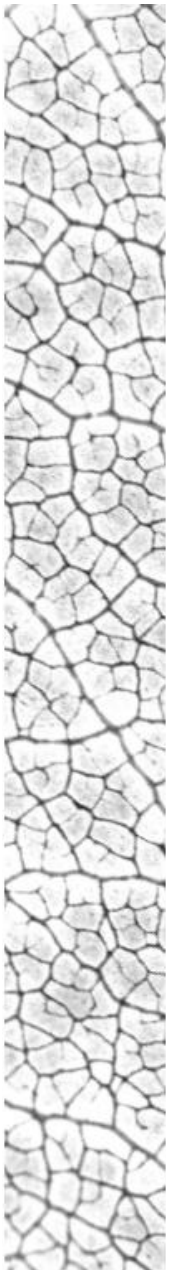

EurAsian Journal of BioSciences
Eurasia J Biosci 14, 4245-4249 (2020)

In vitro biochemical evaluation the effect of (Cobalt and Nickel-Zinc) ferrite Nanoparticles on beta -thalassemia major erythrocytes

Alaa Anwer Ali [1], Dina Abd-Alkareem [2], Israa Ghassan Zainal [1*], Suzan Jameel Ali [3]

[1] Department of Chemistry, College of Science, Kirkuk University, Kirkuk, IRAQ
[2] Department of Chemistry, College of Science, University of Mustansirya, Baghdad, IRAQ
[3] Department of Chemistry, College of Education and pure Science, Tikrit University, Tikrit, IRAQ
*Corresponding author: israaz@uokirkuk.edu.iq

Abstract

Objective: this study evaluate the interaction of Cobalt Ferrite ($CoFe_2O_4$) and Nickel-Zinc Iron Oxide ($Ni_{0.5}Zn_{0.5}Fe_2O_4$) nanoparticles with human erythrocytes in relation to the hemolytic activity and the effect of the above nanoparticles on human plasma albumin of patients with β – thalassemia major compared to healthy subjects. **Design and Methods:** the study include 40 β – thalassemia major patients and 20 apparently healthy subjects. The hemolysis % assay and *in vitro* anti-inflammatory effect of the above nanoparticles were used. **Results:** The results indicated that Co ferrite nanoparticles increases the fragility (the hemolysis % was higher) of the erythrocytes more readily in the case of β – thalassemia patients compared to healthy subjects and the Ni-Zn ferrite nanoparticles increase the fragility of healthy subjects erythrocytes while in the case of thalassemia patients the hemolysis % were decrease with increasing the concentration of nanoparticles. The anti-inflammatory effect of the above nanoparticles was evaluated using the inhibition of denaturation method for human plasma albumin, the results have shown significant *in-vitro* anti-inflammatory effect of Co ferrite nanoparticles on plasma albumin but lower than the effect of Ni-Zn ferrite nanoparticles. **Conclusions:** Changes in the morphological features of erythrocytes were noticed due to the interaction of the above nanoparticles. Stability of erythrocytes was observed at lower concentration of these nanoparticles and the possible mechanisms of interaction have been described.

Keywords: cobalt ferrite nanoparticles, nickel-zinc ferrite nanoparticles, β-thalassemia major and erythrocytes

Ali AA, Abd-Alkareem D, Zainal IG, Ali SJ (2020) *In vitro* biochemical evaluation the effect of (Cobalt and Nickel-Zinc) ferrite Nanoparticles on beta -thalassemia major erythrocytes. Eurasia J Biosci 14: 4245-4249.

INTRODUCTION

The solid colloidal particles with size ranges from (1to 100) nm were defined as nanoparticles (NPs). The dimensions of NPs were very small compared to (viruses, cells, genes and proteins) which enable these NPs to interact with the fundamental biological processes (McNamara, 2015). Among nano-medical materials: the most studied magnetic NPs are magnetite (Fe_3O_4) and magnetic nanoparticles (MNPs) like (Fe, Co or Ni) oxides, display special properties including: (high surface/volume) ratio and high magnetic moment, which give them potential manipulation by an external magnetic field (Cardoso, 2018). In the last decades Fe_3O_4 became easier to develop new and more effective types of MNPs (Song, 2006). The general formula MFe_2O_4 of spinel ferrite NPs, where, (M is divalent cation of Zn, Ni, Mn, Mg or Co).These materials are important due to of their interesting magnetic and electrical properties with good chemical and thermal stabilities (Willard, 2004). The biomedical applications of these NPs are limited because of the limited knowledge on biological response of these NPs(Hafeli, 2009 ; Lartigue, 2012). For this reason, it is important to explore the biological fate and possible toxic response of MNPs for their successful implications in biomedical field. The data from most literature devote to the therapeutic NPs indicate to *in vitro* toxicological assessments(Kawata, 2009; AshaRani, 2008). Hemolysis test was used to evaluate the effect of NPs exposure on erythrocytes(Teodora, 2013). Erythrocytes have a lifespan of (~120 days)before their immune-suppression by the body(Tarnok, 2009; Huang, 2016), the most abundant cells in the blood which showed a remarkably engineered biological entity designed for complex

Received: September 2019
Accepted: March 2020
Printed: October 2020

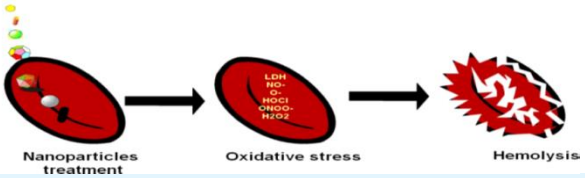

Fig. 1. Schematic illustration of oxidative stress induced mechanisms of hemolysis following administration of various NPs (Teodora, 2013)

biological functionality including oxygen delivery (Surgenor, 1073). Erythrocytes have unique physical and chemical properties including: chemical composition, flexibility, shape and size, these properties are essential to the biological functions of erythrocytes (Discher, 1994; Mohandas,1093). Oxidative stress products were released after erythrocytes exposure to the NPs which cause hemolytic effect to the erythrocytes (Teodora, 2013), as presents in **Fig. 1**.

The destruction of erythrocytes (erythrocytes hemolysis) may outcome in different diseases including anemia and renal failure (Dobrovolskaia, 2008). Studies the risk effects of ferrite NPs on human erythrocytes are rare (Martinez- Rodriguez, 2019). To evaluate the hemolytic effect of biomedical NPs on erythrocytes, the present study focused to assess the potential toxicity of (cobalt ferrite and nickel-zinc ferrite) NPs *in vitro* with different concentrations by determining erythrocytes hemolysis% and then testing the anti-inflammatory effect of these NPs using the inhibition of denaturation method for human plasma albumin (HPA).

MATERIALS AND METHODS

Nanoparticles (NPs)

The company ALDRICH/ Germany were supplied the chemicals and reagents which is used in this study. Cobalt Ferrite ($CoFe_2O_4$) NPs and $Ni_{0.5}Zn_{0.5}Fe_2O_4$ were supplied from Dr.Wajeeh Kaji Obeed /chemistry department, Faculty of science, Mustansiriyah university. The above NPs were used with range of concentrations (0.002, 0.004, 0.006, 0.008 and 0.01) %.

Preparation of washed human erythrocytes suspension

Five milliliter of whole blood on the day of each experiment was drawn from the antecubital vein of (20) healthy volunteers who were free from any medication for at least two weeks and (40) patients with β-thalassemia major patients with aged ranges (19 - 48) years to all studied groups. Samples were collected by vein puncture into tubes containing EDTA. Whole blood was centrifuged at 1500 xg for 12 min, and the buffy coat, and top layer of cells were decanted. The plasma separated from the remaining packed erythrocytes which washed three times with saline. After washing, 500 µl of packed erythrocytes were diluted to (1.5 ml) with phosphate buffer saline (PBS).The plasma samples keep at -20C◦ until used.

Haemolysis assay

The procedure of Oyewale (Oyewale, 1993) & Oladele *et al (*Oladele 2003) was utilized to evaluate the hemolysis assay. The diluted erythrocytes (0.3ml) was mixed with (1.2ml) of NPs in PBS with different concentrations (final hematocrit, 5%). The suspension of erythrocytes with PBS (1.2 ml) without NPs was used as a control. The final combined suspensions were gently mix and incubate at room temperature (25±1) C◦ on a shaking plate for 1 hour, then centrifuged at (1500 xg) for (15 min) and the supernatant was read at (540nm).For each tube, the

hemolysis % was expressed as a percentage, taking as 100% the maximum value of absorbance of distilled water.

In-vitro anti-inflammatory effect

Cobalt Ferrite NPs and the Nickel-Zinc Iron Oxide were shaded for their anti-inflammatory effect by using HPA inhibition by denaturation technique. Test solution (1 ml) containing different concentrations (20,40,60, 80, 100 &120)µl of NPs were mixed with 1 ml of 1% HPA solution in phosphate buffer and incubated at RT (25±1)C° for 15 min. The reaction mixture kept in (60±1)C° for 10 min to induce denaturation, cooled and finally read the turbidity of solution at 660 nm. Denaturation inhibition % was calculated using control (no NPs was added). Each experiment was done in triplicate and average was taken.

Inhibition % = $\{(A_{Control} - A_{test}) / A_{Test}]\}10^2$.

Where, $A_{Control}$ and A_{Test} are absorbance values (mean) of control and test group respectively (Mizushima 1968; Grant 1970; Sangeethe 2011).

RESULTS

This study investigated the cytotoxic effect of [synthetic Co ferrite and purchased Ni-Zn ferrite] NPs on the human RBCs as a function of increasing the concentration of NPs. **Fig. 1A**-**1D** shows the hemolysis curve of the above NPs, for healthy subjects and patients groups.

The results above indicated that with increase the concentration of Co ferrite NPs the hemolysis % of the erythrocytes were increase (with 45 and 95 hemolysis % for healthy and β-thalassemia major patients respectively) this indicate that the effect of Co ferrite NPs on the erythrocytes of β-thalassemia major patients more than to healthy subjects. The results of Ni-Zn ferrite NPs represented that there were increase in the hemolysis % of the erythrocytes with increase the concentration of NPs of healthy subjects while in the case of β-thalassemia major patients the hemolysis % results was decrease with increase the concentration of NPs and highest hemolysis % were obtained with 4% of Ni-Zn ferrite NPs.

The effect of the above NPs on HPA also studied for the two groups, as presented in **Fig. 2A**-**D**:The results

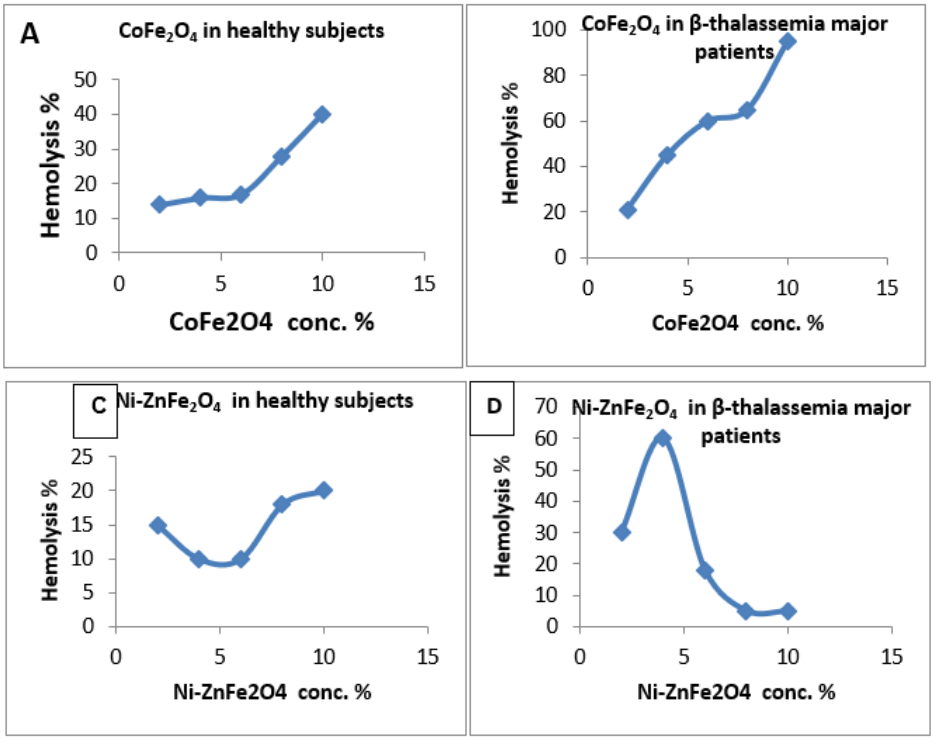

Fig. 1. A, B, C and D: hemolysis curves of the effect of the cytotoxic effect of [Co and Ni-Zn] NPs for healthy subjects and patients groups

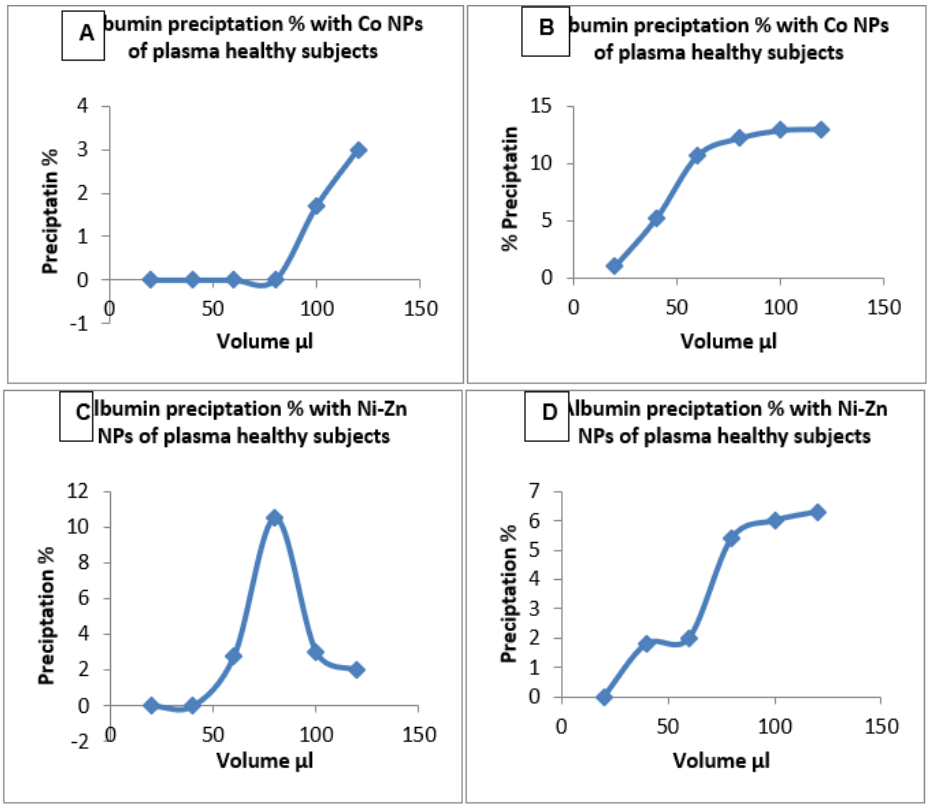

Fig. 2. Precipitation % of BSA after treated with (CO and Ni-Zn) NPs for all studied groups

indicated that the effect Co ferrite NPs on HPA were lower than that the effect of Ni-Zn ferrite NPs for healthy subjects while for β-thalassemia major patients the precipitation % by Co ferrite NPs were higher than that by Ni-Zn ferrite NPs.

DISCUSSION

The mechanisms of interaction at cellular and molecular level are important. However, the interaction between NPs and (active biological molecules, cells and tissues) and its sequels at physiological level are yet to be understood, for this reason it is important to understand the mechanisms of the above interaction. It is essential to study the biosafety concern of the biomedical applications of the prelude of NPs in blood and their interaction with plasma proteins and blood cells(PP Shirekar, 2016). It's evident to study the toxic effect of ferrite NPs to human erythrocytes are increasing (Saquib, 2013)|. Therefore, the toxicological effects of [synthetic Co ferrite and purchased Ni-Zn ferrite] NPs on human erythrocytes of patients with β – thalassemia major patients and healthy subjects were examined. The effect of synthetic Co ferrite NPs indicated from **Fig. 1A** and **1B**, that the hemolysis % were higher for β- thalassemia major patients than healthy subjects, while the purchased Ni-Zn ferrite NPs have inverse effect on the studied groups. The toxic effects of the above NPs may be due to their tiny size and unique physiochemical properties (Murdock, 2008). The stress on the membrane of human erythrocytes (Fragility of erythrocytes) due to the osmotic and mechanical stress (which is due to the pressure caused by the flow of hypotonic solution) and osmotic fragility concerning to the (size, composition and integrity of the cell and/or (surface area / volume ratio). Many diseased conditions related to the fragility of erythrocytes including) hereditary spherocytosis, hypernatremia, anemia, sickle cell anemia and thalassemia (Fischback 2008). The *in vivo* complexity behavior of NPs may be attributed to the interaction with biomolecules in biological fluids which result to enveloped of NPs by dynamic protein coronas, protein coronas act as mediator between NPs and their environment including (blood, tissues and cells). The interaction between protein corona and NPs depends on the (physical, biological and chemical) properties of NPs(Ivona, 2017). Proteins in plasma play a critical role in creating bio – nano interfaces, by opsonize NPs and form coronas (Lane, 2015). It is important to evaluate the effects of corona protein on the (metabolism, distribution, and elimination) of NPs in the body before used in clinical practice (Ivona, 2017). The *in vitro* albumin corona adsorption on NPs was excessively used to suppress the adsorption of opsonin protein and increase the blood circulation capability (Liu, 2015;Lennart,2014).The(good stability,low-immunogenicity & local circulation capacity) of the most abundant protein in plasma albumin, with 55% was tremendously explored (Murdock,2008;Fischbak 2008;MalinBern 2015;Liu 2016). The effect of the NPs on human plasma albumin also studied for the two groups.

ACKNOWLEDGEMENTS

We thank Dr. Wajeeh Khagi Obeed (University of Mustansirya, Faculty of science, Chemistry department) for his assistance by providing us with the studied nanoparticles.

FUNDING

This work was supported by the Ministry of higher education- University of Kirkuk- Faculty of science – chemistry department

CONCLUSION

The results indicated that the effect of Co ferrite NPs on healthy subjects HPA less than on patients group.

REFERENCES

AshaRani P, Low Kah Mun G, Hande MP, Valiyaveettil S(2008) Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS nano. 3,279-290.

Cardoso V F, Francesko A, Ribeiro C, Bañobre-López M, Martins P, Lanceros-Mendez S(2018) Advances in Magnetic Nanoparticles for Biomedical Applications. Adv. Healthc. Mater. 7, doi:10.1002/adhm.201700845.

Discher D, Mohandas N, Evans E(1994) Molecular maps of red cell deformation: Hidden elasticity and in situ connectivity. Science 266,1032–1035.

Dobrovolskaia, MA, Clogston, JD, Neun, BW, Hall, JB, Patri, AK, McNeil, SE(2008). Method for analysis of nanoparticle hemolytic properties in vitro. Nano Lett. 8, 2180–7.

Fischback, Frances Talaska Dunning Marshall (2008) 'A manual of laboratory and diagnostic Tests', 8th Ed.

Grant N H, Alburn H. KryzanauskasE (1970) Stabilization of serum albumin by anti- inflammatordrugs. Biochem.Pharmacol, 19,715-722.

Hafeli UO, Riffle JS, Harris-Shekhawat L, Carmichael-Baranauskas A, Mark F,Dailey JP, et al(2009) Cell uptake and in vitro toxicity of magnetic nanoparticles suitable for drug delivery. Mol Pharm. 6, 1417–28.

Huang YX, et al. Restoring the youth of aged red blood cells and extending their lifespan in circulation by remodelling membrane sialic acid. Journal of cellular and molecular medicine. 20(2016)294–301. doi: 10.1111/jcmm.12721.

Ivona Capjak, Sandra Šupraha Goreta, Darija Domazet Jurašin, and Ivana Vinković Vrček(2017)How protein coronas determine the fate of engineered nanoparticles in biological environment. Arh Hig Rada Toksikol.68,245 253.

Kawata K, Osawa M, Okabe S(2009) In vitro toxicity of silver nanoparticles at noncytotoxic doses to HepG2 human hepatoma cells. Environ Sci Technol. 43,6046-6051.

Lane LA, Qian X, Smith AM, Nie S(2015) Physical chemistry of nano-medicine: understanding the complex behaviours of nanoparticles in vivo. Annu Rev Phys, Chem.66,521-47.

Lartigue L, Wilhelm C, Servais J, Factor C, Dencausse A, Bacri J-C, et al(2012) Nanomagnetic sensing of blood plasma protein interactions with iron oxide nanoparticles: impact on macrophage uptake. ACS Nano. 6,2665–78.

Lennart T, Stefan B, Pauline M, Sarah W, Li Shang G and Ulrich Nienhaus(2014) Impact of Protein Modification on the Protein Corona on Nanoparticles and Nanoparticle–Cell Interactions. ACS Nano. 8,503-513.

Liu Z and Chen N(2016) Simple bio-conjugate chemistry service great clinical advances: albumin as a versatile platform for diagnosis and precision therapy. Chem. Soc. Rev. 45,1432- 56.

Liu Z, Chen N, Dong C, et al(2015) Facile Construction of Near Infrared Nanoprobe with Amphiphilic Protein – Plymer Bioconjucate for Targeted Cell Imaging.ACS Appl. Mater. Interfaces.7,34.

Malin B, Kine M, Knudsen S, Jeannette N, Inger S and Jan T A (2016) The role of albumin receptors in regulation of albumin homeostasis: Implications for drug delivery. Journal of Controlled Release. 211X,144-162.

Martínez-Rodríguez NL, Tavárez S, González-Sánchez ZI(2019) in vitro toxicity assessment of zinc and nickel ferrite nanoparticles in human erythrocytes and peripheral blood mononuclear cell. Toxicol In Vitro. 57,54-61. doi: 10.1016/j.tiv.

McNamara K, Tofail S A M (2015) Nanosystems: The use of nanoalloys, metallic, bimetallic, and magnetic nanoparticles in biomedical applications. Phys. Chem. Chem. Phys. 17,27981–27995, doi:10.1039/c5cp00831j.

Mizushima Y, Kobayashi M(1968) Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins, J. Pharm. Pharmacol, 20,169.

Mohandas N, Chasis J(1993) Red blood cell deformability, membrane material properties and shape: Regulation by transmembrane, skeletal and cytosolic proteins and lipids. Semin Hematol 30,171–192.

Murdock RC, Braydich-Stolle L, Schrand AM, Schlager JJ, Hussain SM(2008) Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. Toxicol Sci. 101,239–53.

Oladele S B, Ayo J O, Esievo K A N and Ogundipe S O(2003) Seasonal and species variations in erythrocyte osmotic fragility of indigenous poultry species in Zaria, northern Guinea Savannah zone of Nigeria, Bull. Anim. Hlth. Prod. Afr.51, 204.

Oyewale J.O(1993) Effects of storage of blood on the osmotic fragility of mammalian erythrocyte, J. Veter. Med. A, 40 258.

Sangeetha M(2011) In-vitro Anti-Inflammatory Studies of 3-(1-Benzofuran-2-yl)-5-(substituted aryl) isoxazole, International J. of Research in Pharmaceutical and Biomedical Sciences, 2, 1203-1205.

Saquib Q, Al-Khedhairy AA, Ahmad J, Siddiqui MA, Dwivedi S, Khan ST, Musarrat J(2013) Zinc ferrite nanoparticles activate IL-1b, NFKB1, CCL21 and NOS2 signaling to induce mitochondrial dependent intrinsic apoptotic pathway in WISH cells. Toxicol Appl Pharmacol. 273, 289–97.

Shirsekar P P, Kanhe N, Mathe V L, Lahir Y K and Dongre P M(2016) Interaction Of Zinc Oxide Nanoparticles With Human Red Blood Cells. Bionano Frontier., 9.

Song Q, Zhang ZJ(2006) Correlation between spin-orbital coupling and the superparamagnetic properties in magnetite and cobalt ferrite spinel nanocrystals. J Phys Chem B. 110,11205–9.

Surgenor D (1974)The Red Blood Cell(Academic, New York, NY), 2nd Ed, pp 13–24.

Tárnok A(2006) Flow and image cytometry side by side for the new frontiers in quantitative single-cell analysis. Cytometry Part A: The Journal of the International Society for Advancement of Cytometry. 75,169–171. doi: 10.1002/cyto.a.20709.

Teodora M (2013) Hemolysis as expression of nanoparticles – induced cytotoxicity in red blood cells. Biotechnology, Molecular Biology And Nanomedicine. 1, 7-12.

Willard MA, Kurihara LK, Carpenter EE, Calvin S, Harris VG(2004) Chemically prepared magnetic nanoparticles. Int Mat Rev. 49,125–70.