

# Cobalt Zinc Ferrite Nanoparticles as a Potential Magnetic Resonance Imaging Agent: An *In vitro* Study

Zeinab Ghasemian<sup>1</sup>, Daryoush Shahbazi-Gahrouei<sup>1\*</sup>, and Sohrab Manouchehri<sup>2</sup>

1. Department of Medical Physics, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

2. Department of Physics, Nano-center, Maleke-ashtar University of Technology, Shahin-shahr, Isfahan, Iran

## Abstract

**Background:** Magnetic Nanoparticles (MNP) have been used for contrast enhancement in Magnetic Resonance Imaging (MRI). In recent years, research on the use of ferrite nanoparticles in T<sub>2</sub> contrast agents has shown a great potential application in MR imaging. In this work, Co<sub>0.5</sub>Zn<sub>0.5</sub>Fe<sub>2</sub>O<sub>4</sub> and Co<sub>0.5</sub>Zn<sub>0.5</sub>Fe<sub>2</sub>O<sub>4</sub>-DMSA magnetic nanoparticles, CZF-MNPs and CZF-MNPs-DMSA, were investigated as MR imaging contrast agents.

**Methods:** Cobalt zinc ferrite nanoparticles and their suitable coating, DMSA, were investigated under *in vitro* condition. Human prostate cancer cell lines (DU145 and PC3) with bare (uncoated) and coated magnetic nanoparticles were investigated as nano-contrast MR imaging agents.

**Results:** Using T<sub>2</sub>-weighted MR images identified that signal intensity of bare and coated MNPs was enhanced with increasing concentration of MNPs in water. The values of 1/T<sub>2</sub> relaxivity (r<sub>2</sub>) for bare and coated MNPs were found to be 88.46 and 28.80 (mM<sup>-1</sup>s<sup>-1</sup>), respectively.

**Conclusion:** The results show that bare and coated MNPs are suitable as T<sub>2</sub>-weighted MR imaging contrast agents. Also, the obtained r<sub>2</sub>/r<sub>1</sub> values (59.3 and 50) for bare and coated MNPs were in agreement with the results of other previous relevant works.

*Avicenna J Med Biotech 2015; 7(2): 64-68*

**Keywords:** Magnetite Nanoparticles, MR imaging, Prostatic neoplasm

## Introduction

One of the most powerful non-invasive techniques for diagnosis is MR imaging which has excellent temporal and spatial (25-100 μm) resolution<sup>1-3</sup>. The limitation of MR imaging technique is its low sensitivity that leads to unclear diagnosis of healthy tissues from abnormal tissues. Theoretically, the sensitivity in MR imaging is due to the relaxivity of magnetic spins in water protons<sup>4</sup>. In the last decades, nanoparticle agents continue to receive considerable attention in the field of medical imaging as potential MR imaging contrast agents<sup>5,6</sup>. Recently, magnetic nanoparticles have attracted growing interest as high performance biomaterials which are used for the diagnosis and treatment of cancers. Nowadays, more studies focus on magnetic nanoparticles as potential MR imaging contrast agents. Magnetic nanoparticles should have features such as uniform particle size<sup>6</sup>, a uniform and high superparamagnetic moment, high colloidal stability, low toxicity and high biocompatibility<sup>7</sup>. Magnetic nanoparticles can alter longitudinal and transverse relaxation times and thereby affect the MR image signal<sup>8</sup>. Studies on the application of ferrite nanoparticles in T<sub>2</sub> MR imaging contrast agents has shown great potential<sup>5,9-11</sup>.

Saturation magnetization (Ms) value is one of the most dominant parameters that affect T<sub>2</sub> relaxivity<sup>12</sup>. Jun *et al* reported that the values of Ms are dependent on the size and composition of nanoparticles<sup>13</sup>. Metallic nanoparticles such as iron, nickel, cobalt, or their alloys have higher magnetization value than that of oxide nanoparticles<sup>14</sup>. For this reason, in this study, cobalt zinc ferrite (Co<sub>0.5</sub>Zn<sub>0.5</sub>Fe<sub>2</sub>O<sub>4</sub>) magnetic nanoparticles were used for further investigation.

In another study, Barcena *et al* found that zinc ferrite nanoparticles increased the 1/T<sub>2</sub> relaxivity and improved the sensitivity of detection by MR imaging<sup>3</sup>. In previous studies, special attention was given to particles coated with Dimercaptosuccinic acid (DMSA) for biomedical applications<sup>15-17</sup>. Recently, the cytotoxicity effect of DMSA-Fe<sub>2</sub>O<sub>3</sub> by tetrazolium dye assay on human aortic endothelial cells was reported which revealed its low cytotoxicity effect<sup>15</sup>. Also, the cytotoxicity of CZF-MNPs and CZF-MNPs-DMSA was also demonstrated on prostate cancer cells (PC3 and DU145)<sup>18</sup>.

The aim of this paper was to evaluate the influence of coated and uncoated magnetic nanoparticles, in par-

\* Corresponding author:  
Daryoush Shahbazi-Gahrouei,  
Ph.D., Department of Medical  
Physics, Faculty of Medicine,  
Isfahan University of Medical  
Sciences, Isfahan, Iran  
Tel: +98 31 37922495  
Fax: +98 31 37922432  
E-mail:  
shahbazi24@yahoo.com &  
shahbazi@med.mui.ac.ir  
Received: 11 Oct 2014  
Accepted: 23 Dec 2014

ticular CZF-MNPs and CZF-MNPs-DMSA, on the relaxivity of water protons as MR imaging contrast agent or molecular imaging for clinical diagnosis.

### Materials and Methods

Preparation and synthesis, characterization, coating with DMSA, and cytotoxicity of cobalt zinc ferrite nanoparticles were described in previous published papers<sup>16,18</sup>. Briefly, cobalt zinc ferrite nanoparticles were prepared using a co-precipitation method and their characterization was assessed using of TEM (Transmission Electron Microscopy), FT-IR (Fourier Transformation Infrared Spectroscopy) and magnetization measurements.

#### Cell culture

Human prostate cancer cells, HPCs, (DU145 and PC3) were purchased from the National Cell Bank of Iran, Pasteur Institute of Iran. They were cultured in Roswell Park Memorial Institute (RPMI) media supplemented with 1% antibiotics (100 units/ml penicillin and 100 µg/ml streptomycin) and 10% Fetal Bovine Serum (FBS) until the third passage before performing the experiments. All the cell culture materials were from Gibco, USA. Cells were grown to confluence at 37°C in 5% CO<sub>2</sub>/air. *In vitro* cytotoxicity (MTT assay) of coated and bare magnetic nanoparticles was evaluated using standard 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) based colorimetric assay as described elsewhere<sup>18</sup>. The cell viability was determined by the following formula:

$$\% \text{ Cell viability} = \frac{\text{Mean absorbance in test wells}}{\text{Mean absorbance in control}} \times 100$$

#### Incubation of cells with the bare and coated MNPs

First, gelatin suspension 1 g was prepared from powder gelatin dissolved in 50 ml PBS solution, and put in water bath for 30 min to obtain uniform solution. Two prostate cancer cell lines (PC3 and DU145) were incubated with different concentrations (0.5, 1 and 1.5 mmol) of CZF-MNPs and CZF-MNPs-DMSA for 2 hr at room temperature in RPMI 1640 culture medium. In order to separate the particles stuck together, before adding bare and coated MNPs suspension with different concentrations into RPMI culture medium, they were sonicated for 20 min. Then, the cells were washed three times with PBS solution. In the next step, 1 ml of gelatin suspension was added to 1.5 ml Eppendorf tubes with different concentrations and stirred to obtain uniform solution. Then, the Eppendorf tubes with different concentrations were placed on ice powder until solidity was obtained in the solution. Control groups received only 1 ml of gelatin suspension.

#### *In vitro* MR imaging

These Eppendorf tubes with different concentrations were used for *in vitro* MR imaging characterizations. T<sub>2</sub>-weighted images were obtained using a 1.5T MRI scanner (1.5 Tesla, GE Medical system).

#### Relaxivity (r<sub>1</sub> and r<sub>2</sub>) measurements

For MR imaging studies, different concentrations of coated and bare MNPs were prepared in 1.5 ml Eppendorf tubes. The longitudinal and transverse relaxation times (T<sub>1</sub> and T<sub>2</sub>) were measured using 3 Tesla field strength (Siemens MR scanner with head coil). MR images of each sample were obtained using a standard spin-echo sequence with two following parameters: Repetition time (T<sub>R</sub>)=4000 ms, echo time (T<sub>E</sub>)=12, 24, 36, 48, 60 and 72 ms, number of echoes (NE)=6, matrix size=512×384, pixel band width=230, slice thickness=2.5 mm, number of excitations (NEX)=3 and field of view=25 cm.

Repetition time (T<sub>R</sub>)=100, 250, 500, 1000, 2000 and 4000 ms, echo time (T<sub>E</sub>)=15 ms, number of excitations (NEX)=3, matrix size=512×384, pixel band width=130, slice thickness=2.5 mm, number of echoes (NE)=1 and field of view=25 cm.

### Results

The surface morphology of CZF-MNPs and CZF-MNPs-DMSA was evaluated by TEM and their results are shown in previous publications<sup>18</sup>. The findings showed that particles had almost spherical structures. Indeed, the average particle sizes of CZF-MNPs and CZF-MNPs-DMSA (co-precipitation) were 16 and 40 nm, respectively.

#### *In vitro* MR imaging

The capability and suitability of synthesized nanoparticles as MR contrast agent was confirmed using a T<sub>2</sub>-weighted image protocol by 1.5 Tesla (GE Medical system) MR imaging system. The results are shown in figure 1. This result demonstrated that both bare and coated nanoparticles have been imported in PC3 and DU145 cell lines and resulted in grating signal intensity reduction.

The T<sub>2</sub>-weighted MR image in figure 1 also shows that CZF-MNPs and CZF-MNPs-DMSA induce a negative contrast. In addition, for the same Fe content, the enhancement in intensity using CZF-MNPs is the same as the amount of CZF-MNPs-DMSA.

#### Relaxivity (r<sub>1</sub> and r<sub>2</sub>) measurements

Figure 2 shows T<sub>2</sub>-weighted MR images of different concentrations of bare and coated MNPs (CZF-MNPs and CZF-MNPs-DMSA) by 3 Tesla, MR imaging system with standard spin-echo sequence (T<sub>R</sub>=1000 ms, T<sub>E</sub>=12 ms, room temperature). As can be seen from this figure, significant differences are observed between signal intensity at different concentrations of bare and

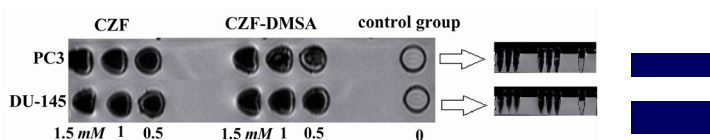


Figure 1. T<sub>2</sub>-weighted magnetic resonance images at various Fe concentrations, CZF-MNPs and CZF-MNPs-DMSA (1.5 T, Fast spin-echo sequence: T<sub>R</sub>=2520 ms, T<sub>E</sub>=102 ms, room temperature).

## Cobalt Zinc Ferrite Nanoparticles as a Potential MRI Agent

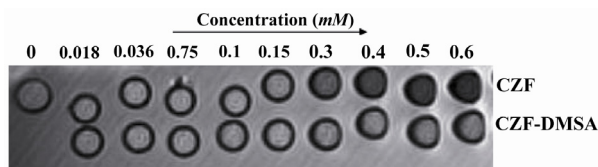


Figure 2. MR imaging signal intensity of bare and coated MNPs for different concentrations of Fe (*mM*) using 3 T, standard spin-echo sequence:  $T_R=1000$  ms,  $T_E=12$  ms, room temperature).

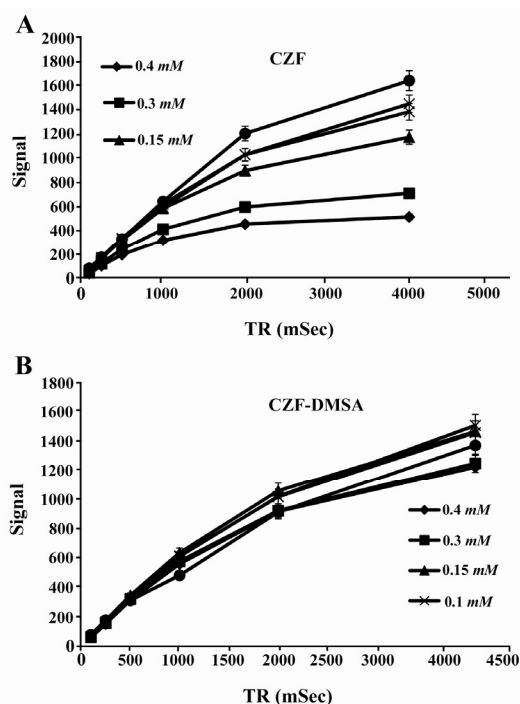


Figure 3. Plots of signal intensity for (A) CZF-MNPs and (B) CZF-MNPs-DMSA as a function of  $T_R$  for different Fe concentrations (*mM*) of bare and coated MNPs. The signal intensity for each sample was measured three times.

coated nanoparticles. Also, the reduction of signal intensity in CZF-MNPs sample is more than that of CZF-MNPs-DMSA sample (in the same concentration). In addition, the signal intensity of CZF-MNPs was enhanced by decreasing CZF-MNPs concentrations in water solution. Two samples were prepared in dark state in the MR image where the composites (contrast agents) were collected.

Relaxivity ratio ( $r_2/r_1$ ) is an important index to determine the ability of nanoparticles as  $T_2$  MR imaging contrast agent.  $T_1$  and  $T_2$  relaxivity values, size (*nm*), saturation magnetization (*Ms*) and  $r_2/r_1$  values of CZF-MNPs and CZF-MNPs-DMSA are presented in table 1. Plots of signal intensity for CZF-MNPs and CZF-MNPs-DMSA as a function of  $T_R$  and  $T_E$  for different

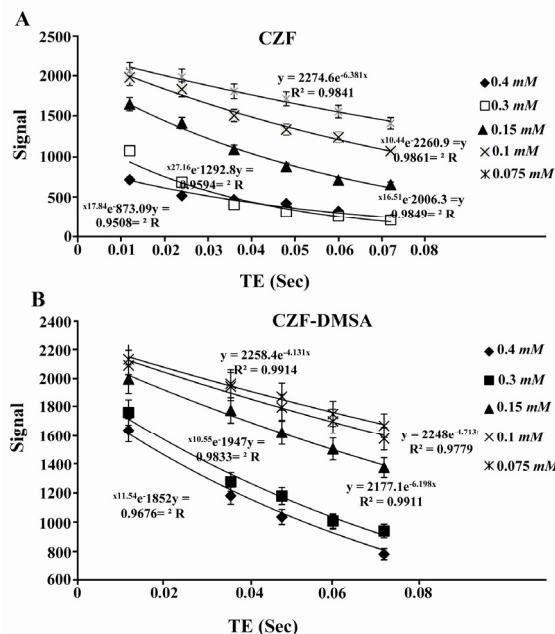


Figure 4. Plots of signal intensity for (A) CZF-MNPs and (B) CZF-MNPs-DMSA as a function of  $T_E$  for different Fe concentrations (*mM*) of bare and coated MNPs.

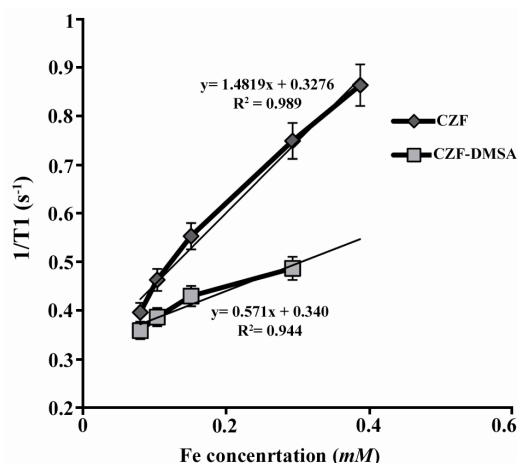


Figure 5. Plot of longitudinal relaxivity ( $1/T_1$ ) for CZF-MNPs and CZF-MNPs-DMSA as a function of different Fe concentrations (*mM*).

Fe (*mM*) concentrations of bare and coated MNPs was measured three times and their results are shown in figures 3 and 4, respectively. Two  $1/T_2$  and  $1/T_1$  relaxivity values of samples have been calculated and are presented in figures 5 and 6.

$R_2$  values of relaxation rates ( $r_1$  and  $r_2$ ) were higher than 0.9, which indicated a good fitness for regression due to high values of  $r_2/r_1$  for bare and coated MNPs. These two magnetic nanoparticles are classified as

Table 1. Relaxivities  $r_1$  and  $r_2$  (at 3 T), size and saturation magnetization (*Ms*) of bare and coated MNPs

MNPs formulation	Size ( <i>nm</i> )	Saturation magnetization ( <i>Ms</i> ) ( <i>emu/g</i> )	$r_1$ ( $mM^{-1} s^{-1}$ )	$R^2$ value	$r_2$ ( $mM^{-1} s^{-1}$ )	$R^2$ value	$r_2/r_1$
CZFMNPs (co-precipitation)	16	43.1	1.490	0.987	88.46	0.972	59.3
CZF-MNPs@DMSA (co-precipitation)	40	42.4	0.577	0.940	28.80	0.999	50.0

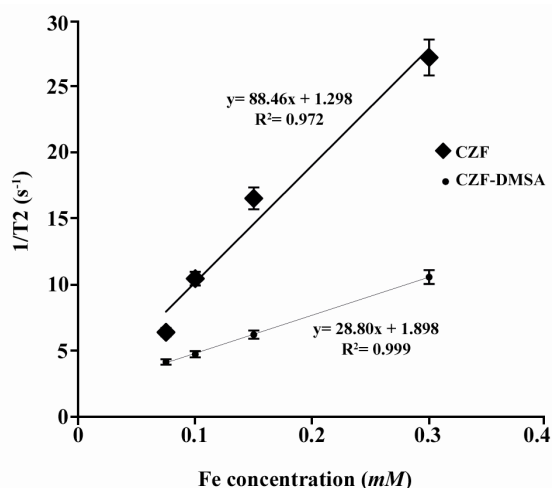


Figure 6. Plot of transverse relaxivity  $R_2$  ( $1/T_2$ ) for CZF-MNPs and CZF-MNPs-DMSA as a function of different Fe concentrations ( $mM$ ).

negative MR imaging contrast agents.

The lower graphs in figures 5 and 6 are related to  $r_1$  and  $r_2$  for coated MNPs which were compared to the bare MNPs. In other words, the relaxation curves for bare MNPs are steeper than that of the coated MNPs, either longitudinal or transverse relaxivity.

### Discussion

The ability of both CZF and CZF-DMSA nanoparticles was assessed in order to decrease the longitudinal ( $T_1$ ) and transverse ( $T_2$ ) relaxation times of water protons. The MR imaging study showed that both  $T_1$  and  $T_2$  relaxation times were reduced with increasing Fe ( $mM$ ) concentration; hence, CZF-MNPs and CZF-MNPs-DMSA may be more favorable as negative MR imaging contrast agents. The transverse and longitudinal relaxivity ( $r_1$  and  $r_2$ ) of water protons were increased by increasing Fe ( $mM$ ) concentration of bare and coated MNPs compared to the control group (gelatin suspension).

The values of the saturation magnetization of bare and coated nanoparticles were approximately the same and found to be 43.1 and 44.4, respectively. But, regarding  $r_2$  relaxivity of both studied magnetic nanoparticles, bare nanoparticle (CZF-MNPs) has  $r_2$  of 3-fold higher than that of coated nanoparticle (CZF-MNPs-DMSA). Although the saturation magnetization of both bare and coated MNPs was so similar but many differences between  $r_2$  relaxivities of two studied samples was observed. Therefore, the other factors such as particle size and shape of nanoparticles influence the relaxation time.

According to literature, factors such as synthesis method of nanoparticles and the type of their coating can affect both  $T_1$  and  $T_2$  relaxation times<sup>9</sup>. The study of Hoque *et al* showed that synthesized nanoparticles using sonochemical technique has  $r_2$  relaxivity values

higher than that of co-precipitation technique<sup>10</sup>. Findings of this study showed that  $r_2/r_1$  values for CZF-MNPs and CZF-MNPs-DMSA turned out to be 59.3 and 50, respectively. MR imaging results also showed that transverse and longitudinal relaxivities were increased with increasing Fe ( $mM$ ) concentration. For this reason, our findings showed that CZF-MNPs and CZF-MNPs-DMSA are desirable negative nano contrast MR imaging agents.

### Conclusion

The values of  $r_2$  relaxivity and  $r_2/r_1$  ratio showed that bare and coated MNPs may be suitable  $T_2$  contrast agents. But, the low values of  $r_1$  relaxivity, and also the low values of  $r_2/r_1$  ratio indicated that bare and coated MNPs are not acceptable  $T_1$  MR imaging nano-contrast agents. Further study should be done to evaluate whether cobalt zinc ferrite nanoparticles with different coatings can be used as  $T_2$  MR imaging contrast agents. In addition, this magnetic nanoparticle should be synthesized with other methods and be coated with other different suitable materials.

### Acknowledgement

This work was supported by Isfahan University of Medical Science (IUMS).

### Conflict of Interest

None declared.

### References

1. Massoud TF, Gambhir SS. Molecular imaging in living subjects: seeing fundamental biological processes in a new light. *Genes Dev* 2003;17(5):545-580.
2. Shahbazi-Gahrouei D, Williams M, Rizvi S, Allen BJ. In vivo studies of Gd-DTPA-mono-clonal antibody and gadoporphyrins: potential magnetic resonance imaging contrast agents for melanoma. *J Magn Reson Imaging* 2001; 14(2):169-174.
3. Corti M, Lascialfari A, Micotti E, Castellano A, Donativi M, Quarta A, et al. Magnetic properties of novel superparamagnetic MRI contrast agents based on colloidal nanocrystals. *J Magn Magn Mater* 2008;320(14):e320-e323.
4. Abdolahi M, Shahbazi-Gahrouei D, Laurent S, Sermeus C, Firozian F, Allen BJ, et al. Synthesis and in vitro evaluation of MR molecular imaging probes using J591 mAb-conjugated SPIONs for specific detection of prostate cancer. *Contrast Media Mol Imaging* 2013;8(2):175-184.
5. Shahbazi-Gahrouei D, Abdolahi M. Superparamagnetic iron oxide-C595: Potential MR imaging contrast agents for ovarian cancer detection. *J Med Phys* 2013;38(4): 198-204.
6. Laurent S, Forge D, Port M, Roch A, Robic C, Vander Elst L, et al. Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical charac-

- terizations, and biological applications. *Chem Rev* 2008; 108(6):2064-2110.
7. Zeng C, Shi X, Wu B, Zhang D, Zhang W. Colloids containing gadolinium-capped gold nanoparticles as high relaxivity dual-modality contrast agents for CT and MRI. *Colloids Surf B Biointerfaces* 2014;123:130-135.
  8. Shahbazi-Gahrouei D, Abdolahi M. Detection of MUC1-expressing ovarian cancer by C595 monoclonal antibody-conjugated SPIONs using MR imaging. *Scientific World Journal* 2013;2013:609151.
  9. Barcena C, Sra AK, Chaubey GS, Khemtong C, Liu JP, Gao J. Zinc ferrite nanoparticles as MRI contrast agents. *Chem Commun (Camb)* 2008;(19):2224-2226.
  10. Hoque SM, Srivastava C, Srivastava N, Venkateshan N, Chattopadhyay K. Synthesis and characterization of Fe- and Co-based ferrite nanoparticles and study of the T1 and T2 relaxivity of chitosan-coated particles. *J Mater Sci* 2013;48(2):812-818.
  11. Arsalani N, Fattahi H, Nazarpour M. Synthesis and characterization of PVP-functionalized superparamagnetic Fe<sub>3</sub>O<sub>4</sub> nanoparticles as an MRI contrast agent. *Exp Polym Lett* 2010;4(6):329-338.
  12. Tong S, Hou S, Zheng Z, Zhou J, Bao G. Coating optimization of superparamagnetic iron oxide nanoparticles for high T2 relaxivity. *Nano Lett* 2010;10(11):4607-4613.
  13. Jun YW, Huh YM, Choi JS, Lee JH, Song HT, Kim S, et al. Nanoscale size effect of magnetic nanocrystals and their utilization for cancer diagnosis via magnetic resonance imaging. *J Am Chem Soc* 2005;127(16):5732-5733.
  14. Seo WS, Lee JH, Sun X, Suzuki Y, Mann D, Liu Z, et al. FeCo/graphitic-shell nanocrystals as advanced magnetic-resonance-imaging and near-infrared agents. *Nat Mater* 2006;5(12):971-976.
  15. Ge G, Wu H, Xiong F, Zhang Y, Guo Z, Bian Z, et al. The cytotoxicity evaluation of magnetic iron oxide nanoparticles on human aortic endothelial cells. *Nanoscale Res Lett* 2013;8(1):215.
  16. Manouchehri S, Ghasemian Z, Shahbazi-Gahrouei D, Abdolahi M. Synthesis and characterization of cobalt-zinc ferrite nanoparticles coated with DMSA. *Chem Xpress* 2013;2(3):147-152.
  17. Cabrera LI, Somoza A, Marco JF, Serna CJ, Morales MP. Synthesis and surface modification of uniform MFe<sub>2</sub>O<sub>4</sub> (M=Fe, Mn, and Co) nanoparticles with tunable sizes and functionalities. *J Nanopart Res* 2012;14(6):1-14.
  18. Shahbazi-Gahrouei D, Ghasemian Z, Abdolahi M, Manouchehri S, Javanmard SH, Dana N. In vitro evaluation of cobalt-zinc ferrite nanoparticles coated with DMSA on human prostate cancer cells. *J Mol Biomark Diagn* 2013; 4(3):154.