Characterization of Nanoparticles in an Aqueous Solution with Bound Water Molecules Using Pulsed Field Gradient Nuclear Magnetic Resonance Spectroscopy

Haruhisa Kato,* Kayori Takahashi, Takeshi Saito, and Shinichi Kinugasa
Polymer Standards Section Japan (PSSJ), National Metrology Institute of Japan (NMIJ),
National Institute of Advanced Industrial Science and Technology (AIST),
Tsukuba Central 5, Higashi 1-1-1, Tsukuba 305-8565

(Received August 11, 2008; CL-080779; E-mail: h-kato@aist.go.jp)

The slow diffusion of water molecules in a polystyrene latex (PS-latex) nanoparticle solution was observed using the pulsed field gradient nuclear magnetic resonance (PFG-NMR) method. The measured diameter of the PS-latex nanoparticle was approximately 32 nm, and the slowly diffusing water was attributed to the small amount of water molecules bound strongly by PS-latex particles.

Over the past decade, drug-targeting technologies using nanosized drug carriers, such as polymeric micelles, including anticancer drugs, have been of increasing interest.[1] Controlling the size of the drug carrier is a solution to the problem of selective drug delivery to specific physiological sites through the bloodstream, using the enhanced permeability and retention (EPR) effect.[2] Therefore, an appropriate characterization of nanomaterials is essential in this field.

Pulsed field gradient nuclear magnetic resonance (PFG-NMR) spectroscopy has not been commonly employed to determine the size of nanoparticles because of the very low local reorientation mobility of hard nanosphere type molecules such as latex, gold colloids, and silica. The short T_2 relaxation time also makes it difficult to determine the size of hard-core materials and large molecules. The diffusion coefficients of large macromolecules are also difficult to determine using the PFG-NMR since an attenuation of the signal intensities over PFG gradients decreases for increasing macromolecular size. However, the PFG-NMR requires no special handling or preparation of the sample. A small sample volume, typically 20 μL, is enough for measurements of expensive or rare substances. In addition, the individual self-diffusion coefficients in a multicomponent system can be obtained by simultaneously monitoring NMR signals at different chemical shifts.[3–5]

The evaluation of the number of water molecules bound on nanoparticles is of particular interest because adsorbed water is expected to exhibit unusual properties.[6,7] Furthermore, the adsorption of a polar solvent makes the diffusion of a particle slower and produces an enlargement of the apperaent particle diameter proportional to the number of bound water molecules.

In this study, we succeeded in the size determination of a hard-core sphere particle in aqueous solution. Additionally, the slow diffusion of the bound water was found to be clearly distinct from that of the bulk water by PFG-NMR measurements.

PFG-NMR measurements were performed (see detail in Supporting Information).[8] Figure 1a is the ^{1}H NMR spectrum of the latex nanoparticles measured with the PFGSTE pulse sequence at a gradient strength of 1.66 T/m. A resonance from H_2O appears at 4.7 ppm since the latex sample was dissolved in water. A spectral peak of $COOCH_3$ at 3.54 ppm was observed.

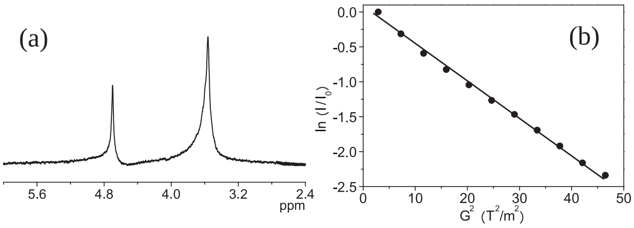

Figure 1. PFG-NMR results. (a) ^{1}H NMR spectrum of nanoparticles collected with the PFGSTE pulse sequence at a gradient strength of 1.66 T m^{-1}. (b) Example of PFG-NMR spin-echo signal attenuation plots PS-latex for $\Delta = 30$ ms and $\delta = 1$ ms.

Other resonances from the latex nanoparticles were negligible or broadened out owing to limited mobility. However, the spectral peak of $COOCH_3$ was significant. This component could exhibit a high mobility since it is considered to be located on the surface of the latex and may induce the latex particle to dissolve in water without any surfactants. We, therefore, carried out the analysis of PFG-NMR diffusion data using this peak. An example of a plot obtained from the PFGSTE echo signal attenuation is illustrated in Figure 1b. The attenuation plot is approximately a straight line, indicating narrow size distribution obtained from the line distribution of the particle size. The calculated diffusion coefficient of the nanoparticles from Figure 1b was $1.54 \pm 0.03 \times 10^{-11}$ m^2 s^{-1}.

The convection in NMR tubes makes the diffusion of the particle faster, therefore, reducing the observed size of a particle.[9–11] As shown in Figure 2, the dependence of the apparent diffusion coefficient D_{app} of the nanoparticles was examined. There is no obvious dependence observed within experimental uncertainties, proving that the convection effect would not affect the apparent diffusion coefficients in this measurement according to ref 12. The averaged value of the diffusion coefficient

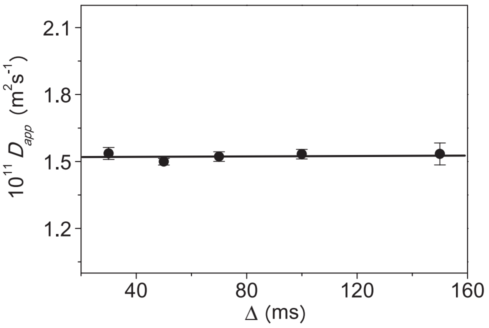

Figure 2. Diffusion coefficients of PS-latex as a function of the corresponding diffusion time.

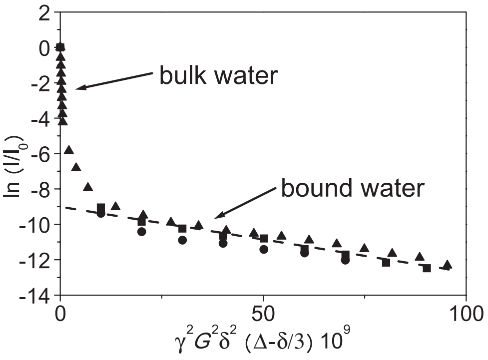

Figure 3. PFG-NMR attenuation plots for observed diffusion times: $\Delta = 50$ ms (●), $\Delta = 70$ ms (■), and $\Delta = 100$ ms (▲), respectively. The straight dash line represents linear regression and the extrapolation to $\gamma^2 G^2 \delta^2 (\Delta - \delta/3) = 0$ in order to determine the diffusion coefficient of very slow diffusing water molecules.

of PS-latex from Figure 2 is $1.53 \pm 0.03 \times 10^{-11}\ \mathrm{m^2\,s^{-1}}$. The determination of the particle size of the PS-latex nanoparticles was performed using the Stokes–Einstein relationship. The calculated value of the diameter of the nanoparticle (d) is 32.2 ± 1.2 nm. The size of the nanoparticle determined using dynamic light scattering (DLS) is 31.0 nm,[13] which agrees well with the PFG-NMR value. This result indicates that the size determination of nanoparticles using the PFG-NMR method is reliable.

Figure 3 shows the PFG-NMR attenuation plots for observed diffusion times. The observed decays were not linear, indicating some distribution of the diffusion coefficients of the water molecules. There is no dependency of the spin-echo attenuation on the observed diffusion time range, and this indicates that distribution of the diffusion coefficients is independent during the time scale of the PFG-NMR measurements. Adsorbed water molecules have different adsorbent mobilities depending on their position of intra- or intermolecular PS-latex space. Assuming two-state model of the water molecules (bulk and bound), significantly slow diffusing water molecules were found as shown by the straight dash line in Figure 3, and the calculated diffusion coefficient is $5.1 \pm 1.4 \times 10^{-11}\ \mathrm{m^2\,s^{-1}}$, clearly slower than that of bulk water ($2.3 \pm 0.1 \times 10^{-9}\ \mathrm{m^2\,s^{-1}}$) but faster than the diffusion of PS-latex.

This diffusion coefficient value supported the diffusional exchange between fast and slow water molecules during the observed diffusion time ($\Delta = 50$–100 ms). Such behavior was previously found in the study of MOF-5 crystals, where the different mobilities of the adsorbed molecules in intra- and intercrystalline space were observed as a fast exchange between the crystal and the surrounding gas phase.[14] Interestingly, the observed diffusion coefficients of the slow diffusing water molecules were independent of the observed diffusion time, indicating they were strongly adsorbed by the PS-latex particle. The number of such bound water molecules during these diffusion time ranges was calculated by extrapolation of the attenuation of the intensity for the slow diffusion component in Figure 3. The extrapolated $\ln(I/I_0)$ value was -8.87, thus, the mole fraction of the water bound by the latex is around 0.014% of all water molecules in aqueous PS-latex solution, and the number of water molecules was calculated to be approximately 6.7×10^3. The slowest diffusion could be attributed to the water molecules bound strongly on the surface of the PS-latex or in the inner space of PS-latex. The observed number of slowly diffusing water molecules was too small to affect the determined size of particles by PFG-NMR.

In conclusion, we determined the size of PS-latex and estimated the number of molecules adsorbed strongly on the surface or in the inner space of PS-latex by PFG-NMR. These results highlight that PFG-NMR is an effective new methodology for determining the size and dynamics of nanoparticles and solvent molecules in solution.

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