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Nanoparticle Size Determination by ^{1}H NMR Spectroscopy

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In a recent paper,[1] we showed that the ^{1}H NMR integral values of the innermost protons of dendrimers containing encapsulated Pd nanoparticles decrease significantly more than those corresponding to the terminal methylene protons. This is a consequence of the well-known loss of ^{1}H NMR signals of protons residing in close proximity to metal nanoparticles.[1−6] Coupled with other evidence, this allowed us to conclude that single nanoparticles are encapsulated within individual dendrimers.[1] This finding led us to hypothesize that the magnitude of the decrease of specific proton signals could be correlated to the size of an encapsulated nanoparticle. Indeed, as we show here, a straightforward 1D ^{1}H NMR experiment can be used to readily distinguish between dendrimer-encapsulated nanoparticles (DENs) differing in size by just a few tens of atoms.

Pd DENs are synthesized in two steps.[1,7−9] First, $PdCl_4^{2-}$ is complexed with interior tertiary amines of the dendrimer. Second, the dendrimer/Pd^{2+} complex is reduced with BH_4^- to yield DENs. By controlling the Pd^{2+}-to-dendrimer ratio, the average size of the DENs can be tuned. In the present case, we examined Pd DENs within sixth-generation, hydroxyl-terminated poly(amidoamine) (PAMAM) dendrimers (G6-OH(Pd$_n$)). DENs are not perfectly monodisperse in size, and the synthetic procedure actually yields a statistical mixture of Pd nanoparticles having an average size whose standard deviation, as measured by TEM, is typically less than 15%.[8] TEM is usually considered the gold standard for determining the size of metal nanoparticles like DENs. However, as we show here, NMR spectroscopy is also useful for this purpose. Importantly, it is the effect of the nanoparticles on their dendritic host, rather than the nanoparticles themselves, that is observed in this experiment. NMR experiments probe the entire population of DENs and thus can provide more representative information about average particle size compared to TEM. Additionally, the NMR data provide information about particle size in situ, which opens the possibility, for example, of studying how the size and environment of nanoparticles change during catalytic reactions.

A series of Pd DENs was synthesized following a procedure given in previous reports from our group.[1,8] Assuming a spherical shape, and taking into account the molar volume of Pd (8.56 cm^3/mol), the G6-OH(Pd$_n$) (n = 55, 147, 200, and 250) DENs have calculated diameters of 1.14, 1.59, 1.76, and 1.89 nm, respectively. These values can be compared to the diameters measured by TEM: 1.0 ± 0.2, 1.3 ± 0.2, 1.5 ± 0.2, and 1.7 ± 0.3, respectively (Supporting Information, Figure S1).

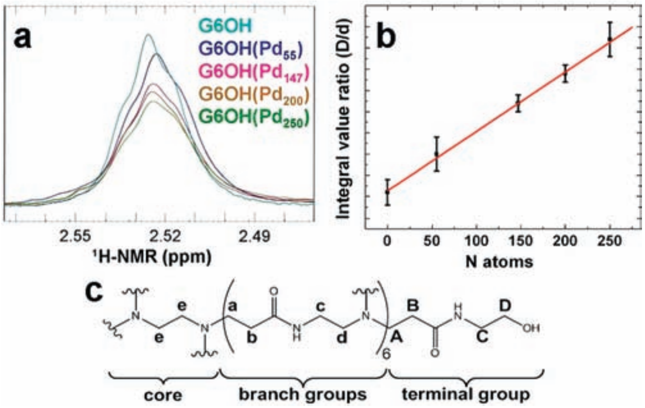

Figure 1. (a) Expansion of ^{1}H NMR region corresponding to the d proton resonances of G6-OH(Pd$_n$). (b) I_D/I_d plotted versus the average number of atoms in each Pd DEN. (c) Schematic representation of G6-OH indicating the lettering scheme used to identify the methylene groups.[1] Numerical values corresponding to the data in this figure are provided in Table S1 (Supporting Information).

^{1}H NMR spectra of the PAMAM dendrimer (G6-OH), the precursor complex (G6-OH(Pd^{2+})$_n$), and G6-OH(Pd$_n$) are provided in Figures S2−S5, respectively. The NMR signals were assigned using 1D and 2D NMR experiments, as described previously for the G4-OH system.[1] The ^{1}H NMR spectrum of G6-OH, a 58 kDa macromolecule containing over 4000 protons, reveals just five signals in the 2−4 ppm range (Figures S2−S5). The relatively small number of resonances is a consequence of the high symmetry of the molecule and the corresponding overlap of signals. Following complexation of Pd^{2+}, the spectra of G6-OH(Pd^{2+})$_n$ (Figures S2−S5) indicate severe broadening, shifting, or even complete disappearance of the proton peaks. This confirms the presence of the metal ions within the dendrimer and their coordination to donor atoms (principally tertiary amines).[1,10] Finally, reduction of the complex to yield the DENs results in recovery of the proton signals (Figures S2−S5), indicating that the configuration of the dendrimers is similar with and without the encapsulated nanoparticles. However, consistent with our previous findings for G4-OH and G4-OH(Pd$_{55}$),[1] some peaks decrease in height and broaden in the presence of the nanoparticle. Importantly, this effect is more pronounced for the signals of the innermost protons and increases as the size of the Pd DENs increases. Because most of the proton resonances in the dendrimer overlap,[1] we focus here on the one inner-proton resonance at 2.52 ppm that is well resolved.

Figure 1a shows an expanded view of the region where resonances of the d protons, which are those located α to the inner

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tertiary amines (Figure 1c), are present. There is a small upfield shift (<0.01 ppm) observed for peak *d* in the spectra of G6-OH(Pd_n) DENs compared to G6-OH. This shift results from the change in pH from 7.0 ± 0.2 to 9.1 ± 0.2 that occurs upon reduction of the G6-OH$(Pd^{2+})_n$ precursor with BH_4^-.[1] More importantly, Figure 1a indicates a qualitative decrease in the intensity of the *d* proton resonances as the nanoparticle size increases. This observation is quantified in Figure 1b, which is a plot of the integral ratio of the *D* and *d* protons (I_D/I_d) as a function of the average number of atoms in the DENs (N_{atoms}). Here, the integral of the peak of the *D* protons, which are on the periphery of the dendrimer and hence least likely to be affected by the presence of the DEN, act as an internal standard to normalize the intensity of the *d* proton resonances (Figure S6). In essence, Figure 1b is a calibration curve correlating a parameter that can be measured by NMR to the size of the DENs.

The linear increase of I_D/I_d as a function of increasing DEN size is attributed to the (locally) restricted motion of the dendrimer and the heterogeneity of the methylene groups near the encapsulated nanoparticles. Specifically, there is a 17% decrease in the integral intensity of the *D* protons for the largest DENs (G6-OH(Pd_{250})) relative to G6-OH. However, this change is accompanied by a larger decrease (37%) in the intensity of the peak integral of the *d* protons. The absolute integral intensities for both the *d* and *D* protons for all four DENs are plotted as a function of the numbers of atoms in the nanoparticles in Figure S6.

The best-fit line in Figure 1b can be represented by eq 1. Therefore, if the Pd DENs are assumed to be spherical in shape, then eq 2 can be combined with eq 1 to yield the size of the DENs.

$$\frac{I_D}{I_d} = (1.16 \pm 0.01) + (1.40 \pm 0.03) \times 10^{-3} \times N_{atoms} \tag{1}$$

$$r_{Pd}^3 \times N_{atoms} = R_{DEN}^3 \tag{2}$$

In eq 2, r_{Pd} is the radius of a Pd atom (150 pm, based on its molar volume) and R_{DEN} is the radius of the corresponding DEN. Although we have chosen to use the *D* and *d* NMR peaks for this analysis, the intensities of other outer and inner proton peaks could also be used if their overlap is not too severe.

The observed positive slope of the I_D/I_d plot provides confirmation of the formation of DENs rather than dendrimer-stabilized nanoparticles (DSNs),[11] which are somewhat larger nanoparticles stabilized on their surface by multiple dendrimers. For DSNs one would expect the outermost protons to be nearest the metal surface, and therefore their intensity would be more influenced by the Pd nanoparticle. This would lead to a negative slope of the I_D/I_d vs N_{atoms} plot.

Pulsed-field gradient spin−echo (PFGSE) experiments are useful for measuring diffusion coefficients, which can be correlated to the hydrodynamic radius. We used this approach to distinguish between DENs, DSNs, and aggregates, which would be expected to have easily distinguishable diffusion coefficients. The results (Figure S7) reveal similar values for all the Pd DENs as well as for G6-OH: $(0.54 \pm 0.04) \times 10^{-10}$ m^2/s. Assuming homogeneous, center-of-mass spheres, and using the Stokes−Einstein equation,[1] this diffusion coefficient corresponds to a hydrodynamic radius of 3.3 ± 0.3 nm, which is within the range reported for G6-NH_2 at high pH (2.6 nm[12a] to 3.7 nm[12b−d]).

In conclusion, we have reported a new method for measuring the size of metal nanoparticles encapsulated within dendrimers. This approach should be appropriate for measuring the size of any encapsulated object as long as the following two conditions are met. First, the host must have a regular structure. Second, the guest must affect a property of the host in a way that can be detected by NMR. The advantages of this method for the DEN system are the remarkable sensitivity of the proton resonances to nanoparticles size and the fact that the average nanoparticle size can be measured in situ. The latter point could be particularly important for understanding how catalytic reactions affect the size, shape, and environment of DENs. In contrast, TEM measurements are made on a tiny fraction of the nanoparticles present within an ensemble, and in situ measurements are not possible. Finally, highly accurate TEM measurements are difficult to obtain for particles in the 1−2 nm size range.

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Supporting Information Available: TEM images, 1D ^{1}H NMR spectra for all the samples, absolute integral values of the *D* and *d* signals plotted vs the average number of atoms in each Pd DEN for G6-OH(Pd_n), PFGSE experiments, a table of integral value ratios (*D*/*d*) for each Pd DEN corresponding to the data shown in Figure 1b, and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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