IN SITU SYNTHESIS OF BIOMINERALIZED MAGNETIC NANOPARTICLE WITHIN HYDROGEL

RAY Ayan[1], SAHA Nabanita*[1], SAHA Tomas[2], SAHA Petr[1]

[1] *Tomas Bata University in Zlin, Centre of Polymer System, Zlin, Czech Republic, EU*
[2] *Tomas Bata University in Zlin, University Institute, Zlin, Czech Republic, EU*
* nabanita@cps.utb.cz

Abstract

Magnetic nanoparticle (MNP) is of high practicle interest in the domain of biomedicine and biomedical application e.g., drug delivery, magnetic fluid hyperthermia, magnetic resonance imaging, biosensing. The magnetic nanoparticles are explored due to its high tunability of its physical and magnetic properties. A considerable effort has been devoted for the synthesis of MNP e.g., ball milling, arc melting, co-precipitation, microwave and microfluidics techniques. However, hydrogels can offer a new platform for *in situ* synthesis of biomineralized MNP as well as magnetic hydrogels (MH), because of its an advantageous controled network structure. It is an emerging and promising concept, as most of the biopolymer are supposed to be biodegradable that can be considered as an alternative eco-friendly material too. In this work, "PVP-CMC Hydrogel" is used as matrix for synthesis of biomineralized MNP, where the *unique porous networks structure* exhibited within swollen/wet PVP-CMC hydrogel. Here, PVP-CMC hydrogel function as a chemical reactor, where inorganic salts ($FeCl_2$ and $FeCl_3$) are reacting with precipitating agents ammonium hydro-oxide (NH_4OH) to generate biomineralized MNP. This method prevents from aggregation and shows a narrow particle size distribution. X-ray diffraction studies reveal the formation of magnetite phase. Structural and morphological analysis using transmission electron microscopy shows the spherical shaped magnetic nanoparticle with size of 10 ± 2 nm. The saturation magnetization of the MNP was 78 emu/g and 12 emu/g for magnetic hydrogel. This synthesis process can be used to synthesize "bare" MNP, MH and extended to other oxides by changing the salts for the biomedical applications.

Keywords: Magnetic nanoparticle, hydrogels, biomineralized MNP, magnetic hydrogel, PVP-CMC

1. INTRODUCTION

In recent years, extensive effort has been devoted to the synthesis methods and techniques of magnetic hydrogel and nanoparticles. Synthesis of these magnetic hydrogels and nanoparticles are of practical interest in biochemical and biomedical applications [1]-[4]. The physiochemical stability, high surface to volume ratio, low level of toxicity, biocompatibility and good magnetic response make these MH and MNP ideal for a wide variety of biomedical applications: drug delivery, magnetic resonance imaging (MRI), tissue engineering and biosensing [5]-[8]. Apart from biomedical applications MH and MNPs are used in magnetic filtration, dampers, shock absorbers, thermal absorbers etc [6].

Various synthesis approaches has been adapted to the synthesis of MNPs. Primarily, physical or wet-chemical process are the main synthesis pathways to prepare Fe_3O_4 MNP [9]-[11]. Wet chemical process requires fine pH control of the medium, external surfactant to reduce nanoparticle aggregation while nucleation and high temperature with pressure to obtain desired magnetic phases. Furthermore, vigorous stirring is required while nucleation to obtain narrow size distribution of the MNPs. Bulk chemical co-precipitation reaction of Fe_2 and Fe_3 do not provide good control over the size distribution and crystallinity of the particles [12], [13]. Nonmagnetic phases and poor size distribution is often obtained. However, physical approaches are simple with low cost, mostly used for bulk MNP preparation but the size distribution is difficult to control and contamination is often observed.

On the other hand, in situ synthesis of MNP in bio-based hydrogel is an alternative promising synthesis process for biomineralized hydrogel. The network structure of the biopolymer based hydrogel is used as nanoreactors,to avoid aggregation during nucleation and they keep the production of the MNP with structural hierarchy. Compared to conventional batch synthesis, magnetic biomineralized hydrogel allows better control of reaction conditions e.g. reaction time, reaction concentration and stoichiometry. Synthesis of MNP within the hydrogel helps in reducing of polydispersity in size. Moreover, biomineralized hydrogels are known to be ecofriendly due to its biodegrability in nature. The extraction of the MNPs from the biomineralized hydrogel can be readily processed with thermal decomposition of the hydrogel or by addition of respective enzymes to decompose the hydrogel network structure.

In this study, polyvinylpyrrolidone, polyethylene glycol, agar, sodium carboxymethyl cellulose and glycerine was used to synthesize the freshly prepared hydrogel [14][15]. This hydrogel was then soaked in salt solution of Fe^{2+} and Fe^{3+}. Reducing agent (NH_4OH) was added for the in situ synthesis of the MNP inside the hydrogel network. As a whole finally achieved a black, magnetic property based biomineralized hydrogel film. This biomineralized (Fe_3O_4) hydrogel can be used directly as MH and/or the synthesize MNPs (after isolation from the matrix) can be used for the purpose of various medical applications.

2. EXPERIMENT AND METHOD

2.1. Materials

For the preparation of *in situ* magnetic hydrogel film and magnetic nanoparticles, polyvinylpyrrolidone K 30 (PVP: molecular weight 40,000), polyethylene glycol 3000 (PEG: average molecular weight 3015-3685) and agar were purchased from Fluka, Switzerland; sodium carboxymethyl cellulose (CMC) was purchased from Sinopharm Chem. Reagent Co., Ltd, China; glycerin was obtained from Lachema Ltd, Czech Republic. Iron(II) chloride tetrahydrate, 98% ($FeCl_2 \cdot 4H_2O$), Ammonium hydroxide 30% v/v aq. Soln. (NH4OH) and Iron(III) chloride hexahydrate 98% ($FeCl_3 \cdot 6H_2O$) was purchased from Sigma-Aldrich, Czech Republic.

2.2. Methodology

Water based PVP-CMC hydrogel was used for the preparation of in situ biomineralized PVP-CMC magnetic hydrogel and magnetic nanoparticles. The hydrogel film was prepared by solution casting method using aqueous solution of PVP (0.2%), CMC (0.8%), PEG (1%), agar (2%) and Glycerin (1%) under the physical stimulation of pressure and heat (15 lbs and 120 °C for 15 minutes). Freshly prepared PVP-CMC hydrogel solution was incubated at room temperature (25-30) °C to obtain the hydrogel film as shown in **Figure 1a**. Circular films were extracted from the hydrogel film for biomineralization process.

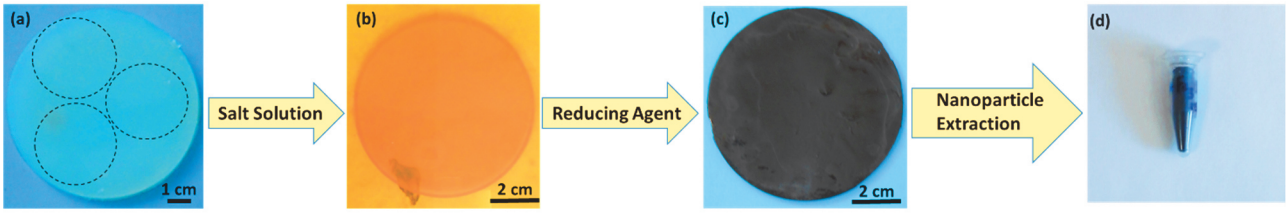

Figure 1 Experimental setup (optical view) showing the process of in situ synthesis of biomineralized magnetic nanoparticles within hydrogel: (a) PVP-CMC aqueous hydrogel, (b) salt solution absorbed PVP-CMC hydrogel and (c) Biomineralized (magnetic property based) PVP-CMC hydrogel, (d) image of magnetic nano particles (MNP) extracted from the hydrogel matrix

The freshly prepared hydrogel was soaked in the salt solution of Fe^{3+} and Fe^{2+} solution. The PVP-CMC hydrogel turns yellow due to diffusion of salt solution into the porous structure of PVP-CMC hydrogel as observed **Figure 1b**. Reducing agent (NH_4OH) is added to the salt solution absorbed PVP-CMC hydrogel. The yellow colored PVP-CMC hydrogel turns to black due to the onset of the nucleation process of the magnetic nanoparticles within the hydrogel. Finaly, Biomineralized (Fe_3O_4) PVP-CMC hydrogel is formed, designated as magnetic hydrogel (MH) as shown in the **Figure 1c**. The obtained biomineralized hydrogel was showing magnetic properties due to the formation of MNP's, which were extracted from these MH films by applying heat. Consecutive cleaning with deionized water results in "bare" MNP's (as shown in **Figures 1d** and **4**).

2.3. Characterization of MH and MNPs

MH and MNPs were characterized by X-ray diffraction, scanning electron microscopy, transmission electron microscopy and vibrating sample magnetometer.

2.3.1. X-ray diffraction

X-ray scans were performed on dried MH and MNPs using PANalytical X-Ray powder diffractometer equipped with a PIXcel RTMS detector with Cu Kα radiation of wavelength 1.54 Å over the 2theta range from 28° ≤ 2θ ≤ 65° at a scan rate of 1 °/min. Phase identification was matched with the reference ICSD reference file (Code: 26410). All the peaks were in good agreement with the standard known diffraction pattern of Fe_3O_4.

2.3.2. Scanning Electron Microscopy

The morphology of the MH was investigated using Scanning Electron Microscopy (SEM). Bright field SEM images were obtained from VEGA II LMU (TESCAN) was operated at 5-20 kV. Samples were prepared from dried freeze dried MH and placed on the sample holder.

2.3.3. Transmission Electron Microscopy

The morphology of the magnetic hydrogel and magnetic nanoparticles was investigated using Transmission Electron Microscopy (TEM). Bright field TEM images were obtained from JEOL, JEM 2100 TEM operated at 200 kV. For the analysis of MH, a thin slices of MH was obtained using microtome technique and drop cast technique was used for MNPs on carbon coated copper grid for 24 h. Image J, the image processing software was used to measure the particle size from the micrograph.

2.3.4. Vibrating Sample Magnetometer

Vibrating Sample Magnetometer (VSM; Lake Shore 7407) was used to measure the magnetic properties of the MH and MNPs. Field dependent magnetization curves at room temperature were measured for both MH and MNP.

3. RESULTS

3.1. X-ray Diffraction

The diffraction peaks of 220, 311, 400, 422, 511, and 440 were observed and could be indexed to cubic inverse spinal structure for both MH and MNP (ICSD FIZ Karlsruhe, Coll. Code: 26410). The intense peak corresponds to the 311 plane that appears at an angle of 35°. Peak broadening is observed for the MH due to the presence of some impurity in the form of PVP-CMC hydrogel.

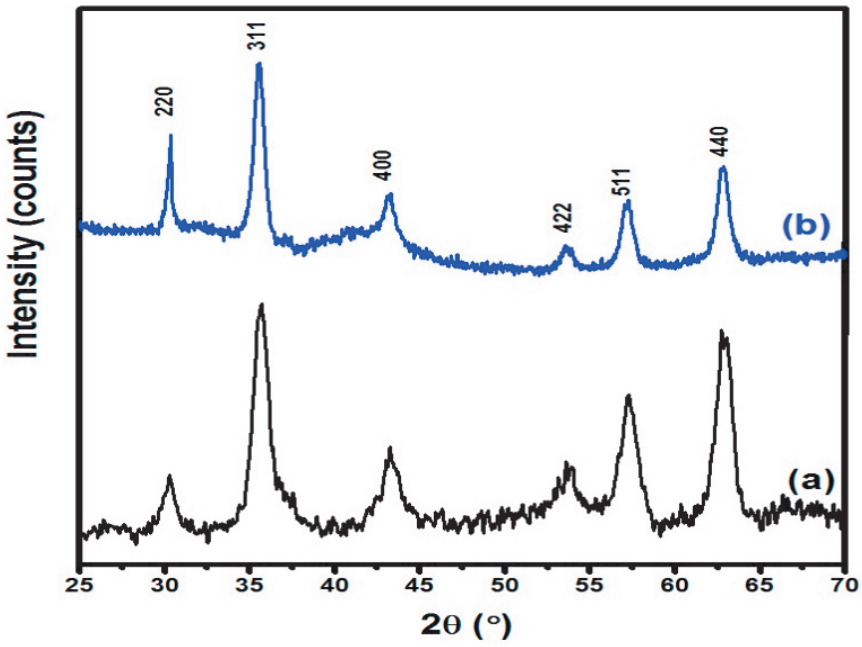

Figure 2. XRD profile of Fe_3O_4 (a) MH and (b) MNP's at room temperature

3.2. Scanning Electron Microscopy

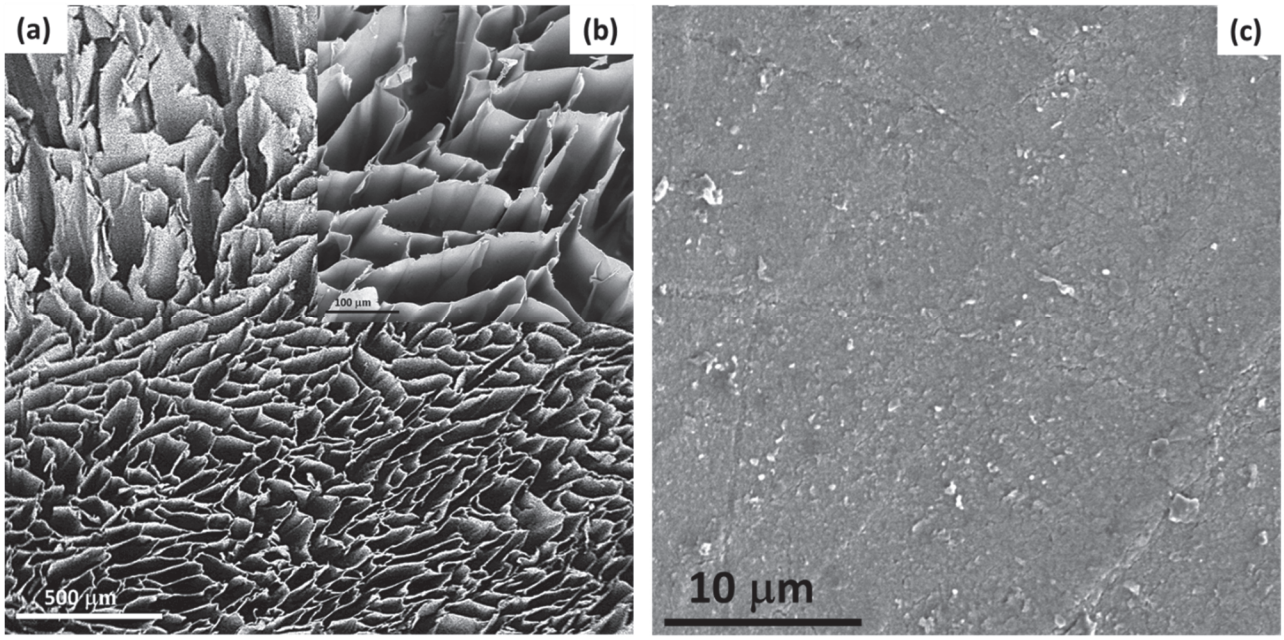

Figure 3 SEM image (a) Hydrogel, (b) high resolution image of the hydrogel (internal struc-morphology) showing the network structure and (c) Surface morphology of biomineralized (Fe_3O_4) hydrogel

Figure 3(a-b) shows SEM micrograph of the network like structure in the PVP-CMC hydrogel when freeze dried. The Fe_3O_4 nanoparticles are synthesized in this network like structure of the hydrogel. Fe_3O_4 nanoparticles nucleates in the hollow like structure. **Figure 4c** shows the image of the biomineralized (Fe_3O_4) PVP-CMC hydrogel.

3.3. Transmission Electron Microscopy

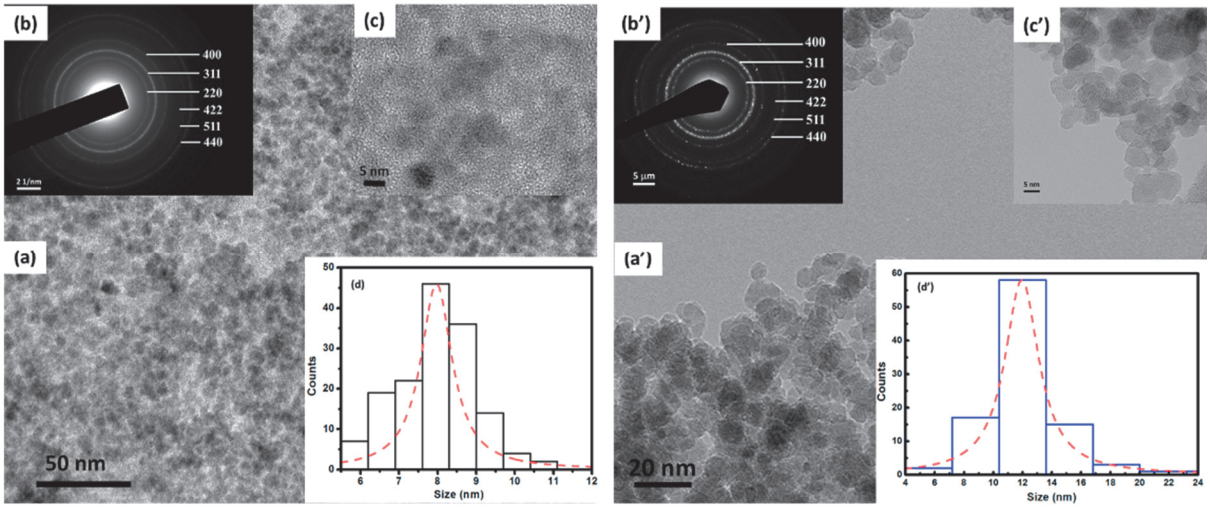

Figure 4 TEM image (a-a') MNP in PVP-CMC Hydrogel, (b-b') SAED pattern of MH, (c-c') high resolution TEM image of spherical biomineralized (Fe_3O_4) magnetic nanoparticles and (d-d') size distribution

Figure 4a shows the TEM image of the spherical MNP embedded in the PVP-CMC hydrogel. Selected area electron diffraction (SAED) patterns for the MH and MNPs was recorded. A ring pattern, with spots showing a large number of randomly oriented crystalline particles was obtained, **Figure 4b**. The high resolution TEM **Figures 4c-c'** shows the lattice fringes corresponding to the (311) planes. **Figures 4d-d'** shows the size distribution for the diameter of the MNP. The edge length of the spherical shaped MH and MNP was measured. It was found that the size ranges between 8 ± 2 nm for MH and 10 ± 2 nm for MNP shown in **figures 4d-d'**.

3.4. Vibrating Sample Magnetometer

The field dependent magnetization (M-H) curves of the MH and MNP at room temperature. Magnetization was measured in the field range of -10000 Oe to 10000 Oe, shown in the **Figure 5**. The saturation magnetization for the MH 13 emu/g whereas, for MNP 77.8 emu/g. This decrease in the saturation magnetization is due to the PVP-CMC polymer network that affects the magnetic property of the biominerilazed (Fe_3O_4) hydrogel.

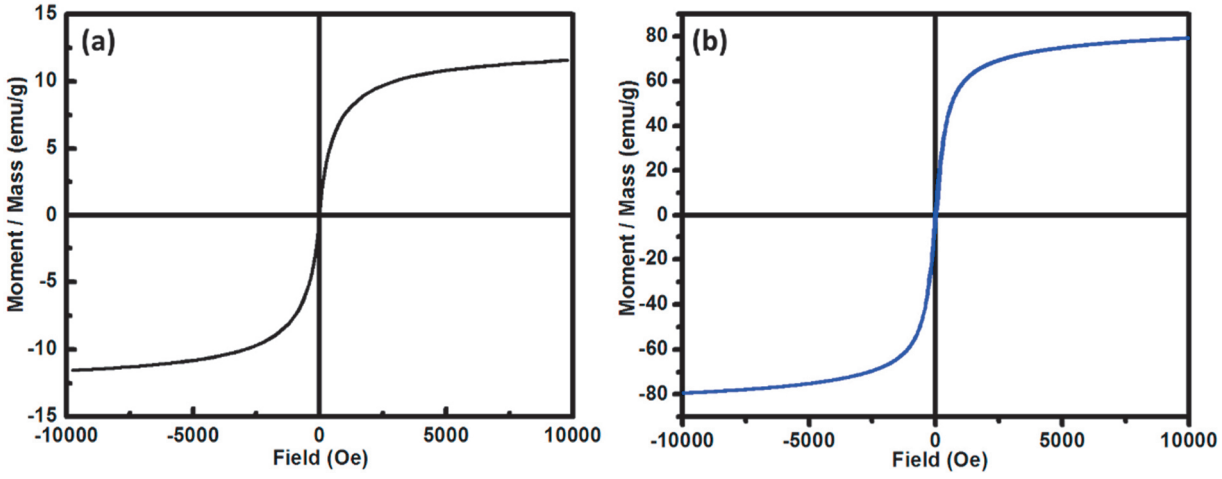

Figure 5 M-H loop for (a) MH and (b) MNP's

4. CONCLUSION

PVP-CMC hydrogel function as a matrix for the preparation of biomineralized (Fe_3O_4) hydrogel / MH as well as perform as a nanoreactor for synthesis of MNPs. In-situ synthesis of MNP and preparation of MH, PVP-CMC hydrogel was used without any surfactant and implementation of any external parameters such as: temperature, pressure, stirring or sonication. By controlling the pore structure of hydrogels, it will be possible to synthesize more narrow size distribution of Fe_3O_4 nanoparticles. The PVP-CMC hydrogel based MNPs synthesis process has practical advantages over the conventional approaches.

ACKNOWLEDGEMENTS

The work was supported by MŠMT ČR-USA Kontakt II (LH14050) and NPUI(LO1504) Ministry of Education, Youth and Sports of the Czech Republic.

REFERENCES

[1] HUANG Z., F. TANG, Preparation, structure, and magnetic properties of polystyrene coated by Fe 3 O 4 nanoparticles, *J. Colloid Interface Sci.*, 2004, vol. 275, no. 1, pp. 142-147.

[2] PEPPAS, N. A., BURES, P. , LEOBANDUNG, W., ICHIKAWA, H., Hydrogels in pharmaceutical formulations, *Eur. J. Pharm. Biopharm.*, 2000, vol. 50, no. 1, pp. 27-46.

[3] LI, Y., HUANG, G., ZHANG, X., LI, B., CHEN, Y., LU, T., LU, T. J., XU, F., Magnetic hydrogels and their potential biomedical applications, *Adv. Funct. Mater.*, 2013, vol. 23, no. 6, pp. 660-672.

[4] LIU, T.-Y., HU, S.-H., LIU, K.-H., LIU, D.-M., CHEN, S.-Y., Preparation and characterization of smart magnetic hydrogels and its use for drug release, *J. Magn. Magn. Mater.*, 2006, vol. 304, no. 1, pp. e397-e399.

[5] GAO, D., XU, H., PHILBERT, M. A., KOPELMAN, R., Bioeliminable nanohydrogels for drug delivery, *Nano Lett.*, 2008, vol. 8, no. 10, pp. 3320-3324.

[6] RAJ, K. , MOSKOWITZ, B., CASCIARI, R., Advances in ferrofluid technology, *J. Magn. Magn. Mater.*, 1995, vol. 149, no. 1, pp. 174-180.

[7] FELFOUL, O., MOHAMMADI, M,. MARTEL, S., Magnetic resonance imaging of Fe $_3$ O $_4$ nanoparticles embedded in living magnetotactic bacteria for potential use as carriers for in vivo applications, *29th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, 2007, pp. 1463-1466.

[8] RAY, A., ALIASKARISOHI, S., FISCHER, T. M., Dynamics of self-assembly of flower-shaped magnetic colloidal clusters, *Phys. Rev. E*, 2010, vol. 82, no. 3, p. 31406.

[9] REDDY, L. H., ARIAS, J. L., NICOLAS, J., COUVREUR, P., Magnetic nanoparticles: design and characterization, toxicity and biocompatibility, pharmaceutical and biomedical applications, *Chem. Rev.*, 2012, vol. 112, no. 11, pp. 5818-5878.

[10] PARMAR, H., DESAI, R., UPADHYAY, R. V, Structural characterization of microwave-synthesized zinc-substituted cobalt ferrite nanoparticles, *Appl. Phys. A*, 2011, vol. 104, no. 1, pp. 229-234.

[11] CHAUDHARY V., RAMANUJAN, R. V, Iron oxide-based magnetic nanoparticles for high temperature span magnetocaloric applications, in *MRS Proceedings*, San Fransisco, 2014, vol. 1708, pp. mrss14-1708.

[12] XIAO, L. LI, J. BROUGHAM, D. F., FOX, E. K., FELIU, N., BUSHMELEV, A., SCHMIDT, A., MERTENS, N., KIESSLING, F., VALLDOR, M., Water-soluble superparamagnetic magnetite nanoparticles with biocompatible coating for enhanced magnetic resonance imaging, *ACS Nano*, 2011, vol. 5, no. 8, pp. 6315-6324.

[13] TRESILWISED, N., PITHAYANUKUL, P., PLANK, C., Factors affecting sizes of magnetic particles formed by chemical co-precipitation, *Mahidol Univ. J. Pharm. Sci.*, 2005, vol. 32, no. 3-4, pp. 71-76.

[14] ROY, N. , SAHA, N. , KITANO, T., SAHA, P., Novel hydrogels of PVP--CMC and their swelling effect on viscoelastic properties, *J. Appl. Polym. Sci.*, 2010, vol. 117, no. 3, pp. 1703-1710.

[15] SAHA, N., ALTOE, V., SHAH, R., BABAYAN, V., ALONI, S., ZUCKERMANN. R. N, SAHA, P., PVP-CMC Hydrogel as a matrix for Biomineralization of Fe3O4 micro-nano particles, *1st Int. Conf. nano FIS 2014-Functional Integr. nano Syst.*, 2014.