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Optical protein detection based on magnetic clusters rotation

Research Paper

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Abstract

In this paper we present a simple method to quantify aggregates of 200 nm magnetic particles. This method relies on the optical and magnetic anisotropy of particle aggregates, whereas dispersed particles are optically isotropic. We orientate aggregates by applying short pulses of a magnetic field, and we measure optical density variation directly linked to this reorientation. By computing the scattering efficiency of doublets and singlets, we demonstrate the absolute quantification of a few % of doublets in a well dispersed suspension. More generally, these optical variations are related to the aggregation state of the sample. This method can be easily applied to an agglutination assay, where target proteins induce aggregation of colloidal particles. By observing only aligned clusters, we increase sensitivity and we reduce the background noise as compared to a classical agglutination assay: we obtain a detection limit on the C-reactive protein of less than 3 pM for a total assay time of 10 min.

Introduction

Immuno-detection on nanoparticles is a simple and widely used method in diagnostic tests [1]. In these assays, latex beads functionalized with antibodies are mixed with the sample. If antigens are present in the sample, they link to a first bead and by linking to a second one they form doublets or larger aggregates. As light scattering is linked to the size of aggregates, turbidimetric measurements reveal the presence of clusters and thus antigens. This method is rapid and very easy to operate, but is not sensitive enough for some analytes.

To lower the detection limit and improve the robustness of the method, various evolutions have been proposed. One approach involved the application of ultrasound to create local higher bead densities [2]. Another approach involved replacing latex particles by superparamagnetic particles. Under a 1-D magnetic field, the particles become magnetized, attract each other and assemble into chains. By bringing beads into contact, the magnetic field increases the probability of doublet formation, and so it decreases the overall test duration. Indeed, it can be shown that these latex nanoparticle based tests are diffusion limited. Microfluidic devices can also be used to locally concentrate superparamagnetic beads in order to accelerate the recognition kinetics [4] but they are less easy to implement.

In this paper, we show that by using the intrinsic optical and magnetic anisotropy of clusters, it is possible to use a magnetic field to detect scattering associated only with aggregates. Clusters are aligned using a short pulse of a low magnetic field, and this rotation induces a variation of turbidity. The amplitude of the pulses is low enough to avoid the formation of new clusters and chains. Moreover, there is no contribution from non-aggregated beads.

In the following, we first present the method and its optical basis. We will then present its application to assay the concentration of

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C-reactive protein (CRP) in solution, and compare results between the previous and the new method.

Materials and methods

Magnetic particles were purchased from Ademtech, Pessac, France. The method was first validated with *Bio-Adembeads Streptavidin* (200 nm). In order to minimize the presence of aggregates in the sample, the solution was mildly centrifuged (2000 × *g* during 10 s) prior to the test and beads were taken from the upper part of the sample. Beads were then diluted in phosphate buffered saline (PBS) + surfactant Pluronic PF127 0.3%; initial optical density indicates that the beads concentration is approximately 50 pM.

The same beads are used for the titration of biotinylated Bovine Serum Albumine (BSA-biotin). Bead concentration is 0.04% w/w—approximately 90 pM. The beads and target are diluted in PBS 0.1× + PF127 0.3%. PBS tablets, PF127 and BSA-biotin were purchased from Sigma. For the samples containing 1000 pM and 2000 pM, the optical length was 4 mm instead of 1 cm and the optical density (OD) measured was multiplied by 2.5 to get the equivalent to 1 cm.

In order to evaluate the method on a real antibody-antigen system, we have developed a C-reactive protein assay. The mean diameter of *Carboxyl-Adembeads* particles is 190 ± 60 nm, measured using dynamic light scattering (Zetasizer Nano from Malvern). Polyclonal antibodies directed again CRP are purchased from Interchim and grafted onto beads by Horiba Medical. CRP is purchased from Euromedex, concentration in stock solution is measured with a Pentra 400 (Horiba Medical).

The final cuvette volume is 150 µL: 7.5 µL of beads stored at 0.8% w/w, 7.5 µL of concentrated CRP in saponin and 135 µL of buffer containing BSA 1% w/w. The final concentration of beads is 0.04% w/w. CRP physiological concentration in plasma of healthy patients is typically less than 10 mg/L [5], corresponding to 85 nM. After a dilution 75× in saponin, CRP concentration is 1.1 nM and so the final CRP concentration in the cuvette is 56 pM. In order to produce an assay response curve and to estimate the detection limit of CRP, 11 blanks and 2 measurements at each concentration were obtained.

The light source is a very stable Coherent's ULN-Series diode laser (typical 0.06% RMS noise), emitting polarized light at 635 nm. Behind the cuvette, the beam is analyzed by a photodiode – Thorlabs, PDA36A-EC – and the voltage is measured by a multimeter Agilent 34410A. A first coil – inner diameter 10 cm – generates an axial high magnetic field B_A in the axis of the laser. Transversal field B_T, perpendicular to the laser, is generated by two smaller coils located inside the previous coil. This second field is also perpendicular to the vertical polarization of the laser. The sample is placed in a spectrometer cell; the optical path length is 10 mm. No analyzer is placed after the cuvette; i.e. dichroism is also considered. The setup is represented in Fig. 1.

The optical density is defined as $OD = \log_{10}(I_0/I)$, where I_0 is the laser intensity and I the intensity after the cuvette. After 1 min of sample stabilization, a first optical measurement is performed. Then, 5 magnetizations at 20 mT are applied – from 10 to 160 s – for a total magnetization of 5 min. Each magnetization is followed by 20 s of resting time. The field amplitude must be large enough to allow chain formation, and it can be either axial or perpendicular to the laser (it is axial in our setup). During this step, specific bonds are formed between beads. At the end, when the field is switched off, a final relaxation time of 100 s enables a fraction of non-specific doublets to dissociate. The second measurement is performed after this relaxation step. The total duration of the test is 10 min.

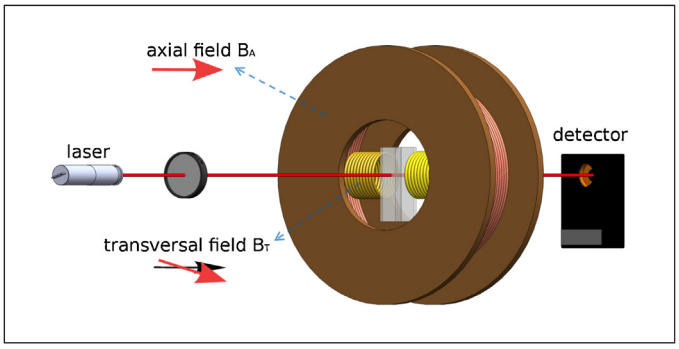

FIG. 1

Experimental setup. The two coils generate magnetic fields perpendicular to laser polarization.

Two methods are compared in this paper. First, the classical optical density method. Here, the difference between the initial and final values, noted ΔOD is measured, and the value recorded provides information on the number of clusters formed under magnetization and also on the possible drift of optical density of the system.

Second, the new method. Here, the ''Clusters Orientation Measurement'' (COM) consists of applying 250 ms of an axial magnetic field – 5 mT – and 250 ms of a transversal field – 5 mT – separated by a relaxation of 250 ms. The COM amplitude is the difference between the maximum and the minimum of optical density during the sequence of magnetic pulses—an example is shown in Fig. 2. The difference between the initial (before magnetization) and final measurement (after magnetization) is called ΔCOM. We will show that this parameter is proportional to the number of clusters created during the high magnetic field phase. We will also show that our method is of particular interest for the smallest aggregates created—e.g. the doublets.

Results and discussion

COM measurements

The sample analyzed in Fig. 2 is composed of pure *Bio-Adembeads Streptavidin* beads. The time response of the optical density during the sequence is shown on Fig. 2(b). We record an increase in the optical density during the application of the axial field and a reduction during the application of the transversal field. When the fields are switched off, OD relaxes to the initial value, indicating no aggregation due to these magnetic fields. We will show that this observation can be qualitatively explained by the reorientation of small aggregates, i.e. doublets already in the solution. In a second part, we will show that this method can also be used to assay antigen concentration as ΔCOM is proportional to the amount of binding proteins.

Orientation of doublets: Magnetic anisotropy

The magnetic beads used here are superparamagnetic. When an external magnetic field is applied, beads in solution rapidly acquire a magnetic moment parallel to the external field, and proportional to its amplitude. In the second step, due to anisotropy of the