# Structural characterization of nanoparticulate magnetic resonance contrast agents for MR relaxivity determination

C. Kremser[1], I. Paschkunova-Martic[2], K. Mistlberger[3], N. Shcherbakova[3], M. Galanski[2], W. Buchberger[1], B. Keppler[2], P. Debbage[3]

[1]Department of Radiology, Innsbruck Medical University, Innsbruck, Tyrol, Austria, [2]Institute of Inorganic Chemistry, University of Vienna, Vienna, Vienna, Austria, [3]Department of Histology and Molecular Cell Biology, Innsbruck Medical University, Innsbruck, Tyrol, Austria

**Introduction**

In molecular imaging nanoparticles will in future play an important role. Several groups have reported successful application of nanoparticles as magnetic resonance imaging (MRI) contrast media ([1],[2],[3])). In MRI, targeted nanoparticle contrast media (CM) with very high relaxivities are required in order to obtain adequate signal enhancement, due to the low number of target sites. The determination of relaxivity r is usually based on $\Delta R = R_i - R_{ref} = r\,c$ where R represents the longitudinal or transversal relaxation rate and c the concentration of the contrast agent. I.e., to measure relaxivity values the concentration of the used nanoparticles has to be known, which is not easily determined. In addition homogenous particle populations are assumed. The aim of this work was to apply structural nanoparticle characterization with special focus on nanoparticle counts to enable relaxivity determination.

**Matrials and Methods**

As model nanoparticles polystyrene beads were selected, because these have been well characterised for use in numerous industrial and biomedical applications and are available accurately calibrated in a wide range of nanometer and micrometer sizes. As targeting group the tomato lectin LEA was used which is capable of binding to all endothelial cells after intravenous application. The preparation of lectin-nanoparticle conjugates was based on the method described by Irache et al. [4]. The obtained lectin nanoparticles were conjugated with Gd-DTPA complexes yielding Latex/LEA/Gd-DTPA nanoparticles with diameters of 100nm and 400nm. Nanoparticle size distributions were determined by photon correlation spectroscopy (PCS, Submicron particle sizer nicomp 380, Particle Sizing Systems, Santa Barbara, California, USA). Size and structure of the nanoparticles was assessed by Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). TEM was also used to obtain nanoparticle counts based on a method described by Kato et al. [5]. The obtained nanoparticle counts served as basis to determine nanoparticle concentrations.

MR relaxivity measurements were performed on standard clinical 1.5 T whole body MR systems (Magnetom VISION and Magnetom Symphony, Siemens, Germany) using a linear polarized small loop receive coil. Serial dilutions of the lyophilized nanoparticles were prepared using distilled water, starting with a typical nanoparticle concentration of 50 mg/ml which was then serially diluted. As a reference substance pure distilled water was used. The individual suspensions were filled in 100 µl tubes (Eppendorf AG, Germany) and placed within the MRI receive coil using a special sample holder. $T_1$ measurements were performed using an inversion recovery sequence (TR=3.6 ms, TE=2.1 ms, flip angle= 8°, receive bandwidth=490 Hz/pixel, FOV=67 mm, acquisition matrix=64x128, 1 slice, slice thickness=5 mm) with 15 different inversion times between 140ms and 4000ms. $T_2$ measurements were performed using a CPMG-type multi echo spin-echo sequence (TR=1000 ms, TE=n*13.5 ms with n=1...16, receive bandwidth=130 Hz/pixel, FOV=51 mm, acquisition matrix=128x128, 1 slice, slice thickness=3 mm). T1 and T2 parameters were calculated by a 2 parameter fit to the obtained signal intensities. Relaxivities were calculated from the obtained relaxation rates of the dilution series by means of linear regression.

**Results**

The derivatised nanoparticles were compared with the non-derivatised nanoparticles that had served as starting material. In PCS, non-derivatised (control) latex nanoparticles exhibited single peaks within approx. 10% of nominal size. In direct visualisation by SEM and TEM their measured sizes were approximately 25% smaller than nominal size, partly due an immediate shrinkage observed to occur in the electron beam in TEM. Counts made on non-derivatised 400nm nanoparticles ($2x10^{10}$ nanoparticles/mg) were in good agreement with data derived from the technical data sheets supplied by the manufacturer ($2.86x10^{10}$ nanoparticles/mg). For the 100nm nanoparticles the TEM count yielded a value ($6.7x10^{10}$ nanoparticles/mg) which was approx. a factor of ten from the value given by the manufacturer ($1.83\ x10^{10}$ nanoparticles/mg). In contrast, latex nanoparticles conjugated to both protein and to Gd-DTPA generally exhibited two peaks in PCS. Electron microscopical analysis indicated for both 100 nm latex-LEA/Gd-DTPA nanoparticles and 400 nm latex-LEA/Gd-DTPA nanoparticles that this resulted mainly from nanoparticle aggregation and occasionally from nanoparticle fusion. For use in estimating MR relaxivity values counts of the total number of particles in the suspensions therefore had to be used. The so obtained relaxivity data at room temperature are summarised in Fig. 1, together with data acquired for a standard Gd-DTPA contrast agent, which is widely used in clinical routine, as a control for comparison. The relaxivity values obtained with our method for Gd-DTPA (r1=4600 1/Ms, r2=5790 1/Ms for 64MHz and 24°C) were in good agreement with published data. For the nanoparticles, extremely high relaxivity values were found which increased, as expected, with size.

**Conclusions**

By imaging nanoparticles with TEM, nanoparticle counts can be obtained enabling calculation of nanoparticle concentrations and thus MR relaxivity values. In addition to counting, TEM also allows visualisation of inhomogenous nanoparticle populations. Since relaxivity is usually defined in terms of homogenous particle populations, values obtained for heterogenous populations of derivatised nanoparticles are inexact. In particular, precise values would only be attained by separation of the different size populations of derivatised nanoparticles, and independent determination of the relaxivity for each size class. Based on these findings thorough structural analysis of nanoparticle contrast media using PCS, SEM or TEM should be mandatory.

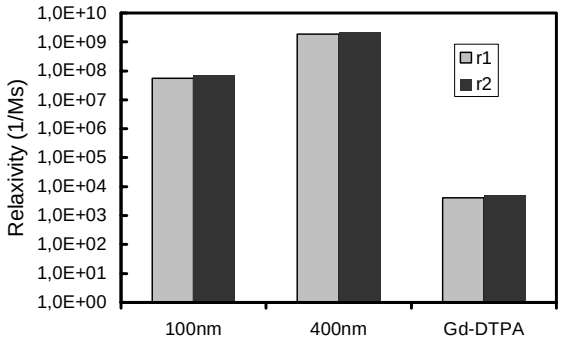


**Figure 1:** Summary of relaxivity values obtained for latex-LEA/Gd-DTPA nanoparticelse at room temperature

**References**

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