Diffusion Limited Field Induced Aggregation of Magnetic Liposomes

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Magnetic liposomes are spherical vesicles containing magnetic particles. Their practical interest relies in their potential use as "intelligent" drug delivery systems, with magnetic driving or targeting possibilities. In the presence of a field, the vesicles become magnetized and interact. A strong interaction leads to aggregation and could in extreme lead to undesirable clogging in physiological conditions. We investigated the formation of chain aggregates in liposome dispersions in the presence of magnetic fields by light scattering measurements. Data analysis showed that aggregation number increased linearly with elapsed time, as for ordinary isotropic colloid diffusion limited aggregation. The rate of aggregation increased with the field also linearly for low field strengths, and saturates at higher fields. These results are in contrast with measurements for the pure magnetic fluid, i.e., non-encapsulated magnetic particle dispersions.

I Introduction

Liposomes have been used as a means to carry and protect drugs even in harsh body environments as through gastrointestinal fluids. The ability to deliver encapsulated molecules only at final target increases efficiency while at the same time reduces collateral effects. As a further step in drug delivery research, magnetic particles were incorporated into liposomes in such a way that use of magnets could guide or localize the field-responsive vehicles [1], [2]. As another issue in anti-cancer research, magnetic particles or liposomes were tailored to target cancer cells, and the application of an AC magnetic field caused cell death by thermolysis [3].

The behavior of aqueous dispersions of magnetic liposomes under a magnetic field has the complexity typical of nanostructured materials. Transient phenomena range from the microsecond orientation of individual magnetic grains and vesicle deformation up to their macroscopic aggregation. The latter phenomena may take up hours to grow depending on a broad range of physico-chemical parameters such as field strength and concentrations of vesicles and magnetic material. The understanding of aggregation dynamics is relevant to the determination of efficient concentration of vesicles, as well as to the control of clogging in thin arteries. The problem of aggregation under a field is itself a challenging problem of non-equilibrium statistical physics that remains mostly undisclosed. Non-universal cluster time-size scaling for diffusion limited aggregation has been proposed [4] but scarcely verified outside isotropic models. Anisotropic aggregation is expected for dipolar interactions as those prevailing in magnetic fluids. Magnetic fluids have on their side been classified as ferrofluids or magnetorheological fluids according respectively to the ferromagnetic or paramagnetic nature of their dispersed particles [5]. Field-induced aggregation in magnetorheological fluids is expected since the interaction energy between two magnetic particles is proportional to the square of the induced magnetic moments. The dipolar nature of the interaction strongly favors the formation of strings of particles. In a latter stage, columns made of string aggregates have been also reported. This interesting phenomenon has long attracted attention from experimenters (for example [6], [7]) and has been studied with the help of model simulations ([8], [9]), or mean field theories ([10], [11]). Previously, we reported investigations on ferrofluid field induced aggregation [12]. Here, we investigated the time evolution of aggregation (mean aggregation number) in a magnetorheological fluid composed of an aqueous dispersion of ferrofluid-containing liposomes, since the application of a magnetic field.

II Characterization and sample preparation

Magnetic fluid of coated water-soluble magnetic particles (EMG707) was purchased from Ferrofluidics Corporation, Nashua, NH, USA. Their mean size was characterized by photon correlation spectroscopy (PCS). PCS measurements of diluted samples gave effective (mass average) hydrodynamic radius of 35±5nm at small wave-vector limit. Data analysis shows that the samples are quite polydisperse and the mean (number average) hydrodynamic radius should be much smaller. As a comparison, the high-q estimate of this radius gave 26±2nm. Since the magnetic cores should be smaller than 10nm in order to be stable against sedimentation, we infer that coating is comparatively thick.

Liposomes were prepared from the following lipids (Avanti Polar Lipids Inc., Alabaster, AL, USA): L-α-distearoylphosphatidylcholine, cholesterol and distearoylphosphatidylethanolamine-polyethylene glycol 2000 at a molar ratio of 5:4:0.3. We have chosen a lipid composition containing polymer-bearing lipid to increase liposome stability against spontaneous aggregation [13]. Briefly, a chloroform solution of 50mg of lipids was submitted to evaporation that led to the formation of a thin lipid film. Multilamellar vesicles (MLVs) were obtained following hydration at 60°C of the thin lipid film with 0.7ml of ferrofluid solution (EMG707). MLVs were transformed into frozen and thawed MLVs (FATMLVs) by freezing MLVs suspension in liquid nitrogen and thawing the sample in a water bath at 60°C. The freeze-thaw protocol was repeated 10 times to increase trapping efficiency [14]. FATMLVs were then extruded at 70°C (Extrusion device, Lipex Biomembranes, Vancouver, B.C.) through two stacked polycarbonate membranes of 200nm pore size [15]. An extruded suspension of magnetic liposomes was thereby obtained.

Photon correlation spectroscopy characterization of hydrodynamic radius of the magnetic liposomes gave finally Rh $\sim$ 105±10nm with low polydispersity, in good accordance with the extrusion step in preparation.

III Experiment

A sample of about 10^{-4} volume concentration was prepared by dilution in deionized water. The concentration was roughly estimated from the absorption coefficient of the sample against the original solution. The glass scattering cell had a 5mm optical path and was located between the flat poles of an electromagnet. An HeNe laser source was used with power under 1mW. Polarized light intensity (I_{VV}) was measured at a fixed scattering angle of 12.5^0 (scattering wavenumber $q = 2.87\mu\mathrm{m}^{-1}$) with careful alignment so that the magnetic field (H_V) is perpendicular to the scattering plane. This condition was necessary, since the anisotropic growth generates strings of particles whose length becomes comparable to the micrometer along the field direction, imposing strict bounds for the small scattering wave vector limits in the parallel direction. With a perpendicular geometry, the gyration radius R_g along q is only weakly dependent on aggregation number (as far as thermally induced lateral wiggling of the stretched chains is considered). Besides, $(qR_g)^2/3 \sim .02$ becomes a small and fixed Guinnier correction to the hydrodynamic limit ($q \to 0$) of the structure factor [16]. From these considerations, and light scattering theory, measured intensity, I, should be essentially given as the product of the fixed particle (low) concentration, a particle scattering constant, and the "z-average" (mass average) aggregation number:

$$I/I_0 = N_z = < N^2 > / < N > \qquad (1)$$

throughout. The excess mean aggregation number is then:

$$\Delta N = I/I_0 - 1. \qquad (2)$$

Intensity as well as correlation functions were measured in logarithmically increasing time intervals up to 4 hours since the field was turned on. The field was set to 0.01, 0.02, 0.05, 0.10, 0.20 and 0.40T (H up to 3.2 10^5 A/m) at the start of each run.

The scattered intensity grew continuously as depicted in Fig.1. The growths are well fit by the linear relation

$$\Delta N = \Gamma t \qquad (3)$$

with a field dependent growth rate $\Gamma(H)$.

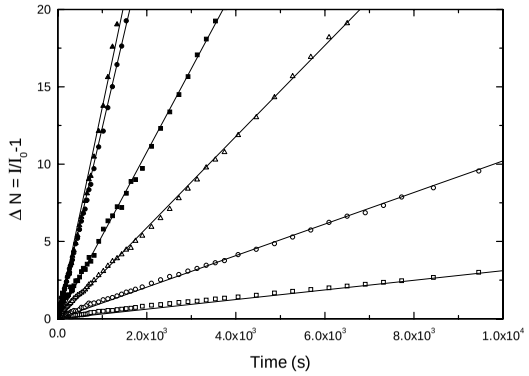

Figure 1. Time evolution of aggregation number ΔN, for 6 different field strengths (H = .8, 1.6, 3.2, 8, 16 and 32 10^4A/m).

For D-dimensional fractal objects one would expect the ideal mass-size scaling

$$N \sim R_h^D. \quad (4)$$

Eq.(4) combined with eq.(1) shows that a plot of the intensity against effective hydrodynamic radius (also z-average) allows for determination of fractal dimension of the aggregates. The increase displayed in Fig. 2 as a log-log plot shows that the clusters consist of almost linear or stretched chains. The small deviation from the ideal $D = 1$ law can be accounted for by the details of complex phenomena as either particle or chain length polydispersity (change of shape for the instantaneous distributions), or thermal wiggling. Polydispersity could lead to subtle chain mass-size correlation effects which analysis is beyond the scope of this paper. On the other side, the extreme wiggling of a long string (entropy comparable to aligning energy) would shrink the chain and thus slightly increase the effective or observed fractal dimension. The limiting case of a self-avoiding polymer should apply to a chain of ferromagnetic particles (ferrofluid) in the absence of an external field. The analysis of such problem has been pioneered by Flory and results in a fractal dimension $D \sim 1.7$ [17]. See also [8] for a discussion on the fractal dimension of dipolar aggregates.

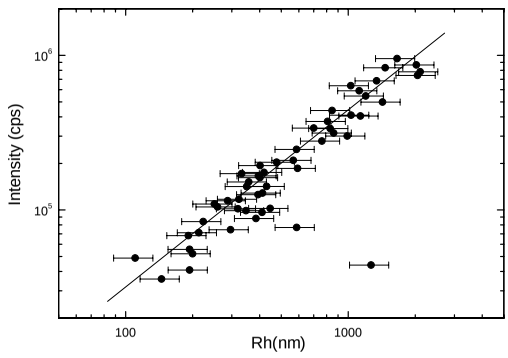

Figure 2. Log-log plot for the intensity versus hydrodynamic radius in a single aggregation run (H = 8 10^4A/m). The straight line is a power law giving $I \sim R_h^D$ with $D = 1.1 \pm .1$. The power gives the fractal dimension of aggregates (see text).

IV Discussion

The growth scenario we invoke for these results is the diffusion limited aggregation process [4]. The diffusing liposomes may get close to each other to such an extent that the induced dipoles will interact. In a head to tail configuration, this interaction is attractive and its strength or interaction range depends on the magnetic to thermal strength parameter λ:

$$\lambda = M^2 d^3 / k_B T \quad (5)$$

where $M = \mu H$ is the liposome magnetization due to the field H, d is its diameter, and $k_B T$ is the thermal energy. The rate of aggregation will then depend on particle mobility, density, and interaction range. An estimate of a random walker collision rate can be easily obtained from first principles. From the diffusivity $D \sim 2.1\ 10^{-8}$ cm^2/s of particles with diameter $d \sim 200$nm at volume fraction $\phi \sim 10^{-4}$, it gives [8]:

$$\gamma = 6(d/D^2)\phi \sim .03Hz. \quad (6)$$

This estimate is of the right order of magnitude as compared to the highest measured rates (At saturation, $\Gamma \sim .014$Hz). Of course, a fraction of the collisions are not effective, as they may even be repulsive. In Fig. 3 we plot the field dependence of the growth rates. Since neither diffusion, nor size and concentration are field dependent, we are led to the conclusion that for smaller fields one or both the following phenomena play. An increasing fraction of the pairwise collisions may not be binding efficient (due to the angular nature and decreasing strenght of dipolar interactions); and at weak binding, competition with dissociation rates becomes increasingly important.

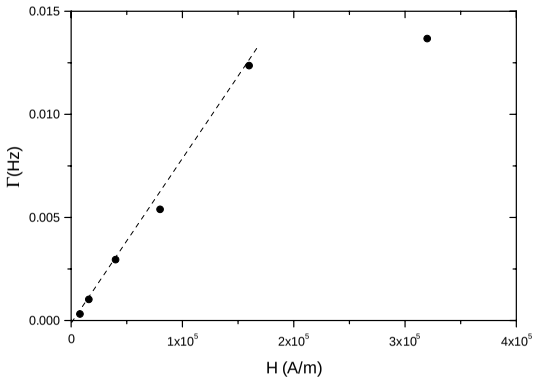

Figure 3. Growth rate as a function of field strength. The saturation value is compatible with an estimate for a pairwise diffusion limited aggregation process. Dashed line is a linear behavior at low fields. At strong fields the rate saturates.

The rates increase almost linearly with the field but seem to saturate at some point around 2 10^5 A/m. This correlates to a Langevin type susceptibility, increasing interaction strength up to saturation (all ferrofluid particles in a liposome fully aligned). As a matter of fact, the lowest fields used could already saturate the greatest ferromagnetic cores. Using at maximum a 10nm magnetite particle dipole moment, $m \sim 2\ 10^{-19}$ Am^2, the saturation fields can be estimated from the field-dipole particle interaction energy:

$$H_s \sim k_B T/m > 2\ 10^4 A/m. \quad (7)$$

Similar experiments were performed by Hagenbüchle and Liu [7] on ferrofluid emulsions of 466nm droplets at lower concentrations, and by Fermigier and Gast [6] on 1.5μm paramagnetic latex spheres, both at lower field strengths. Their results contrast to the ones reported here in two aspects. First, a stronger (quadratic) field dependent rate was reported. This may be related to their measurements being done well below saturation magnetization. They have also observed non-linear growth $N \sim t^z$ with $z \sim .4$ to .8. It is observed that this power increases with both volume fraction and interaction parameter λ. Clearly, further measurements at broader field strengths and different concentrations should be pursued to clarify these points.

Finally we note that the magnetic liposome dispersion aggregation properties are in strong contrast to a pure ferrofluid dispersion of same magnetic content [12]. First, the ferrofluid grows non-linearly ($z > 1$), i.e., cooperative effects are more important in these systems, increasing the growth rate as aggregates get bigger. Second, liposomes grow much faster, since particles are already condensed inside the vesicles from the beginning.

Acknowledgments

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