Proposed biosensors based on time-dependent properties of magnetic fluids

Joan Connolly, Timothy G. St Pierre*

Biophysics Programme, Department of Physics, The University of Western Australia, Nedlands, Western Australia 6097, Australia

Abstract

A new method of detection of biomolecules in aqueous solution is proposed. The method is based on the detection of shifts in the frequency-dependent magnetic susceptibility of magnetic colloids due to increase in hydrodynamic radius on specific binding with biomolecules. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Magnetic fluids comprise suspensions of magnetic particles 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. Particle–solvent interactions and interparticle repulsions must become strong enough to overcome Van der Waals attraction between the particles [1] and magnetic attraction in the case of particles with a permanent magnetic moment.

One method of stabilization is coating of the magnetic particles with organic polymers [2–4]. This has been shown to stabilise 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 [5,6] thus creating composite structure particles that can act as magnetic labels in aqueous media.

Here we propose a novel methodology for the detection of binding of biomolecules to colloidal magnetic particles in suspension. The methodology exploits the time-dependent magnetic properties of magnetic colloidal suspensions and could form the basis for new types of biosensor. The methodology could be applied to the general class of assays based on the streptavidin–biotin interaction, for example.

The time-dependent magnetic properties of aqueous media containing magnetic particles are altered when biomolecules such as proteins bind to the nanoscale particles in suspension [7,8]. The cause of the change in magnetic relaxation times is the larger hydrodynamic radius of the biomolecule-magnetic particle composite compared with the magnetic particle alone.

Theory shows that the relaxation time is proportional to the hydrodynamic volume of the particle in suspension. Time-sensitive magnetic
measurement devices such as AC magnetic susceptometers are able to measure a spectrum of relaxation times within a suspension of magnetic particles thus enabling particle sizes to be measured.

2. Theoretical background

There are two different mechanisms by which the magnetic moment of a particle suspended in a fluid may relax after removal of a magnetic field. The first mechanism involves the bulk rotation of the particle within the fluid owing to Brownian motion. This mechanism applies mainly to particles whose magnetic moments are fixed relative to the crystal axes of the particles. They are often referred to as magnetically blocked particles. This Brownian rotational diffusion time is given by

\[ \tau_B = \frac{4\pi\eta r^3}{kT}, \]  

(1)

where \( r \) is the hydrodynamic radius of the particle, \( \eta \) is the dynamic viscosity of the fluid, \( k \) is Boltzmann’s constant, and \( T \) is the absolute temperature. In an unblocked particle, the magnetic moment vector rotates, while the particle remains stationary. This rotation is known as Néel relaxation. Generally speaking, there is a critical volume above which the Brownian relaxation mechanism becomes dominant [9]. For an iron oxide particle at room temperature this corresponds to a typical critical particle size of approximately 25 nm.

The response of the magnetisation of a dilute suspension of spherical magnetic particles to an alternating magnetic field can be modelled by the theory developed by Debye to describe the dielectric dispersion in dipolar fluids [10]. The magnetisation of the fluid lags behind the applied magnetic field owing to the finite rate of change of magnetisation with time. In small applied fields, the magnetisation is a linear function of the field and so the response of the magnetisation to an alternating field can be described in terms of the complex magnetic susceptibility, \( \chi = \chi' + i\chi'' \). \( \chi' \) is the in-phase component of the magnetic susceptibility while \( \chi'' \) is the quadrature component. Inertial effects do not need to be taken into account for low-frequency (less than 10 MHz) fields [11].

Debye’s theory leads to an expression for the frequency dependence of the complex magnetic susceptibility of the fluid, \( \chi(\omega) = \chi_0/(1 + i\omega\tau) \), where \( \chi_0 \) is the magnetic susceptibility of the fluid in a DC field. This results in the following expressions for \( \chi' \) and \( \chi'' \):

\[ \chi'(\omega) = \frac{\chi_0}{1 + (\omega\tau)^2}, \]  

(2)

\[ \chi''(\omega) = \frac{\chi_0\omega\tau}{1 + (\omega\tau)^2}. \]  

(3)

Note that \( \chi'(\omega) \) decreases monotonically with increasing frequency, whereas \( \chi''(\omega) \) has a maximum at \( \omega\tau = 1 \). Therefore, by measuring the frequency at which \( \chi'(\omega) \) is a maximum, it is possible to gain information on the hydrodynamic radius of blocked particles using Eq. (1). Any increase in the hydrodynamic radius of the particle, such as the binding of biological macromolecules to it, will cause a corresponding decrease in the frequency of the maximum of \( \chi''(\omega) \). The greater the increase in hydrodynamic radius, the greater will be the frequency shift. It is this phenomenon that we propose to exploit as the basis for the detection of the binding of different sized biological molecules to magnetic colloidal particles in suspension.

3. Experimental method

Measurement of \( \chi'(\omega) \) and \( \chi''(\omega) \) can be achieved by measuring the changes in the inductance and resistance of an induction coil when a magnetic fluid is inserted into its field [12]. When the magnetic fluid is inserted into the coil, the inductance will change by an amount proportional to \( \chi' \) while the resistance increases by an amount proportional to \( \chi''\omega \).

These measurements can be made using the method described in detail by Fannin et al. (1988) to measure \( \chi' \) and \( \chi'' \) as functions of frequency [13].

A mumetal toroid with a narrow slit is wound with 20 turns of wire. An alternating current through this wire produces an alternating magnetic
field in the toroid and its slit. The sample under investigation is held in the slit by the surface tension of the fluid. The resistance and impedance of the toroid are measured using a Hewlett-Packard low-frequency impedance analyser. This has a frequency range from 5 Hz to 13 MHz, with a resolution of 1 mHz for frequencies below 10 kHz. Above this frequency range, the resolution decreases with frequency to a minimum resolution of 1 Hz above 1 MHz.

The resistance and reactance of the coil-toroid system with empty slit, with magnetic fluid in the slit, and with magnetic fluid plus biomolecules in the slit, can be measured as a function of frequency. The magnetic fluid is held by surface tension in the slit in the toroid, with a volume of less than 0.1 cm³ needed for measurement. To ensure that there is no magnetostatic aggregation of the particles in the fluid, the particles need to have a low magnetic moment. They must also be blocked on the time scale of the measurement so that Brownian rotation is the only rotation mechanism.

In practice, magnetic fluids do not contain monodisperse magnetic particles but particles having a distribution of particle radii [14,15]. The in-phase (Eq. (1)) and quadrature (Eq. (2)) susceptibilities then become

\[ \chi'(\omega) = \int_0^\infty \frac{\chi_0 r p(r)}{1 + (\text{cor} r)^2} \, dr, \]

\[ \chi''(\omega) = \int_0^\infty \frac{\chi_0 \text{cor}^2 p(r)}{1 + (\text{cor} r)^2} \, dr, \]

where \( p(r) \, dr \) is the probability of a particle having a radius between \( r \) and \( (r + dr) \) and \( \text{cor} = 4\pi\eta/kT \).

Magnetic iron oxide particles produced by co-precipitation in the presence of organic polymers are found to have a Gaussian particle size distributions with small standard deviations [16]. Although hydrodynamic radii can only be measured indirectly (for example, by magnetic birefringence relaxation measurements [17,18]), it is reasonable to assume that these will also follow a normal distribution as described by

\[ p(r) = \frac{1}{\sigma \sqrt{2\pi}} \exp \left[ -\frac{(r - r_m)^2}{2\sigma^2} \right], \]

where \( \sigma \) is the standard deviation and \( r_m \) the mean hydrodynamic radius of the distribution.

4. Discussion

The frequency at which the maximum quadrature susceptibility is calculated to occur for monodisperse particles in aqueous suspension at a temperature of 20°C is shown in Fig. 1. The viscosity of the solution is taken to be that of pure water at a temperature of 20°C (1.002 \( \times \) 10⁻³ Ns m⁻²). For radii over 200 nm, a change in the hydrodynamic radius of 1 nm causes the frequency peak to shift by approximately 0.1 Hz. For smaller radii, the same change in hydrodynamic radius causes a larger frequency shift. These shifts are significantly larger than the resolution limit of the experimental method described above thus making the technique feasible. It is worth noting that to obtain a 0.1 Hz shift through temperature-dependent viscosity changes in water, a temperature change of approximately 1°C would be required. Thus, temperature stabilisation of the system better than approximately ± 0.1°C is required if confident detection of a 1 nm increase in hydrodynamic radius is to be achieved.

A suspension of magnetic particles with a normal distribution of hydrodynamic radii with a mean of 75 nm and a standard deviation of 7.5 nm is calculated to have a frequency-dependent

Fig. 1. Frequency of peak quadrature susceptibility \( \chi'' \) for an aqueous suspension of blocked magnetic particles at 20°C as a function of particle hydrodynamic radius calculated from Eqs. (1) and (3).
susceptibility at 20°C as shown in Fig. 2. Total coverage of these particles with a monolayer of 1 nm biomolecules increases the mean radius to 76 nm. This is calculated to shift the peak in the quadrature susceptibility by approximately 10 Hz as seen in Fig. 3. For distributions of particle sizes with larger standard deviations, this binding will also be measurable. For example, distributions with mean hydrodynamic radii of 75 and 76 nm but with standard deviations of 25 nm produce curves comparable with those in Fig. 3, with the separation in the peaks being around 3 Hz.

In addition, calculations show that the coverage of only some particles in the fluid will be detectable. For example, a 1:1 mixture of two populations of particles with mean radii 75 nm (SD 7.5 nm) and 76 nm (SD 7.5 nm), is measurably different from a pure sample of either population (Fig. 3). For mixtures of particles from populations with two very different distributions of hydrodynamic radii, the quadrature susceptibility will show two peaks corresponding to the rotational relaxation times of the two distributions.

The experimental method described above enables measurement at frequencies down to 5 Hz and hence detection of particles up to approximately 350 nm in hydrodynamic radius. The range of target molecule size detectable is dependent on the original size of the magnetic particle. For a large range of detectable biomolecule sizes, the magnetic particles will need to be as small as possible while still remaining magnetically blocked. It will also be necessary to ensure that the magnetisation of these particles is small to prevent aggregation due to magnetic dipole interaction effects. Thus, materials with weak ferrimagnetic character and large magnetocrystalline anisotropies would be appropriate for the magnetic component of the particles. It may also be possible to engineer the shape and surface of the particles in order to enhance shape and surface anisotropies.

It may be possible to detect biological entities with radii larger than those shown in Fig. 1, as binding of these will also shift the susceptibility curves. However, in this case, the size of the entity would not be directly measurable; instead, the system could only assay for its presence. Techniques based on measurements with a superconducting quantum interference device microscope [19] may be more suited to objects in this larger size range.

It is often desirable to detect biomolecules in solution without changing their activity. Since the method of biomolecule detection proposed here will keep the molecule suspended in a biocompatible solution at all times, the activity of the molecule is less likely to change as it may in measurements involving aggregation of particles or binding to a substrate.
References