

Effect of flowrate on the size segregation of magnetic nanoparticles by using continuous flow magnetic separator

Chung Wei Khew¹, Wei Ming Ng², Sim Siong Leong^{1*}, Ling Yong Wong¹, and Pey Yi Toh¹

¹Faculty of Engineering and Green Technology (FEGT), Universiti Tunku Abdul Rahman, 31900 Kampar, Perak, Malaysia

²School of Chemical Engineering, Universiti Sains Malaysia, Nibong Tebal, Penang 14300, Malaysia

Abstract. Magnetic Nanoparticles (MNPs) possess significant potential across various sectors due to their versatility. However, specific MNP sizes are crucial for effective utilization. Traditional methods for synthesizing monodispersed MNPs are often expensive, complex, or environmentally harmful. This study proposes the effect of flow rate on the size segregation of MNPs using a continuous flow low gradient magnetic separator (CF-LGMS). To produce MNPs that are monodispersed, firstly, MNPs undergo functionalization with the polyelectrolyte, poly (sodium 4-styrene sulfonate) (PSS), to enhance colloidal stability. Employing PSS molecules as splicing agents effectively inhibits MNP aggregation. To further enhance the monodispersity of MNPs, continuous flow is facilitated by configuring three separator columns in series, with permanent magnets (NdFeB) attached to the sides. As larger MNPs exhibit stronger magnetic field attraction, resulting in their capture by magnets first, followed by smaller MNPs, and finally the smallest ones. Consequently, MNPs captured in Column 1 possess the largest size, while those in Column 3 have the smallest size. Additionally, this paper investigates the impact of solution flowrate on size segregation efficiency. By varying the solution flow rate (10 mL/min, 15 mL/min, and 20 mL/min), it is found that the performance of the size segregation system is higher under the lower solution flowrate.

1 Introduction

Magnetic nanoparticles (MNPs) is one of the most widely used material in various industries such as medicine, cancer therapeutics, biosensing, catalysis, environment and etc [1]. To effectively employ MNPs in various applications, the ability to synthesis relatively monodispersed MNPs is crucial. For instance, Xu and co-researchers found that to effectively separate tumour cells from fresh whole blood using antibody conjugated magnetic iron oxide nanoparticles, MNPs with size 27 ± 5 nm is crucial [2]. Furthermore, MNPs with a hydrodynamic size of less than 50 nm are indispensable for their effective utilization in theragnostic applications. Ensuring their capability for extravasation and stability against uptake by the reticuloendothelial system (RES) hinges on hydrodynamic size <50 nm [3].

*Corresponding author: leongss@utar.edu.my

Thus, it is incontrovertibly true to state that the functionalization of MNPs that is relatively monodispersed is significant for the effective implementation of MNPs.

As mentioned, studies showed the necessity and significance of specific sizes of MNPs in various applications but ways to synthesize monodispersed MNPs are usually expensive, complicated, or not environmentally friendly. One of the most popular synthesis methods for the MNP system is the co-precipitation method, as a case in point, serves as support for the above statement. As co-precipitation method involves reacting magnetite formation in an alkaline solution. An anaerobic reaction condition must be created to prevent the conversion of magnetite to iron (III) oxide and then iron (III) hydroxide. Thus, an inert atmosphere must be created in this method [4]. Moreover, the thermal decomposition method is another famous method to synthesize MNPs. In the thermal decomposition method, complex synthesis procedure which is time-consuming is required. To make the condition worse, the thermal decomposition method is said to be more difficult to scale than the co-precipitation reaction. Also, thermal decomposition involves a lot of reagents and playing around with temperature. In other words, precise control of the proportions of reagents and temperature is required. Most importantly, the MNPs prepared using this method are only dispersible in non-polar solvents, for example, hexane and are not miscible with water [4]. Although surface functionalization of commercially available MNP powder with polyelectrolyte can produce MNPs with a moderate degree of polydispersity, it is a relatively eco-friendly and low-cost method to obtain MNP system with a sufficiently good degree of monodispersity. Thus, in this work, the magnetophoresis process is proposed to segregate the polyelectrolyte-functionalized MNPs according to their size using a low gradient magnetic separator (LGMS).

In contrast to a high gradient magnetic separator (HGMS) which usually involves a more complicated setup, LGMS does not require an intricate setup of an electromagnet, merely a handheld permanent magnet with a magnetic field gradient of less than 100 T/m is required [5]. Additionally, LGMS also has been proven to be feasible in performing size segregation of MNPs under a batchwise mode [6]. However, LGMS that is incorporated with continuous flow (CF) feature is expected to enable this segregation process to be conducted in a less hassle manner and decrease the workforce demand. As the separation pathway of MNPs is governed by its size, a continuous flow-low gradient magnetic separator (CF-LGMS) process is employed to segregate MNPs according to the size in this project. Unlike batch processing, it does not require intensive manpower to closely monitor the process and to start up the process after one batch of solution is segregated. Hence, it has the higher productivity as it can process more solutions within shorter time. Apart from that, the implementation of continuous flow in the LGMS process to perform MNP size segregation also can provides a better good quality control on the MNP fractions produced. Despite of the great advantages, the major disadvantage of continuous flow processing is that there is a higher risk of contamination of materials, which usually occurs due to improper implementation and use. However, due to the decreased cost, increased quality and increased productivity, continuous flow mode outperformed the batch wise LGMS process in performing MNP size segregation, hence, CF-LGMS is chosen as the system to execute MNP size segregation in this work.

2 Methodology

Functionalization of poly(sodium-4-styrenesulphonate) (PSS) powder onto the surface of MNPs were performed using grafting-to-technique [6]. First, 0.05 g of PSS powder and 0.05 g of MNPs were dispersed into ultrapure water. The solutions were sonicated for 20 minutes before undergoing pH adjustment to 3.5 ± 0.5 . The solutions were then whirl mixed in a 50 mL container. The mixed solutions were then being put into end-to-end rotator for 24 hours. The PSS-grafted MNPs were magnetically decanted, and the excess PSS were discarded.

Next, it was followed by washing step of resuspending the PSS-grafted MNPs into ultrapure water, ultrasonicated for 20 minutes, and centrifuging at 4000 rpm for 40 minutes. The washing steps were repeated for 3 times.

To obtain the PSS-functionalized MNP with the highest colloidal stability for the subsequent size segregation study, surface functionalization of MNPs using PSS (poly-sodium-4-styrenesulphonate) powder was performed in mass ratio of MNPs to PSS of 1:1. For the examination of the colloidal stability of PSS-functionalized-MNPs, two actions were taken: sedimentation test and dynamic light scattering (DLS) tests. Sedimentation tests were performed and functionalized MNPs which exhibits the longest sedimentation time was chosen as the optimum ratio. To further verify the results of sedimentation tests, DLS was performed to have a precise result on the hydrodynamic size (h_d) of the functionalized MNPs.

Figure 1 shows the experiment setup used in this study, which involves 3 separator columns connected in series form with magnets attached to the side. PSS-functionalized-MNP solution was pumped into the system using a peristaltic pump connected with tubes. After that, the columns were removed from the systems and with magnets detached, the MNPs that stayed in the column will be flushed out using ultrapure water. MNPs collected was sent for characterization such as Scanning Electron Microscopy (SEM), Energy Dispersive X-ray (EDX), and Vibrating Sample Magnetometer (VSM). Table 1 summarises the parameters used in the experiment.

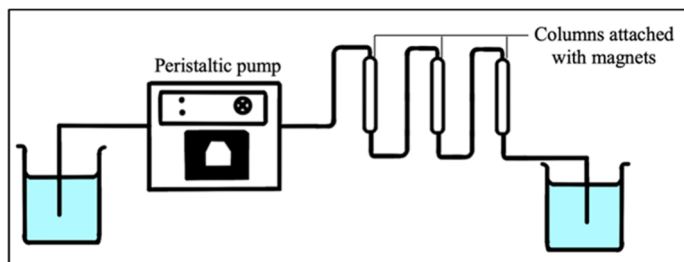


Fig. 1. Schematic diagram of experiment setup.

Table 1. Parameters used in the experiment.

Separator column dimension (internal diameter; length of column)	25 mm; 128 mm
Concentration of PSS-functionalized MNPs used in experiment	50 mg/L
Number of magnets attach on 1 column	4
Magnet arrangement	Opposite but misaligned (2;2)
Flowrate of PSS-grafted MNP solution	10, 15, and 20 mL/min

3 Results and discussion

By comparing both SEM images of pre- and post-functionalized MNPs, it can be found that there is a decrease in the amount of aggregate for the post-functionalized MNPs, as shown in Figure 2a and Figure 2b. This is due to the functionalization of PSS onto the surface of MNPs that obscure the magnetic attraction between MNPs particles to form larger MNP aggregates, which in turn reducing the average hydrodynamic size and at the same time enhancing the colloidal stability of the MNPs.

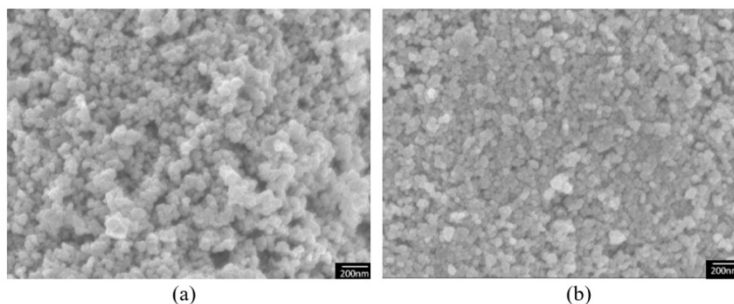


Fig. 2. SEM image of MNPs used in this study: (a) before functionalization (b) after functionalized with PSS.

Table 2 shows that the weight percentage of the element carbon (C) and sulphur (S) increased. This indicates the successful functionalization of PSS given that the linear formula of PSS is $(C_8H_7NaO_3S)_n$. This scenario subsequently leads to the decrease in weight percentage of oxygen (O) and ferrite (Fe) elements. Figure 3 shows the EDX spectrum of pre- and post-functionalized MNPs.

Table 2. Details of EDX results.

Element	Before functionalization		After functionalization	
	Approximate Concentration	Weight Percentage	Approximate Concentration	Weight Percentage
Ca	1.11	13.35	2.41	19.74
O	13.32	52.79	15.02	52.00
Na	0.00	0.00	0.00	0.00
S	0.03	0.09	0.07	0.16
Fe	12.20	33.77	13.58	28.11

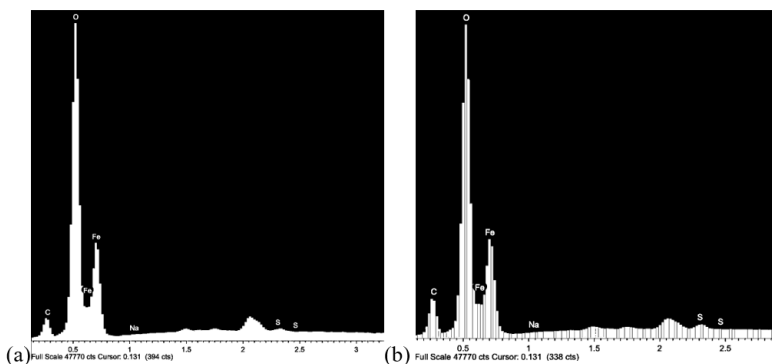


Fig. 3. EDX Spectrum of MNPs (a) before functionalization and (b) after functionalized with PSS.

According to the VSM results in Figure 4, it can be noticed that the unmodified MNPs present a clear hysteresis loop with coercivity (H_c) of 108.18 G and retentivity (M_r) of 12.777 emu/g, which indicates that the particles are non-superparamagnetic in nature. The hysteresis loop can be clearly observed in the magnetization curve of functionalized MNPs too, but it is slightly narrower with H_c of 105.39 G and M_r of 13.235 emu/g. The saturation magnetization of unmodified MNPs and functionalized MNPs was measured as 89.215 emu/g and 77.292 emu/g, respectively. The slightly lower saturation magnetization values of functionalized MNPs as compared to unmodified MNPs is due to the decrease in the mass fraction of the magnetic material (MNPs) after the incorporation of non-magnetic macromolecule during the functionalization process [6].

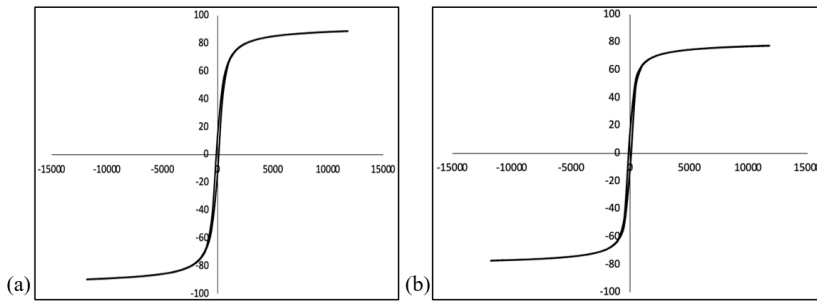


Fig. 4. The magnetization curve of (a) unmodified MNPs and (b) PSS-functionalized MNPs is derived from the VSM measurement.

The effectiveness of the CF-LGMS setup in the size segregation of MNPs is examined using the standard deviation of the MNP fractions collected in each column, which is calculated by the following equations:

$$\mu = \frac{\sum Ix}{\sum I} \tag{1}$$

$$\sigma = \sqrt{\frac{\sum Ix^2}{\sum I} - \mu^2} \tag{2}$$

where μ is the average of the hydrodynamic size of MNP fraction, I is the intensity of MNP clusters with size of x and σ is the standard deviation of hydrodynamic size of MNP fraction. The average and standard deviation of the hydrodynamic size of the MNP fractions collected in each column are shown in Tables 3 and 4, respectively. The size distribution of MNPs from each fraction is illustrated in Figure 5.

Table 3. Average hydrodynamic size of MNP clusters (in nm) in each column after size segregation.

	10 mL/min	15 mL/min	20 mL/min
Column 1	536.1	375.7	619.5
Column 2	419.6	354.5	504.8
Column 3	273.4	283.5	374.4

Table 4. Standard deviation of hydrodynamic size of MNP clusters (in nm) in each column after size segregation.

	10 mL/min	15 mL/min	20 mL/min
Column 1	98.41	273.54	312.00
Column 2	96.98	157.99	208.06
Column 3	79.73	83.08	83.73
Total	360.59	514.61	603.79

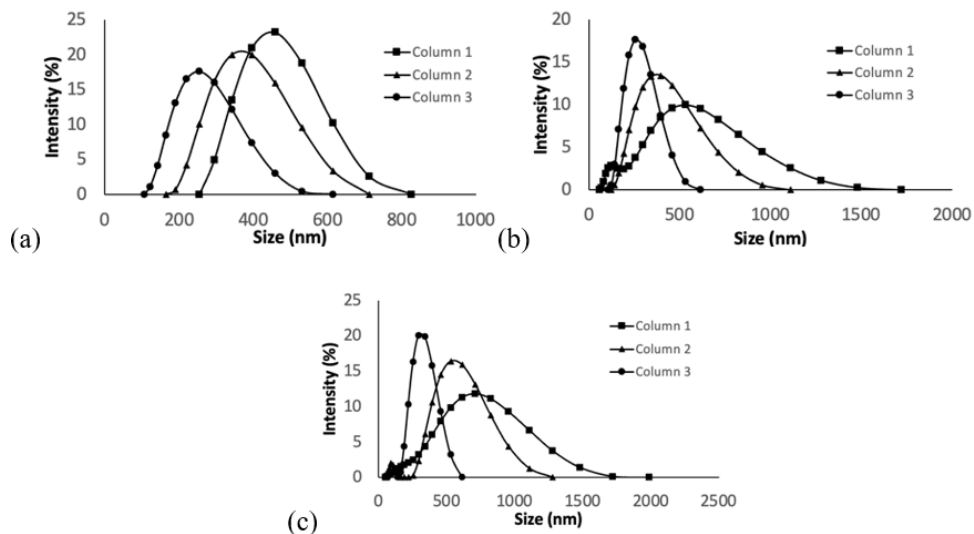


Fig. 5. Size distribution graphs of (a) 10 mL/min, (b) 15 mL/min, and (c) 20 mL/min.

Table 3 illustrates a noticeable trend of decreasing average hydrodynamic size for PSS-functionalized-MNPs after size segregation. As depicted in Figure 5, the peak of the size distribution graph gradually shifts leftward from Column 1 to 3. This indicates a gradual reduction in the hydrodynamic size of the PSS-functionalized-MNPs as they pass through Columns 1 to 3.

From Table 4, it is evident that the standard deviation of size distribution of MNP fraction captured in the downstream column (Column 3) is smaller as compared to those being captured in the upstream column (Column 1). This suggests an increase in the monodispersity of the size distribution of PSS-functionalized-MNPs after passing through the CF-LGMS.

Finally, to facilitate a more intuitive comparison on the effect of flowrate on the performance of CF-LGMS in MNP size segregation, the standard deviation of the MNP fractions captured in each column serves as the judgement criterion. This parameter was calculated by summing up the standard deviation of MNP fractions retained in each column of the CF-LGMS setup, as demonstrated in the last row of Table 4. By this comparison, the optimum flow rate for size segregation is determined to be 10 mL/min based on this study. This conclusion is drawn from Table 4, where the total standard deviation at 10 mL/min is the lowest (360.59 nm) as compared to 15 mL/min and 20 mL/min, with 15 mL/min being next (514.61 nm), and 20 mL/min being the highest (603.79 nm). This trend is attributed to the fact that a lower flow rate allows for a longer retention time of MNPs within the separator column, which gives the longer duration for the size segregation to occur within the LGMS column. Conversely, a higher flow rate results in a shorter retention time, leading to MNPs being flush out from the column before adequate segregation can occur.

The bumps on the graphs may be attributed to human errors during the sample collection process. Given that the separator columns are of different sizes, ensuring complete rinsing of all collected MNPs during the washing process was challenging. Consequently, there may have been residual material from previous experiments left in the separator column, resulting in impurities in the sample.

4 Conclusion

This study successfully demonstrates the effectiveness of the continuous flow low-gradient magnetic separator (CF-LGMS) in achieving size segregation of poly(sodium 4-styrene

sulfonate) (PSS)-functionalized magnetic nanoparticles (MNPs). The functionalization of MNPs with PSS significantly reduced aggregation, enhancing the colloidal stability of the nanoparticles. Size segregation was influenced by the solution flow rate, with the system exhibiting higher monodispersity at lower flow rates. The results indicate that a flow rate of 10 mL/min achieved the most efficient size segregation, yielding MNPs with the smallest standard deviation in size distribution. This is attributed to the longer retention time of the particles in the columns, allowing for more effective separation based on size. Conversely, higher flow rates of 15 mL/min and 20 mL/min resulted in larger standard deviations, likely due to the reduced retention time, leading to incomplete segregation. Overall, CF-LGMS proves to be a feasible and efficient approach for continuous size segregation of MNPs, with potential applications in various industrial and medical fields where precise control of nanoparticle size is critical. Future work could explore optimizing magnet configurations and refining flow conditions to further enhance the system's segregation capabilities.

References

1. A. Ali, T. Shah, R. Ullah, P. Zhou, M. Guo, M. Ovais, Z. Tan, Y. Rui, Review on recent progress in magnetic nanoparticles: Synthesis, characterization, and diverse applications. *Front. in Chem.*, **9**, (2021). <https://doi.org/10.3389/fchem.2021.629054>
2. H. Xu, Z.P. Aguilar, L. Yang, M. Kuang, H. Duan, Y. Xiong, H. Wei, A. Wang, Antibody conjugated magnetic iron oxide nanoparticles for cancer cell separation in fresh whole blood, *Biomat.*, **32**, 9758–9765, (2011). <https://doi.org/10.1016/j.biomaterials.2011.08.076>
3. D. Ho, X. Sun, S. Sun, Monodisperse magnetic nanoparticles for Theragnostic Applications, *Acc. of Chem. Res.*, **44**, 875–876, (2011).
4. K. Mylkie, P. Nowak, P. Rybczynski, M. Ziegler-Borowska, Polymer-coated magnetite nanoparticles for protein immobilization, *Mat.*, **14**, 248, (2021). <https://doi.org/10.3390/ma14020248>
5. S.S. Leong, S.P. Yeap, J. Lim, Working Principle and application of magnetic separation for biomedical diagnostic at high- and low-field gradients, *Inter. Foc.*, **6**, 20160048, (2016). <https://doi.org/10.1098/rsfs.2016.0048>
6. S.P. Yeap, S. S. Leong, A.L. Ahmad, B.S. Ooi, J. Lim, On size fractionation of iron oxide nanoclusters by low magnetic field gradient, *The J. of Phys. Chem. C*, **118**, 24042–24054, (2014). <https://doi.org/10.1021/jp504808v>
7. General Kinematics, The differences between batch processing and continuous flow processing, *Gen. Kinematics*. (2019). <https://www.generalkinematics.com/blog/batch-processing-vs-continuous-flow/>