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Rate of Dimer Formation in Stable Colloidal Solutions Quantified Using an Attractive Interparticle Force

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Supporting Information

ABSTRACT: We describe an optomagnetic cluster experiment to understand and control the interactions between particles over a wide range of time scales. Aggregation is studied by magnetically attracting particles into dimers and by quantifying the number of dimers that become chemically bound within a certain time interval. An optomagnetic readout based on light scattering of rotating clusters is used to measure dimer formation rates. Magnetic field settings, that is, field rotation frequency, field amplitude, and on- and off-times, have been optimized to independently measure both the magnetically induced dimers and chemically bound dimers. The chemical aggregation rate is quantified in solutions with different pH and ionic strengths. The measured rates are extrapolated to effective dimer formation rates in the absence of force, showing that aggregation rates can be quantified over several orders of magnitude, including conditions of very low chemical reactivity.

INTRODUCTION

Colloidal solutions are metastable systems containing particles with a size between the nanoscale and microscale. The particles are made of numerous materials and are found in many applications, because of their large surface-to-volume ratio, their versatile mechanical and optical properties, and because they allow a wide range of functionalization strategies. Colloids are used in biomedical applications, for example, as carriers for drug delivery, as contrast agents in magnetic resonance imaging, and as labels to facilitate diagnostic assays. In biophysical research, colloidal particles function as optical or magnetic tweezers for studies on proteins and DNA. Self-assembly and directed-assembly of colloidal solutions find applications in, for example, 3D photonic crystals. In these applications, interparticle interactions play an important role. Biomedical applications are hampered by corona-induced particle aggregation, causing low efficiencies in drug delivery, and low sensitivity and limit-of-detection in biosensing. The optical properties of photonic crystals depend on the 2D and 3D particle arrangements, which are determined by the interparticle forces. Thus, it is crucial to understand and control the interactions between particles, on short as well as long time scales.

In this work, we focus on investigating the early stages of particle aggregation, when dimers are formed in a solution that still dominantly consists of monomers. The dimer formation process is important, for example, in diagnostic agglutination assays. Agglutination assays, also known as aggregation or cluster assays, are used to quantify biomolecular concentrations via particle aggregation. Clusters of particles are formed in dependence of (bio)chemical reactivity between the particles and the aggregation is typically measured by turbidimetry, nephelometry, or dynamic light scattering (DLS). As colloidal solutions exist both in equilibrium and far-from-equilibrium, the time scale at which aggregation occurs can vary from micro seconds or less, up to many years. Advances in the synthesis of antifouling coatings are leading to colloidal particles that are stable also in complex solutions.

Cluster assays based on the thermal diffusion of particles are slow and can therefore operate only with relatively unstable colloidal systems, that is, particles with a high chemical reactivity. Baudry et al. demonstrated that the assay time can be significantly reduced using superparamagnetic particles in combination with external magnetic fields. Particles become magnetized in the external field and self-organize into chains by attractive magnetic dipole interactions, which accelerates cluster formation.

Here, we study how attractive magnetic forces can be used to quantify the early stages of aggregation, in colloidal systems with a relatively low chemical reactivity. We use the optomagnetic cluster (OMC) experiment of Ranzoni et al. to measure the amount of dimers in solution. In this method, a rotating magnetic field is applied that rotates clusters of particles, causing an oscillating optical signal because of their
orientation-dependent scattering cross section. Single particles, due to their spherical shape, do not contribute to the oscillating scattering intensity, making this optomagnetic method suited to detect low concentrations of dimers against a background of monomers. We describe in this paper how time-dependent data in the OMC experiment can be used to quantify dimer formation rates in colloidal systems with low chemical reactivity. The experimental approach is corroborated by calculations, showing how experimental parameters can be tuned to obtain control of the aggregation kinetics. Subsequently, nonspecific particle aggregation rates are measured in varying electrostatic conditions (pH and ionic strength). Finally, the measured rates are extrapolated to aggregation rates without applied attractive forces, in order to determine the chemical aggregation rates of colloidal solutions with low reactivity.

■ MATERIALS AND METHODS

Materials. Carboxylated superparamagnetic Masterbeads were purchased from Ademtech [nominal size 0.5 μm, hydrodynamic diameter from DLS is 528 nm with coefficient of variation (CV) 25%]. Buffer components: phosphate-buffered saline (PBS) tablets, citric acid anhydrous, sodium citrate dihydrate, potassium chloride, Pluronic F-127 and Protein LoBind Eppendorf tubes were all obtained from Sigma-Aldrich. Borosilicate glass 3.3 cuvettes with a square cross section, inner dimensions of 1.00 ± 0.05 mm, outer dimensions of 1.23 ± 0.05 mm, and length of 20 ± 1 mm were obtained from Hilgenberg.

pH Buffer Preparation. Buffers with different pH values were prepared using two citrate salts: citric acid anhydrous (HOC-(COOH)(CH₂−COOH)) and sodium citrate dihydrate (HOC-(COONa)(CH₂−COONa)·2H₂O). The buffer strength was kept at 10 mM in all experiments of this paper, and the molar ratio of the two salts determined the pH of the buffer. In several experiments, potassium chloride (KCl) was added to increase the salt concentration of the buffer solution, without affecting the pH. After adding all salts to deionized water, the pH of the buffer was measured with a WTW Inolab pH 720 pH probe (precision of 0.1). The exact composition of the used buffers can be found in Table S1 in the Supporting Information.

Zeta Potential Measurement. The average surface charge of the particles was quantified by measuring the zeta potential of the...
particles with a Malvern Zetasizer Nano ZS. Particles were diluted to 0.1 mg/mL, and triplicate measurements were performed using either citric acid buffers of varying pH (10 mM citric acid buffer, ionic strength 150 mM) or using deionized water to disperse the particles. At the high salt concentrations, the operating voltage of the zetasizer was limited to max. 10 V in order to prevent electrolysis at the electrodes, which decreases the signal-to-noise ratio in the measurements. The uncertainty in the zeta potential measurement is relatively large because of the low absolute value of the zeta potential of the measured particles ($\Delta \zeta \approx 2$ mV).

**Experimental Setup.** The OMC experiment is schematically depicted in Figure S2 of the Supporting Information. In the middle of the setup, a square glass cuvette containing a particle solution is located. Around the cuvette four electromagnets are positioned in a cross arrangement. With this quadruple setup, in-plane rotating magnetic fields are created by flowing a sinusoidal current through each of the four coils with a phase lag of 90° between neighboring coils, using a homemade LabVIEW program. A 660 nm laser (Single Mode Hitachi HL6545MG laser, Thorlabs) is focused into a square glass cuvette containing the particle solution by a positive lens (AC254-150-A-ML $f = 150.0$ mm lens, Thorlabs). The light scattered by the rotating particles (monomers, dimers, trimers, etc.) is collected at an angle of 90° with respect to the laser beam. A positive lens (AC254-075-A-ML $f = 75.0$ mm lens, Thorlabs) focusses the scattered light onto a photodetector (PDA36A-EC Si amplified detector, Thorlabs) which is read out by the same LabVIEW program. MATLAB analysis software has been developed to further analyze the scattering signals.

**Mie Scattering Simulation.** Mie scattering simulations were performed on two- and three-particle clusters, using the MSTM v. 3.2 code developed by Mackowski. The simulations were performed using a monochromatic 660 nm light source with s-polarization, as used in the experiments. The particles were simulated as smooth spheres with a diameter that is normally distributed around an average of 500 nm, with a CV equal to 25%. The distance between the particles was kept at 10 nm. The refractive index of the particles was calculated according to eq 1 from van Vliembergen et al., giving a value of 1.7 ± 0.1.

**OMC EXPERIMENT**

Figure 1a sketches the process of dimer formation without and with an attractive interparticle force. In both cases, the clustering of particles is a multistep process, containing a transport step that leads to an encounter complex, and subsequently, a chemical aggregation step in which a chemical bond is formed between the particles. In this paper, we study the formation of nonspecific bonds, that is, interparticle bonds due to general physicochemical interactions such as van der Waals interactions or hydrophobic interactions between particle surfaces (so not bonds due to selective biomolecular interactions). We assume that the particles are homogeneously reactive and therefore neglect rotational alignment.

For stable colloidal systems without attractive interparticle forces, the thermal aggregation rate $k_{agg}^{th}$ is much smaller than the separation rate $k_{sep}$. The effective rate of dimer formation $k_{agg}^{th, eff}$ can be written in terms of the encounter, separation, and aggregation rates

$$k_{agg}^{th, eff} = k_{enc}^{th}k_{agg}^{th}k_{agg}^{th} + k_{enc}^{th}k_{agg}^{th} \approx k_{enc}^{th}k_{agg}^{th} \quad \text{for } k_{agg}^{th} \ll k_{sep}$$

The process of thermal dimer formation can take months or longer for stable colloidal solutions. To bring the aggregation process into time scales that are more suited for measurements, we propose to apply an attractive interparticle force, in the form of a dipolar magnetic field resulting from magnetic particles and an applied magnetic field. The magnetic dipole–dipole interaction accelerates the primary encounter step $k_{agg}^{mag}$ to make it no longer diffusion limited. Additionally it prevents the separation of magnetic dimers, that is, $k_{agg}^{mag} = 0$. In this way, we will demonstrate that the OMC experiment can be used to quantify the rate of chemical dimer formation in the presence of an external magnetic field, that is, parameter $k_{agg}^{mag}$.

To quantify the number of dimers formed over time, we use the optomagnetic readout principle developed by Ranzoni et al. that allows to measure dimer concentrations in the picomolar range. Briefly, a laser is focused inside a cuvette containing a solution of superparamagnetic particles, which is situated in the center of a quadrupole electromagnet (Figure 1b). The scattered light is collected by a photodiode at an angle of 90° with respect to the incoming laser beam. To distinguish clusters from single particles, an in-plane rotating magnetic field is applied. The scattered light of a rotating single particle is constant as a function of time, whereas the scattered light from a rotating cluster yields an oscillating signal as a function of time because of its asymmetry. Figure S3a shows the measured photodiode signal as a function of time. When the magnetic field is off, a baseline signal is measured because of scattering of both single particles and clusters. When the rotating field is turned on, an oscillating signal is measured on top of the baseline. As each rotating cluster contributes to the amplitude of the oscillating signal, this amplitude represents a measure of the cluster concentration. To extract the amplitude of the oscillation, the Fourier spectrum of each pulse train is analyzed (Figure S3b). The peak in the Fourier spectrum at twice the field rotation frequency ($A2f$) is used as a measure of the cluster concentration. Figure S3c shows a calibration measurement in which a stock solution of Ademtech particles was titrated into several dilutions and the $A2f$ peak was measured. The stock solution consists almost completely of single particles, with only a few dimers being present as verified by microscopy, see Figure S3d (one dimer per 12–15 monomers). The linear relation between dimer concentration and mean $2f$ amplitude proves that the dimer concentration can sensitively be quantified with the OMC experiment, without the interference of magnetic cluster formation, because of the application of sufficiently long field-free time intervals.

In order to quantify particle aggregation rates, we developed a four step protocol shown in Figure 1c. During the first step, the initial cluster concentration is measured using a pulsed rotating magnetic field with a short on-time ($t_{on} = 0.2$ s) and a long off-time ($t_{off} = 10$ s). The long off-time is used to allow diffusive particle redisperision during the measurement and avoid build-up of magnetic clusters. During the second step, a rotating field is turned on continuously. This causes the particles to form magnetic clusters that rotate with the field and causes the $A2f$ signal to increase linearly in time. This step aims to create magnetic clusters and keep the particles in close proximity for a certain interaction time. During this interaction time, a fraction of the magnetic clusters will form a nonspecific noncovalent chemical bond and thus become a chemical cluster. During the third step, the magnetic field is turned off. This functions as a waiting time, so that all free particles can diffuse and redistribute homogeneously throughout the solution. Finally, in step four, the resulting chemical cluster concentration is measured, using the same protocol as described for step one.

During the actuation time $t_{act}$ more and more magnetic dimers are formed. This means that the interaction time is not...
the same for all magnetic dimers and that the average interaction time of dimers is smaller than \( t_{\text{act}} \). The fact that the number of magnetic dimers increases linearly over time during the actuation phase (see Figure 1c) makes that the average interaction time of magnetic dimers is equal to \( \frac{1}{2} t_{\text{act}} \).

During the interaction time, the particles in a dimer are in close proximity, that is, a nanometer-scale surface-to-surface distance, which enhances the possibility to form a nonspecific chemical bond. Of all magnetic dimers formed (\( N_{\text{mag,tot}} \)), a fraction reacts to become a chemical dimer. The number of chemical dimers \( \Delta N_{\text{chem}} \) is quantified after the waiting time \( t_{\text{wait}} \). Finally the aggregation rate \( k_{\text{agg}}^{\text{mag}} \) is calculated by eq 2.

\[
k_{\text{agg}}^{\text{mag}} = \frac{\Delta N_{\text{chem}} / N_{\text{mag,tot}}}{\frac{1}{2} t_{\text{act}}} \tag{2}
\]

To increase statistics, multiple actuation cycles are applied (see Figure 1d). The aggregation rate is quantified for every cycle, and the average and standard deviation are calculated (see Figure 1e).

**TUNING EXPERIMENTAL SETTINGS**

In step one and four of the OMC experiment (Figure 1c), measurement pulses are used to quantify the number of chemical dimers in the solution \( \Delta N_{\text{chem}} \). For an accurate quantification, the pulse should not induce additional magnetic or chemical dimers. For this purpose, several experimental parameters have been optimized: field on-time and off-time, field amplitude, field frequency, and particle concentration.

The influence of the field on-time on the measured number of dimers was investigated by performing 50 measurement pulses for a varying field on-time and a constant intermittent off-time of 10 s. Figure 2a shows the measured \( |A_{2f}| \) signal normalized to the \( |A_{2f}| \) of the first measurement pulse. For an on-time of 1 s or more, the \( |A_{2f}| \) signal significantly increases with the number of measurement pulses, whereas for an on-time of 0.2 s, the measured value does not increase as a function of time. The fluctuations in the measured \( |A_{2f}| \) are caused by dimers diffusing in and out of the focus volume of the laser, changing the local dimer concentration. For the chosen experimental settings (\( B = 4 \) mT, \( f = 5 \) Hz and \( [\text{particle}] = 1.0 \) pM), the on-time should be 0.2 s to prevent the formation of additional dimers during an individual measurement pulse.
In the previous experiment, the off-time was chosen to be long enough to avoid any influence on the measurement; however, decreasing the off-time can also lead to magnetic aggregation because particles may not have enough time to redisperse in between measurement pulses. Figure 2b shows the normalized LA2fl signal for measurement pulses with an on-time of 0.2 s and a varying off-time. For off-times longer than 5 s, the chemical dimers can be measured without inducing additional magnetic dimers.

Increasing the magnetic field amplitude accelerates the kinetics of magnetic dimer formation by quadratically increasing the attractive dipole–dipole force (Figure S4a). The field rotation frequency does not have a significant influence on the measured LA2fl, as long as the frequency is below the break down frequency for dimers.21 The field rotation frequency has only minor influence on the dimer formation kinetics (Figure S4c). In the remainder of this paper, the following experimental parameters are used for the measurement pulses: \( t_{\text{on}} = 0.2 \text{ s}, t_{\text{off}} = 10 \text{ s}, B = 4 \text{ mT}, f = 5 \text{ Hz} \) and \( [\text{particle}] = 1 \text{ pM} \).

During step two of the OMC experiment, the magnetic field is turned on continuously during the actuation time \( t_{\text{actuation}} \). Initially, the sample contains mainly monomers and a few chemical dimers, as has been observed by microscopy (1 dimer per 12–15 monomers). During actuation, the number of dimers increases and eventually also larger clusters (trimers, tetramers, etc.) are formed.28 The total number of dimers that can be created is limited by the maximum shifting to shorter times. Figure 2d shows the dependence of the LA2fl for several particle concentrations during the actuation pulse. Higher particle concentrations do not only increase the total number of dimers that can be created but also accelerate the formation of magnetic dimers. The field rotation frequency has only minor influence on the dimer formation kinetics (Figure S4c).

Figure 2e shows the evolution of the normalized LA2fl signal as a function of time for an actuation time of 90 s. The scattered light is measured simultaneously at an angle of 16° and 90° with respect to the incoming laser. The scattering intensity at 90° reaches a maximum first while the scattering intensity at 16° still increases. This seems to indicate a higher sensitivity for larger clusters at a scattering angle of 16°.

To interpret the experimental results of Figure 2e and to get an upper limit of the percentage of two-particle clusters over time, we performed simulations as reported in Figure 2f. The simulations are performed simulations as reported in Figure 2f. The simulations are based on two aspects, namely, the cluster growth dynamics and the scattering cross sections of the clusters. For each cluster size (\( i = \text{dimer, trimer, tetramer, etc.} \)), the number of clusters is calculated as a function of time \( N_i(t) \) and also the corresponding complex 2f scattering cross section at the detector angle \( \alpha_f \). The total complex 2f signal is the product of the number of clusters multiplied by the complex scattering cross section summed over all cluster sizes. The LA2fl signal is the absolute value of this complex number

\[
|\text{LA2fl}| = |N_{\text{d}} 2f_{\alpha_f} + N_{\text{tr}} 2f_{\alpha_f} + N_{\text{tet}} 2f_{\alpha_f} + \ldots |
\]  

The cluster growth dynamics is modeled using the Smoluchowski population balance equations describing the reaction of two monomers (m) becoming a dimer (d), a monomer and a dimer becoming a trimer (tr), and so on.28 For tetramers (te), for example, there are two production terms and two loss terms when cluster sizes up to hexmers (h) are included, see eqs 4–7. For each cluster size, the population balance equations yield a differential equation for the rate of cluster formation, as shown for tetramers in eq 8. Here, \( k_i \) is the \( i \)th reaction rate of an \( i \)-particle cluster and \( N_i \) is the number of \( i \)-particle clusters. By numerically solving the system of coupled differential equations up to and including hexmers, the cluster distribution was calculated as a function of time. Note that the initial cluster distribution and all of the reaction rates need to be predefined. The initial cluster distribution was estimated from microscopy images of the stock solution but is difficult to accurately determine. The dimer reaction rate \( k_d \) is calculated from the initial slope of the actuation curve, and an upper limit for the reaction rates \( k_i (>\text{dimer}) \) follows from \( k_d \) and the number of particles in the reacting clusters (described in full detail in Section S5 of the Supporting Information).

\[
m + tr \rightarrow te \ (\text{rate } k_{\text{tr}})
\]  
\[
d + d \rightarrow te \ (\text{rate } k_{\text{d}})
\]  
\[
te + m \rightarrow p \ (\text{rate } k_p)
\]  
\[
te + d \rightarrow h \ (\text{rate } k_h)
\]

In order to find the complex scattering cross section of the clusters, Mie scattering simulations have been performed.22 The scattering intensity of clusters with various numbers of particles, particle sizes, interparticle distances, and orientations has been calculated at the detector angles of 16° and 90°. The oscillating scattering signal of a dimer, trimer, and tetramer are shown in the Supporting Information (Figure S5a). The calculations show that because of the size dispersion of the particles (CV ∼25%), the characteristic peaks of dimers, trimers, and tetramers are broadened. Taking the Fourier transform of the simulated scattering signals yields the complex scattering cross section for dimers, trimers, and tetramers. Figure S5b shows that the amplitude of the complex scattering cross sections of larger clusters increases more for detection at 16° than for detection at 90°. Also, the phases of the complex scattering cross sections are different. This explains why the total scattering signal (Figure 2e) increases sublinearly for both 16° and 90° and why the sub-linearity is stronger for the 90° signal. A description of the scattering simulations and the comparison with the measurements is given in Section S6 of the Supporting Information.

Using the complex scattering cross sections obtained from the Mie scattering simulations and the calculated evolution of the cluster distribution as a function of time, the normalized LA2fl signal can be estimated as a function of time for both detector angles, as shown in Figure 2f. The calculated time dependence of the LA2fl signals shows similar shape and trends as the measured LA2fl signals, although this calculation is dependent on many input parameters like particle size distribution, refractive index, detector angle, and so forth. The simulated signal shows faster kinetics than the measured signal, especially the 16° signal, which could be caused by overestimating the reaction rates for larger clusters. A full Brownian dynamics simulation of the magnetic clustering process could be a next step, but this lies outside of the scope of the present paper. Using the simulation data, we can estimate the percentage of clusters that is a dimer at each point in time, see Figure 2f. This shows that for actuation times of less than 30 s, at least 85% of clusters is a dimer. This result justifies the procedure to derive the rate constant of dimer formation from the measurement as described in eq 2.

With the above found experimental settings, the total experiment time is 5–15 min dependent on the amount of actuation cycles that is performed. As such, gravitational effects can be neglected in the OMC experiment. A full calculation of the typical time scale of sedimentation is given in Section S7 of the Supporting Information.

**PARTICLE AGGREGATION AS A FUNCTION OF pH AND IONIC STRENGTH**

In order to test the validity of the OMC experiment for determining rate constants, we measured the influence of electrostatic interactions on the dimer formation rate \( k_{\text{agg}} \). The electrostatic interaction between particles was varied in two ways: first, by changing the surface charge of the particles via

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the pH of the solution and second, by changing the Debye length via the ionic strength of the solution, see Figure 3.

To control the particle surface charge density, the pH of the citrate buffer was varied between 4 and 7 (see Materials and Methods section). Carboxyl-functionalized superparamagnetic Ademtech Masterbeads were used with a nominal diameter of 500 nm. The effect of pH on particle surface charge was quantified by zeta potential measurements shown in Figure 3a (right y-axis). Increasing the pH from pH 4 toward pH 7 leads to a more negative zeta potential, as a higher fraction of carboxyl groups is deprotonated. The absolute value of the zeta potential decreases at low pH, which implies that the isoelectric point of the particles is approached. The aggregation rate of the Ademtech Masterbeads was measured in each of these solutions. The left y-axis in Figure 3a shows the aggregation rate (averaged over four cycles) as a function of the pH of the citric acid solution. A clear decrease in the aggregation rate of more than an order of magnitude was measured for increasing pH (more negative zeta potentials). This demonstrates that electrostatic charge is an important factor for particle aggregation kinetics and shows the ability to quantify the aggregation kinetics with the OMC experiment.

The influence of ionic strength on the particle aggregation rate was measured by varying the amount of KCl added to a citrate buffer at pH 4.3. Figure 3b shows that the aggregation rate increases by more than an order of magnitude with increasing ionic strength, underlining the importance of electrostatic interactions for the aggregation rate. In summary, the measured trends of the particle aggregation rate as a function of zeta potential and ionic strength are consistent and provide proof of concept for the aggregation experiment. A quantitative interpretation of the data will be addressed in the next section.

TRANSLATION TO AGGREGATION RATES IN ABSENCE OF MAGNETIC ATTRACTION

Figure 4a sketches the potential energy landscape of a dimer as a function of the interparticle distance $x$, in the presence of an attractive interparticle force. At large interparticle distances ($x \gg d$), the magnetic dipole–dipole attraction is very weak and the potential energy is close to zero (not included in the graph). For somewhat shorter interparticle distance ($x > d$), the particles attract each other, which causes the formation of magnetic dimers. Once a magnetic dimer is formed, the two particles are in close proximity and a chemical bond can be formed. In order for the particles to chemically react, the energy barrier $U_b$ needs to be overcome. The presence of the
attractive interparticle force lowers the energy barrier compared to the situation of particles free in solution. The aggregation rate that is measured with the OMC experiment, $k_{agg}^{\text{mag}}$, describes the average rate by which a magnetic dimer crosses the energy barrier to become a chemical dimer, for a certain magnetic field amplitude.

The energy barrier $U_0$ depends on magnetic field strength but is dominated by steric, electrostatic, and van der Waals interactions. A complete calculation of the potential energy landscape is outside of the scope of this paper. Here, we assume that the magnetic interaction gives a weak reduction of the energy barrier, so that the rate of dimer formation $k_{agg}^{\text{mag}}$ equals the thermal aggregation rate $k_{agg}^{\text{th}}$ with a field-dependent correction factor $\alpha(B)$ (with $\alpha(B) < 1$). Using eq 1, the thermal dimer formation rate for particles free in solution ($k_{agg}^{\text{th,eff}}$) can now be expressed as

$$k_{agg}^{\text{th,eff}} = \frac{k_{agg}^{\text{th}}}{k_{sep}^{\text{th}}} = \frac{k_{agg}^{\text{th}} \cdot \alpha(B) k_{agg}^{\text{mag}}(B)}{k_{sep}^{\text{th}}}$$

If $k_{agg}^{\text{mag}}$ is measured in the OMC experiment as a function of the applied magnetic field, then extrapolation to zero field (where $\alpha(B) = 1$) provides a convenient way to estimate $k_{agg}^{\text{th}}$, as will be shown later. This leaves us with the need to estimate the thermal encounter rate $k_{enc}^{\text{th}}$ and thermal separation rate $k_{sep}^{\text{th}}$.

In absence of an attractive interparticle force, particles are free in solution and move solely due to Brownian motion. The average encounter rate $k_{enc}^{\text{th}}$ of spherical particles in a solution with viscosity $\eta$ can be calculated using the diffusion limited rate equation.  

$$k_{enc}^{\text{th}} = \frac{4k_BT}{3\eta}$$

(10)

The thermal encounter rate for particles in an aqueous solution with $\eta = 1$ mPa·s is $5.5 \times 10^{-14}$ m³ s⁻¹ or $3 \times 10^{-9}$ M⁻¹ s⁻¹.

The separation rate $k_{sep}^{\text{th}}$ describes the typical rate at which two particles in an encounter complex diffuse away from each other. In order to find an estimate for $k_{sep}^{\text{th}}$, an interparticle distance needs to be defined at which an encounter complex will be considered as two separate particles (Figure 1a). At this separation distance, the encounter complex can no longer become a chemical dimer. We define the separation distance as the interparticle distance at which the potential energy is less than $k_BT$. The energy landscape for the particles used here is unknown and will vary for different particles, coatings, and solvents. However, Biancaniello and Crocker 30 and Wang et al. 31 succeeded in measuring the potential energy landscape of two particles inside an optical trap and of a particle near a surface, respectively. Both energy landscapes tail off at an interparticle distance of about 40 nm. The separation rate can now be calculated as the typical time in which a particle with radius $R$ (250 nm in our experiments) diffuses $\Delta x = 40$ nm.

$$k_{sep}^{\text{th}} = \frac{k_BT}{\pi\eta(\Delta x)^2}$$

(11)

This gives a typical time of 300 μs, and thus, $k_{sep}^{\text{th}}$ is estimated to be $3 \times 10^{-9}$ s⁻¹.

In order to experimentally determine the effect of the magnetic field on the aggregation rate, we measured the aggregation rate of streptavidin-coated Ademtech Masterbeads in PBS at different field amplitudes (Figure 4b). The data show a dependence that appears linear on lin-log axes. The fitted magnetic field correction factor $\alpha(B)$ is given by the following expression

$$\alpha(B) = \exp[-(3.0 \pm 0.8) \times 10^2 \times B]$$

(12)

Combining $k_{agg}^{\text{th,eff}}$ and $\alpha(B)$ gives an expression for $k_{agg}^{\text{th,eff}}$, the effective dimer formation rate of particles free in solution, as a function of $k_{agg}^{\text{mag}}$, the aggregation rate measured with the OMC experiment. The resulting relationship is shown in Figure 4c. For example, a measured $k_{agg}^{\text{mag}} = 2 \times 10^{-3}$ s⁻¹ in the OMC experiment using a magnetic field of 4 mT corresponds to a thermal aggregation rate $k_{agg}^{\text{th,eff}} \approx 2 \times 10^{-4}$ M⁻¹ s⁻¹. This means that a solution with a particle concentration of 1 pM thermally shows significant aggregation on a time scale of $5 \times 10^7$ s ≈ 2 years. The shelf life of these particles is indeed about a few years, after which severe aggregation is observed. In comparison, in case the particles would immediately aggregate upon a single collision (hit-and-stick behavior), then the characteristic aggregation time would be drastically shorter, namely, about 5 min. This example clearly demonstrates that the OMC experiment is able to quantify aggregation rates in stable colloidal solutions with very low reactivities.

The range of rates that can be measured with the OMC experiment has an upper limit, which is determined by the maximum fraction of magnetic dimers that can react to a chemical dimer during the shortest possible actuation pulse. If all magnetic dimers become a chemical dimer during a mean interaction time of 2 s, it would correspond to $k_{agg}^{\text{mag}} = 5 \times 10^{-1}$ s⁻¹. The lowest measurable rate is determined by the standard deviation of the fraction of chemically converted magnetic dimers and the longest possible actuation pulse. Estimating this fraction to be about 0.02 after an interaction time of 30 s leads to $k_{agg}^{\text{mag}} = 5 \times 10^{-3}$ s⁻¹. Figure 4c shows the corresponding range in $k_{agg}^{\text{th,eff}}$ that can be measured. By varying the magnetic field amplitude, aggregation can even be accelerated, extending the measurable range of dimer formation rates from about $10^3$ to $10^7$ M⁻¹ s⁻¹.

**CONCLUSION**

We described an experiment that allows quantifying the dimer formation rate of submicrometer magnetic particles with low surface reactivity. Dimer concentrations are measured using an optomagnetic detection principle and attractive magnetic forces are used to accelerate chemical aggregation by bringing particles in close proximity. The aggregation rate is determined from the fraction of dimers that chemically aggregate during a certain interaction time.

The magnetic field settings to quantify aggregation rates were extensively studied and tested. The nonspecific aggregation rate of carboxylated 500 nm particles was measured for varying pH and ionic strength of the aqueous buffer. The aggregation rate increases over 2 orders of magnitude when decreasing the absolute zeta potential of the particles (by decreasing the pH of the buffer solution) or when increasing the ionic strength of the solution, in both cases caused by a reduction of the interparticle electrostatic repulsion.

Aggregation rates measured with the OMC experiment are significantly faster than the aggregation rate of identical particles in the absence of a magnetic field. The aggregation rates measured in the presence of attractive magnetic forces
were extrapolated to chemical aggregation rates in the absence of force, taking into account the thermal encounter and separation rates due to Brownian motion. The rates measured with the OMC experiment translate to thermal dimer formation rates \( k^{\text{dil}} \) in the range of \( 10^4 \) to \( 10^5 \) M\(^{-1}\) s\(^{-1}\). Thus, the described methodology makes a range of very low aggregation rates experimentally accessible, for fundamental studies on colloidal stability as well as optimizations with respect to surface chemistries and performance in complex matrices.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.9b01461.

Citic acid buffer compositions; experimental setup of OMC experiment; OMC readout; tuning experimental settings; calculated clustering process; scattering signal shape; and sedimentation in the OMC experiments (PDF).

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Notes

The authors declare no competing financial interest.

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**REFERENCES**

(1) Sun, C.; Lee, J.; Zhang, M. Magnetic nanoparticles in MR imaging and drug delivery. *Adv. Drug Delivery Rev.* 2008, 60, 1252–1265.

(2) Indira, T. K.; Lakshmi, P. K. Magnetic nanoparticles - A review. *Int. J. Pharm. Sci. Nanotechnol.* 2010, 3, 105–1042.

(3) Vuong, Q. L.; Gillis, P.; Roch, A.; Gossuin, Y. Magnetic resonance relaxation induced by superparamagnetic particles as contrast agents in magnetic resonance imaging: a theoretical review. *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.* 2017, 9, e1468.

(4) Howes, P. D.; Chandrawati, R.; Stevens, M. M. Colloidal nanoparticles as advanced biological sensors. *Science 2014*, 346, 1247390.

(5) van Reenen, A.; Gutierrez-Meija, F.; van IJzendoorn, L. J.; Prins, M. W. J. Torsion profiling of proteins using magnetic particles. *Biophys. J.* 2013, 104, 1073–1080.

(6) Lipert, J.; Kerssemakers, J. W. J.; Jager, T.; Dekker, N. H. Magnetic torque tweezers: measuring torsional stiffness in DNA and RecA-DNA filaments. *Nat. Methods 2010*, 7, 977–980.

(7) Min, Y.; Akbulut, M.; Kristiansen, K.; Golan, Y.; Israelachvili, J. The role of interparticle and external forces on magnetic particles using magnetic particles. *Biophys. J.* 2013, 104, 1073–1080.

(8) Shenhar, R.; Norsten, T. B.; Rotello, V. M. Polymer mediated nanoparticle assembly: Structural control and applications. *Adv. Mater. 2005*, 17, 657–669.

(9) Inan, H.; Poyraz, M.; Inci, F.; Lifson, M. A.; Baday, M.; Cunningham, B. T.; Demirici, U. Photonic crystals: emerging biosensors and their promise for point-of-care applications. *Chem. Soc. Rev. 2017*, 46, 366–388.

(10) Subramanian, G.; Manoharan, V. N.; Thorne, J. D.; Pine, D. J. Ordered macroporous materials by colloidal assembly: A possible route to photonic bandgap materials. *Adv. Mater. 1999*, 11, 1261–1265.

(11) Xia, Y.; Gates, B.; Park, S. H. Fabrication of three-dimensional photonic crystals for use in the spectral region from ultraviolet to near-infrared. *J. Light. Technol. 1999*, 17, 1956–1962.

(12) Müller, J.; Bauer, K. N.; Prozeller, D.; Simon, J.; Malländer, V.; Wurm, F. R.; Winzen, S.; Landfester, K. Coating nanoparticles with tunable surfactants facilitates control over the protein corona. *Biomaterials 2017*, 115, 1–8.

(13) Suk, J. S.; Xue, Q.; Kim, N.; Hanes, J.; Ensign, L. M. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Adv. Drug Delivery Rev. 2016*, 99, 28–51.

(14) Ranzon, A.; Schleip, J. H. B.; van IJzendoorn, L. J.; Prins, M. W. J. Frequency-selective rotation of two-particle nanoactuators for rapid and sensitive detection of biomolecules. *Nano Lett. 2011*, 11, 2017–2022.

(15) Singer, J. M.; Plotz, C. M. The latex fixation test. *Am. J. Med. 1956*, 21, 888–892.

(16) Oppenheimer, S. B.; Odencrantz, J. A quantitative assay for measuring cell agglutination: Agglutination of sea urchin embryo and mouse teratoma cells by concanavalin A. *Exp. Cell Res. 1972*, 73, 475–480.

(17) Cohen, R. J.; Benedek, G. B. Immunoassay by light scattering spectroscopy. *Immunochimistry 1975*, 12, 349–351.

(18) Virella, G.; Waller, M.; Fudenberg, H. H. Nephelemetric method for determination of rheumatoid factor. *J. Immunol. Methods 1978*, 22, 247–251.

(19) Fischer, K.; Schmidt, M. Pitfalls and novel applications of particle sizing by dynamic light scattering. *Biomaterials 2016*, 98, 79–91.

(20) Baudry, J.; Rouzeau, C.; Goubault, C.; Robic, C.; Cohen-Tannoudji, L.; Koenig, A.; Bertrand, E.; Bibette, J. Acceleration of the recognition rate between grafted ligands and receptors with magnetic forces. *Proc. Natl. Acad. Sci. U.S.A 2006*, 103, 16076–16078.

(21) Ranzon, A.; Schleip, J. H. B.; van IJzendoorn, L. J.; Prins, M. W. J. Frequency-selective rotation of two-particle nanoactuators for rapid and sensitive detection of biomolecules. *Nano Lett. 2010*, 11, 2017–2022.

(22) Mackowski, D. W. A general superposition solution for electromagnetic scattering by multiple spherical domains of optically active media. *J. Quant. Spectrosc. Radiat. Transfer 2014*, 133, 264–270.

(23) van Vliembergen, R. W. L.; van IJzendoorn, L. J.; Prins, M. W. J. Distance within colloidal dimers probed by rotation-induced oscillations of scattered light. *Opt. Express 2016*, 24, A123.

(24) van Reenen, A.; de Jong, A. M.; Prins, M. W. J. Accelerated particle-based target capture the role of volume transport and near surface alignment. *J. Phys. Chem. B 2013*, 117, 1210–1218.

(25) Delcanale, P.; Miret-Ontiveros, B.; Arista-Romero, M.; Pujals, S.; Albertazzi, L. Nanoscale mapping functional sites on nanoparticles by points accumulation for imaging in nanoscale topography (PAINT). *ACS Nano 2018*, 12, 7629–7637.

(26) Jayaraman, A. Polymer grafted nanoparticles: Effect of chemical and physical heterogeneity in polymer grafts on particle assembly and dispersion. *J. Polym. Sci., Part B: Polym. Phys. 2013*, 51, 524–534.

(27) Reynolds, C. P.; Klop, K. E.; Lavergne, F. A.; Morrow, S. M.; Aarts, D. G. A. L.; Dullens, R. P. A. Deterministic aggregation kinetics of superparamagnetic colloidal particles. *J. Chem. Phys. 2015*, 143, 214903.

(28) Smoluchowski, M. Drei Vorträge über Diffusion, Brownsche Molekularbewegung und Koagulation von Kolloidteilchen. *Z. Phys. 1916*, 2, 530–594.

(29) Nekrasov, V. M.; Polshchitsin, A. A.; Yurkin, M. A.; Yakovleva, G. E.; Maltsev, V. P.; Chernyshev, A. V. Brownian aggregation rate of colloid particles with several active sites. *J. Chem. Phys. 2014*, 141, 064309.
(30) Biancaniello, P. L.; Crocker, J. C. Line optical tweezers instrument for measuring nanoscale interactions and kinetics. *Rev. Sci. Instrum.* 2006, 77, 113702.

(31) Wang, Z.; He, C.; Gong, X.; Wang, J.; Ngai, T. Measuring the surface-surface interactions induced by serum proteins in a physiological environment. *Langmuir* 2016, 32, 12129–12136.