2011 11th IEEE International Conference on Nanotechnology
Portland Marriott
August 15-18, 2011, Portland, Oregon, USA

# Effects of Surface Functional Groups on the Aggregation Stability of Magnetite Nanoparticles in Biological Media Containing Serum

Hilda T. R. Wiogo[1], May Lim[1], Volga Bulmus[2], and Rose Amal[1,*]
[1]ARC Centre of Excellence for Functional Nanomaterials, School of Chemical Engineering, The University of New South Wales, Sydney, NSW 2052, Australia
[2]Department of Chemical Engineering, İzmir Institute of Technology, Gülbahçe Urla 35430, Turkey
Email: h.wiogo@student.unsw.edu.au, m.lim@unsw.edu.au, r.amal@unsw.edu.au

***Abstract* — Size stability of magnetite nanoparticles (MNP) with different surface functional groups in biological media was achieved by the addition of fetal bovine serum (FBS). The stability of the particles was attributed to the formation of protein coronas around the particles, which provides sufficient steric hindrance to prevent aggregation of the particles. The stability of different modified MNP were also studied in biological media containing bovine serum albumin (BSA) to further understand the stabilization mechanism. BSA was found to stabilize polyethyleneimine (PEI) modified MNP and polymethacrylic acid (PMAA) coated MNP, but not the bare MNP. These results indicate a difference in interactions between serum protein and the MNP that is govern by the type of functional groups on the MNP surface, with positively charged surface groups resulting higher protein adsorption and better stability.**

***Index Terms – Magnetite nanoparticles, Stabilization, Serum, Albumin, Protein corona, Gel electrophoresis***

## I. INTRODUCTION

One key limitation to the application of magnetic nanoparticles in biomedicine is the tendency of the particles to aggregate, particularly in high ionic strength solutions such as biological media. While considerable success has been achieved in stabilizing magnetic nanoparticles in water, the challenge to produce magnetite nanoparticles with long term aggregation stability in high ionic strength media, particularly in biological fluid, still remains [1].

As such, considerable efforts are being made to produce dispersion of nanoparticles with long term size stability in biological fluid. The stabilisation of magnetic nanoparticles in biological media, however, is particularly challenging due to the additional magnetic force which attracts the particles together and the high ionic strength of the fluid which suppress the electrical double layer [2].

Several recent studies showed that presence of biological serum can improve the aggregation stability in biological conditions [3-6]. In this work, further studies on the long-term stability of magnetite nanoparticles having different surface functional groups in RPMI-1640 solution with the presence of FBS was conducted, focusing on the different stabilization mechanism provided by the interaction between serum protein and different surface functional groups on the particle surface, leading to the design of a simpler, biocompatible, and stable magnetic nanoparticles in biological media.

## II. EXPERIMENTAL

The synthesis of magnetite nanoparticles (MNP) was described in detail elsewhere [7]. Functionalization of MNP were conducted by mixing 3 mg/mL of MNP in Milli-Q water with 20 mg/mL of either cationic polyethylenimine (PEI, molecular weight 25 kDa, PDI 2.5) or anionic polymethacrylic acid (PMAA, molecular weight 20 kDa, PDI 1.12) in equal volume. The pH of particles suspension was adjusted to 10 and 6 for PEI and PMAA modified MNP, respectively, and the suspension was sonicated to improve the dispersion of MNP. Particles-polymer suspension was then mixed in rotating wheel for 24 h, followed by washing of the modified particles three times with Milli-Q water. PMAA used in this work was prepared with living radical polymerisation known as RAFT (reversible addition fragmentation chain transfer) following a similar method described elsewhere [8], with some modification. Briefly, methacrylic acid, RAFT agent, and radical initiator with molar ratio of 280:1:0.1 respectively, were dissolved in 5 mL of methanol. The suspension was purged with nitrogen in an ice bath for 30 min, followed by heating the suspension in an 70°C oil bath for 6 h. Resulting PMAA was purified by repetitice precipitation (at least 3 times) in a vigorously stirred anhydrous diethyl ether.

Adsorption of serum protein onto the surface of MNPs was described in detail elsewhere [6]. Briefly, 125 ppm magnetite particles were prepared in RPMI-1640 solution containing different amount of FBS. The particles suspension was then mixed in rotating wheel for 24 h. Dynamic light scattering (DLS, Brookhaven BI-90 PALS) was used to measure and observe their hydrodynamic size over 16 h period and Transmission Electron Microscope (TEM, JEOL JEM 1400, 100 kV, 55 μA) was used to visualize MNPs suspended in the various media. Proteins on the surface of MNPs that withstand washing were released and analyzed using 1-D gel electrophoresis.

## III. Results and Discussion

Magnetite nanoparticles (MNP) synthesized using co-precipitation method in alkaline conditions resulted in nanoparticles with primary diameter between 10 and 20 nm. The surface modified MNP using either positively charged polyethylenimine (PEI) or negatively charged polymethacrylic acid (PMAA) could be confirmed by the shift of isoelectric point (pI) of particles from 7.7 for the bare MNP to 11.3 and 3.5 for PEI-MNP and PMAA-MNP, respectively (Fig. 1). The tendency for bare MNP to aggregate in water at pH of 7.4 (biological pH) had been successfully prevented by modification of the surface of the particles with polymer, which could maintain the aggregate size of approximately 200 nm for at least 16 h. This is expected to be due to the high absolute zeta potential introduced by the polymeric coating which resulted in stronger electrostatic repulsion between the particles to prevent aggregation of particles, as well as steric hindrance introduced by the presence of polymer. However, the aggregation stability could not be maintained when the modified particles were transferred to a cell culture medium, RPMI-1640, which simulate the ionic strength of biological media (Fig. 2a-c), and the highly aggregated particles were also shown by TEM images of MNPs (Fig. 3a-c).

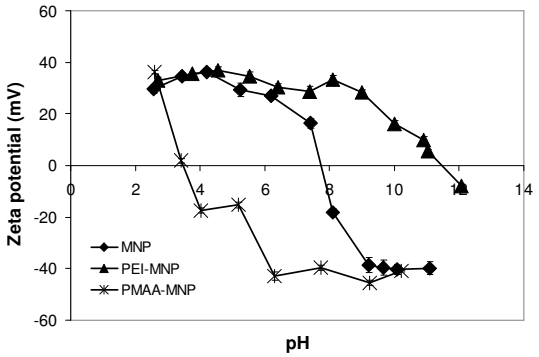



Fig. 1. Isoelectric point (pI) of MNPs measured in 1.5 mM NaCl ionic strength.

The presence of fetal bovine serum (FBS), which contain mixtures of different protein, in RPMI-1640 however, were shown to interact with the surface of particles, forming a protein corona layer around the particles (Fig. 2b), which provide steric hindrance for preventing aggregation of nanoparticles and maintained the aggregate size at approximately 250 nm in RPMI-1640 solution (Fig. 1) for different MNPs. The amount of FBS required for preventing aggregation of particles depends on the functional groups presence on the surface of MNP. The bare MNP, PEI-MNP, and PMAA-MNP required at least 2%, 0.5%, and 6% of FBS in RPMI-1640 solution, respectively, to achieve stable aggregates.

The bare and modified MNPs were also studied in RPMI-1640 solution containing bovine serum albumin (BSA), which is a major component of FBS. The data shows that in the presence of BSA, the aggregation stability of unmodified MNP could not be maintained and the hydrodynamic diameter of particles increased from approximately 300 nm to 600 nm over time (Fig. 2d), although the presence of BSA could slow down the aggregation rate compared to the MNP suspended in RPMI-1640 solution (compared with Fig. 2a). Aggregation of polymer coated MNP however, could be prevented in the presence of BSA only (Fig. 2d). Stabilisation of PEI-MNP in the presence of BSA can be attributed to the different affinity of BSA for the positively charge PEI-MNP due to the negative charge of the BSA molecules. The stability of PMAA-MNP in presence of BSA however, also indicated the interaction between the negatively charged carboxylic acid on the surface of PMAA-MNP and BSA.

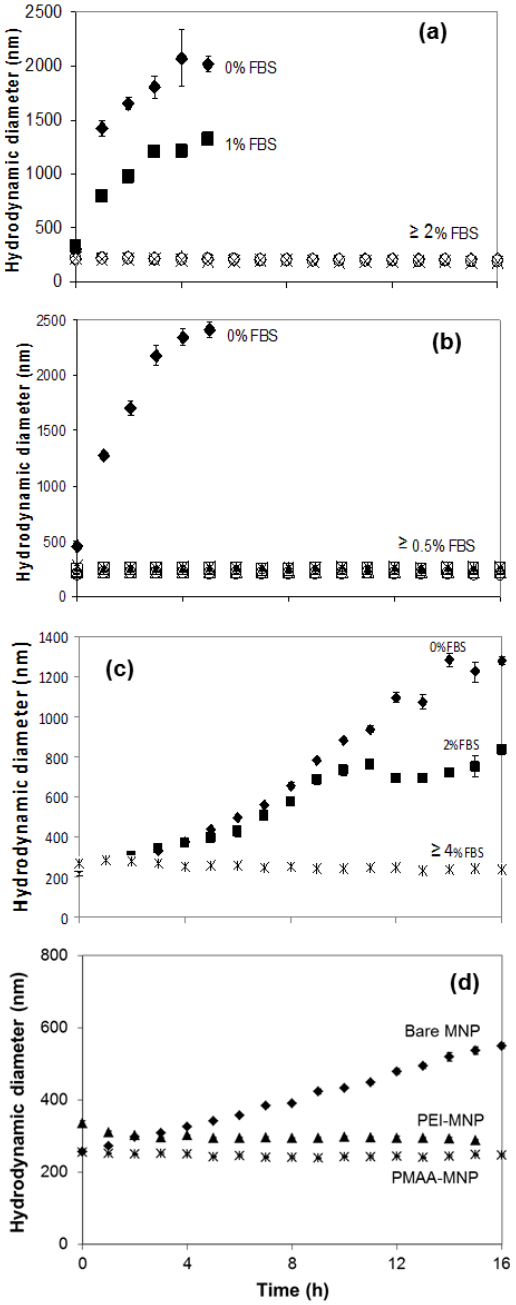



Fig 2. DLS average diameter of (a) unmodified MNP, (b) PEI-MNP, (c) PMAA-MNP in RPMI-1640 solution containing different amount of FBS, and (d) MNPs in RPMI-1640 containing 5% BSA.

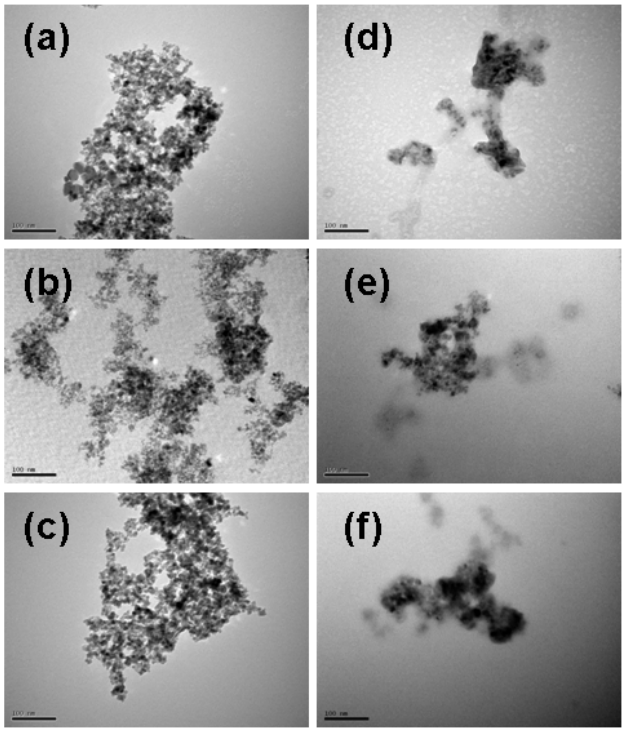
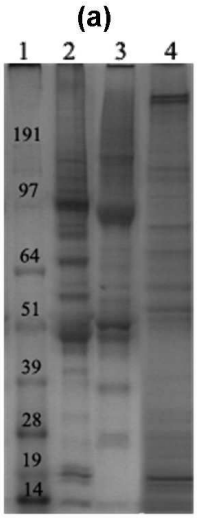



Fig 3. TEM images of (a) unmodified MNP, (b) PEI-MNP, (c) PMAA-MNP in RPMI-1640 solution, and panel (e) to (f) shows the corresponding MNP in RPMI-1640 containing 10% FBS. Scale bars represent 100 nm.

These data suggest that the degree of attraction between the serum proteins and the MNP is governed by the type of functional groups on the MNP surface. When one-dimensional (1-D) gel electrophoresis was carried out on the protein released from comparable amount of MNPs (Fig. 4), the protein bands corresponding to the PEI modified MNP is the most intense, which suggest that it has the highest amount of protein attached, followed by the bare, and then the PMAA-MNP. Note that Fig. 3b shows a three times dilution of the protein released from PEI-MNP as the concentration of protein in the original solution was too high. These results are expected as most serum proteins (e.g. BSA) are negatively charged, and therefore a positive charged surface such as PEI modified MNP would favour protein adsorption. However, in addition to the electrostatic interaction which causes adsorption of protein on oppositely charged surfaces [9], proteins have amphiphilic character which will cause the adsorption of protein onto similar charged surfaces [10], which explain the interaction between BSA and negatively charged PMAA-MNP surface. The slower rate of aggregation of bare MNP indicated the there is also an interaction between BSA and surface of bare MNP, although the aggregation of bare MNP could not be fully prevented. Different molecular weight protein being released from the surface of MNPs is listed in Fig. 4b which showed that different functional groups presents on the surface of MNP affect the type of protein attached.

The amount of protein strongly adsorbed onto the particles surface also determines the aggregation stability of the MNP in biological media. We have previously shown that proteins which have a high affinity will attach more strongly to the MNP surface, forming an enduring "hard" protein corona [6, 11]. A secondary protein layer that interact weakly with the "hard" corona is then adsorbed to form an additional steric layer around the particles ("soft" corona), and it is the latter which lead to the aggregation stability of MNPs in biological fluids [6].

This work shows the different serum protein interaction resulting as the different surface MNPs were introduced into a biological media. Although all of those MNPs showed better stability in serum containing medium, the different interaction between MNPs and protein were evident from the different amount of the protein required for achieving stability of particles and also the amount of protein released after the washing of serum protein conjugated particles. Better understanding on the different protein interaction with MNP having different surface functional groups would allow for a better design of particles surfaces to obtain a stable particles in biological media.




(b)

| Molecular Weight of Protein Released from Different MNPs (kDa) | | |
|---|---|---|
| Bare MNP | PEI-MNP | PMAA-MNP |
| 97 | 97 | 140 |
| 80 | 76 | 80 |
| 66 | 65 | 66 |
| 58 | 60 | 58 |
| 50 | 51 | 51 |
| 43 | 39 | 19 |
| 19 | 28 | 14 |
| 14 | 14 | |

Fig. 4. (a) 1-D gel electrophoresis analysis of molecular weight standard (lane 1), and protein released from surfaces of bare MNP (lane 2), PEI-MNP (3 times diluted, lane 3), PMAA-MNP (lane 4), and (b) tabulated molecular weight of proteins released from different MNPs' surfaces.

## IV. Conclusion

Presence of serum in high ionic strength cell culture medium, RPMI-1640, is shown to improve the stability of magnetite nanoparticles having different surface functional groups. Serum protein adsorption was found highest for PEI-MNP and least for the PMAA-MNP which affects the amount of FBS required for stability of various MNPs in RPMI-1640 solution, with PEI-MNP required the least amount of FBS, followed by bare MNP and PMAA-MNP. The presence of BSA to replace FBS was found to prevent the aggregation of PEI-MNP and PMAA-MNP, but not bare MNP. It is shown that the interaction between protein and particles surface is due to both electrostatic and non-electrostatic factors. The understanding of the different interaction between serum protein and magnetite nanoparticles having different surface functional groups

which had been subjected to numerous research for biomedical applications are required to provide better insight of stabilisation mechanism by serum protein, which would allow for the design of a simpler and safer magnetic nanoparticles.

## ACKNOWLEDGEMENT

This work was financially supported by the Australian Research Council and NanoMaterials Technology Pte. Ltd. through the ARC Linkage program (LP0882720). The authors thank Centre of Advanced Macromolecular Design (CAMD, UNSW) for the GPC facility, Dr. Christopher Marquis and Miss Roslyn Tedja from School of Biotechnology and Biomolecular Sciences (BABS, UNSW) for protein gel electrophoresis facility.

## REFERENCES

[1] S. Laurent, et al., "Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications", *Chem. Rev.*, vol. 108, no. 6, pp. 2064-2110, 2008.

[2] A. K. Gupta, and M. Gupta, "Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications", *Biomaterials*, vol. 26, no. 18, pp. 3995-4021, 2005.

[3] Z. P. Chen, R. Z. Xu, Y. Zhang, and N. Gu, "Effects of proteins from culture medium on surface property of silanes-functionalized magnetic nanoparticles" *Nanoscale Res. Lett.*, vol. 4, no. 3, pp. 204-209, 2009.

[4] Z. P. Chen, et al, "Stability of hydrophilic magnetic nanoparticles under biologically relevant conditions", *J. Nanosci. Nanotechnol*., vol. 8, no. 12 , pp. 6260-6265, 2008.

[5] A. Petri-Fink, M. Chastellain, L. Juillerat-Jeanneret, A. Ferrari, and H. Hofmann, "Development of functionalized superparamagnetic iron oxide nanoparticles for interaction with human cancer cells", *Biomaterials*, vol. 26, no. 15, pp. 2685-2694, 2005.

[6] H. T. R. Wiogo, M. Lim, V. Bulmus, J. Yun, and R. Amal, "Stabilization of magnetic iron oxide nanoparticles in biological media by Fetal Bovine Serum (FBS)", *Langmuir*, vol. 27, no. 2, pp. 843-850, 2011.

[7] Y. S. Kang, S. Risbud, J. F. Rabolt, and P. Stroeve, "Synthesis and characterization of nanometer-Size Fe3O4 and γ-Fe2O3 particles" *Chem. Mater*., vol. 8, no. 9, pp. 2209-2211, 1996.

[8] J. M. Pelet, and D. Putnam, "High molecular weight poly (methacrylic acid) with narrow polydispersity by RAFT polymerization", *Macromolecules*, vol. 42, no. 5, pp. 1494-1499, 2009.

[9] L. Ionov, et al., "Stimuli-responsive command polymer surface for generation of protein gradients", *Biointerphases*, vol. 4, no. 2, pp. FA45-FA49, 2009.

[10] A. Wittemann, and M. Ballauff, "Interaction of proteins with linear polyelectrolytes and spherical polyelectrolyte brushes in aqueous solution", *Physical Chemistry Chemical Physics*, vol. 8, no. 45, pp. 5269-5275, 2006.

[11] I. Lynch and K. A. Dawson, "Protein-nanoparticle interactions", *Nano Today*, vol. 3, no. 1-2, pp. 40-47, 2008.