ON USING OF NANO-SIZED ROD-LIKE MAGNETITE PARTICLES FOR DETERMINATION OF BIOLOGICAL BINDING REACTIONS♦

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Abstract: Rod-like nano-sized magnetite particles have been prepared by direct precipitation from aqueous solutions in the presence of an external magnetic field. Using these particles we prepared ferrofluids which become birefringent when a magnetic field is applied perpendicular to the optical axis of light impinging the fluid. After switching off the magnetizing field, the birefringence relaxes. We observed that for ferrofluids containing rod-like magnetite nanoparticles the dominant relaxation mechanism is the Brownian motion. Since the constant of the Brownian relaxation time depends on the hydrodynamic size of the particles it can be determined by transient magnetic birefringence measurements.
Considering the fact that biological binding reactions are always connected to changes in the particle size of the reaction components, our work focuses

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on the monitoring of binding reactions. For the determination of the binding reaction between an antibody and its antigen the magnetic nanoparticles were conjugated with streptavidin. The biotinylated antibody against human immunoglobulin M (hIgM) was attached to the nanoparticles via the binding between biotin and streptavidin. The presented experiments confirm that the determination of the relaxation of the transient field-induced birefringence of rod-like magnetic nanoparticles can be used for the investigation of binding reactions of antibodies to their antigens.

Keywords: *ferrofluids; rod-like nanoparticles; birefringence relaxation; biological binding.*

INTRODUCTION

Magnetic nanoparticles (MNP) possess increasing importance as diagnostic and therapeutic tools in medicine, as well as in cellular biology [1, 2]. The combination of MNP with biologically active molecules, such as proteins, peptides, receptor ligands or antibodies builds the most promising concepts of biomedical applications. A wide variety of magnetic immunoassays are applied in vitro, using the magnetic forces to track and separate magnetically labeled biomolecules, cells or targeted organelles.

A methodology for the detection of binding of biomolecules to magnetic nanoparticles in a suspension exploits the time-dependence of magnetooptic properties (birefringence) of a magnetic colloidal suspension (ferrofluid) [3, 4]. Magnetic fluids comprise suspensions of (MNP) in either aqueous or organic fluids. In order to obtain a stable dispersion of magnetic particles in an aqueous medium, the characteristics of the particle surface have to be tailored to the medium. This has been shown to stabilize the magnetic particles against aggregation and can produce a biocompatible fluid. Enzymes and other biomolecular recognition elements and receptors can be covalently bound to the organic polymers thus creating composite structure particles that can act as magnetic labels in aqueous media.

It is known that ferrofluids become birefringent when a magnetic field is applied perpendicular to the optical axis, as the MNP contained in the ferrofluid tend to align in the direction of the external field. This causes an optical anisotropy (Cotton-Mouton effect). After switching off the magnetizing field a relaxation of the optical birefringence can be observed. The mechanism of birefringence relaxation is the Brownian rotation of MNP. Recently it was demonstrated that magneto-optical relaxation measurements can be used for the determination of binding reactions of biological molecules attached onto MNP [5].

Based on the fact that biological binding reactions are always connected to changes in the particle size of the reaction components, the aim of the present study was to evaluate this novel approach, using bioconjugated ferrofluids and consisting in measurements of birefringence relaxation.

THEORETICAL BACKGROUND

The origin of the magnetobirefringence (Cotton-Mouton effect) in ferrofluids has been mainly associated to the combined effects of field induced orientation of anisotropic isolated nanoparticles and pre-existing particle agglomerates in suspension, and of field-dependent particle chain formation. From a statistical mechanical viewpoint, the birefringence Δn is proportional to H^2 when the interaction between the magnetic moment of each colloidal particle and the external field is dominant for the magnetic birefringence of the fluid [6], but Δn is linearly proportional to H in the case that the interaction among the particles themselves must be taken into account [7].
We prepared a diluted ferrofluid containing in suspension rodlike magnetite nanoparticles. Due to predominant shape anisotropy the particles' magnetic moments are linked to an easy axis and in the presence of an applied magnetic field a preferred individual orientation may be induced, resulting in an optical birefringence. When the applied field is set equal to zero, the orientations of the rodlike MNP randomize, resulting in a decay of the optical birefringence. Assuming that the MNP are cylindrical with all principal axes of the optical anisotropy and rotational diffusion tensor coinciding, the birefringence relaxation is determined by a single exponential term

$$\Delta n = \Delta n_0 \exp(-6D_r t) \tag{1}$$

where Δn_0 is a function of the intensity of the applied field before its turning off and D_r is the rotational diffusion constant about an axis perpendicular to the cylindrical axis. The diffusion constant D_r can be written as [8]:

$$D_r = \frac{3kT}{\pi\eta L^3}\left(\ln\frac{L}{d} - \gamma\right) \tag{2}$$

where k is Boltzmann's constant, T is temperature, η is the viscosity of the medium, L the length of the cylinder, and d the width. The parameter γ refers to end effects and depends upon the aspect ratio L/d. For sufficiently large L/d, the γ term becomes inconsequential; for small aspect ratios it can be used in the form [9]:

$$\gamma = 0.662 - 0.92\left(d/L\right) \tag{3}$$

With known values for the viscosity η of the ferrofluid, the temperature T, Boltzmann's constant k and D_r determined from the magnetobirefringence relaxation measurement it is possible to calculate the aspect ratio L/d of the particles and thus to monitor the biological binding reactions.

MATERIALS AND METHODS

The aqueous ionic ferrofluid, used in this work, was prepared by coprecipitation of Fe(II) hydroxide followed by heating at 90 ^{0}C in the presence of a uniform magnetic field of 400 kA/m [10]. The obtained magnetite (Fe_3O_4) particles was then acidified, oxidized at maghemite (γ Fe_2O_3) and dispersed into water leading to an acidic ferrofluid composed of magnetic particles positively charged with nitrate counterions. Scanning

electron microscopy images were performed to analyze the morphology of the particles and the dimensional distribution. For the determination of the binding reaction between an antibody and its antigen the MNP of the ferrofluids were conjugated with streptavidin. The biotinylated antibody against human immunoglobulin M (hIgM) was attached to the nanoparticles via the binding between biotin and streptavidin. For the magneto-optical measurements different amounts of hIgM (10^{-1} – 10^{4} μg) were added to the streptavidin conjugated MNP (10 μg, calculated as Fe) at each case.

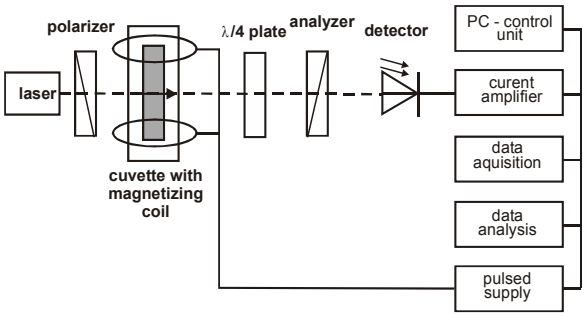

Figure 1. *The measurement setup*

The measurement setup (Figure 1) consists of a He-Ne laser of weak power (~ 5 mW) and wavelength 632.8 nm, a polariser, aligned orthogonal to an analyser at 45^{0} to the magnetic field lines, a retardation plate with its slow axis parallel to the polariser and a cuvette containing the sample, placed into a pair of Helmholtz coils generating a variable pulsed magnetic field of up to 10 kA/m (0.1 Hz, 2 s magnetization time). After switching off the magnetising field the relaxation of the birefringence is measured by a PIN-photodiode connected to a variable-gain low-noise current amplifier. The system is controlled by a PC. A high-speed multifunction data acquisition board records and processes the data.

RESULTS

Figure 2 shows a typical transient magnetization pulse and the corresponding transient birefringence signal of a simple ferrofluid.

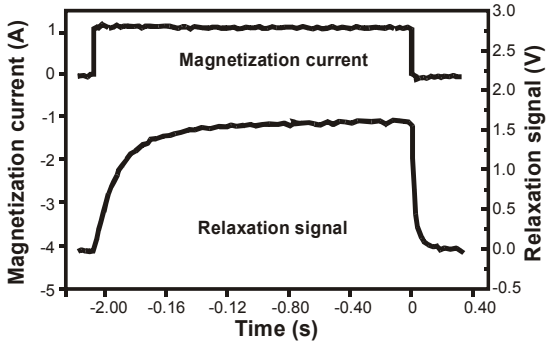

Figure 2. *Transient measurement of magnetic field and the corresponding relaxation signal of a simple ferrofluid*

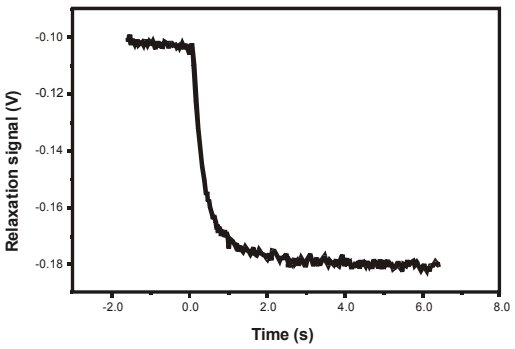

Figure 3. *Relaxation signal of a ferrofluid sample containing 4.75 nmol Fe in 0.5 mL dilution*

The measured signal is the light flux I impinging the detector, which is for our configuration related to the phase shift ϕ of the birefringence through

$$I(t) = I_0 \sin^2 \frac{\phi}{2} \tag{4}$$

$$\phi = \frac{2\pi}{\lambda} d\left(n_{\text{II}} - n_{\perp}\right) = \frac{2\pi}{\lambda} d\Delta n \tag{5}$$

with d being the thickness of the sample and λ the wavelength of the laser light. Intensity changes are solely attributed to birefringence, other effects, e.g. magnetically induced dichroism are neglected. For small ϕ, caused by relatively weak concentrations of the ferrofluid, one can write $I \propto \Delta n$. Thus, the measured signal can be analyzed using a single exponential decay fit function

$$y(t) = y_0 + Ae^{-\frac{2t}{\tau}} \tag{6}$$

yielding the mean relaxation time. This is a first simple approximation as due to the particle size distribution the measured signal is not a pure exponential decay. Assuming a cylindrical particle shape with $L \cong 50$ nm, a viscosity η = 1.0 mPa·s and a temperature T = 293 K the mean diameter of the nanoparticles contained was calculated as d = 15 nm. For the nanoparticles used, the detection limit of the system was found for a nanoparticle concentration of 9.5 μmol Fe/L (Figure 3). The given volume of the sample was 0.5 mL. Accordingly, 4.75 nmol Fe could be detected.

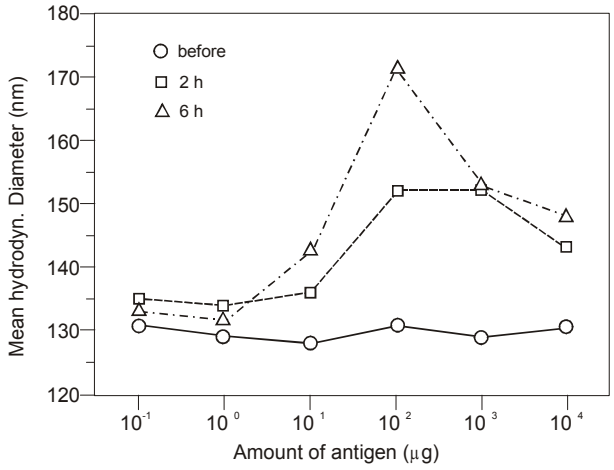

Figure 4. *Rodlike particle diameters calculated from magneto-optical measurements before, 2 h and 6 h after addition of different amounts of antigen (hIgM) to magnetic nanoparticles conjugated with an antibody against hIgM*

The mean hydrodynamic particle diameters obtained by magnetic birefringence relaxation measurements for the binding experiments are shown in figure 4. The data show that during the observed incubation time of 6 h the mean particle sizes of the samples incubated with 10 μg, 100 μg, 1 mg and 10 mg of hIgM are increasing. The maximum increase in particle size was found for an added amount of 100 μg. After the addition of higher amounts of hIgM (1 mg and 10 mg, respectively) the particle sizes tend to decrease compared to the maximum seen at 100 μg. This decrease is most probable due to the saturation of binding sites by an excess of the hIgM molecules, which leads to reduced cross linking between the magnetic particles.

CONCLUSIONS

The presented experiments confirm that the determination of the relaxation of the transient field-induced birefringence of magnetic nanoparticles can be used for the investigation of binding reactions of antibodies to their antigens. The separation of stimulation (magnetic) and signal detection (optical) is of great advantage, as the optical measurement system is comparatively simple, robust and compact. On the other hand, the application of optical measurements is restricted by optical properties of the sample due to scattering or absorption of the laser beam. Consequently, magneto-optical nanoparticle relaxation measurements are a novel tool for the determination of binding reactions *in vitro*.

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REFERENCES

1. Halbreich, A., Roger, J., Pons, J.N., Geldwerth, D., Da Silva, M.F., Roudier, M., Bacri, J.C.: Biomedical applications of maghemite ferrofluid, *Biochimie*, **1998**, **80**, 379-390;
2. Pankhurst, Q.A., Connolly, J., Jones, S,K., Dobson, J.: Applications of magnetic nanoparticles in biomedicine, *J. Phys. D: Appl. Phys.*, **2003, 36,** R167–R181;
3. Wilhelm, C., Gazeau, F., Roger, J., Pons, J.N., Salis, M.F., Perzynski, R., Bacri, J.C.: Binding of biological effectors on magnetic nanoparticles measured by a magnetically induced transient birefringence experiment, *Phys. Rev. E: Stat. Nonlin. Soft Matter. Phys.*, **2002, 65**, 031404(9 p.);
4. Fosa, G., Bădescu, R., Călugăru, Gh., Bădescu, V.: Measuring the transmittivity of light: a tool for testing the quality of magnetic liquids, *Optical Materials*, **2006**, **28**, 461–465;
5. Romanus, E., Groß, C., Glöckl, G., Weber, P., Weitschies, W.: Determination of biological binding reactions by field-induced birefringence measurements, *J. Magn. Magn. Mat.*, **2002**, **252**, 384–386;
6. Scholten, P.C.: Magnetic Measurements on Partricles in Suspensions, *IEEE Trans. Magn.,* **1975**, **MAG-11**, 1400-1402;
7. Taketomi S., Ukita, M., Mizukami, M., Miyajima, H., Chikazumi, S.: Magnetooptical Effects of Magnetic Fluid, *J. Phys. Soc. Jap.*, **1987**, **56**, 3362-3374;
8. Elias, J.G., Eden, D.: Transient electric birefringence study of length and stiffness of short DNA restriction fragments, *Biopolymers*, **1981**, **20**, 2369-2380;
9. Tirado, M.M., de la Torre J.G.: Rotation dynamics of rigid, symmetric top macro-molecules: Application to circular cylinders, *J. Chem. Phys.*, **1980**, **73,** 1986-1993;
10. Vereda, F., de Vicente, J., Hidalgo-Álvarez, R.: Colloidal characterization of micron-sized rod-like magnetite particles, *Colloids and Surfaces A: Physicochem. Eng. Aspects*, **2008**, **319** (1), 122-129.