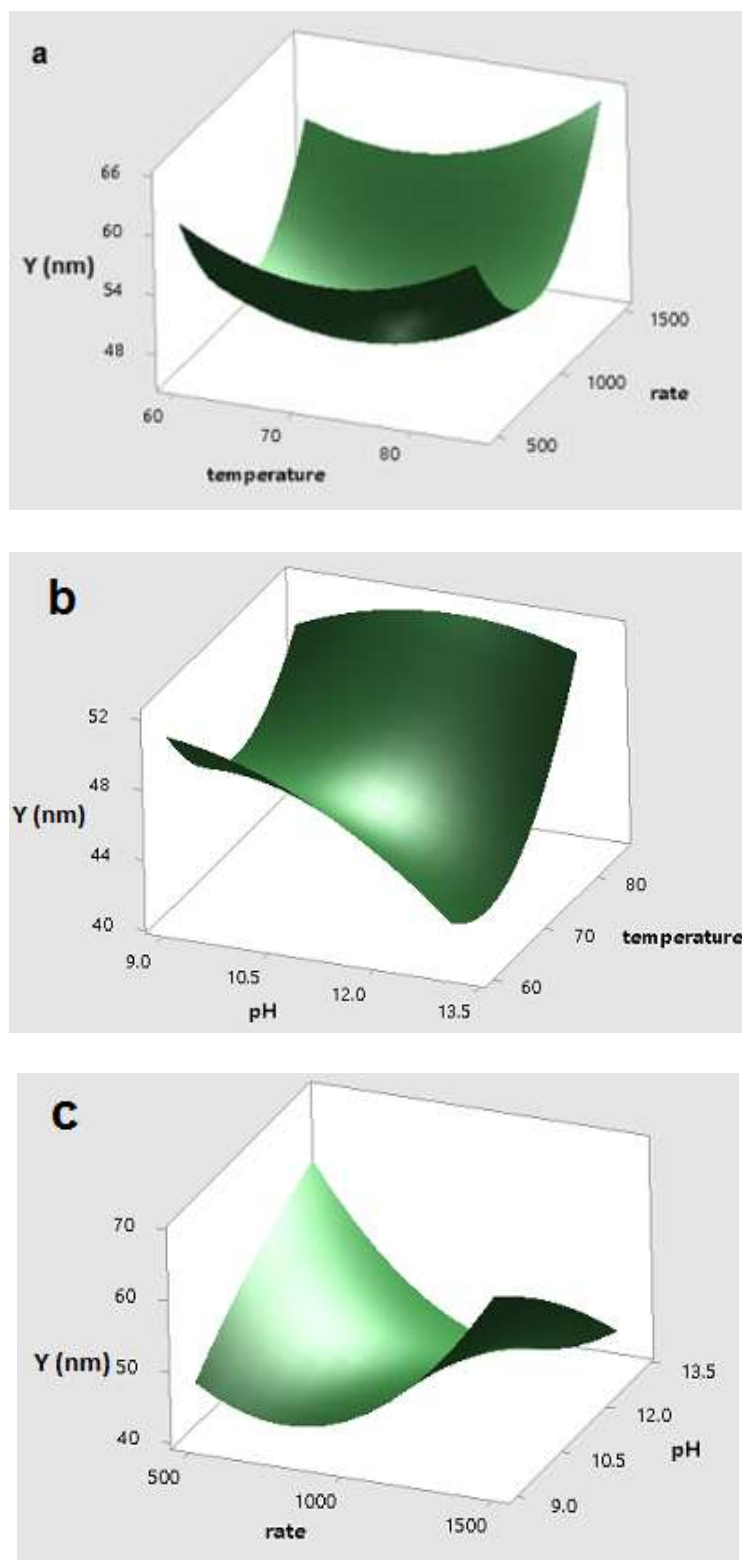
Figure 1.3-D response of (a) nanoparticle size vs tempreture and stirring rate at the central level of pH, (b) nanoparticle size vs pH and tepreture at the central stirring rate,(c) nanoparticle size vs stirring rate and pH at the central level of tempreture.

3.3 Effect of three factors on synthesis of MNPs

3.3.1. Stirration Rate

The stirration rate in preparation of MNPs was found to be important for achieving complete reduction. As shown in Table 2, the optimal PS of 43.05 nm was obtained using a rate of 1000 rpm at an oil bath temperature. This result indicated that variation in stirration rate may affect PS. Both decreasing and increasing the rate from 1000 rpm resulted in an increase in PS. The rate-temperature interaction shows that at 72 ˚C in rate of 1000 rpm, both values minimized PS (Fig 1a). Studying the effect of varying the pH-rate relationship indicates that pH has a good impact on PS (Fig 1b).

3.3.2. Temperature

The influence of temperature factor between 60 ˚C and 85 ˚C on size of MNPs was studied. By increasing the temperature to 72 ˚C, a reduction in the size of MNPs was obtained, although the largest size was observed at higher temperatures. The bigger MNPs were obtained when temperature factor increased from 72 ˚C to 85 ˚C. Previous research has shown that the size of MNPs increases at higher temperature. Therefore, the best condition to form optimal MNPs in size was found at 72 ˚C (Fig. 1c).

3.3.3. pH

A digital pH meter was used to measure the pH values of the solutions. The interactions between pH and two other factors are shown in figures 1b and 1c. It can be concluded that increasing the amount of pH in any temperature decreases the size of MNPs. At high pH, a small PS was produced compared to lower pHs, which may be due to the MNPs' tendency to bind to each other and agglomerate. Other research suggested that the optimal pH for producing the smallest PS must be in the range of 9.7–10.6 [23]. Synthesis with final pH 13 under other synthetic conditions yielded the smallest PS (Table 2). Therefore, run 15 was selected as the optimal condition and the MNPs produced in that run were used for further experiment.

3.4 Comparative study on structure and morphological properties of MNPs prepared by combined method and pechini sol-gel

XRD was used to determine the size and crystal structure of MNPs. As shown in figure 2(a and b), the diffraction peaks of the XRD patterns were found to be consistent with $Li_2NiFe_2O_4$ structures for both methods. Average crystallite size was obtained 44.24 for combined method and 57.42 for pechini sol-gel.
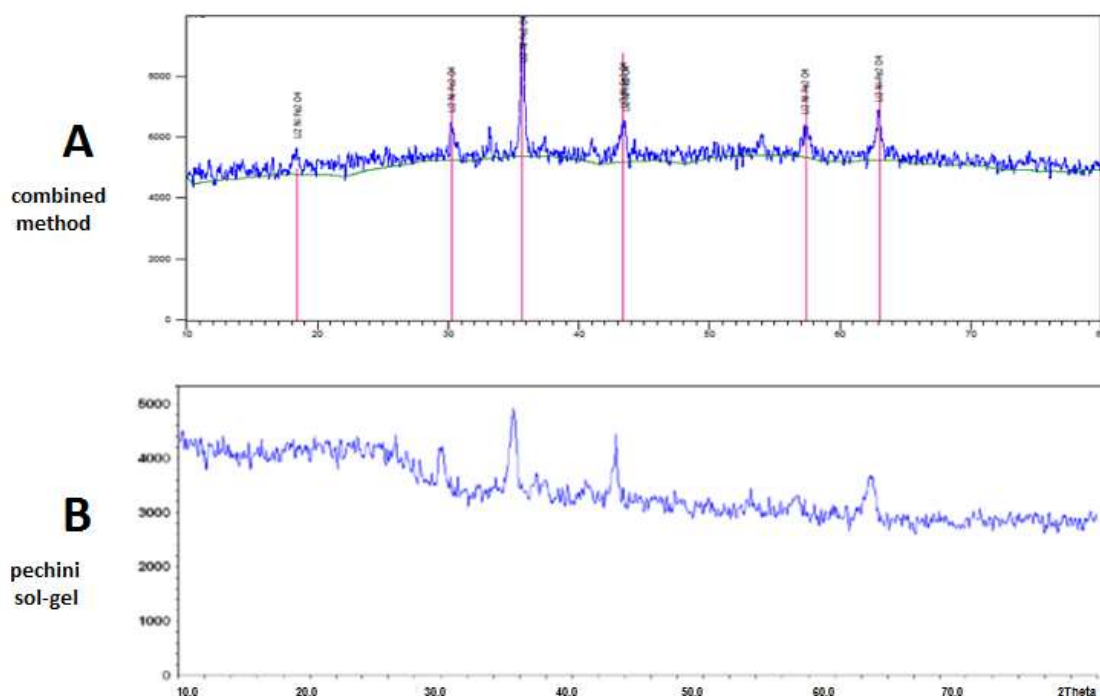
Figure 2. X-ray diffraction pattern showed formation of $Li_2NiFe_2O_4$

Scanning electron microscopy is known as a common technique to determine the morphology and size distribution of prepared particles in scales ranging from micro to nano. The SEM image in Fig. 3 shows the minimum size of MNPs was observed in the sufficient range for both methods. Comparing SEM images, it was found that MNPs which were prepared by combined methods of co-precipitation and hydrothermal have spherical-shaped in their surface morphology and qualified agglomeration.
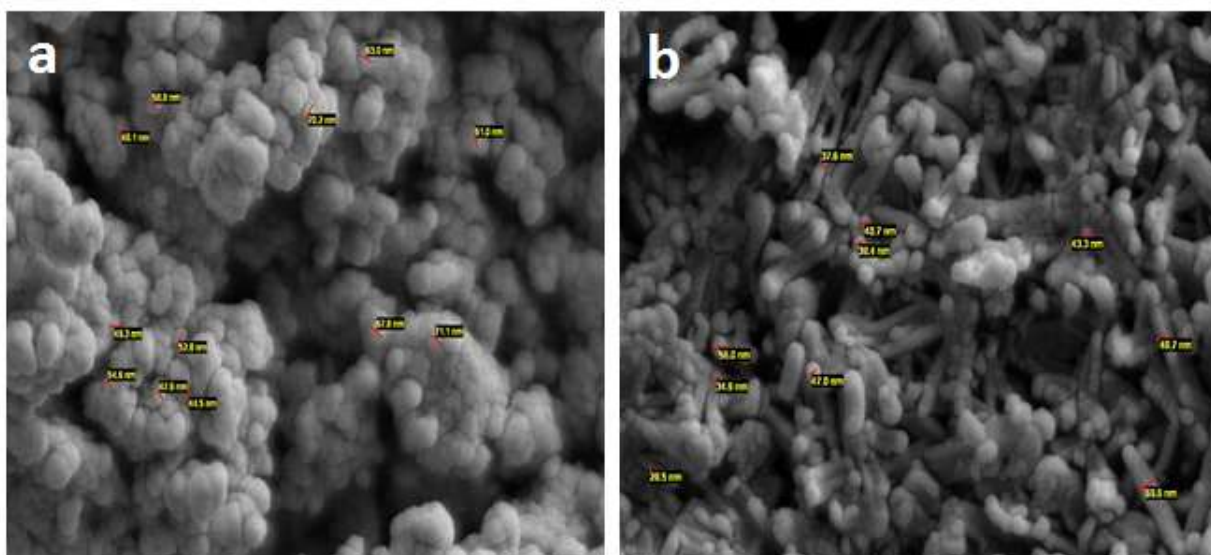
Figure 3. Comparative SEM images of MNPs. (a) by combined method (b) pechini sol-gel.

3.5. Analysis of particle size in combined method

FT-IR spectroscopy is known as a useful tool used to show the functional group of any organic molecule. FT-IR has been widely employed to confirm the attachment of different functional groups to each step of functionalization. In the FT-IR spectra (Fig. 4), magnetic nanoparticles are shown in the lower peaks.

Peak broadening in the area around 1700 and 3400 could be related to the silica coating. Since FT-IR spectrum could be more relevant to the operating groups, the functional groups were as follows: Area of NH was in the area between 2500- 4000, C-N was in the area 1000 – 1350.
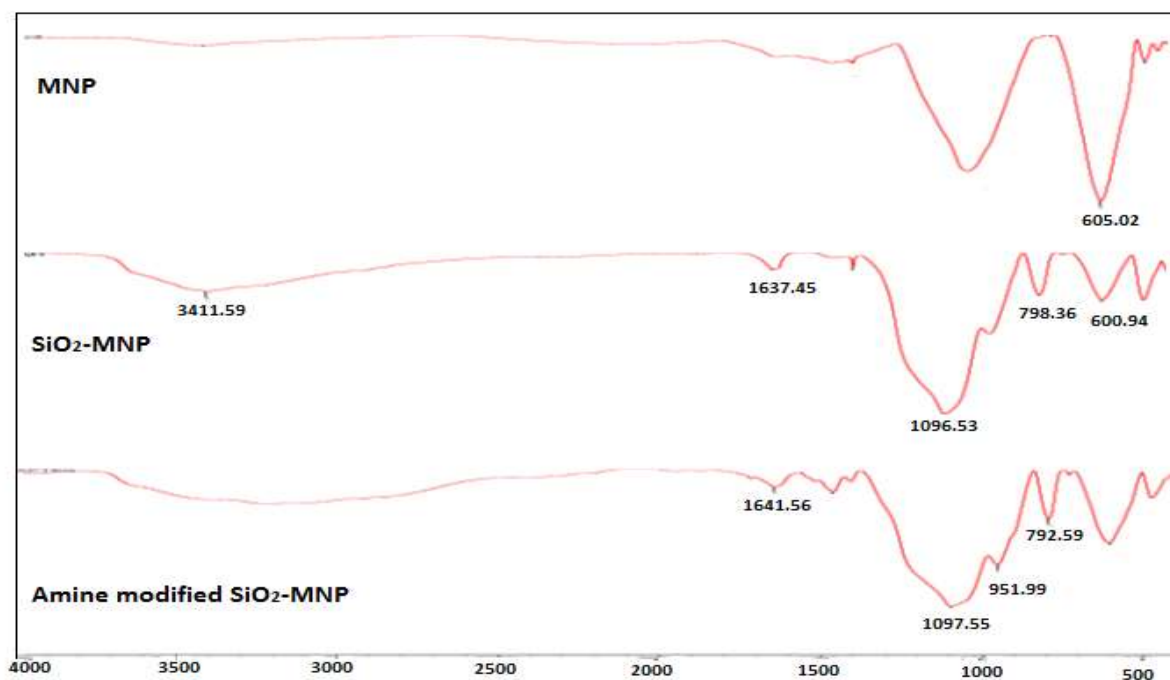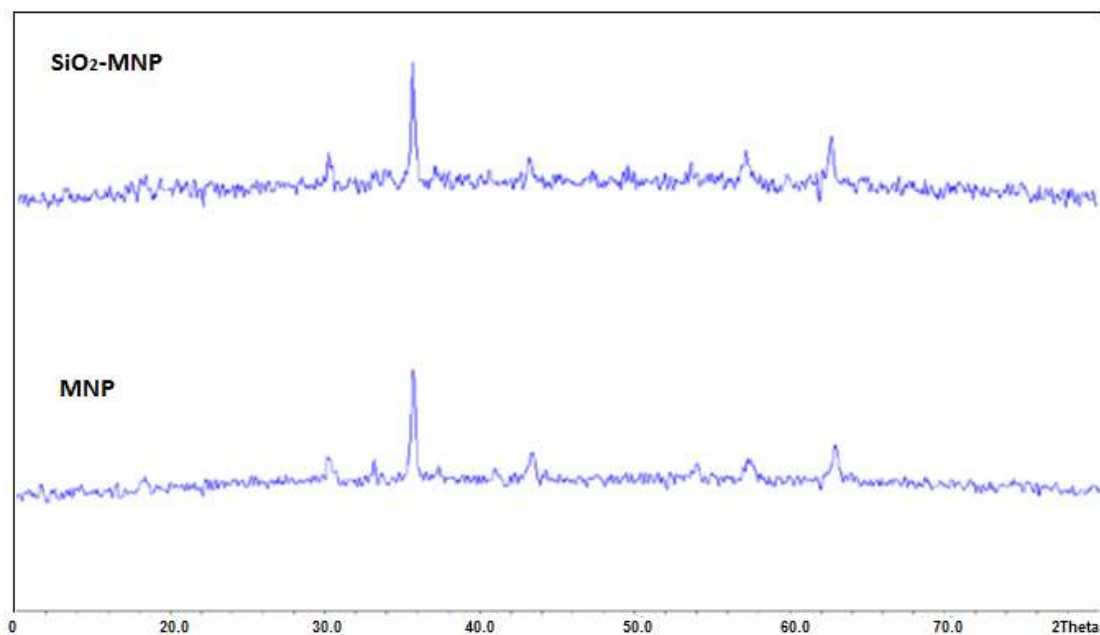

Figure 4. Fourier transform infrared spectroscopy.

As shown in Figure 5, the diffraction peaks of the XRD patterns of MNP samples and SiO$_2$-MNPs were compared. There is minimum difference in X-ray

diffraction patterns of SiO$_2$-MNP and MNP, which means silica coating, has no significant impact on MNPs size.

Figure 5. X-ray diffraction patterns showing minimum difference between SiO_2-MNP and MNP

VSM is meant to evaluate the magnetization of the MNPs as a function of an applied external magnetic. The results related to the magnetization of the samples at room temperature (Fig. 6) revealed that the saturation magnetization value of SiO_2-MNP was lower than that of MNPs, owing the diamagnetic contribution of the silica shell surrounding the magnetite.
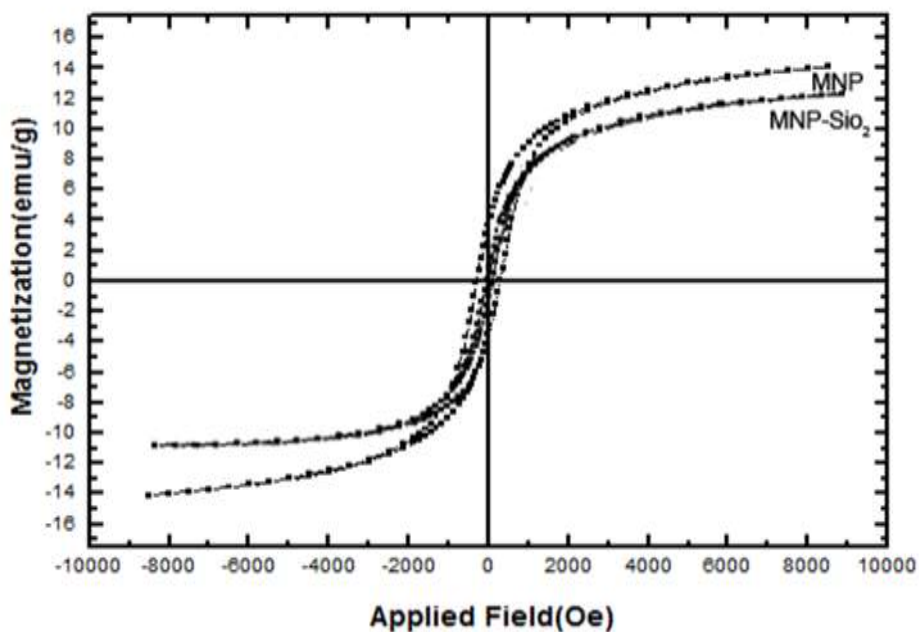
Figure 6. Superconducting quantum interference device magnetization curves. The silica coated MNPs are compared with uncoated MNP

4. Conclusion

In this study, two methods of sol-gel pechini and combined technique were compared with each other. The novel $Li_2NiFe_2O_4$ magnetic nanoparticle is easily synthesized with low cost. SEM images revealed the spherical shaped morphology for nanoparticles. The minimum sizes of MNPs gained from the XRD and SEM were in good agreement. Based on these results,

the composite nanoparticles were observed to have a spherical shape and a uniform size distribution; therefore, they could be considered for targeted drug delivery. In other research, glutaraldehyde was introduced as an effective cross linking agent in formation of imide bonds between the amine groups of SiO_2-MNPs or the aldehyde groups of glutaraldehyde.

Reference

[1] M. Ferrari, Cancer nanotechnology: Opportunities and challenges, Nature Reviews. Cancer **5** (2005) 161.

[2] N. Sanvicens, M.P. Marco, Multifunctional nanoparticles - properties and prospects for their use in human medicine, Trends in Biotechnology **26** (2008) 425.

[3] O.C. Farokhzad, R. Langer, Nanomedicine: Developing smarter therapeutic and diagnostic modalities, Advanced Drug Delivery Reviews **58** (2006) 1456.

[4] J. Dobson, Magnetic nanoparticles for drug delivery, Drug Dev. Res. **67** (2006) 55.

[5] Sun, Conroy, Jerry SH Lee, and Miqin Zhang. "Magnetic nanoparticles in MR imaging and drug delivery." Advanced drug delivery reviews **60** (2008) 1252.

[6] O. Veiseh, W.Jonathan, and M. Zhang. "Design and fabrication of magnetic nanoparticles for targeted drug delivery and imaging." Advanced drug delivery reviews 62.**3** (2010) 284.

[7] J.Andreas, et al. "Inductive heating of ferromagnetic particles and magnetic fluids: physical evaluation of their potential for hyperthermia." International Journal of Hyperthermia 25.**7** (2009) 499.

[8] T. Nhiem, and Thomas J. Webster. "Magnetic nanoparticles: biomedical applications and challenges." Journal of Materials Chemistry 20.**40** (2010): 8760.

[9] Y. Fang, et al. "Superparamagnetic iron oxide nanoparticle-embedded encapsulated microbubbles as dual contrast agents of magnetic resonance and ultrasound imaging." Biomaterials 30.**23** (2009): 3882.

[10] C.Miriam, et al. "Biological applications of magnetic nanoparticles." Chemical Society Reviews 41.**11** (2012) 4306.

[11] Q. A. Pankhurst, et al. "Progress in applications of magnetic nanoparticles in biomedicine." Journal of Physics D: Applied Physics 42.**22** (2009): 224001.

[12] W. Jiang, H. Yang, S. Yang, H. Horng, J. Hung, Y. Chen, C.-Y. Hong, Preparation and properties of superparamagnetic nanoparticles with narrow size distribution and biocompatible, J. of Mag. and Mag. Mat. **283** (2) (2004) 210.

[13] V. Pramod Kumar, et al. "Superparamagnetic fluorescent nickel–enzyme nanobioconjugates: synthesis and characterization of a novel multifunctional biological probe." Journal of Materials Chemistry 20.**18** (2010): 3722.

[14] A. Tadayon, R. Jamshidi, A. Esmaeili, International Journal of Pharmaceutics, Delivery of tissue plasminogen activator and

streptokinase magnetic nanoparticles to target vascular diseases,(November 2015) 428.

[15] S.PoornimaBudime, and N. PoklarUlrih. "Multifunctional superparamagnetic iron oxide nanoparticles: promising tools in cancer theranostics." Cancer letters 36.**1** (2013) 8.

[16] TK. Thanh, Nguyen, and Luke AW Green. "Functionalization of nanoparticles for biomedical applications." Nano Today 5.**3** (2010) 213.

[17] H. Rui, et al. "Synthesis, functionalization, and biomedical applications of multifunctional magnetic nanoparticles." Advanced Materials 22.**25** (2010) 2729.

[18] B.J. Boyd, Past and future evolution in colloidal drug delivery systems, Expert Opinion on Drug Delivery **5** (2008) 69.

[19] V.P. Torchilin, Multifunctional nanocarriers, Advanced Drug Delivery Reviews **58** (2006) 1532.

[20] E. Allemann, R. Gurny, E. Doelker, Drug-loaded nanoparticles: preparation methods and drug targeting issues, Eur. J. of Pharm. and Biopharm. **39** (1993) 173.

[21] N. Kohler, Fryxell GE, Zhang M. A bifunctional poly (ethylene glycol) silane immobilized on metallic oxide-based nanoparticles for conjugation with cell targeting agents. J Am Chem Soc. **126** (2004) 1267206.

[22] A.H. Abdullah, H.J. Melanie Moey and N. Azah, Response surface methodology analysis of the photocatalytic removal of Methylene Blue using bismuth vanadate prepared via polyol route. J Environ Sci .**24** (2012).1694.

[23] CQ. Yang, G. Wang G, et al. Effect of ultrasonic treatment on dispensability of Fe3O4 nanoparticles and synthesis of multi-core Fe3O4/SiO2 core/shell nanoparticles. J Mater Chem.**15** (2005) 4252.