Nanostructure of Materials Determined by Relayed Paramagnetic Relaxation Enhancement

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S *Supporting Information*

ABSTRACT: Particle and domain sizes strongly influence the properties of materials. Here we present an NMR approach based on paramagnetic relaxation enhancement (PRE) relayed by spin diffusion (SD), which allows us to determine lengths in the nm–μm range. We demonstrate the method on multicomponent organic polymer mixtures by selectively doping one component with a paramagnetic center in order to measure the domain size in a second component. Using this approach we determine domain sizes in ethyl cellulose/hydroxypropyl cellulose film coatings in pharmaceutical controlled release formulations. Here we measure particle sizes ranging from around 50 to 200 nm.

Particle and domain sizes strongly influence the physical properties of (multicomponent) materials. This is especially true in multiphase systems, which have applications in a wide range of materials such as alloys, composites, and polymer blends, and where sizes can be tailored to provide enhanced physical, mechanical, optical, thermal, electrical, or magnetic properties.[1−3] To rationalize the performance of multicomponent materials, it is important to elucidate the phase morphology, notably by determining the domain sizes of each component in the mixture.

Depending on the nature of the sample and the size of the particles or domains, they may be measured by laser diffraction or scattering methods. However, with these methods it is very difficult to determine sizes in complex multicomponent mixtures. In that case NMR could provide an advantageous alternative since it often allows distinction between the different components based on chemical shift differences or their unequal relaxation properties and therefore opens the possibility to study particle or domain sizes *in situ*. Such NMR domain size measurements are usually carried out with proton spin diffusion experiments,[4−6] where a spatially inhomogeneous nonequilibrium distribution of magnetization is created in which different types of domains are polarized differently. The return to equilibrium driven by spin diffusion is monitored, and the dynamics of this process can be interpreted in terms of models of the domain size and structure.[7] Different procedures to select proton magnetization from particular

domains have been proposed including filters based on differences in dipolar couplings,[5,8,9] differences in relaxation properties,[7] or proton chemical shift differences.[10] These methods can be applied to two-component polymers where the components exhibit significant differences in the properties chosen for selection. However, for more complex multiple component systems these methods are usually not feasible. It has recently been shown that domain sizes can be determined in complex systems using dynamic nuclear polarization (DNP) where the nonequilibrium distribution of magnetization is obtained by locally enhanced polarization.[11]

Here we propose an alternative conventional NMR method where the selection process is replaced by selective doping of one of the domains of the diamagnetic system with paramagnetic species. The method is demonstrated with the measurement of known particles sizes in a model polymer nanoparticle system and then is used to measure the domain sizes in ethyl cellulose (EC)/hydroxypropyl (HPC) cellulose film coatings.

The presence of a paramagnetic species induces very short T_1 relaxation for nearby nuclear spins, well-known as paramagnetic relaxation enhancement (PRE). Measuring PREs is very well established as a tool for structure determination of proteins,[12] even in solid samples,[13−18] where the $r^{−6}$ dependence of the PRE is used to determine electron to nuclear distances, typically in the range of a few angstroms. PRE is thus not *a priori* useful to directly determine particle or domain sizes in materials, usually in the nm–μm range. Here we propose to combine PRE and spin diffusion to measure relayed PRE (R-PRE) effects. To achieve this, as illustrated schematically in Figure 1, we use selective doping (here with stable organic radicals). By doping a particular domain we introduce a significant reduction of T_1 values throughout that domain and at the surface of the other particle/domain. Apart from the (often negligible amount of) nuclear spins in the undoped domain that are in close vicinity to the radical (a few angstroms from the surface) which will relax very rapidly, most of the spins in the undoped domain will not undergo a modification in their T_1. However, spin diffusion will transport magnetization from the center of the undoped domain to the surface, where it
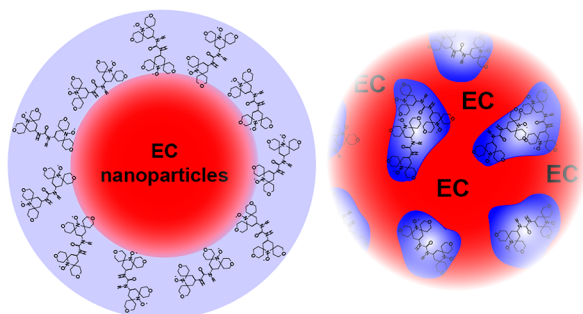
Figure 1. Scheme of domain selective doping of the samples used here. (Left) For EC nanoparticles, radical containing solution (light blue) impregnates the surface of the insoluble EC nanoparticles. (Right) For EC in films covering pellets in controlled release formulations, the (soluble) HPC domains (blue) are selectively doped.

will relax. The smaller the domain size of the undoped domain, the quicker the polarization reaches the surface, and the more the apparent T_1, here dubbed T_1*, of the spins in the undoped domain will be accelerated (note that the T_1 recovery in this regime will appear multiexponential). Thus, a comparison of apparent T_1* between an undoped and a doped sample should allow determination of the domain size of the undoped domains.

We propose that a classical diffusion model, similar to that used for relayed DNP experiments,[19] can be used to quantitatively model the evolution of the magnetization and extract domain sizes from saturation recovery build-up curves of doped and undoped samples.

Note that the effect we propose to induce here exploits the mechanisms postulated in the very first description of spin diffusion by Bloembergen, who invoked paramagnetic sinks connected by spin diffusion to explain anomalously short relaxation in crystalline substances doped with paramagnetic impurities.[20] Since then it has been shown that T_1 measurements in samples with locally different T_1 values can be used to obtain qualitative structural information in paramagnetic materials, to discriminate surface layers.[21] Changes in T_1 in pharmaceutical materials have been observed, induced by crystal defects and production of amorphous material during formulation.[22] The relative determination of the amorphous particle sizes based on this reduction of relaxation time is described in ref 23. The authors however note that the method is unable to provide an absolute measurement of grain sizes due to too many unknowns. It has been shown[24,25] that from multiexponential behavior of water in cells, or liquid in pores of porous materials, it is possible to determine the cell/pore size based on a diffusion model proposed by Brownstein and Tarr.[25]

We demonstrate the R-PRE method on selectively doped organic samples of known particle size and on multicomponent polymers used in film-coatings for controlled release formulations in the pharmaceutical industry.

[1]H polarization build-up curves were obtained from carbon-13 detected saturation recovery [1]H−[13]C CP experiments at slow spinning rates (8 kHz) for all samples (experimental details given in SI). The difference in build-up behavior between the doped and undoped samples is highlighted by plotting the integral ratio of the signals obtained with and without radical doping (Figure 2). Following DNP practice, we will henceforth call this ratio the R-PRE enhancement, $\varepsilon_{\text{R-PRE}}$.
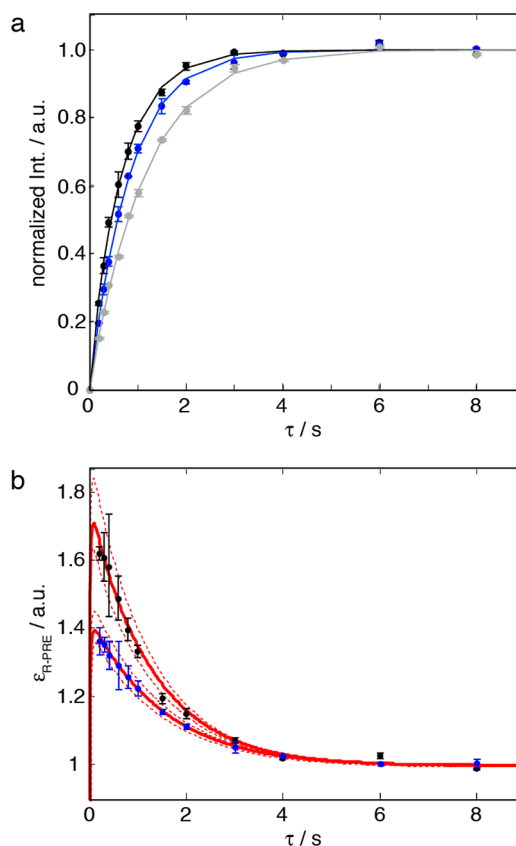

Figure 2. R-PRE build-up (a) and R-PRE enhancement (b) curves measured for the EC component of the pellet sample as a function of the recovery delay τ. Curves are obtained from the intensity of the methyl ^{13}C resonance of EC at 15 ppm. Blue dots: impregnation with 15 mM AMUpol; black dots: 30 mM AMUpol; gray dots: without radical. Experimental data are the mean values of three experiments. Error bars on the experimental data represent the standard deviation. Experimental details, the pulse sequence, and a spectrum can be found in SI. Red lines are from the fit using the model described in the text. Dashed red lines indicate error margins (obtained domain size from fit ±10 nm).

To model $\varepsilon_{\text{R-PRE}}$, a classical diffusion process is used with the diffusion equation considering the influence of spin−lattice relaxation being[20,26]

$$\frac{\partial P(r, t)}{\partial t} = D\Delta P(r, t) - \frac{P(r, t) - P_0}{T_1(r)} \qquad (1)$$

with $P(r,t)$ the polarization as a function of position r inside the domain at time t, and D being the spin diffusion coefficient. For polymers spin diffusion coefficients are usually around 1 nm^2/ ms (10^{-15} m^2/s).[7] P_0 is the equilibrium polarization normalized to $P_0(r) = 1$. In the experiment carried out without radical, T_1 is the same for all positions in the domain which we will call $T_{1,\text{core}}$.[19] With radical doping, the spin−lattice relaxation time at the surface of the domain, in close proximity to the radical becomes very short, $T_{1,\text{surface}}$ and increases with r^{-6}. The initial and boundary conditions are

$$P(r, 0) = 0; \quad \frac{\partial P(r_{\text{max}}, t)}{\partial t} = 0 \qquad (2)$$

At $t = 0$ the detectable polarization is assumed to be 0 over the whole sample, corresponding to saturation. The boundary condition eq 2 corresponds to no polarization diffusing out of

the system. Note that different diffusion models and boundary conditions can be used depending on the system. Other possible models to obtain surface to volume ratios could for example be based on models proposed by Brownstein and Tarr,[25] however, a more detailed study of the exact models is well beyond the scope of this work. To obtain relative signal intensities the polarization is integrated over the domain at each point in time. To account for the quenching effects of the radical in very close proximity to the surface, we assume that protons located within a very small layer (2 Å) at the surface of the domain/particle do not contribute to the spin diffusion process and the detected signal, due to the induced paramagnetic shift and broadening. (The MatLab code used for the calculations is given in the SI.)

To verify that the method is valid in the regime of interest for pharmaceutical excipients, we first use a water-based suspension of EC nanoparticles (Aquacoat ECD) where the particle size has been evaluated from light scattering measurements (z-average, diameter = 169 nm, intensity average, diameter = 180 nm). Figure S2 shows measured R-PRE enhancements and fits using the model described above, which leads to the determined domain sizes for EC given in Table 1. In the fits

Table 1. Experimentally Determined Sizes of EC Nanoparticles and EC/HPC Domains in Aquacoat and Pellet Samples as Obtained by the Fits Described in the Text

[AMUPol, mM]	EC aquacoat EC particle size [p_{EC}, nm]	film-coated pellets EC/HPC domain size [nm]
15	144 ± 30	$d_{EC} = 90 \pm 10$
		$d_{HPC} = 182 \pm 20$
30	140 ± 30	$d_{EC} = 70 \pm 10$
		$d_{HPC} = 142 \pm 20$

for all samples $T_{1,surface}$ was set to 13 and 19 ms for the experiments with 30 and 15 mM AMUpol, respectively. This is the measured solvent T_1, which depends on radical concentration. We note that being able to measure the relaxation rate at the surface of the domain is a big advantage over previously published[23] methods to determine particle sizes using local T_1 differences. The experiment can thus be repeated for a given sample using solutions with different radical concentrations with the only changing parameter in the spin diffusion model being (the measurable) $T_{1,surface}$, leading to more accurate fitting results. The doping method thus allows us to introduce a significant difference of relaxation rates between the surface and the core of a domain/particle. $T_{1,core}$, as determined from the reference experiment carried out without the radical, was found to be 1.9s for the EC nanoparticles. D was set to 0.8 nm²/ms. (The influence of the choice of the value of D is discussed in more detail in the Supporting Information.) Thus, the only variable parameter in the fit is r_{max}, which is doubled to obtain the particle size p_{EC} or domain size d_{EC}. We note that in the case of the nanoparticles we chose a three-dimensional, spherical spin diffusion model since the particle shape is thought to be spherical. The experimental data and fits for the EC nanoparticle sample are shown in the SI.

The results are summed up in Table 1, and it can be seen that the particle sizes obtained for the EC nanoparticles sample are similar to the values obtained from light scattering (z-average, diameter = 169 nm, intensity average, diameter = 180 nm).

Ethyl cellulose/hydroxypropyl cellulose film coatings are a much more complex system. Drug release behavior is governed primarily by the phase structure of the water-soluble HPC phase,[27] which is defined by the number of domains, their size, and their connectivity[28,29] within the insoluble EC matrix. Determination of the structure and sizes of the domains in film coatings on pharmaceutical samples (pellets) would lead to the possibility to control the coating structure, tune the functionality, and better control drug release. Pellet samples consist of multiple, similar components (the EC/HPC film-coating, an active pharmaceutical ingredient and a core material, such as microcrystalline cellulose) which makes it nearly impossible to use classical spin-diffusion experiments or other approaches usually used to determine domain sizes in EC/HPC free films such as scanning electron microscopy[30] or confocal laser scanning microscopy.[31] The size of the domains within the film-layer has so far previously only been characterized by SANS.[32] Here, we use R-PRE by selectively doping one of the domains, i.e., the water-soluble HPC domains with aqueous radical solutions as shown in Figure 1 (AMUPol,[33] 15 and 30 mM) can provide the domain sizes in the film.

The experimental data and fits for the EC domain sizes in the pellets are shown in Figure 2, and the domain sizes determined for both components are given in Table 1. R-PRE data were modeled with a 1D diffusion model since the EC domains are not expected to have spherical shape. The models used here for both samples allow us to obtain a single (average) characteristic length. In principle more sophisticated models could be used, for example, by modeling minimal surface structures.[32,34] HPC domain sizes can then be deduced assuming spherical HPC domains surrounded by EC and based on the fact that the volume ratio of EC/HPC in these films is measured to be 70/30.

In summary, we have shown here that domain sizes can be obtained in complex materials, such as pharmaceutical polymer formulations, by a simple procedure, at room temperature, and without DNP. The approach should be widely applicable to determine domain sizes in multicomponent mixtures when domains can be selectively doped with radicals during production or, as shown here, *a posteriori*, based on the different solubility and swelling properties of the components. In particular, the method is sensitive to domain sizes from around 10 nm to at least (here) 200 nm (note that the upper limit depends mainly on the $T_{1,core}$ of the investigated system), corresponding to a size window that is otherwise often hard to study.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b08249.

> Experimental details and the Matlab program to simulate R-PRE enhancement curves (PDF)

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Notes

The authors declare no competing financial interest.

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