Observation of the dynamics of magnetically induced chains of sub-micron superparamagnetic beads in aqueous solutions by laser light scattering

Y Tanizawa1,*, T Tashiro1, P J Ko2, and A Sandhu1,2
1 Department of Electrical and Electronic Information Engineering, Toyohashi University of Technology, 1-1 Hibarigaoka, Tempaku-cho, Toyohashi, Aichi 441-8580, Japan
2 Electronics-Inspired Interdisciplinary Research Institute (EIIRIS), Toyohashi University of Technology, 1-1 Hibarigaoka, Tempaku-cho, Toyohashi, Aichi 441-8580, Japan
E-mail: tanizawa-y@eiiris.tut.ac.jp

Abstract. Optical monitoring the behaviour of magnetically induced self-assembled chains of superparamagnetic beads (SPBs) are of interest for biomedical applications such as biosensors. However, it is difficult to directly monitor magnetically induced self-assembly of sub-micron nano-beads with conventional optical microscopes. Here, we describe the optical observation of the dynamics of magnetically induced self-assembled rotating chains of 130 nm SPBs in aqueous solutions by laser light scattering. Magnetic fields of ~1 kOe were applied to control the self-assembly chains of SPBs and their behaviour analyzed by monitoring the intensity of laser light scattered from the chain structures. We compared the light scattering from chains that were formed only by the application of external fields with chains formed by beads functionalized by EDC, where chemical reactions lead to the bonding of individual beads to form chains. The EDC experiments are a precursor to experiments on molecular recognition applications for biomedical diagnostics.

1. Introduction
Superparamagnetic beads (SPBs) are used for a wide range of biomedical applications including drug delivery, magnetic separation, magnetic resonance imaging (MRI), and enzyme-linked immunosorbent assay [1, 2]. The size of SPBs can be controlled to be comparable to actual biological materials such as proteins (10 –100 nm), viruses (20 –450 nm), and bacteria (0.2 μm –5 μm). Notably, SPBs offer the following unique properties for medical diagnostic applications: SPBs can be manipulated by external magnetic field gradients; external magnetic fields applied to the SPBs are not screened by biological materials or liquids; and it is easy to functionalize SPBs with antibodies, enzymes, proteins, and ligands [2]. To-date, research on the dynamics of SPBs in aqueous solutions has been limited to optical observations of micrometer sized SPBs with conventional white light microscopes [1, 3]. However, the spatial resolution of such methods is limited to the micrometer range, thus optical microscopes are not applicable for monitoring the behavior of sub-micrometer magnetic beads.

Here, we describe the use of laser light scattering for monitoring COOH-functionalized 130 nm sized SPBs (nanobeads), where changes in the amplitude of magneto-optical scattering (MS) from magnetic beads in an aqueous solution enabled the quantitative determination of the concentration of
1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) in the solution. Furthermore, we tracked single SPBs in solution to monitor the interaction of small numbers of SPBs under external magnetic fields. These experiments enabled the determination of the concentration of EDC by monitoring rotating SPBs functionalized with carboxylic acid (COOH) in less than five minutes.

2. Methods and Experimental

The experiments were conducted using a modified laser light scattering microscope with integrated electromagnetic coils for producing rotating magnetic fields (Figure 1). The laser light scattering microscope enabled the real-time tracking nano-SPBs dispersed in aqueous solutions. The 635 nm line of the laser was guided to pass through the sample of liquid containing the magnetic nanobeads. The laser beam is refracts at the interface between the liquid and the optical element through which the light passes, with the light travelling parallel to the glass-sample interface. Nano-SPBs in the path of the laser beam, which is approximately 100 μm wide and 15 μm deep, are visualized by x20 microscope objective lens of an optical microscope aligned at 90 degrees to the laser beam axis. The microscope is fitted with a CCD camera, which collects the light scattered by particles in the field of view [4, 5]. During the experiments, we collected the intensity of scattered light and monitored the structures of the diffraction patterns, frame-by-frame during the measurements. The measurements were made using in-plane rotating fields up to ~1100 Oe with rotation frequencies of 0–5 Hz.

The experiments were conducted using 130 nm diameter superparamagnetic beads (Nanomag-D, Micromod) with concentrations of $15.8 \times 10^8$ and $6.10 \times 10^8$ particles/mL. The SPBs were functionalized with carboxyl groups.

After dilution and ultrasonic cleaning the nano-SPBs suspensions were slowly introduced into the observation chamber. Next, the field was applied, the focus adjusted, and images of 100 μm × 80 μm area recorded with a 640 × 480 pixel (8 bit) CCD camera operating 30 frames per second. The images were processed using ImageJ software [6] to determine the scattering area and intensity of light scattered by each SPB and chains formed by the external field.

In the 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) experiments, the nano-SPBs were dispersed in deionized (DI) water at 2000-times dilution, where the concentrations of the EDC aqueous solution were 0.001, 0.01 and 0.2 M in DI water. The EDC aqueous solution was added to the nano-SPBs solution until the ratio was SPBs : EDC = 10 : 1. The mixture was shaken for five times and introduced into measurement chamber of the laser scattering microscope focused on the nano-SPBs in solution. Next, the external magnetic field applied and images recorded. The external magnetic field was rotated at $f = 0.1$ Hz, $H = 400$ Oe and was turned off after recording for 3, 5, and 7 min after mixing the solutions. The video data were converted to the scattering area data by processing the images with ImageJ software. We compared the results of the scattering area data with and without EDC.

![Figure 1. Schematic of the laser light scattering microscope integrated with four electromagnetic coils.](image-url)
3. Results and Discussion

3.1. Observation of two nano-SPBs

Figure 2 (a) shows tracking of two nano-SPBs in solution without an external rotating magnetic field for several video frames. Frame 4 shows two 130 nm SPBs (A and B), getting close to each other. In frame 5, the center to center distance approaches the sum of the individual diameters of SPBs A and B, resulting in the aggregation into structure C. In frame 8 the structure C separates back into the single SPBs A and B. The movement of SPBs in the liquid suspension is due to Brownian motion. The variation of scattered light intensity with function of time is shown in Figure 2 (b).

![Figure 2](image)

**Figure 2.** Tracking of two nano-SPBs in solution without an external rotating magnetic field. (a) Image of tracking of two SPBs at frame-by-frame, (b) Intensity of scattered light from the two SPBs being tracked as function of number of frames.

3.2. Comparison of with and without EDC under an external rotating magnetic field

Figure 3 shows intensity of light scattered by chains formed by 130 nm SPBs under an external rotating magnetic field of 1000 Oe at 0.1 Hz with respect to angle (θ). The maximum (Smax) and minimum (Smin) optical scattering for a given external rotating magnetic field occurred along the lowest (θ = 0, 180, 360,...) and highest (θ = 90, 270,...) scattering intensity with respect to the incident laser light, respectively. The light scattering by SPBs chains depends on the angle (θ) between
the direction of SPBs chain and the incident laser light. Here, we define the magneto-optical scattering (MS) effect in terms of $\Delta S = (S_{\text{max}} - S_{\text{min}}) / S_{\text{max}} \times 100$, where the $S_{\text{min}}$ and $S_{\text{max}}$ are the optical scattering at $\theta = 0^\circ$ and $90^\circ$, respectively. The video frame images used to monitor the scattering of SPB chains under rotating fields at $\theta = 0^\circ$ and $90^\circ$ are shown in Figure 3 (b).

Figure 3. (a) Optical scattering of 130 nm SPBs with respect to the angle ($\theta$) between the external magnetic field and the incident laser light at $\theta = 0^\circ$ and $\theta = 90^\circ$. (b) Video frames of the optical scattering of SPBs at $\theta = 0^\circ$ and $\theta = 90^\circ$, respectively.

Figure 4 shows $\Delta S$ of SPBs functionalized with carboxylic acid (COOH) containing EDC (0, 0.001, 0.01 and 0.2 M) as a function of the time after applying the external rotating field. The $\Delta S$ of non-EDC increased due to Brownian motion in magnetically-induced chains due to chain-chain interactions and the formation of thicker, column-like chains. Notably, with increasing EDC concentration, $\Delta S$ increased significantly due to forces resulting from COOH-EDC conjugation [7] contributing significantly to enhancing the bead to bead bonds in the rotating SPB chains, which leads to an increase in $\Delta S$. Thus, biomedical applications are possible by monitoring changes in the $\Delta S$ corresponding to COOH-EDC conjugation due to the addition of EDC into the colloidal solution.
4. Conclusions
We described the scattering of laser light from individual and chains of 130 nm SPBs in aqueous solutions under rotating magnetic fields. Coalescence of individual nano-SPBs was clearly observed. Furthermore, we observed a clear relationship between $\Delta S$ and the mean length of chains of 130 nm SPBs in terms of the laser light scattering intensity. The addition of EDC to solutions containing functionalized 130 nm SPBs led to clear increases in $\Delta S$, due to enhanced forces from COOH-EDC conjugation between bead to bead bonds in the rotating SPB chains. Finally, these results may find applications in biomedical diagnostics.

Acknowledgments
This work was supported in part by a grant from the Ministry of Education, Culture, Sports, Science, and Technology, Japan in the form of the Special Coordination Funds for Promoting Science and Technology for the Integrated Research Institute.

References
[1] Park S Y, Handa H and Sandhu A 2010 IEEE Trans. Nano Lett. 10 446-451
[2] Marszał M P 2011 Pharm. Res. 28 480–483
[3] Anik K V, Antonio A G and Mark A H 2003 Langmuir 19 8646-8653
[4] Carr B, Hole P, Malloy A, Nelson P, Wright M and Smith J 2009 European Journal of Parenteral & Pharmaceutical Sciences 14(2) 45-50
[5] Carr R, Smith J, Nelson P, Hole P, Malloy A, Weld A and Warren J 2008 J. Nanopart. Res.
[6] Rasband, W.S., U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2012.
[7] Eric V and Mark B 2009 Chem. Soc. Rev. 38 606–631