Fabrication and Pulse Sequences Evaluation of Iron Oxides Nanoparticles as MRI Contrast Agent

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Abstract

The objective of this study is to assess the in vivo MRI contrast agent of magnetic nanoparticles using pulse sequences at low field Tesla. For this purpose, magnetic nanoparticles were prepared with different weight percentage (2wt. % and 4wt. %) by co-precipitation method. Magnetic nanoparticles were characterized by X-ray diffraction (XRD), scanning electron microscope (SEM), vibrating sample magnetometer (VSM) and magnetic resonance imaging (MRI). XRD results showed that magnetic nanoparticles have an average crystalline size of 7.6nm and 5.1nm. Morphology of iron oxide nanoparticles was evaluated by SEM that elaborated non spherical and spherical structure of magnetic nanoparticle. Elemental analysis by EDX spectrum showed Fe, O and Cl present in iron oxide nanoparticles. VSM studies indicated the superparamagnetic nature of particle the saturation magnetization (Ms) of 2wt. % and 4wt. % were 29.4 emu/cm-3 and 75.8 emu/cm-3 respectively. T1-W and T2-W MRI images of liver and spleen were taken by Spin Echo (SE). Intensity of signal induced by contrast agent of magnetic nanoparticle for control, S.I and S.D for liver is 98 ±22 and, S.I and S.D for spleen is 111±17. For spin echo (SE), S.I and S.D for liver is 207 ±32 and , S.I and S.D for spleen is 189±32.Magnetic resonance imaging(MRI) results demonstrated that superparamagnetic nanoparticles have showed high signal intensity on T2-W images that can be used as capable candidate for T2-W MRI modalities.

Key Words: Fe_3O_4, XRD, SEM, VSM, MRI, Contrast agent

1. Introduction

Magnetic Resonance Imaging (MRI) consists biogenic configuration and control the intrinsic nuclear magnetic moment of endogenous nuclei that enable to increase the spin density along the body through distribution of hydrogen nuclei[1]. Net spin is due to their valence electron in their outermost shell that align the H^1 nuclei (proton and neutron) to provide the better contrast using pulse sequences. This configuration in the water protons will give a low amplitude or low energy wave. When recognize for the echo or return signal as protons in the water are saturated with energy and that can no longer produces from class first magnetization[2]. By applying radio frequency (RF) pulse net magnetization vector flips by a certain angle and results as transverse(T_2) and longitudinal(T_1) recovery occur[3]. Unlike T_1, suppression of T_2 enhance the images to better contrast using MR signals. MR images are the consequence as a contrast through the various extrinsic and intrinsic framework that enhance the difference between different modalities for contrast enhancement[4]. Echo time is the dominant factor for the image contrast mostly based on the contrast agent that are able to target the specific tissue. They are govern to seek the image weighting, though the using of Contrast Agent(CA) that can improve the sensitivity in notable manner[5]. Contrast agent commonly works to enhance the contrast difference among normal and typical tissues. For clinical diagnosis of magnetic nanoparticles(MNPs) are currently uses as MR imaging Contrast agents(CA) that possess highly appreciable magnetic properties for biomedical applications[6]. Usually these nanoparticle contrast agent govern as an intravenous bolus injection that evaluate the bio-distribution affect after administration the body that much crucial to maximize the performance [7]. Contrast agent does not provide any signal from the external or RF field but it effects chemically by improving the water (hydrogen) proton signals from surrounding the tissue in notable manners. [8] Contrast agents gives a significant contrast effect and also hydrogen relaxation rate that helps to acquire the MRI imaging sequence. MR imaging contrast agent are either paramagnetic or superparamagnetic to assess the sensitivity, and specificity of contrast in the tissue[9]. However, paramagnetic behavior acquires the anatomical (brighter images) but the relaxation rate of superparamagnetic nanoparticles(NPs) provide pathological manners(darker contrast) that enhance the

sensitivity when interacts with the resident proton and shortened the relaxation time[10]. In this study signal intensity of T_2-weighted contrast agents of iron oxides nanoparticles have been evaluated at different pulse sequences to see the MRI contrast in vivo for biomedical applications.

2. Experimental Methodology

Iron oxide Fe3O4 nanoparticles have been synthesized using the co-precipitation technique[11]. The magnetic nanoparticles have been made from ferrous chloride tetrahydrate (FeCl2.4H2O), Ferric chloride hexahydrate (FeCl3.6H2O) and sodium hydroxide (NaOH) from Daejung chemicals, Korea were used to prepare the magnetic nanoparticles. The solutions have been made with de-ionized water. [12].The process began with the dissolution of ferric chloride hexahydrate (FeCl3.6H2O) in 200ml water. In 100ml water, ferrous chloride tetrahydrate (FeCl2.4H2O) has been also added to form a solution 2:1, as given in eq. 1[13]. The solution has been continually combined using a magnetic stirrer. Drop by drop, NaOH solution has been added to the solution to bring it to pH-9[14]. The solution was then centrifuged at 4000 rpm for 30 minutes to obtain the black precipitate. Impurities in the precipitates have been removed using deionized water [15]. Later, the precipitate was dried at 80˚C for 8-10 hours and then grinned the magnetic nanoparticles to get powder form.

$$2FeCl_3.6H_2O+FeCl_2.4H_2O+8NaOH \quad Fe_3O_4 + 8NaCl +20H_2O$$

$$2FeCl_3.6H_2O + FeCl_2.4H_2O + 8NaOH \qquad (1)$$

3. Characterization Analysis

3.1 X-Ray Diffraction Analysis

X-Ray diffraction analysis was applied to examine the crystalline structure of magnetic nanoparticle. This XRD pattern of magnetic nanoparticles (MNPs) were analyzed using powder X-ray diffractometer: Bruker D8 Advance laboratory diffractometer) with CuKα radiation (λ = 1.5406 Å) in range of 2θ = 20-85°, with 1°/minute scanning rate, operated at 40 kV/35 mA. The given pattern of iron oxide nanoparticles authenticated the crystal nature of nanoparticle[16]. Five specific diffraction peaks demonstrated their miller indices (200), (220). (311), (400) and (440) presented in fig. 1. The peaks were well matched with JCDP card No. 01-074-1910 and card No 85-436 that confirmed the magnetic nanoparticles[17]. The pattern of Fe3O4 (2 wt. % and 4 wt. %) were analyzed at different angles i.e. 30.29°, 35.54°, 43.22°,58.6, 62.47° and 30.32°, 35.60°, 43.08°,58.2°, 62.71° respectively[18] that contained structural parameters like unit cell volume(v), the average crystalline size(D) that can be measured through the following:

$$D_{hkl} = k\,\lambda/\beta\cos\theta \qquad (2)$$

where 'Dhkl' shows the crystallite size, 'λ' is the wavelength of used XRD Cu-Kα, 'k' shows the shape factor, the broadened Bragg reflection 'θ' is the half angle (in radian) was calculated through the Bragg's equation:

$$2d\sin\theta = n\lambda \qquad (3)$$

The grain size were calculated through the eq.3, which indicated the formation of Fe3O4 magnetic nanoparticles. The particle size were approximately 7.6 nm and 5.1 nm in diameter which were matched with reported data[19].These particles showed the crystalinity in the form of non spherical shape.

Table 1: Grain size of Fe_3O_4

Sr. No	Sample	Lattice spacing [λ]	D [nm]	Strain	Reference
1	Fe_3O_4 [2 wt.%]	0.248	7.67	0.78	[17], [18], [19]
2	Fe_3O_4 [4 wt.%]	0.248	5.1	0.52	[17], [18], [19]

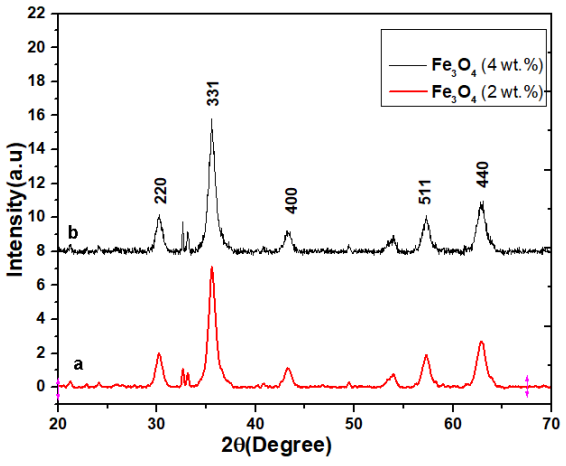

Fig. 1: XRD patterns of Fe_3O_4 magnetic nanoparticles

3.2 Scanning Electron Microscope (SEM) Analysis

The morphological properties of nanoparticles have always stimulate researcher's

interest. SEM is an electron scanning microscopy technique that provides all accessible information about a nanoparticle at the nanoscale level. SEM is utilized in new medication and pharmaceutical research as well as for practical applications to get insight into cellular interactions [20]. The morphology of the Fe3O4 magnetic nanoparticles (MNPs) were examined by scanning electron microscope (SEM) VEGA3 TESCAN machine at 20 KV. The samples were taken in the form of powder for SEM analysis[21]. SEM images demonstrated non spherical shape. The surface of iron oxides (Fe3O4) magnetic nanoparticles (MNPs) showed little agglomeration on images shown in Fig 2. The morphological accumulation was due to their stacked Vander-wall forces that confirmed the pure magnetite behavior[22]. Moreover, these MNPs have high surface to volume ratio due to their small size and enhance their tendency to form accumulation. [23].

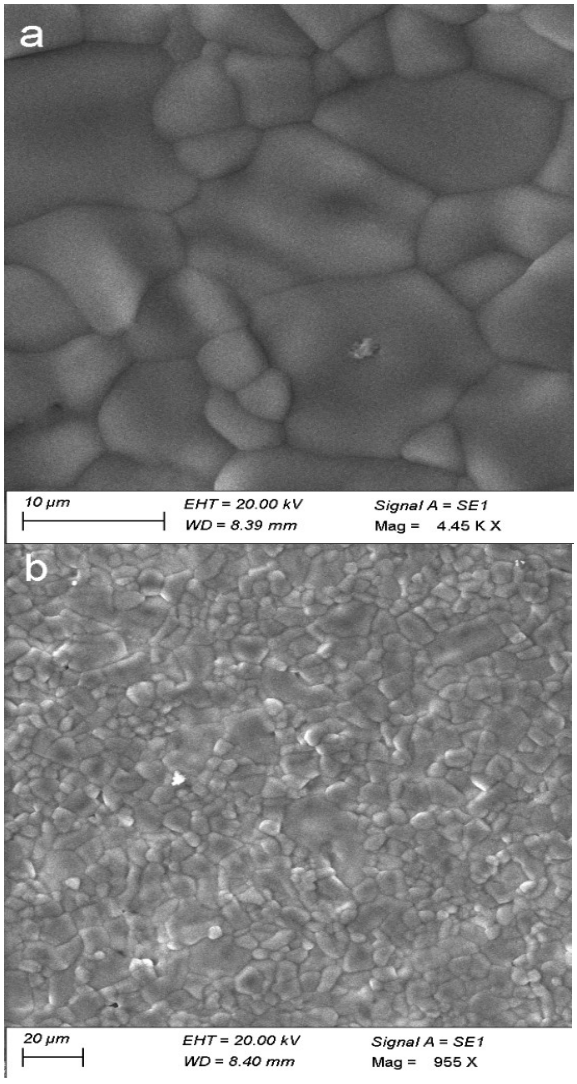

Fig. 2: SEM images of Fe_3O_4

3.3 Vibrating Sample Magnetometer (VSM)

Magnetic properties of Fe3O4 nanoparticle were examined by using a Dexing Magnet Tech Co, Limited (VSM-100). The curves of Fe3O4 (2 wt.% and 4 wt.%) magnetic nanoparticles were obtained at room temperature under fields of -10,000 to 10,000 Oersted, depicted their values for saturation magnetization(Ms), remnant magnetization (Mr) and their high coercivity (Hci)[25]. It could be estimated that the synthesized Fe3O4 nanoparticles acquire a very small hysteresis attitude to the magnetization curve[26]. The value of saturation magnetization demonstrated the ability of a nanoparticle to maintain irreversibility magnetic domains when an external magnetic field applied. Moreover, Fe3O4(4 wt. %) nanoparticles exhibit a small magnitude for the remnant magnetization coercive field which revealed magnetic nanoparticles having superparamagnetic characteristics at room temperature and particularized the particle size that smaller than the critical size of the magnetic domain size[27]. Ms v H hysteresis curve of Fe3O4 in fig.3, showed the saturation magnetization curve with the measured value of 29.4 emu/cm-3 and 75.7 emu/cm-3 respectively. The magnetic characteristic summery illustrated in table. 2[28]. Superparamagnetic behavior showed its flexibility for an applied magnetic field. The important magnetization property is permanent magnetization[23].The negligible coercivity value of samples confirming their superparamagnetic behavior for the application to use as the magnetic resonance imaging[29].

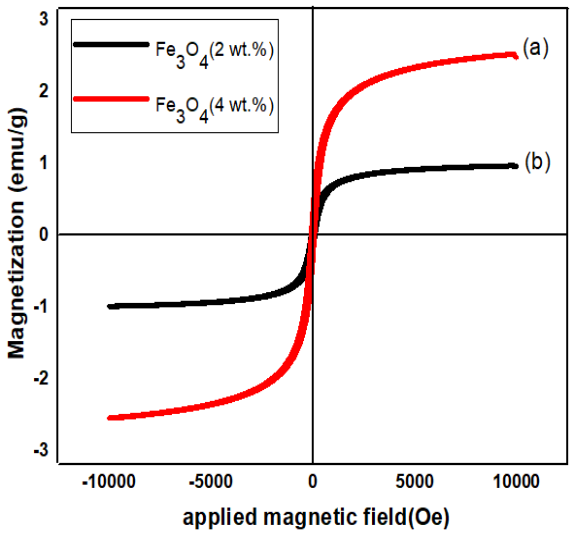

Fig. 3: Saturation magnetization curves for Fe_3O_4 (2 wt.% and 4 wt.%)

3.4 MRI analysis

Iron oxide nanoparticles (IONPs) used on large-scale as contrast agent due to their pharmacokinetics, nontoxic behavior, and clinically applicable manners. Magnetic nanoparticles have been found an excellent prospect to reveal their ability in clinical approach as contrast agent with approachable relaxivity scale**[30]**.The recent MRI study of Fe_3O_4 were executed at very low field MRI band (0.5 Tesla Siemen MAGNETOM) in order to acquire high resolution contrast achieved organ like liver and spleen of different rabbits **[12]**. Pulse sequences: Spin echo, gradient echo and STIR were applied for equally weighted rabbits to examine throughout the in-vivo study as shown in fig.4 and fig.5. The parameter used as follows: CORN and Axial, TR/TE= 400ms/15ms, slice thickness/gap = 6mm, zoom factor = x0.93; and TR/TE= 551/15ms, slice thickness/gap = 6mm, zoom factor = x0.93 respectively**[31]**. Equally weighted rabbits were taken to assess the contrast enhancement on MRI images. Extracted values for Control, signal intensity for liver is 98 and standard deviation ±22 and signal intensity for spleen is 111, and standard deviation ±17. For spin echo (SE) signal intensity for liver is 207 and standard deviation ±32 and signal intensity for spleen is 189, and standard deviation ±32.

Table 2: The magnetic properties of superparamagnetic iron oxide nanoparticles [SPIONs]

Sample	Ms [emu/cm^{-3}]	Coercivity [T]	Remanence [emu/cm^3]	Reference
Fe_3O_4 (4 wt.%)	75.78	16.2	0.03	[28], [24], [23]
Fe_3O_4 (2 wt.%)	29.44	7.2	0.03	[24], [27], [28]

Table 3: Fe_3O_4 MRI contrast agents Signal intensities and standard deviation

Contrast Agent (CA)	**ROI**	**S.I**	**S.D**
Control	Liver	98	22
	Spleen	111	17
T_2-SE (Fe_3O_4)	Liver	207	32
	Spleen	189	32
T_2-STIR (Fe_3O_4)	Liver	119	07
	Spleen	173	17

4. Conclusion

In this study superparamagnetic nanoparticles have been fabricated co-precipitation method. XRD analysis was performed to see the structure of superparamagnetic nanoparticle. The morphology of the magnetic nanoparticles was studied by SEM that showed the zero agglomeration and non-spherical surface of the Fe_3O_4. M_s vs H curve confirmed the properties of superparamagnetic nanoparticles. Saturation magnetization M_s was measured 29.4 emu/cm^{-3} and 75.7 emu/cm^{-3} from the curve. MRI images of the liver and spleen were compared to control MRI images. This study concludes that the signal intensities of T_2-W contrast agent material was found higher and confirmed the better contrast on axial and coronal MRI images of liver and spleen of rabbits using different pulse sequences at low field Tesla.

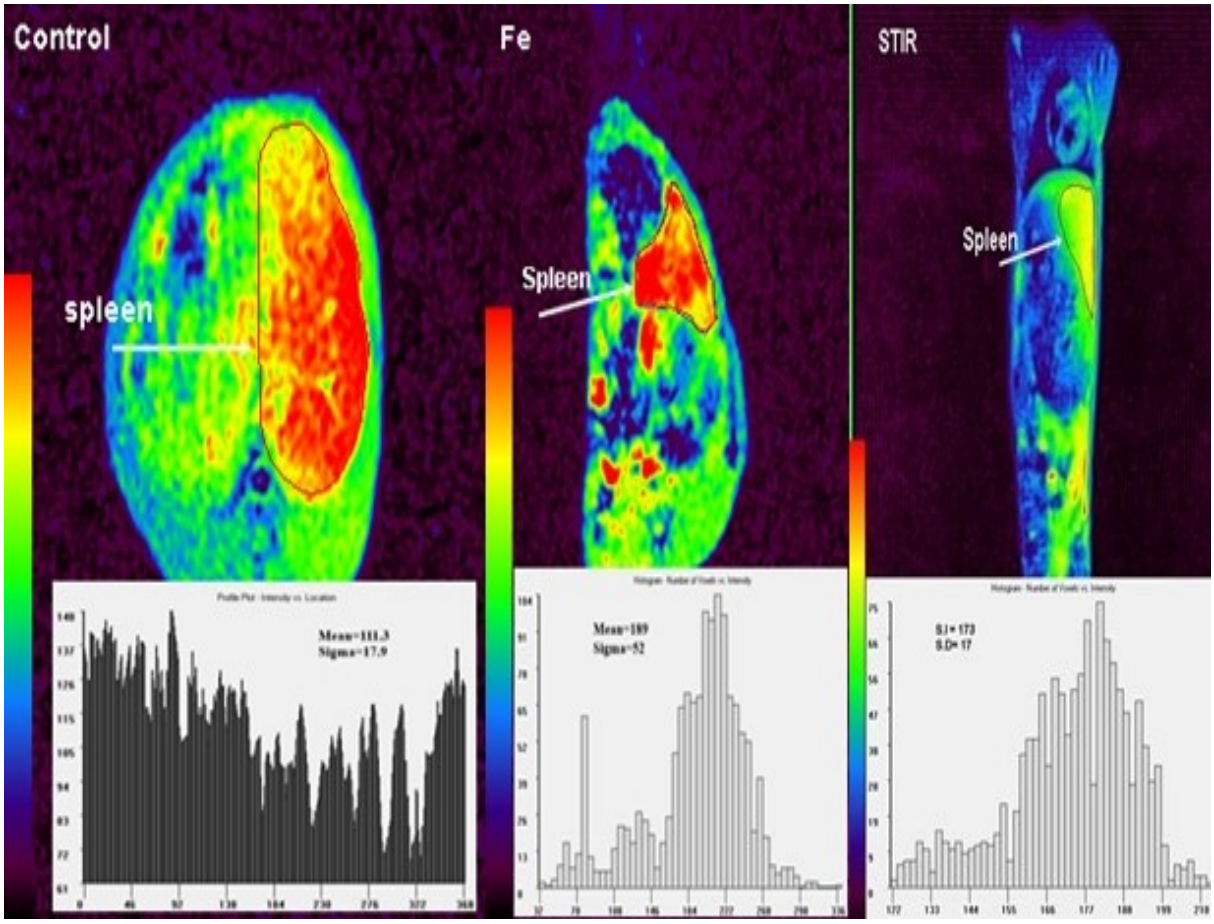

Fig. 4: Contrast effect image of spleen

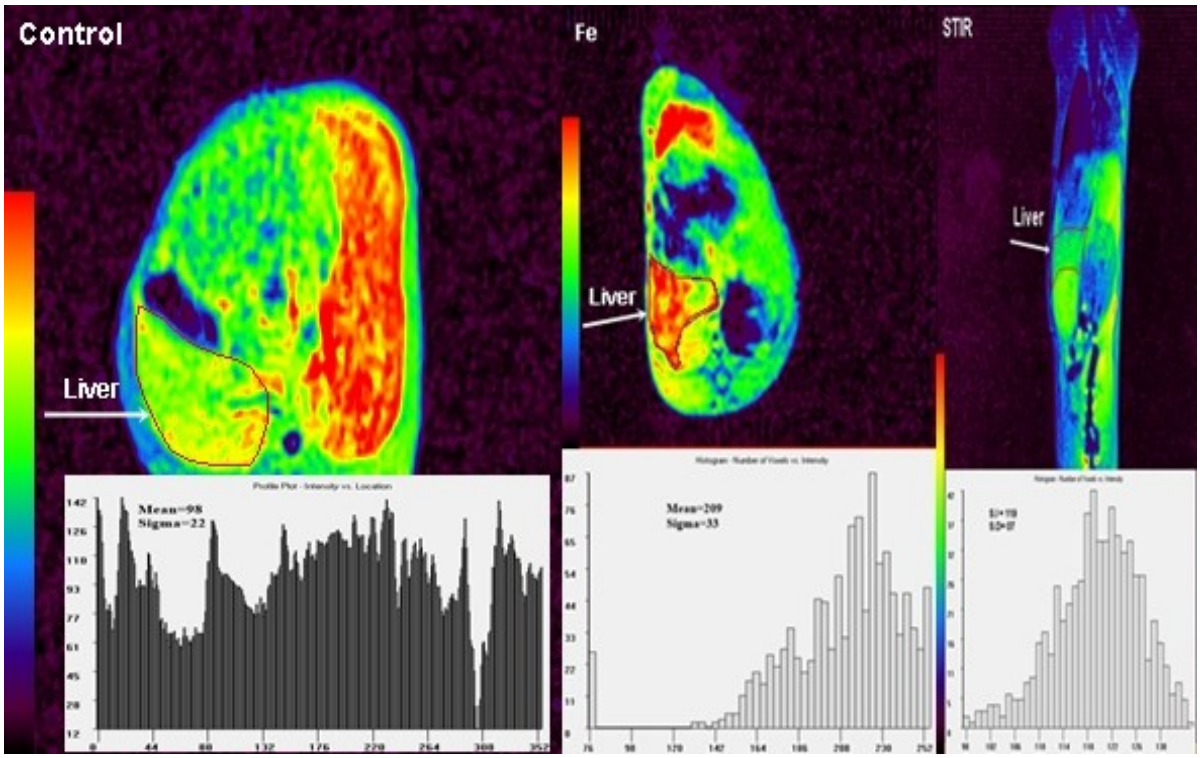

Fig. 5: Comparison the control image and Fe_3O_4 contrast effect image of Liver

5. References

[1] A. Neuwelt, N. Sidhu, C.-A. A. Hu, G. Mlady, S. C. Eberhardt, and L. O. Sillerud, "Iron-based superparamagnetic nanoparticle contrast agents for MRI of infection and inflammation," *American Journal of Roentgenology,* vol. 204, no. 3, pp. W302-W313, 2015.

[2] N. Amin, M. Afzal, M. Yousaf, and M. A. Javid, "Choice of the pulse sequence and parameters for improved signal-to-noise ratio in T1-weighted study of MRI," *JPMA. The Journal of the Pakistan Medical Association,* vol. 65, no. 5, pp. 512-518, 2015.

[3] M. F. Casula *et al.*, "Magnetic resonance imaging contrast agents based on iron oxide superparamagnetic ferrofluids," *Chemistry of Materials,* vol. 22, no. 5, pp. 1739-1748, 2010.

[4] R. E. Walker and S. J. Eustace, "Whole-body magnetic resonance imaging: techniques, clinical indications, and future applications," in *Seminars in musculoskeletal radiology*, 2001, vol. 5, no. 01, pp. 005-020: Copyright© 2001 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New ….

[5] T. Ichikawa, "MRI in the evaluation of hepatocellular nodules: role of pulse sequences and contrast agents," *Intervirology,* vol. 47, no. 3-5, pp. 252-270, 2004.

[6] R. Bitar *et al.*, "MR pulse sequences: what every radiologist wants to know but is afraid to ask," *Radiographics,* vol. 26, no. 2, pp. 513-537, 2006.

[7] M. J. Graves, "Pulse sequences for contrast-enhanced magnetic resonance imaging," *Radiography,* vol. 13, pp. e20-e30, 2007.

[8] J. Wahsner, E. M. Gale, A. Rodríguez-Rodríguez, and P. Caravan, "Chemistry of MRI contrast agents: current challenges and new frontiers," *Chemical reviews,* vol. 119, no. 2, pp. 957-1057, 2018.

[9] G. J. Strijkers, W. J. M Mulder, G. A. F van Tilborg, and K. Nicolay, "MRI contrast agents: current status and future perspectives," *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents),* vol. 7, no. 3, pp. 291-305, 2007.

[10] C. M. Ellis, J. Pellico, and J. J. Davis, "Magnetic Nanoparticles Supporting Bio-responsive T1/T2 Magnetic Resonance Imaging," *Materials,* vol. 12, no. 24, p. 4096, 2019.

[11] A. A. Hernández-Hernández, G. Aguirre-Álvarez, R. Cariño-Cortés, L. H. Mendoza-Huizar, and R. Jiménez-Alvarado, "Iron oxide nanoparticles: synthesis, functionalization, and applications in diagnosis and treatment of cancer," *Chemical Papers,* vol. 74, pp. 3809-3824, 2020.

[12] J. M. Arshad, W. Raza, N. Amin, K. Nadeem, M. I. Arshad, and M. A. Khan, "Synthesis and characterization of cobalt ferrites as MRI contrast agent," *Materials Today: Proceedings,* 2020.

[13] A. Yusoff, M. N. Salimi, and M. F. Jamlos, "Synthesis and characterization of biocompatible Fe3O4 nanoparticles at different pH," in *AIP Conference Proceedings*, 2017, vol. 1835, no. 1, p. 020010: AIP Publishing LLC.

[14] Y. Piñeiro *et al.*, "Hybrid Nanostructured Magnetite Nanoparticles: From Bio-Detection and Theragnostics to Regenerative Medicine," *Magnetochemistry,* vol. 6, no. 1, p. 4, 2020.

[15] S. Ikram, M. I. Arshad, K. Mahmood, A. Ali, N. Amin, and N. Ali, "Structural, magnetic and dielectric study of La3+ substituted Cu0. 8Cd0. 2Fe2O4 ferrite nanoparticles synthesized by the co-precipitation method," *Journal of Alloys and Compounds,* vol. 769, pp. 1019-1025, 2018.

[16] M. J. Arshad *et al.*, "Synthesis, Characterization and Blood Based Toxic Effects of Superparamagnetic Nanoparticles," *Materials Science,* vol. 25, no. 4, pp. 359-364, 2019.

[17] A. Akbarzadeh, M. Samiei, and S. Davaran, "Magnetic nanoparticles: preparation, physical properties, and applications in biomedicine," *Nanoscale research letters,* vol. 7, no. 1, p. 144, 2012.

[18] J. A. Lopez, F. González, F. A. Bonilla, G. Zambrano, and M. E. Gómez, "Synthesis and characterization of Fe3O4 magnetic nanofluid," *Revista Latinoamericana de Metalurgia y Materiales,* vol. 30, no. 1, pp. 60-66, 2010.

[19] S. A. Kulkarni, P. Sawadh, P. K. Palei, and K. K. Kokate, "Effect of synthesis route on the structural, optical and magnetic properties of Fe3O4 nanoparticles," *Ceramics International,* vol. 40, no. 1, pp. 1945-1949, 2014.

[20] Kozenkova E, Levada K, Efremova MV, Omelyanchik A, Nalench YA, Garanina AS, Pshenichnikov S, Zhukov DG, Lunov O, Lunova M, Kozenkov I. Multifunctional Fe3O4-Au nanoparticles for the MRI diagnosis and potential treatment of liver cancer. Nanomaterials. 2020 Sep;10(9):1646.

[21] R. K. Wardani, K. Dahlan, S. T. Wahyudi, and S. G. Sukaryo, "Synthesis and characterization of nanoparticle magnetite for biomedical application," in *AIP Conference Proceedings*, 2019, vol. 2194, no. 1, p. 020137: AIP Publishing LLC.

[22] M. Ebadi, K. Buskaran, S. Bullo, M. Z. Hussein, S. Fakurazi, and G. Pastorin, "Synthesis and Cytotoxicity Study of Magnetite Nanoparticles Coated with Polyethylene Glycol and Sorafenib–Zinc/Aluminium Layered Double Hydroxide," *Polymers,* vol. 12, no. 11, p. 2716, 2020.

[23] M. Mahdavi *et al.*, "Synthesis, surface modification and characterisation of biocompatible magnetic iron oxide nanoparticles for biomedical applications," *Molecules,* vol. 18, no. 7, pp. 7533-7548, 2013.

[24] M. Mashkour and M. Mashkour, "A Simple and Scalable Approach for Fabricating High-Performance Superparamagnetic Natural Cellulose Fibers and Papers," *Carbohydrate Polymers,* p. 117425, 2020/11/23/ 2020.

[25] A. Hamdy, S. H. Ismail, A. Ebnalwaled, and G. G. Mohamed, "Characterization of Superparamagnetic/Monodisperse PEG-Coated Magnetite Nanoparticles Sonochemically Prepared from the Hematite Ore for Cd (II) Removal from Aqueous Solutions," *Journal of Inorganic and Organometallic Polymers and Materials,* vol. 31, no. 1, pp. 397-414, 2021.

[26] B. Wang, Q. Wei, and S. Qu, "Synthesis and characterization of uniform and crystalline magnetite nanoparticles via oxidation-precipitation and modified co-precipitation methods," *Int. J. Electrochem. Sci,* vol. 8, no. 3, pp. 3786-3793, 2013.

[27] M. Abdeen, S. Sabry, H. Ghozlan, A. A. El-Gendy, and E. E. Carpenter, "Microbial-physical synthesis of Fe and Fe3O4 magnetic nanoparticles using Aspergillus niger YESM1 and supercritical condition of ethanol," *Journal of Nanomaterials,* vol. 2016, 2016.

[28] M. Van De Loosdrecht *et al.*, "A novel characterization technique for superparamagnetic iron oxide nanoparticles: The superparamagnetic quantifier, compared with magnetic particle spectroscopy," *Review of scientific instruments,* vol. 90, no. 2, p. 024101, 2019.

[29] J. Sun *et al.*, "Biomed Mater Res," *Part A,* vol. 80, pp. 333-341, 2007.

[30] M. Anbarasu, M. Anandan, E. Chinnasamy, V. Gopinath, and K. Balamurugan, "Synthesis and characterization of polyethylene glycol (PEG) coated Fe3O4 nanoparticles by chemical co-precipitation method for biomedical applications," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* vol. 135, pp. 536-539, 2015.

[31] C. Hsiao, J. Jao, Y. Ting, H. Pan, and P. Chen, "Optimal dose of MR contrast agent in T1-weighted MRI," *Biomedical Engineering: Applications, Basis and Communications,* vol. 16, no. 06, pp. 331-336, 2004.