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Binding kinetics of magnetic nanoparticles on latex beads studied by magnetorelaxometry

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A system of magnetic nanoparticles (MNPs) and modified latex spheres was used serving as a model for studying binding reactions. The binding of MNPs on latex is estimated by the change of the magnetic relaxation properties of the MNP measured by a SQUID-based magnetorelaxometry measurement system. By fitting of subsequently recorded relaxation curves, the kinetics of the binding reactions, i.e. the evolution of the fraction of bound MNPs, was extracted. The signal of bound MNPs scales linearly with the concentration of latex beads. For low latex concentrations the kinetics are described by a simple aggregation model, providing information about the density and probability of bindings to the target surface. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: magnetic nanoparticles; latex; coupling kinetics; binding model; relaxometry; relaxation; concentration

INTRODUCTION

The study of binding reactions between bio-molecules is important for many fields of bio-sciences, e.g. for monitoring the effectiveness of drug applications or the selective coupling of antibodies or particular protein structures to their specific binding sites. Molecular binding is characterized by its stability, i.e. the degree of reversibility, by the relative amount of realized bindings and by the kinetics of the reaction.

A tool for characterizing these aspects of bio-molecular binding reactions is the magnetic relaxation immunoassay (MARIA),[1,2] which is based on the method of magnetorelaxometry (MRX), i.e. the measurement of the magnetic relaxation of magnetic nanoparticles (MNPs) using superconducting quantum interference devices (SQUIDs). For MARIA, MNPs are used to label one of the reaction partners. When a labelled molecule binds to a molecular structure that is fixed to a solid phase, magnetic relaxation behaviour may change significantly due to the suppression of Brownian motion. Thus, MARIA provides a quantitative measure of the amount of bound molecules in the presence of the unbound molecules.

In real biological systems, such as blood, binding to moving targets is often of interest. In this situation Brownian relaxation of bound particles is not fully suppressed, because they still are subjected to the Brownian motion of the moving target. As a consequence, the discrimination between signals of bound and unbound signals may become more difficult. The aim of the present study is to investigate the decomposition of the signals corresponding to bound and unbound magnetic particles experimentally. To this end, we studied a model system composed of a suspension of large latex beads to the surface of which MNPs couple. In particular, we measured the kinetics of the binding reaction by MRX. The results are compared with the predictions of a straightforward aggregation model.

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EXPERIMENT

Particles

As targets for the coupling of MNPs we used latex spheres with a diameter of 5 μm. The latex beads were coated with a polymer presenting an amino group. The MNPs consist of magnetic cores surrounded by a surfactant layer that contains carboxyl groups. These carboxyl groups, loaded with a counterion (Na^+), couple via ion exchange on the amino groups of the latex beads (Fig. 1).

Samples and measurements

Four different water-based ferrofluids, namely Resovist, G328, D446 and DDM128, were used as magnetic probes for

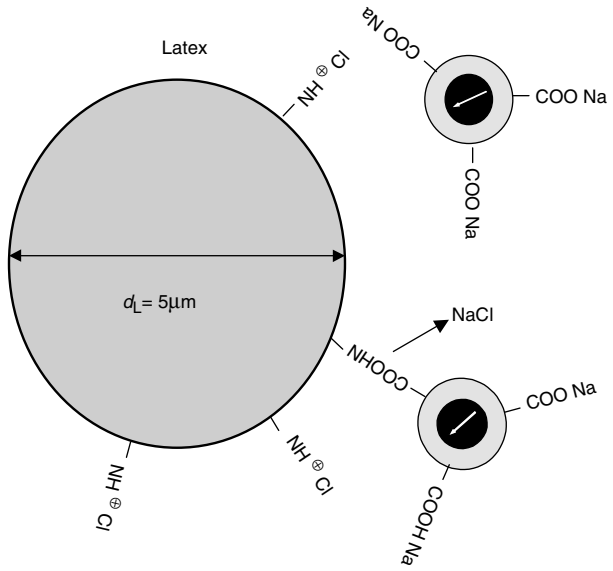

Figure 1. Schematic picture of the binding of a latex bead to MNPs.

investigation of their binding to the latex beads. In order to characterize these nanoparticle preparations, they were first investigated in the freeze-dried state, where the particles are immobilized and relax through the Néel mechanism. From these relaxation curves we have estimated the moments μ and σ of particle core-size distribution (assumed to be a log-normal distribution) by fitting the data with the moment superposition model described Eberbeck *et al.*[3] Using these data, it is possible to estimate the particle concentration $n_{\mathrm{MNP,L}} = V_S c_V/(\frac{\pi}{6} d_V^3)$ of an aqueous suspension of the MNPs considered, where V_S is the volume of the suspension (Table 1).

To start a coupling reaction, 50 µl of the MNP suspensions were placed into a vial containing 100 µl of an aqueous latex suspension. Measurements were performed on latex suspensions of different bead concentrations. Then, the sample was placed into the MRX device, and the measurement of the relaxation began some 30 s later. Relaxation curves were measured every 10 s for several minutes.

Magnetic relaxation was measured using a device and a procedure described earlier in detail.[4] In short, a magnetizing field of $H = 1300$ A m^{-1} was applied for $t = 1$ s. 450 µs after switching off the field, a highly sensitive SQUID recorded the magnetic induction $B_r(t)$ at a distance of 10 mm above the sample. The remanent induction of the sample was determined by the difference between measurement curves of the sample and that of the empty sample holder.

Data treatment

The relaxation signal

$$B(t) = \beta B_b(t) + (1-\beta) B_{ub}(t) \quad (1)$$

consists of two contributions: $B_b(t)$, the relaxation of the MNP bound on latex, and $B_{ub}(t)$, signal of the unbound MNPs. β represents the relative amount of the signal of the bound MNPs. $B_b(t)$ is assigned to the relaxation curves measured on samples with an excess of latex. It is the upper limit for $\beta \to 1$ considering the concentration dependency of the relaxation curves. The curve for the fraction of unbound MNPs may also contain signals of aggregates of MNPs in addition to the relaxation of primer MNPs. It is known from the measurement of MNP suspensions without latex that it can be described fairly well by a stretched exponential function

$$B_{ub}(t) = B_0(t) \exp\left(\frac{-t^\alpha}{\tau}\right) \quad (2)$$

MODEL OF AGGREGATION

For the purpose of the interpretation of the measured binding kinetics we will now calculate the time and concentration dependency of β with the help of a simple model which incorporates the microscopic structure of the particles. According to the model of von Smoluchowski, we consider the diffusion-driven collision of free movable particles. If every collision leads to an irreversible binding then two

Table 1. Concentrations and size parameters of the MNPs and latex beads, d_V denotes the diameter of the particle with the mean volume

	Concentration		Core-size distribution parameter			
	Iron (mol m^{-3})	Magnetite/latex (vol.%)	μ	σ	Mean diameter d_V (nm)	Particle concentration n_L, n_{MNP} (m^{-3})
Latex	—	1.2	—	—	5000	1.84×10^{14}
Resovist	500	0.000 738	6.5	0.38	8	2.75×10^{19}
G328	702	0.001 02	4.78	0.51	7.1	1.95×10^{19}
D446	542	0.0008	2.1	0.48	3.0	52.15×10^{19}
DDM128	476	0.0007	12.0	0.204	12.8	0.67×10^{19}

primer particles per event become aggregated. For the number density of the primer particles we then have

$$\frac{dn}{dt} = -\alpha_i n^2 \tag{3}$$

with the interaction parameter $\alpha_i = 8\pi RD$ where R and D are the radius and the diffusion constant of the primer particles.[5]

We now modify this model for the present situation. A primer particle (in our case the MNP) becomes bound with some probability only if it collides with a latex bead. Collisions among latex beads, as well as among MNPs, will not be taken into account because of their less effective binding. This is why particles of the same type present binding molecules which do not complement among each other, i.e. having the same charge. Equation (3) is modified to

$$\frac{dn}{dt} = -\alpha_i D n_L n \quad n(t=0) = n_0 \tag{4}$$

where n is now the concentration of the primer particle and n_L is the concentration of the target particles, i.e. the latex beads. The interaction parameter becomes

$$\alpha_i = \alpha_B \alpha_C = \alpha_B 4\pi (R + R_L)^2 \frac{D + D_L}{R + R_L} \tag{5}$$

which composes of the collision coefficient α_C and the probability α_B that a collision leads to a binding. Further, we take into account that only a limited fraction of latex surface α_F will be covered by MNPs. This may be related with the density of the binding molecule groups. Additionally, the free target area for the MNPs, i.e. the actual non-occupied surface of the latex beads, reduces during aggregation. Thus

$$\alpha_i = \alpha_B \left[\alpha_F 4\pi (R + R_L)^2 - \frac{n_0 - n}{n_L} \pi R^2 \right] \frac{D + D_L}{R + R_L} \tag{6}$$

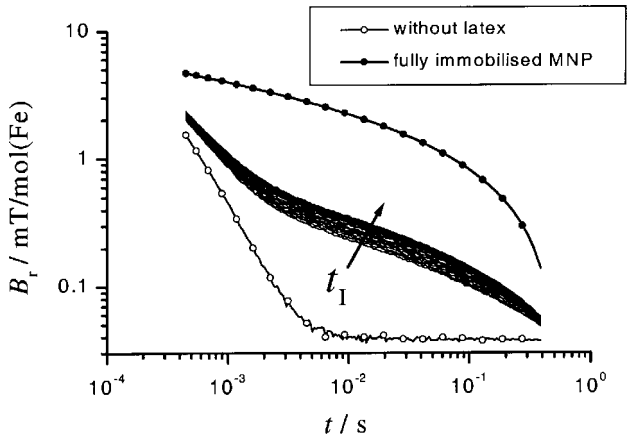

Figure 2. A time series of relaxing magnetic moment of an MNP-suspension added to a 8.5% latex suspension together with a pure MNP suspension and MNPs in excess of latex. t_I indicates the time after incubation.

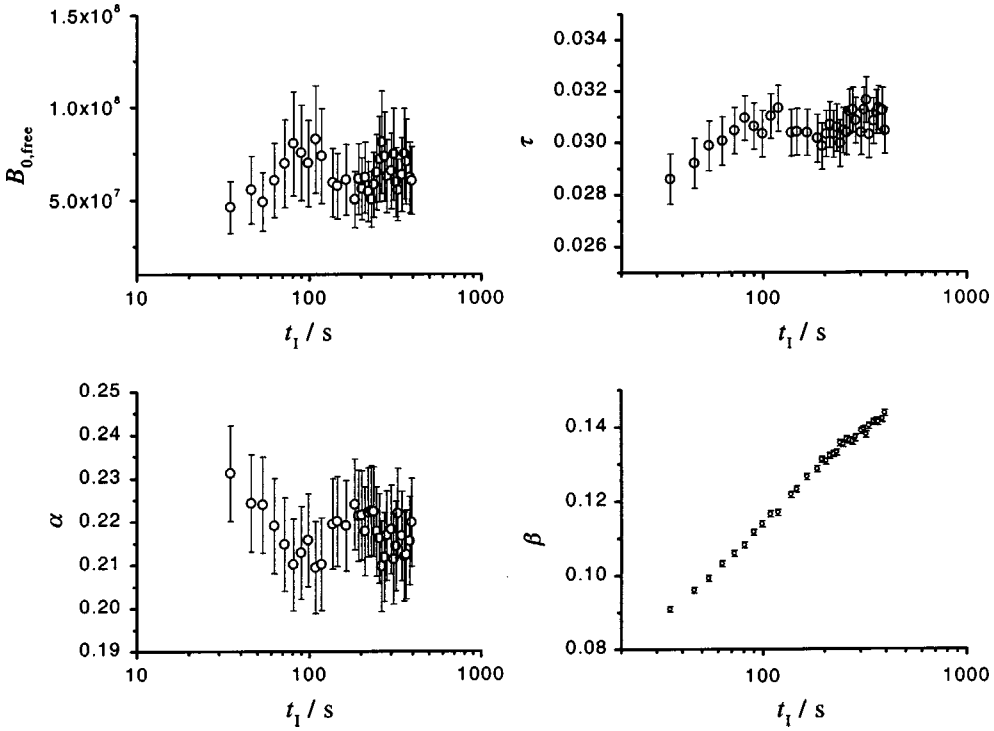

Figure 3. Parameters estimated by fitting of $B(t)$ according to Eqn (1) with Eqn (2) for 8.5 µl latex suspension incubated with Resovist. For β the uncertainties hardly exceed the size of the symbols.

The solution of Eqn (4) with Eqn (6) is

$$\frac{n(t)}{n_0} = \frac{n_0 A_C - \alpha_F n_L q}{n_0 A_C - \alpha_F n_L q \quad \exp\left[-\frac{\alpha_B}{\alpha_F}(n_0 A_C - \alpha_F n_L q)\frac{D + D_L}{R + R_L}t\right]} \tag{7}$$

with $A_C = \pi R^2$ being the cross-sectional area of MNP and $q = 4\pi(R + R_L)^2$ being the cross-section of the collision between an MNP and a latex sphere.

RESULTS AND DISCUSSION

The observed relaxation time of MNPs without latex is in the region of 2 ms. The Brownian relaxation time of a single MNP with a core diameter of $d_{Core} = 10$ nm and a surfactant layer thickness of $\delta = 3$ nm is $\tau_B \approx 10^{-6}{}_s$, which is below the measurement time window. This implies that the observed relaxation is generated by aggregates of MNPs in the sample, rather than by a single MNP. The maximum size of MNP aggregates is deduced from the time at which B_r reaches zero (Fig. 2) to be approximately 130 nm.

At just 30 s After the addition of MNPs to a latex suspension, the relaxation of the magnetic moments of the MNPs is already much slower than that of a pure MNP suspension (Fig 2).

With increasing time the relaxation of the magnetic sample moment becomes slower, i.e. the fraction of bound particles having a longer relaxation times increases. With an excess of latex beads the relaxation reaches a limiting curve. In this situation all the particles have a long Brownian relaxation time, indicating that they bind to the latex beads.

Fitting the relaxation curves according to Eqn (1) with Eqn (2) we obtain a set of parameters for each relaxation curve measured at time t_I. The results (Fig. 3) show that only the

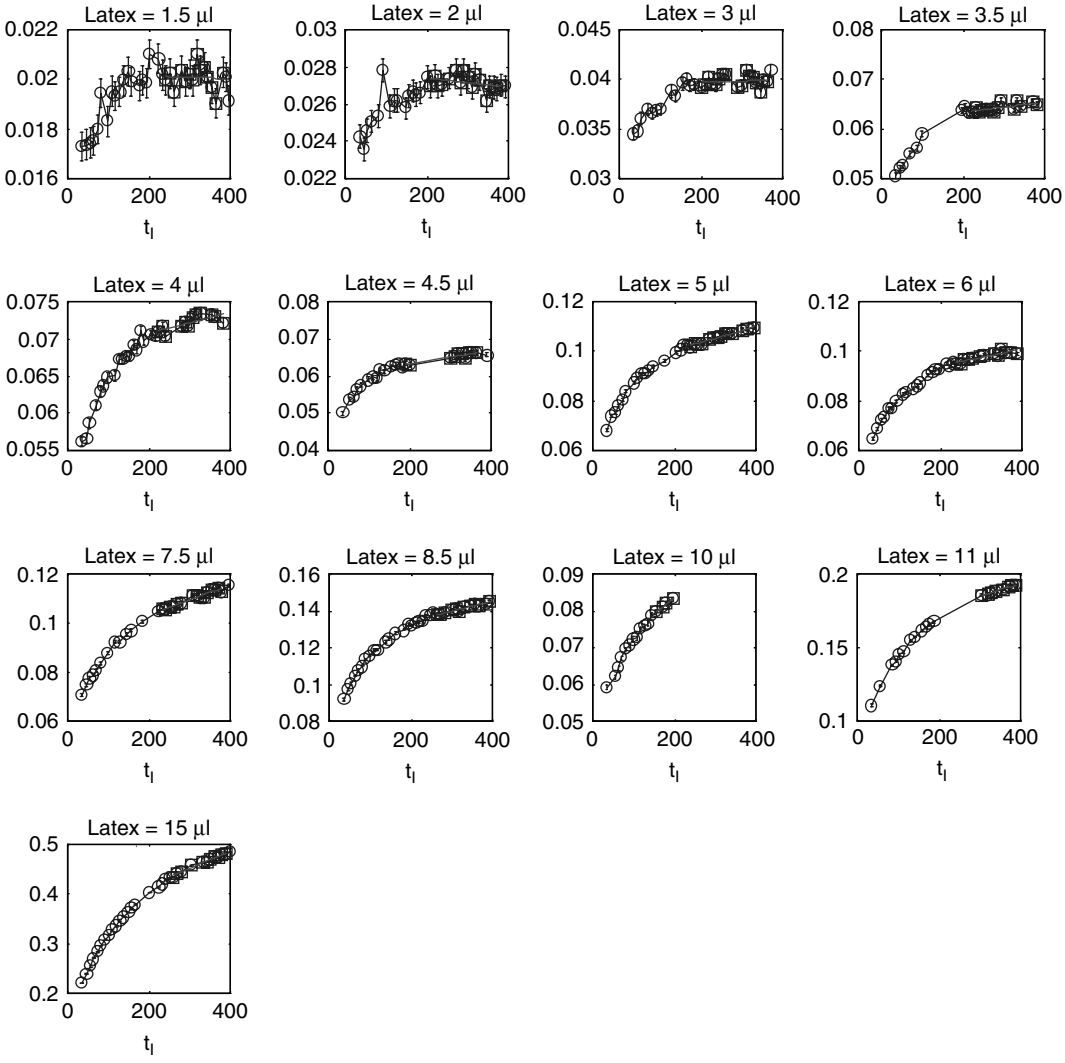

Figure 4. Fraction of bound MNPs β as a function of incubation time t_I estimated by fitting of $B(t)$ according Eqn (1) with Eqn (2) for samples with different latex concentrations. The quantity of original latex suspension is indicated at the top of the diagrams.

fraction of bound particles β changes remarkably. The small uncertainties of β demonstrate a good separability of bound and unbound MNPs.

The parameters $B_{0,\mathrm{free}}$, τ and α, representing the relaxation of the remaining small aggregates of unbound particles, do not vary significantly, indicating that the distribution of these aggregates does not change significantly.

The function $\beta(t_\mathrm{I})$ reflects the kinetics of the binding reaction. Now, the dependency of the relaxation on time t_I was measured on samples with different concentrations of latex beads. Figure 4 shows the results for $\beta(t_\mathrm{I})$ obtained by fitting the corresponding sets of relaxation curves. In the long time limit ($t_\mathrm{I} \to \infty$), $\beta(t_\mathrm{I})$ reaches a time-independent saturation value β_sat for low latex concentrations (Fig. 4). For higher latex concentrations a saturation behaviour does not take place during the observation time. Obviously, the equilibrium time becomes remarkably longer, indicating the emergence of some slow binding processes. This is in contrast to the proposed simple model, where curves calculated according to Eqn (7) do not show a significant dependency of equilibrium time on the concentration (Fig. 5).

For low concentration of latex, the measured binding of Resovist-MNP to latex beads can be described by this model (figure 5(a)) using the core size distribution parameters given in table 1 and a surfactant layer thickness of about 3 nm (R. Lawaczeck, personal communication, 2003).[6] By this fit we determine the reaction parameters to be $\alpha_\mathrm{B} = 0.45$ and $\alpha_\mathrm{F} = 0.52$. With these parameters it is *not* possible to describe the data assigned to higher latex concentrations (Fig. 5b, dashed curve). Fitting the long time (t_I) part of the data we obtain $\alpha_\mathrm{B} = 0.17$ and $\alpha_\mathrm{F} = 0.66$. The significantly smaller value of α_B also seems to be connected with some slower relaxation processes. A fit of these data in the full time range with Eqn (7) is not satisfying. Rather, the data look like a superposition of relaxations according to a faster and a slower binding mechanism.

Now we consider the concentration dependency of the final value β_f calculated by a linear fit of the long time tail of $\beta(t_\mathrm{I})$ (Fig. 4). For the measured aggregation systems with different ferrofluids the relationship between latex concentration and the apparent fraction of bound MNPs is nearly linear (Fig. 6a). This behaviour can be understood in terms of the proposed aggregation model.

Further, the aggregation speed was be parameterized by the initial value of $\mathrm{d}\beta/\mathrm{d}[\log(t_\mathrm{I})]$, i.e. the slope of the first linear part of $\beta(\log t_\mathrm{I})$ in Fig. 3. The concentration dependency of this value shows a slight non-linear behaviour (Fig. 6b). Equation (7), on the other hand, predicts a linear dependency of the kinetic parameter on the latex concentration. Again, we refer this slight discrepancy to supplementary aggregation processes, e.g. among the MNPs.

Finally, we have compared $\beta_\mathrm{f}(n_\mathrm{L}/n)$ with data calculated according Eqn (7) for Resovist and G328 with known structure data (Fig. 7). The horizontal shift of the curves is sensitive to the ratio of the total cross-sectional area of the MNPs to the total surface of the latex beads which can be occupied by MNPs. This ratio depends on α_F as well as on the size of the MNPs. The model describes the data with the structure parameters given. However, the value of the covering parameter α_F can only be determined if the size of the MNPs and especially the thickness of the surfactant layer are well known.

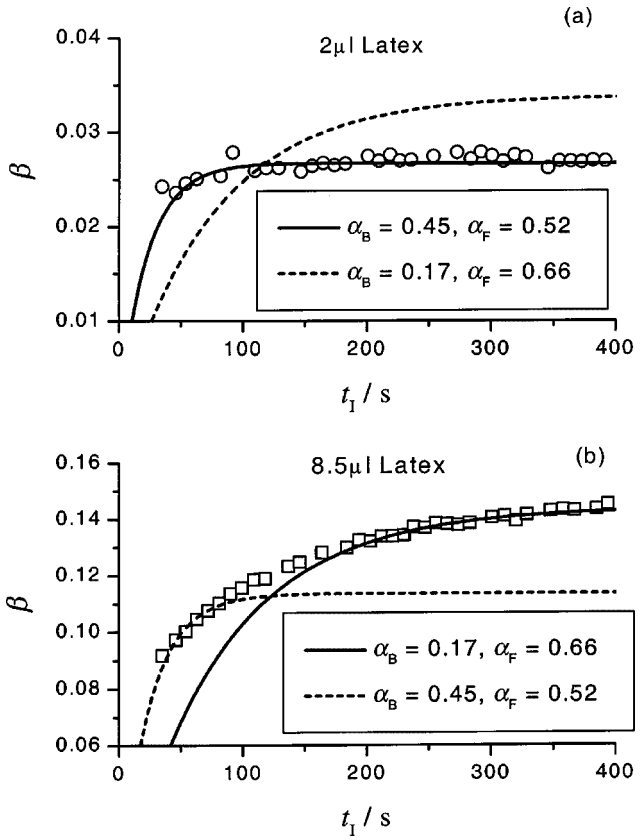

Figure 5. Measured time dependency of binding parameter β compared with the model in Eqn (7), calculated for two latex quantities using two different sets of binding reaction parameters α_B and α_F (see text). The parameters α_B and α_F describe the data for large t_I for one concentration (solid line) but are not appropriate for the data of the other concentration (dashed line).

CONCLUSIONS

We have shown experimentally that the simple two-component particle model system of surface-charged latex and MNPs is well suited to studying binding kinetics in some detail. With the decomposition of magnetic relaxation signals arising from bound and unbound MNPs we could determine a quantitative relationship between the concentration of target particles (latex) and the binding-specific magnetic signal arising from MNPs. Because of the linearity of the relation, the method allows a comfortable estimation of unknown quantities using only a few calibration values. If the target

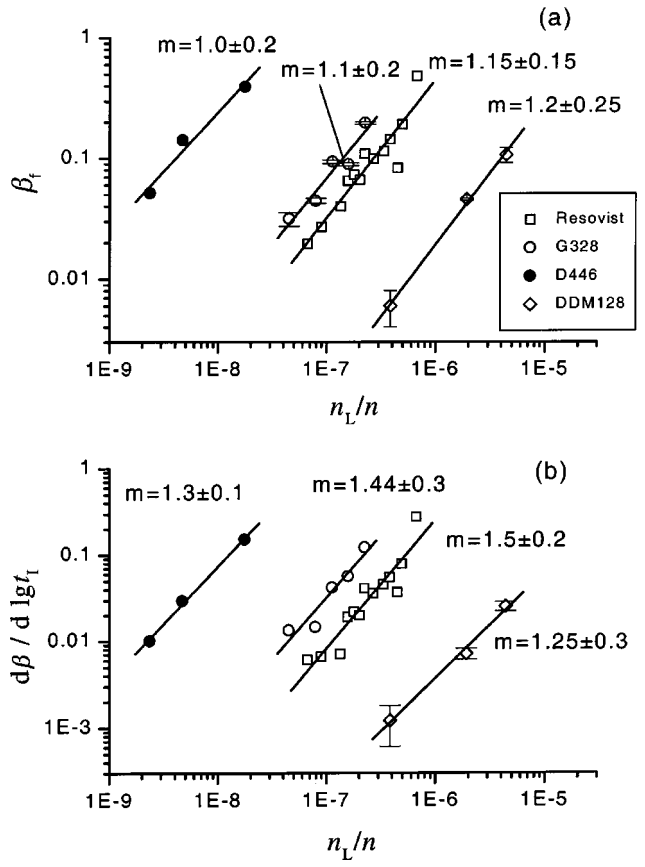

Figure 6. Dependency of (a) final value of $\beta(t_1)$ and (b) the kinetic parameter $d\beta/d(\log t)$ on the particle concentration of latex beads in comparison with that of the MNPs for the different MNP suspensions investigated. The lines are linear fits and m indicates the corresponding slopes.

concentration is well defined one can gain information about the number of couplet MNPs, at least for low target to MNP ratios, which can easily reached by variation of MNP quantity in the experiment. Thus, one might be able to deduce the number of specific binding sites on cells for example.

With this comparatively fast measurement method it was possible to observe the kinetics of the binding reaction. For low target concentrations it was possible to describe the kinetics by a simple model. This allows the determination of further binding-related properties, e.g. the probability of an effective binding after a collision of the probe with the target.

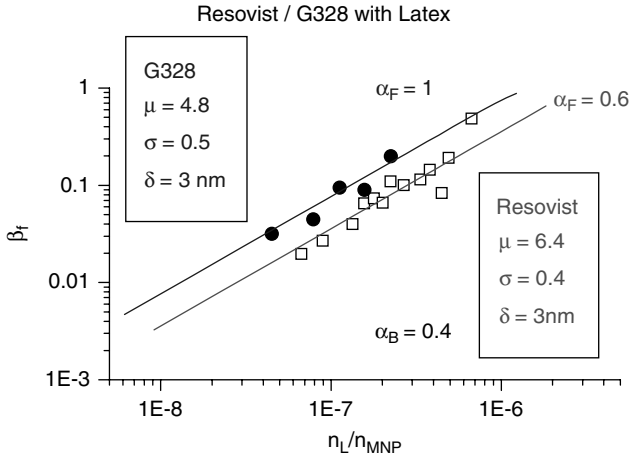

Figure 7. Comparison between measured concentration dependency of β_f and calculated (not fitted) data.

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