INVESTIGATION OF THE BINDING AND AGGREGATION OF FUNCTIONALISED MAGNETIC NANOPARTICLES IN DIFFERENT SUSPENSIONS BY MAGNETORELAXOMETRY

D. Eberbeck[1], *Ch. Bergemann*[2], *F. Wiekhorst*[1], *U. Steinhoff*[1], *S. Hartwig*[1], *L. Trahms*[1]

[1] *Physikalisch-Technische Bundesanstalt, 2-12 Abbestraße, D-10587 Berlin, Germany (dietmar.eberbeck@ptb.de)*
[2] *chemicell GmbH, 6 Wartburgasse, D-10823 Berlin, Germany*

Introduction. Coated magnetic nanoparticles (MNP) serve as probes for the investigation of biological binding reactions in Magnetic Relaxation ImmunoAssays (MARIA) [1, 2]. MARIA is based on MagnetoRelaXometry (MRX) which is an integral and sensitive methode to quantify the binding behaviour of functionalized MNP. In contrast to flourescence and radioactive assays, MRX allows to distinguish between bound and unbound MNP-labeled reagents without washing steps. Non-aggregated well separated probes are required for immunological detection tasks.

MRX also allows to assess quantitatively the aggregation of MNP in suspension. Here, we studied the effect of different MNP coatings (polar, nonpolar, biological) in different suspension media such as aqua dest., phosphate buffered saline (PBS) or serum on the aggregation behaviour of the magnetic probes. This serves to estimate the non-specific binding of functionalized magnetic probes which makes the analysis of the specific bindings more difficult. Specific binding of biological molecules was studied by the coupling between biotinylated latex spheres and streptavidin linked MNP.

Magnetorelaxometry is the measurement of the relaxation of the magnetic moment of magnetic nanoparticles (MNP) after switching off the external polarisation field. There are two mechanisms of the relaxation of the particle moments: (i) Néel-relaxation, i.e., the flipping of the moment inside the particle, (ii) the Brownian relaxation: movement of the entire particle in the suspension. If the particles become immobilised, the latter mechanism is suppressed and the relaxation prolongs [3]. By separation of fast and slow relaxation contributions we can quantify the amount of bound and unbound magnetic probes [4].

The size, here the hydrodynamic diameter d_{hyd}, of the relaxating structures (MNP, aggregates) determines the time constant of the Brownian relaxation

$$\tau_{\mathrm{B}} = \frac{\pi\,\eta d_{\mathrm{hyd}}^3}{2\,k_{\mathrm{B}}T} \quad . \tag{1}$$

For the dynamic viscosity of the fluid, η, the values are 0.001 Pa s and 0.002 Pa s for water and serum were taken, respectively. k_{B} and T are the Boltzmann constant and temperature.

1. Samples and measurement. The MRX-measurement system is described in [5] and bases on Superconducting Quantum Interference Devices (SQUIDs) to achieve a high sensitivity.

The investigated MNP consist of magnetite cores coated with negatively charged molecules like polyacrylic acid (MagAS), carboxymethyl-dextran (Reso-

vist) or silica (SiMag-basic/10) or with nearly neutral molecules like dextran (Mag-DX92). Two types of MNP with biomolecular functionalisation were investigated: (i) MNP coated with carboxymethyl-dextrin (V128) were covalently bound to γ-globuline, (ii) MNP coated with carboxymethyl-dextran were covalently bound to streptavidin (Table 1).

Aqueous suspensions of MNP were prepared by dilution with different media like water, phosphate-bufferd-saline (PBS) or fetal calf serum (FCS) or human serum. For the measurement, 150μl of the suspensions were filled into a polystyrene-well. Samples with fully immobilized MNP were prepared by freeze drying and served as controls.

For the detection of specific binding between biomolecules, streptavidin-linked MNP were incubated with large biotin-latex beads (diameter 4 μm).

2. Results and discussion.

2.1. Aggregation of MNP in different media. The time courses of relaxaion, for Resovist depicted in Fig. 1, are parametrized by the amplitude $\Delta B = B(t_{\mathrm{start}} - B(t_{\mathrm{end}}))$ and the relaxation time τ^* being the time, at which $B(\tau^*) - B(t_{\mathrm{end}}) = 1/e\,\Delta B$ (Table 1). The results clearly demonstrate that the relaxation time of immobilised MNP, τ_{s}^*, is much higher (15 times for Resovist) than for fluid samples τ_{fl}^*.

The hydrodynamic diameters d_{hyd} of the relaxing structures in Table 1 are calculated from the relaxation time of the fluid samples τ_{fl}^* according to (1). Assuming that d_{hyd} of the single particles equals the structural size, i.e., $d_{\mathrm{hyd}} = d_{\mathrm{core}} + \delta_{\mathrm{shell}} \approx 8\,\mathrm{nm} + 5\,\mathrm{nm}$, equation (1) yields $\tau_{\mathrm{B}} = 1\mu\mathrm{s}$ being much less than the lower bound of the measurement time window of $400\mu\mathrm{s} \leq \mathrm{t_m} \leq 0.5\,\mathrm{s}$. Therefore, the presence of a relaxing magnetisation in the given time window indicates that aggregates of MNP are present. The amount of MNP, which is organised in such aggregates, is related to the ratio of the relaxation amplitudes $\Delta B_{\mathrm{fl}}/\Delta B_{\mathrm{s}}$.

The relaxation curves of Resovist in water scale with the concentration of MNP (Fig. 1). This is a prerequisite to apply MRX for binding quantification tasks. A small fraction of MNP is present in aggregates with a mean diameter

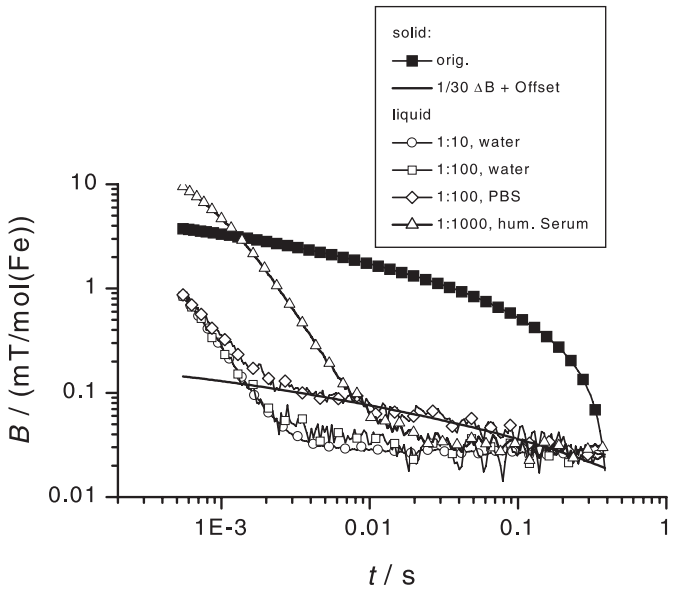

Fig. 1. Relaxation curves, normalised to the magnetite content, of Resovist in water, PBS and human serum.

Table 1. Parameter of the magnetic relaxation of the samples. The uncertainty of relaxation amplitudes is about 10% and that of relaxation times about 5%.

sample	surfac-tant	solvent	agg.		relaxation parameters				
			CS	P	ΔB_{fl} $\frac{mT}{mol}$	$\frac{\Delta B_{\mathrm{fl}}}{\Delta B_{\mathrm{s}}}$	τ_{fl} ms	$\frac{\tau_{\mathrm{fl}}}{\tau_{\mathrm{s}}}$	d_{hyd} nm
polar surfactants									
MagAS11	acrylic	water	+	-	2.3	0.39	1.1	0.08	145
	acid	PBS	-	+					
		FKS	+	-	10.2	1.03	2.0	0.13	139
Resovist	carboxy	water	+	-	0.8	0.22	0.9	0.05	135
	-dextran	PBS	+	-	0.9	0.23	1.0	0.06	141
		serum h.	+	-	7.6	2.04	1.2	0.07	118
		FKS	+	-	3.0	0.80	1.2	0.07	119
SiMag	silicate	water	+	-	5.8	4.20	2.9	0.16	198
-basic/10		PBS	+	+	2.1	1.53	10.5	0.58	305
non-polar surfactants									
Mag-DX92	dextran	water	-	+	11.6	1.35	24	1.00	403
		PBS	+	-	15.6	1.81	3.1	0.13	203
bio-molecular surfactants									
V182	carboxy	water	+	-	3.5	0.58	2.2	0.11	181
	-dextrin	PBS	+	+	11.0	1.83	32.0	1.60	442
	γ-Glob.	FCS		-	25.0	4.17	10.0	0.50	238

Abbreviations: agg.: aggregation, CS: concentration scaling, P: visual precipitation of MNP, γ-Glob.: γ-Globuline

related to $d_{\mathrm{hyd}} \approx 150\,\mathrm{nm}$. By preparation in PBS, on the other hand, a relaxation contribution attributed to aggregates with τ^* beyond Δt_{m} emerges, i.e., with a size $d_{\mathrm{hyd}} > 1000\,\mathrm{nm}$. Comparing the relaxation curve with the scaled curve of immobilised MNP, we can calculate that only about 3% of the MNP are organised in these large aggregates (Fig. 1).

The aggregation of the charged particles can be explained by the reduction of the energy barrier of the resulting interaction forces due to emerging of an electrical double layer in polar media. Thereby, with an increasing ion concentration, the effective range of the electrical repulsion potential reduces [6]. This is supported by the fact that MagAS-MNP, loaded with stronger negatively charged surfaces, floculate apparently completely in PBS, whereas MNP with neutral surfaces like dextran do not. In PBS they become even more stabilised than in water. They tend to agglomerate in water, as indicated by high $\tau^*_{\mathrm{fl}}/\tau^*_{\mathrm{s}}$-ratios and visual observation.

The preparation in FCS leads to higher relaxation times τ_{fl}, which we attribute to the two times higher viscosity of serums. Taking this into account, the hydrodynamic size of the aggregates decreases for all suspensions except for V182 with γ-globuline. However, the fraction of aggregated particles increasess by factors of 2.5 and 7 for MagAS, Resovist and V182, respectively.

2.2. Specific binding of labeled MNP . We incubated streptavidin-linked MNP 4 days after streptavidin coupling with biotin-latex. The measured relaxation curve had nearly a shape and amplitude like that of the sample, which was freeze dried afterwards. We conclude that all particles became immobilised on the latex spheres, for which τ_{B} is much greater than our measurement time. This experiment were repeated with the same probes one month later. The relaxation curve looked like a superposition of contributions of bound and unbound particles. The fraction of bound particles we estimated according [4] to 50%. This indicates an aging process in the sample, presumably a desorption of the streptavidin.

Conclusion. The presented result illustrates that MRX as an integral method is suitable for quantification of the aggregation and functionalisation of MNP. The aggregation depends significantly on the interplay between the surface of the particles and the suspension medium. Therefore, MRX seems to be a powerful tool for immunological detection tasks and checking of magnetic probes.

Acknowledgements. This work was supported by priority program 1104 of the Deutsche Forschungsgemeinschaft and German Ministry of Education and Research, Grant-No.: FKZ 13N8535.

REFERENCES

1. W. Weitschies, R. Kötitz, T. Bunte, L. Trahms. Determination of relaxing or remanent nanoparticle magnetization provides a novel binding-specific technique for the evaluation of immunoassays. *Pharm. Pharmacol. Lett.*, vol. 7 (1997), pp. 1–4.
2. J. Lange, R. Kötitz, A. Haller, L. Trahms, W. Semmler, W. Weitschies. Magnetrelaxometry – a new binding specific detection method based on magnetic nanoparticles. *J. Magn. Magn. Mat.*, vol. 252 (2002), pp. 381–383.
3. R. Kötitz, P.C. Fannin, L. Trahms. Time domain study of Brownian and Néel relaxation in ferrofluids. *J. Magn. Magn. Mat.*, vol. 149 (1995), no. 1-2, pp. 42–46.
4. D. Eberbeck, C. Bergemann, S. Hartwig, U. Steinhoff, L. Trahms. Binding kinetics of magnetic nanoparticles on latex berads studied by magnetorelaxometry. *App. Organometal. Chem.*, vol. 18 (2004), pp. 542–547.
5. H. Matz, D. Drung, S. Hartwig, H. Gross, R. Kötitz, W. Müller, A. Vass, W. Weitschies, L. Trahms. A SQUID measurement system for immunoassays. *Appl. Supercond.*, vol. 6 (1998), no. 10-12, pp. 577–583.
6. H. Sonntag. *Lehrbuch der Kolloidwissenschaft* (VEB Deutscher Verlag der Wissenschaften, Berlin, 1977).