

Probing Diffusional Transport in Redox-Active Dendrimers

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The diffusion coefficients of polyamidoamine (PAMAM) dendrimers (generations 0–4) surface-modified with terpyridyl and bipyridyl ruthenium complexes were determined using cyclic voltammetry (CV), voltammetry at a rotating disk electrode (RDE), and pulsed field gradient spin-echo nuclear magnetic resonance (PFGSE-NMR). In all experiments, the magnitude of the diffusion coefficient decreased with increasing dendrimer size, but each experimental technique led to dramatically different experimental results in terms of the value for a particular molecule. The diffusion coefficients determined by PFGSE-NMR were larger than those determined by RDE, and the diffusion coefficients determined by CV were smaller still. The Stokes–Einstein equation was used to estimate the size of the dendrimers from the measured diffusion coefficients and the results from CV and RDE experiments were found to predict unphysically large diameters for the dendrimer molecules. On the other hand, the diffusion coefficients determined by PFGSE-NMR led to physically reasonable estimations of the dendrimers' sizes. It is proposed that, for macromolecules containing multiple redox centers, electrochemical methods may not provide an accurate measure of diffusion coefficients due to incomplete sampling of all redox sites. Unlike the electrochemical methods, PFGSE-NMR does not rely on electron-transfer occurring, and it appears to be superior to those electrochemical methods for measuring the diffusion coefficients of redox-active macromolecules.

Introduction

The study of hyperbranched dendritic molecules has gained considerable relevance in the past decade. Scores of different dendrimers^{1–9} have been prepared including many functionalized with redox-active moieties.^{3,10–14} Various applications have been envisioned for such molecules, including drug delivery systems^{15–18} and luminescent dyes and contrast agents for use in medical imaging.^{19–22} The use of dendrimers in catalysis has also been explored, since redox dendrimers potentially have the ability to shuttle large numbers of electrons.^{3,11,13,14,23,24} In many of these applications, the dendritic molecule is present in solution, and consequently, it is important to understand the solution behavior of these large and complex molecules. Some of the most important aspects to characterize are the transport properties, since, for many applications, the movement of molecules by diffusion (or by other means) is pivotal. Depending on the specific application, one may want a material that will diffuse readily, such as in a drug delivery system, or a material that will remain in the volume of solution where initially placed, such as an imaging dye that is injected directly into the site under investigation.

There are many electrochemical techniques that are often utilized for measuring the diffusion coefficient of redox-active molecules. An analysis of the variation of peak current (I_p) with sweep rate in a series of Nernstian cyclic voltammograms (CVs) leads readily to the determination of the diffusion coefficient for the redox-active species of interest.²⁵ This requires only a knowledge of the area of the electrode (A), the number of electrons transferred (n), and the bulk concentration of the material under investigation (C_0^*):

$$I_p = (2.69 \times 10^5) n^{3/2} A D_o^{1/2} v^{1/2} C_0^* \quad (1)$$

where D_o is the diffusion coefficient in cm^2/s and v is the sweep rate in volts/s .²⁶ Alternatively, diffusion coefficients can be determined by performing voltammetry at a rotating-disc-electrode (RDE). The rotation rate of the electrode is varied, changing the rate of hydrodynamic transport, and the diffusion coefficient is extracted in an analogous fashion as above from an analysis of the variation of the limiting current (I_l) observed with the rotation rate.^{27,28} In addition to the requirements of the previous method, it is also necessary to know the kinematic viscosity (viscosity/density) of the solvent:

$$I_l = 0.620 n F A D_o^{2/3} \omega^{1/2} \nu^{-1/6} C_0^* \quad (2)$$

where F is Faraday's constant, ω is the rotation rate of the electrode in radians/s , and ν is the kinematic viscosity of the solvent in cm^2/s .²⁹

Both of the techniques mentioned above are conventional electrochemical methods for the determination of diffusion coefficients. However they are both indirect measurements of transport since in both methods, the current flow due to electron transfer is the measured quantity. However, the measured current can be affected by other factors that are not related to transport. There are several possible sources of nonfaradaic current in typical electrochemical experiments, including the charging of the double-layer, and these can lead to currents that are not reflective of transport only. The adsorption of material, either electroactive or non-electroactive, onto the electrode surface can lead to enhanced (or diminished) currents and/or changes in the double-layer capacitance.³⁰ In fact, under certain experimental conditions, *apparent* diffusion that is, in fact, due to electrons hopping between stationary redox sites has been

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observed and intensely investigated.^{31–36} Additionally, in electrochemical experiments, there is typically a large excess of ionic supporting electrolyte present in solution, and the solvation of a charged molecule in such a medium is a complex phenomenon which necessarily affects its transport. For highly charged molecules with multiple redox centers, this effect could be considerable.

An alternative technique, in which many of these complicating factors are abrogated, involves the use of magnetic resonance techniques in a method termed pulsed field-gradient spin-echo nuclear magnetic resonance (PFGSE-NMR).³⁷ This technique uses magnetic field gradients to “encode” spatial information within the NMR signal for a material. After a given period of time has elapsed, the gradient-encoded molecule can be probed again to determine its new spatial location, and its diffusion coefficient can be subsequently determined.^{38,39} In contrast with the electrochemical techniques mentioned above, PFGSE-NMR is useful for determining diffusion coefficients because the results are independent of the concentration of analyte, the number of electrons involved in a redox step or the kinematic viscosity of the solution. Since a conducting solution is not required, the use of PFGSE-NMR allows for the direct measurement of diffusion coefficients without the presence of supporting electrolyte. Moreover, charge screening effects can be estimated by comparing measurements in the presence and absence of supporting electrolyte.

In this work, the materials under investigation are two families of redox-active dendrimers based on polyamidoamine (PAM-AM) cores that are functionalized at the periphery with pendant ruthenium polypyridyl complexes. Generations 0, 1, 2, 3, and 4, containing 4, 8, 16, 32, and 64 pendant groups, respectively, of dendrimers modified with ruthenium bis-terpyridyl moieties [Ru(tpy)₂] as well as the analogous dendrimers with ruthenium tris-bipyridyl pendant groups [Ru(bpy)₃] have been examined (see Figure 1). The diffusion coefficient was determined for each dendrimer using each of the three techniques, CV, RDE, and PFGSE-NMR described above. This not only allows comparisons to be made among dendrimers of different sizes and with different pendant groups, but also allows for a comparison of the analytical techniques themselves.

Experimental Section

Materials. The dendrimers under investigation were synthesized according to previously published procedures.⁴⁰ Acetonitrile was purchased from Burdick and Jackson (distilled in glass) and dried over 4 Å molecular sieves prior to use. Tetra-*n*-butylammonium hexafluorophosphate (TBAH) (G. F. S. Chemicals) was recrystallized three times from absolute ethanol and dried under vacuum for 96 h. All other materials were of at least analytical grade and were used as received.

Apparatus. For all electrochemical measurements, the potential of the working electrode was controlled with a Bio-Analytical Systems (BAS) CV-27 potentiostat, and the experiments were carried out in homemade three-compartment cells of conventional design. A silver wire was used, unless otherwise noted, as the reference electrode to avoid contamination of the nonaqueous medium with water, and the potentials were subsequently referenced to a Ag/AgCl electrode. A coiled platinum wire was used as the counter electrode. A rotated disk electrode (Pine Instruments) with a platinum disk ($A = 0.196 \text{ cm}^2$) was used as the working electrode for both CV and RDE experiments, and voltammograms were recorded on a Soltec X-Y recorder. For RDE experiments, a Pine Instruments analytical rotator and speed controller were utilized.

PFGSE-NMR experiments were carried out on a Varian Unity 500 MHz NMR spectrometer equipped with a triple resonance probe with pulsed-field Z-gradients. Wilmad 535PP NMR tubes were used for all experiments, and the data were processed using VNMR (Varian) on a Sun Ultra 10 workstation.

Procedures. For all electrochemical measurements, the electrode was initially polished with 1 μm diamond paste (Buehler) and rinsed with acetone and water prior to use. The electrode was then pretreated by continuous cycling between -0.20 V and $+1.20 \text{ V}$ vs Ag/AgCl in 0.1 M H₂SO₄ until the characteristic voltammogram of a clean polycrystalline platinum electrode was obtained. The electrode was subsequently rinsed with water and acetone and air-dried for 30 min. All measurements were performed in 0.10 M TBAH/acetonitrile solution containing micromolar concentrations of the dendrimers of interest such that, for all experiments, the concentration of redox sites in solution was approximately constant at 0.2 mM. Cyclic voltammetry was performed at a range of sweep rates from 10 mV/s to 500 mV/s, and RDE experiments were carried out at a constant sweep rate of 20 mV/s at rotation rates from 100 to 3000 rpm. CV and RDE experiments were carried out sequentially on a single sample of dendrimer-containing solution, in the same cell, and with the same electrodes in order to mitigate any concentration effects as well as any other systematic errors. For all electrochemical experiments, the potential was scanned only over the Ru(II/III) peak which for these materials is typically centered around $+1.30 \text{ V}$ vs Ag/AgCl.

The PFGSE-NMR technique involves the application of a standard spin-echo sequence coupled with the use of powerful pulsed magnetic field gradients and is depicted in Figure 2. After the initial 90° radio frequency (RF) pulse of the spin-echo sequence (a), a gradient pulse (b) is applied. This results in an encoding of the spatial position of the species of interest. After a given delay time, the molecules have moved, by diffusion, away from the positions encoded by the first gradient pulse. A 180° RF pulse (c), the second part of the standard spin-echo sequence, is then applied and is followed by a second, identical, magnetic field gradient pulse (d).^{37–39} Those molecules that have been transported by diffusion will experience a magnetic field different from the one initially encoded by the gradient. When a free-induction decay (FID) is acquired (e) and Fourier transformed, the area under the peaks for molecules that have moved (by diffusion) will be diminished in proportion to the distance traveled. The larger the diffusion coefficient, the longer the delay time and the more powerful the magnetic field gradient, the smaller the integrated peak area will be in relation to the standard spin-echo (i.e. without field gradient) result. Experimentally, the magnetic field gradient strength is the parameter that is generally varied.³⁹ From the normalized area of a peak in a series of spectra taken with different field gradients, the diffusion coefficient of the molecule of interest can be determined using eq 3:

$$\ln(I_g/I_0) = -[\gamma^2 \delta^2 G^2 (\Delta - \delta/3)] D_0 \quad (3)$$

where I_g/I_0 is the normalized area under a particular peak (I_g and I_0 are the areas in the presence and absence of the gradient, respectively), δ is the duration (width) of the gradient pulse, γ is the gyromagnetic ratio of ¹H, Δ is the time between the gradient pulses, G is the strength of the magnetic field gradient, and D_0 is the diffusion coefficient of the species of interest (see Figure 2). A plot of $\ln(I_g/I_0)$ versus G^2 yields a line, the slope of which allows the calculation of the diffusion coefficient through eq 3.³⁹

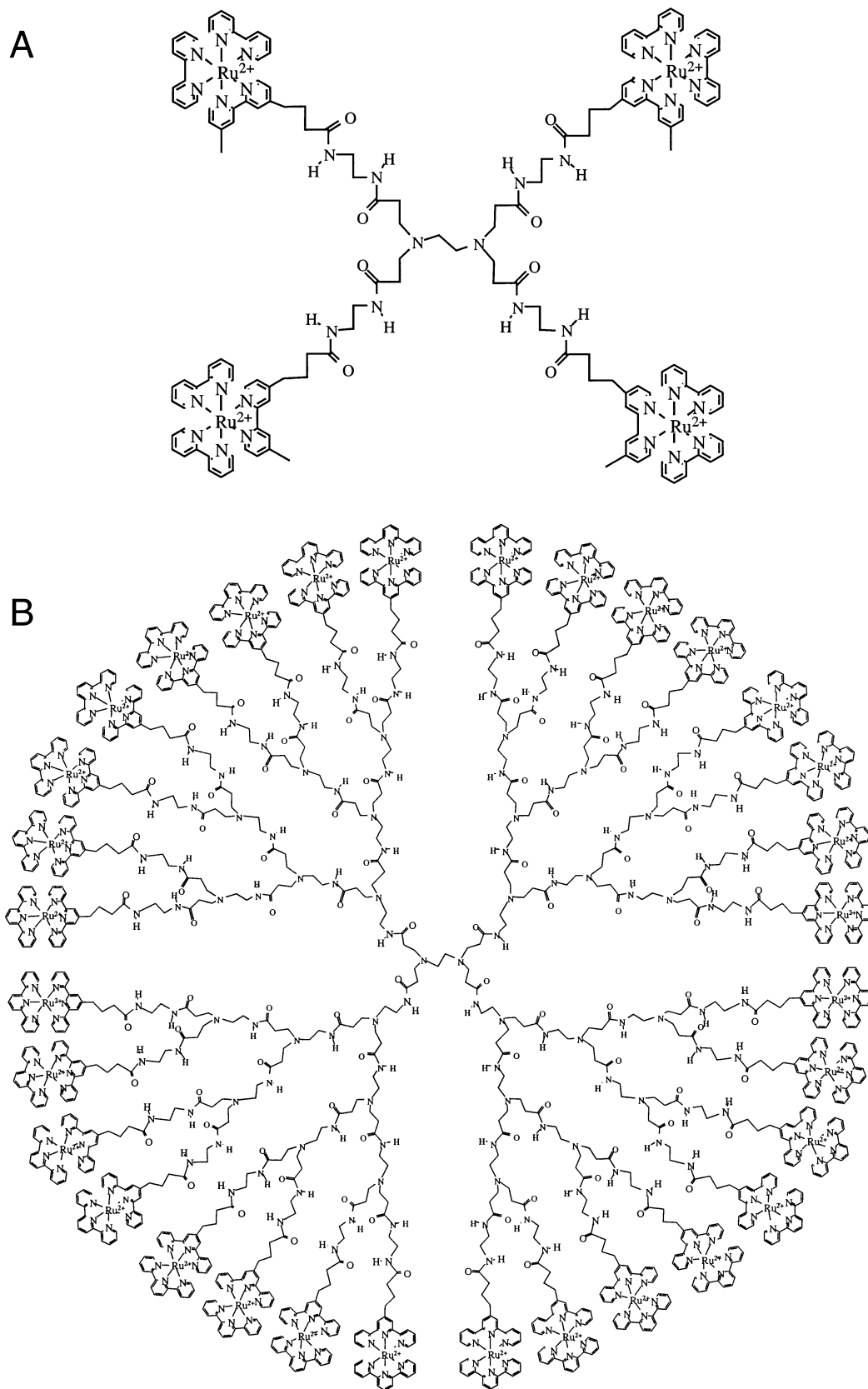


Figure 1. Structures of (A) Dend-4-[Ru(bpy)₃]²⁺ and (B) Dend-32-[Ru(tpy)₂]²⁺.

To test the accuracy of the PFGSE-NMR method, a sample containing ferrocene was analyzed. The calculated diffusion coefficient of $2.5 \times 10^{-5} \text{ cm}^2/\text{s}$ is in good agreement with the literature value of $2.4 \times 10^{-5} \text{ cm}^2/\text{s}$.⁴¹ For PFGSE-NMR experiments, approximately 2 mg of each dendrimer were dissolved in 0.5 mL of 99.9+% acetone-*d*₆ (Aldrich). Spectra

were acquired at a constant temperature of $25.0 \text{ }^\circ\text{C} \pm 0.1 \text{ }^\circ\text{C}$ without spinning the NMR tube. The standard Stejskal-Tanner pulse sequence (see Figure 2) was used.³⁷ The width of each spectrum was 16 ppm and a transmitter offset of -1.25 ppm was used. Data were acquired at 0.2 Hz/point: each FID was acquired for 2.5 s, and there was a 2 s delay before the beginning

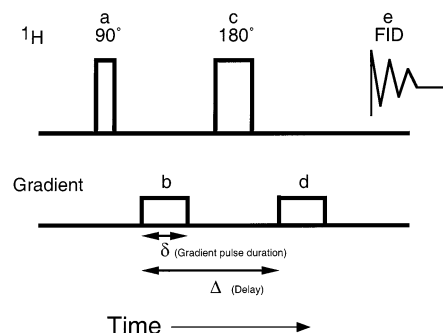


Figure 2. Radio frequency and magnetic field gradient pulse sequences for the pulsed field gradient spin-echo (PFGSE) NMR experiment.

of the next acquisition. The 90° pulse was calibrated before each data acquisition, and 32 to 64 spectra were averaged for each acquisition. To avoid artifacts due to J-coupling, the gradient pulses as well as the delay times, during which diffusion was to occur, were each kept under 5 ms in duration.³⁹ Only the integrals of the terpyridyl and bipyridyl protons (δ 7.0 ppm – δ 9.2 ppm) were used for the analysis because the baseline was flat and well resolved in that region of the spectrum. For each sample, 11 different magnitudes of the pulsed-field gradient were used, from 0 to 0.73 T/m.

Results

Determination of Diffusion Coefficients by Cyclic Voltammetry. The diffusion coefficients of the dendritic species Dend- n -[Ru(tpy)₂]²⁺ and Dend- n -[Ru(bpy)₃]²⁺ (where $n = 4, 8, 16, 32, 64$) were determined by analysis of cyclic voltammograms for the Ru(II/III) oxidation taken at a variety of scan rates. The voltammograms for Dend-16-[Ru(bpy)₃]²⁺ are presented in Figure 3A. Data were acquired at scan rates (ν) from 10 mV/s to 500 mV/s for a solution of 13 μ M dendrimer (0.2 mM of Ru sites) in 0.10 M TBAH/acetonitrile. Similar sets of cyclic voltammograms were obtained for all of the other dendrimers studied. In all cases, well defined cyclic voltammograms were obtained. All of the metal centers appear to undergo oxidation and reduction at the same potential since there is only one wave for the Ru(II/III) process, indicating that there are no significant interactions among the sites on the time scale employed. From a plot of I_p vs $\nu^{1/2}$ it is possible to determine the diffusion coefficient of the dendrimers²⁵ (eq 1), and such plots for all of the molecules studied are presented in Figure 4 (1a–e). The calculated diffusion coefficients for all dendrimers obtained from this method of analysis are shown in the figure and are tabulated in Table 1. As anticipated, the diffusion coefficients were found to decrease with increasing dendrimer size. While these compounds are known to adsorb to platinum electrode surfaces,⁴² the shape of the voltametric wave is that of a freely diffusing material,²⁵ suggesting that the molecules involved in the redox processes are not bound to the surface. Additionally, since the dependence of peak current on scan rate for molecules adsorbed on a surface is $I_p \propto \nu$, not $I_p \propto \nu^{1/2}$ as for molecules in solution,³⁰ any contribution to the peak current from adsorbed species would manifest itself as a positive deviation from linearity, especially at high scan rate values, in the plots seen in Figure 4. Even if adsorbed species were present, we have previously shown that these dendrimers adsorb only up to one monolayer of coverage as long as negative potentials are avoided.⁴² Considering the concentration of material in solution, the contribution to I_p of one monolayer of material would be negligible at the scan rates employed.

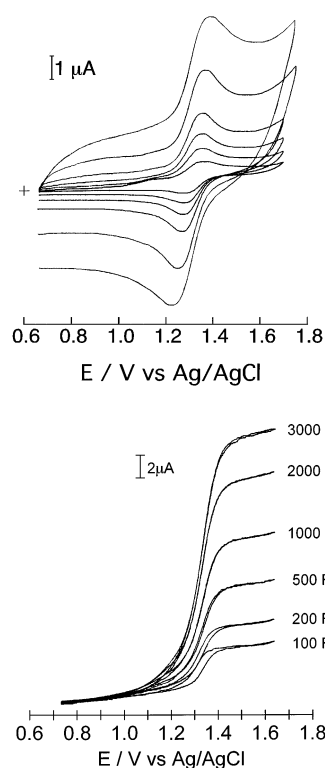


Figure 3. (A) Overlay of six cyclic voltammograms for a platinum electrode in contact with an acetonitrile solution containing 12.5 μ M Dend-16-[Ru(bpy)₃]²⁺ (0.2 mM Ru²⁺ sites) and 0.10 M TBAH at scan rates of 10, 20, 50, 100, 200, and 500 mV/s. (B) Overlay of six rotating disk electrode (RDE) voltammograms for a platinum electrode in contact with an acetonitrile solution containing 25 μ M Dend-8-[Ru(tpy)₂]²⁺ (0.2 mM Ru²⁺ sites) and 0.10 M TBAH at rotation rates of 100, 200, 500, 1000, 2000, and 3000 rpm.

TABLE 1: Diffusion Coefficients of Dend- n -[Ru(tpy)₂]²⁺ and Dend- n -[Ru(bpy)₃]²⁺ Determined by Various Methods

	D_0 determined by CV ^a (cm ² /s)	D_0 determined by RDE ^a (cm ² /s)	D_0 determined by PFGSE-NMR ^b (cm ² /s)
Dend-4-[Ru(tpy) ₂] ²⁺	1.8×10^{-6}	3.7×10^{-6}	4.9×10^{-6}
Dend-8-[Ru(tpy) ₂] ²⁺	1.3×10^{-6}	2.9×10^{-6}	3.7×10^{-6}
Dend-16-[Ru(tpy) ₂] ²⁺	1.2×10^{-6}	3.2×10^{-6}	3.5×10^{-6}
Dend-32-[Ru(tpy) ₂] ²⁺	8.5×10^{-7}	2.1×10^{-6}	3.2×10^{-6}
Dend-64-[Ru(tpy) ₂] ²⁺	3.3×10^{-7}	8.1×10^{-7}	2.3×10^{-6}
Dend-4-[Ru(bpy) ₃] ²⁺	1.4×10^{-6}	5.5×10^{-6}	5.5×10^{-6}
Dend-8-[Ru(bpy) ₃] ²⁺	1.0×10^{-6}	2.5×10^{-6}	4.0×10^{-6}
Dend-16-[Ru(bpy) ₃] ²⁺	3.6×10^{-7}	1.1×10^{-6}	3.6×10^{-6}
Dend-32-[Ru(bpy) ₃] ²⁺	2.4×10^{-7}	8.2×10^{-7}	3.3×10^{-6}
Dend-64-[Ru(bpy) ₃] ²⁺	2.2×10^{-7}	4.8×10^{-7}	3.1×10^{-6}

^a In 0.10 M TBAH/acetonitrile. ^b In acetone-*d*₆.

Determination of Diffusion Coefficients with RDE. The diffusion coefficients of the compounds mentioned above were also determined by rotated disk electrode (RDE) voltammetry. All voltammograms had the expected sigmoidal shape. Figure 3B presents the voltammograms for Dend-8-[Ru(tpy)₂]²⁺ at rotation rates from 100 to 3000 rpm. Well-defined sigmoidal responses were obtained, as would be anticipated. Plots of the limiting current (I_l) vs the square root of the rate of rotation ($\omega^{1/2}$) (Levich plots) are shown in Figure 4 (2a–e).^{27–29} From the slope of the lines, diffusion coefficients were determined through the use of eq 2. The results of those calculations are shown in each panel of the figure and are tabulated in Table 1. A similar trend to that observed previously for cyclic voltammetry was noted. Again, the measured values of the diffusion coefficients decreased as the generation (size) of the dendrimer

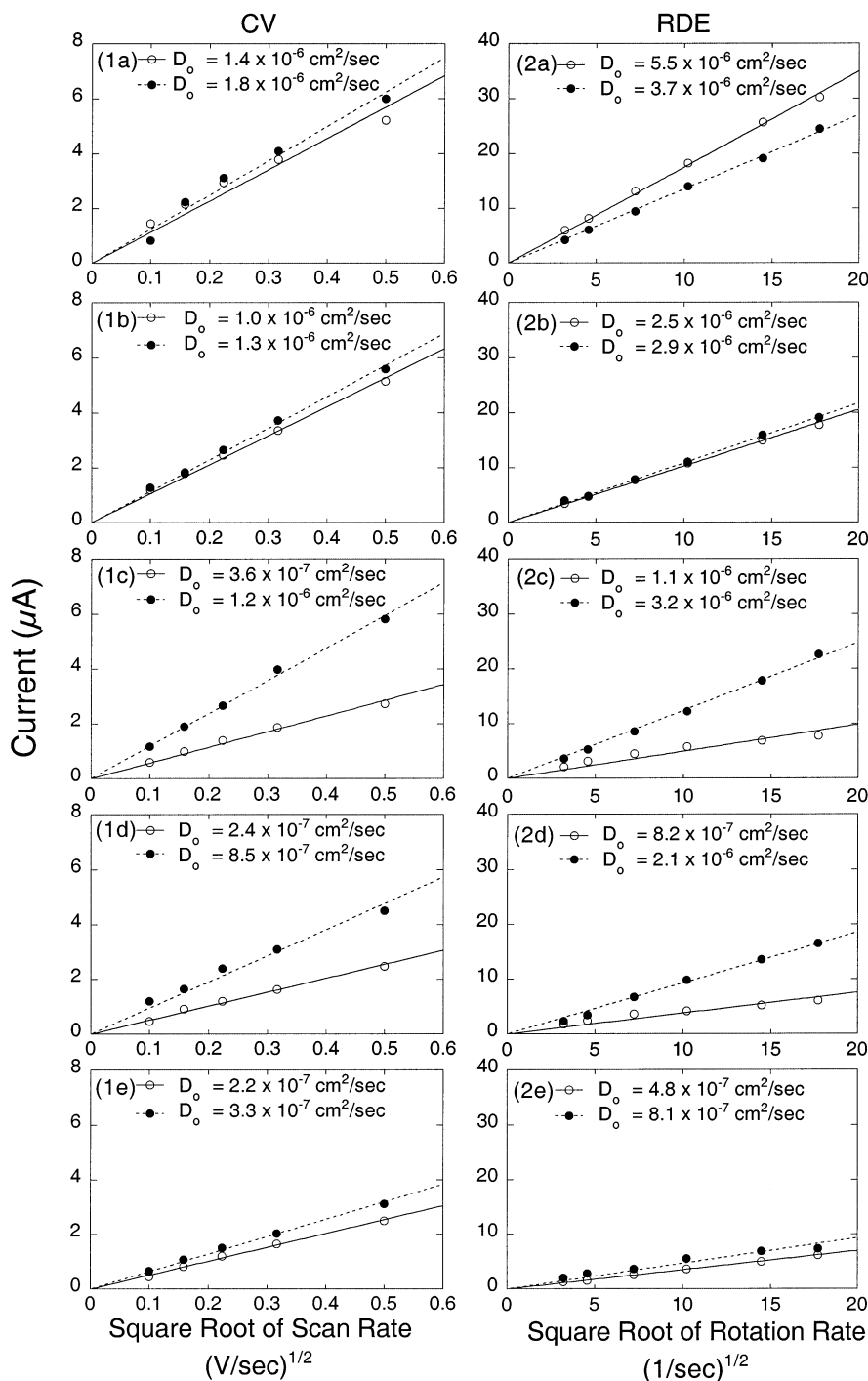


Figure 4. Plots of peak current vs square root of scan rate from CV experiments with $\text{Dend-}n\text{-[Ru(bpy)}_3\text{)]}^{2+}$ (○) and $\text{Dend-}n\text{-[Ru(tpy)}_2\text{)]}^{2+}$ (●) for (1a) $n = 4$, (1b) $n = 8$, (1c) $n = 16$, (1d) $n = 32$, (1e) $n = 64$. Plots of limiting current vs square root of rotation rate from corresponding RDE experiments for (2a) $n = 4$, (2b) $n = 8$, (2c) $n = 16$, (2d) $n = 32$, (2e) $n = 64$. Calculated diffusion coefficients are presented in each panel.

increased. Note, however, the significant difference in the absolute values relative to those determined by cyclic voltammetry.

Determination of Diffusion Coefficients by PFGSE-NMR. Samples of each dendrimer in deuterated acetone were made up in order to carry out the PFGSE-NMR experiments. The raw data from such experiments are a series of FT-NMR spectra at various values of the applied magnetic field gradient, and a representative sample of the data for $\text{Dend-8-[Ru(tpy)}_2\text{)]}^{2+}$ can be seen in Figure 5. In principle, analysis of any portion of the NMR spectrum should lead to the same calculated value of the diffusion coefficient. However, the resonances from the aliphatic protons of the dendrimer are generally quite broad and a good

baseline was difficult to achieve. On the other hand, the baseline of the NMR spectrum in the region of the polypyridyl protons was much flatter, and the resonances were sharp and easily resolved. For this reason, only the integrations of the terpyridyl and bipyridyl protons were used for the determination of the diffusion coefficients. Additionally, since those protons are well resolved and the signals are separable, several independent sets of data could be collected and analyzed from each acquisition. Also, for each dendrimer, experiments were done with two different sets of delay times (2.3 ms and 3.5 ms). In Figure 6, representative plots of $\ln(I_g/I_0)$ vs G^2 (the square of the gradient strength) are shown for all of the dendrimers investigated. The diffusion coefficients determined by PFGSE-NMR are presented

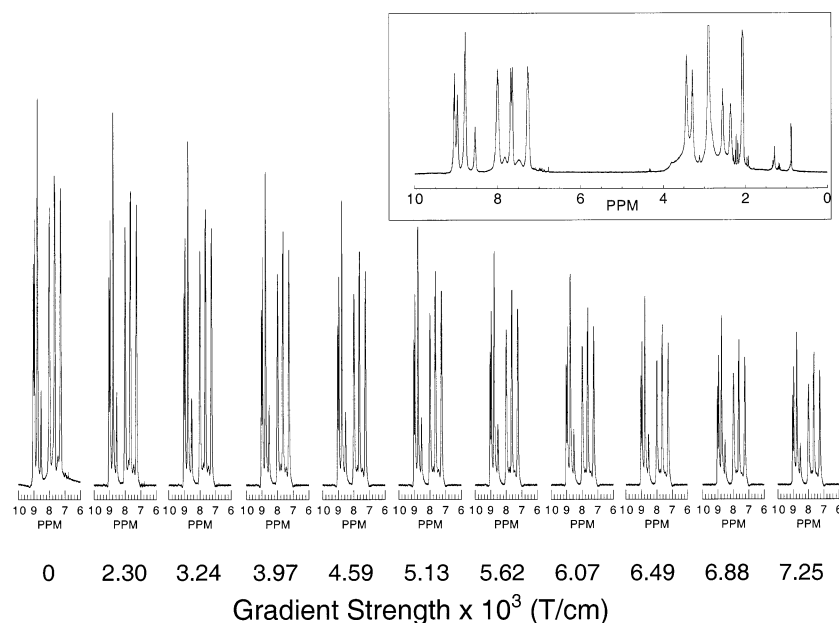


Figure 5. PFGSE-NMR spectra of the terpyridyl protons of Dend-8-[Ru(tpy)₂]²⁺ in acetone-*d*₆ at various magnetic field gradient strengths. The inset is a standard ¹H NMR spectrum of Dend-8-[Ru(tpy)₂]²⁺.

in Table 1 and are each the average of 4–8 measurements. These values are also placed in the appropriate panels of Figure 6. As in the previous two methods of analysis, increasing dendrimer size correlated with decreasing diffusion coefficient.

Discussion

A significant amount of data and results have been presented above and in order to understand the implications of all of these data as well as to draw some conclusions, it is instructive to consider various subsets of the information presented above. The diffusion coefficients determined by electrochemical methods range from 2.2×10^{-7} cm²/s to 5.5×10^{-6} cm²/s, and previous studies of dendritic molecules similar to the ones examined here have reported that the diffusion coefficients of such molecules, determined by cyclic voltammetry, are in the range of 10^{-6} – 10^{-7} cm²/s.^{43–45} Upon first glance, the data from the electrochemical experiments appear reasonable in that there is a monotonic decrease in the value of the diffusion coefficient with increasing dendrimer generation (size). However, for all of the dendrimers studied, there are distinct differences between the diffusion coefficients determined from CV experiments and those from RDE experiments. Even though each analytical method is quantitative and is generally considered reliable to ca. 10–20%, the diffusion coefficients, for any given dendrimer, determined using the RDE technique, are 2–4 times larger than those determined by CV.

When the diffusion coefficients determined by electrochemical methods are compared to those from PFGSE-NMR, significant discrepancies are also noted. For the smaller dendrimers, with 16 or less pendant redox centers, the results from the NMR experiments are very similar to the diffusion coefficients calculated from analysis of the RDE data. However, for the larger dendrimers, there is not good agreement between PFGSE-NMR and RDE with the NMR-determined diffusion coefficients being significantly larger than those determined by RDE. There is no similarity between the NMR-determined diffusion coefficients and those determined from cyclic voltammetry. In all cases, the PFGSE-NMR results are much larger than those from CV. The differences range from ca. 400% for the smaller

dendrimers to ca. 1000% for the larger ones. Clearly all of these differences are well beyond the experimental variations involved.

The three different experimental techniques described have been shown to give dramatically different diffusion coefficients for the families of dendrimers studied. To be able to ascertain whether the use of each (or any) of these methods is appropriate, it is important to consider exactly what is being measured by each technique. Both of the electrochemical techniques measure the current that results from the oxidation of Ru(II) at the surface of the electrode. In both cyclic voltammetry and in RDE, the current is determined by the flux of material to the electrode surface. In the CV experiment, mass transport is purely diffusional, but the RDE method is more complex. The current at the electrode is still controlled by the flux of material to the surface but hydrodynamic transport, controlled by the rotation rate of the electrode, dominates until the material gets very close to the electrode surface. In contact with the electrode is a thin, stagnant layer (Levich layer) of solution with a thickness of the order of microns, through which the redox-active material is ultimately transported by diffusion.^{27–29}

A particular molecule clearly has a unique value for its diffusion coefficient. The Faradaic current at an electrode is determined by the flux of material to that electrode. To measure two different diffusion coefficients for the same molecule (at strictly identical concentrations), the flux of material to the electrode in the RDE experiment must *appear* larger than that in the CV experiment. However, diffusion is the final mode of mass transport in both experiments, and the only difference between the methods is the stirring of the solution and the slow scan rate employed when using a rotating-disk electrode.

Interpreting the diffusion coefficient results is made more complex by the fact that the dendrimers are known to adsorb to platinum surfaces up to (but not more than) a monolayer in coverage as long as negative potentials are avoided.⁴² Species adsorbed to the electrode will contribute to the current observed in the CV method, although the concentration of material in solution was deliberately kept large in an attempt to keep the contribution from the adsorbed species to a minimum. In all experiments, the same exact electrode was used, so there should

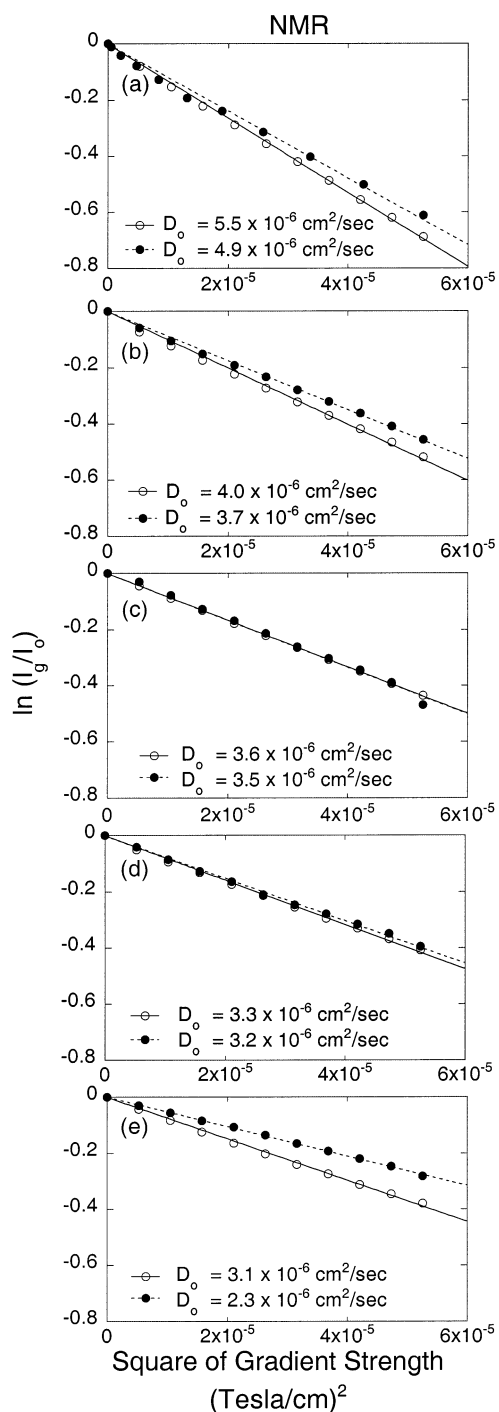


Figure 6. Plots of natural logarithm of normalized peak area vs square of magnetic field gradient strength from PFGSE-NMR experiments with $\text{Dend-}n\text{-[Ru(bpy)}_3\text{]}^{2+}$ (○) and $\text{Dend-}n\text{-[Ru(tpy)}_2\text{]}^{2+}$ (●) for (a) $n = 4$, (b) $n = 8$, (c) $n = 16$, (d) $n = 32$, (e) $n = 64$. Calculated diffusion coefficients are presented in each panel.

not be any difference in the amount of material adsorbed. Whereas there is no contribution from adsorbates in RDE experiments, it would be expected that an enhanced response (adsorbed material plus material in solution) would be observed in CV experiments. An increase in the observed current, due to material on the surface, would lead to a larger calculated diffusion coefficient, but such a result is not seen experimentally. In fact, the diffusion coefficients calculated from the CV experiments are *smaller* than those from the RDE experiments. Clearly, adsorption cannot be responsible for the observed differences.

While the differences among the various methods of analysis have been discussed, it remains to be shown which, if any, of the methods provides the correct value for the diffusion coefficient. To compare all of the experimentally determined values and make some judgments about their relative validity, one can consider the Stokes-Einstein formulation for diffusion of a particle:

$$D = kT/6\pi R\eta \quad (4)$$

where D is the diffusion coefficient, R is the radius of a spherical particle, η is the viscosity of the solution, and the remaining symbols have their usual meaning.⁴⁶ Using this expression to calculate the radius of each dendrimer from the experimentally measured diffusion coefficient normalizes the results for differing solution viscosities, and allows direct comparisons to be made among the experimental methods employed. Additionally, this calculation allows a comparison between the experimentally determined size of the molecule and the size as estimated from modeling studies.⁴⁷ Shown in Table 2, for each of the experimental methods utilized, are the calculated sizes of all dendrimers as determined from eq 4 as well as the estimated values of the molecular diameters.

From the data contained in Table 2, it is clear that the diffusion coefficients determined by cyclic voltammetry are much too small. Especially for the larger dendrimers, the molecular diameters as calculated from diffusion coefficients are nonsensically large. While the estimation of the dendrimer sizes is not exact, an upper limit of 100 Å for the diameter of the largest molecule is very reasonable, and the experimental data does not match such a prediction. In fact, for all of the dendrimers under consideration, the sizes indicated by the CV measurements are larger than the maximum theoretical sizes of the molecules. For the smaller dendrimers, both the RDE measurements and the PFGSE-NMR measurements are in good agreement with the predicted sizes of the molecules. However, for the larger dendrimers, the RDE method overestimates the sizes (i.e., gives diffusion coefficients that are too small) and the PFGSE-NMR method leads to a calculation of molecular size which is smaller than expected. Neither of those two methods, however, approaches the gross inconsistencies noted in the CV measurements.

From the above analysis, it is clear that electrochemical determinations of the diffusion coefficients of the dendrimers under investigation are not satisfactory. While not perfect, the technique of PFGSE-NMR leads to more reasonable results over the entire range of dendrimers studied. Potentially, there are several reasons for the failure of the electrochemical methods, including the roles played by both the solvent and the supporting electrolyte. Depending on the extent to which the dendrimer molecules are solvated, their effective size could be significantly greater than the geometric dimensions of the particular molecule. Since all the dendrimers are highly charged, in addition to solvent molecules, charge compensating counterions will be associated with each molecule, adding to their hydrodynamic size. One could argue that the effects of such solvation and counterion association would be to make the effective size of the dendrimers much larger and the observed diffusion coefficient correspondingly smaller than expected. To test for such effects, a PFGSE-NMR experiment was carried out for $\text{Dend-32-[Ru(bpy)}_3\text{]}^{2+}$ in deuterated acetonitrile with 0.10 M TBAH. The effects of solvation and counterion association, whether the solution is in an NMR tube or in an electrochemical cell, should be identical. However, the diffusion coefficient obtained from the NMR experiment in deuterated acetonitrile with

TABLE 2: Sizes of Dend-*n*-Ru(tpy)₂]²⁺ and Dend-*n*-[Ru(bpy)₃]²⁺ Calculated from Measured Values of Diffusion Coefficients

	diameter determined from CV measurements (Å)	diameter determined from RDE measurements (Å)	diameter determined from PFGSE-NMR measurements (Å)	estimated diameter of dendrimer ^a (Å)
Dend-4-[Ru(tpy) ₂] ²⁺	65	32	28	45–50
Dend-8-[Ru(tpy) ₂] ²⁺	90	40	38	60–65
Dend-16-[Ru(tpy) ₂] ²⁺	97	36	40	75–80
Dend-32-[Ru(tpy) ₂] ²⁺	138	56	43	85–95
Dend-64-[Ru(tpy) ₂] ²⁺	355	144	60	100–110
Dend-4-[Ru(bpy) ₃] ²⁺	84	22	25	45–50
Dend-8-[Ru(bpy) ₃] ²⁺	118	47	35	60–65
Dend-16-[Ru(bpy) ₃] ²⁺	326	106	39	75–80
Dend-32-[Ru(bpy) ₃] ²⁺	488	143	42	85–95
Dend-64-[Ru(bpy) ₃] ²⁺	532	244	45	100–110

^a See Note 47.

supporting electrolyte was 3.4×10^{-6} cm²/s, almost identical to the value of 3.3×10^{-6} cm²/s measured by NMR in pure (i.e., without supporting electrolyte) deuterated acetone. To verify that solvent properties affected the PFGSE-NMR measurements in the predicted fashion, the diffusion coefficient of Dend-64-[Ru(tpy)₂]²⁺ was measured in deuterated DMSO, which is much more viscous than acetone. By NMR, a diffusion coefficient of 0.9×10^{-6} cm²/s was measured in DMSO, which is, as expected, much smaller than the corresponding value (2.3×10^{-6} cm²/s) measured in acetone. The only variable that cannot be examined with the NMR experiments is the electric field present in the electrochemical techniques employed. However, the use of a high concentration of supporting electrolyte is the accepted manner of eliminating the effects of migratory transport. Even if transport due to an electric field is a factor in the electrochemical experiments, the effect would be expected to be similar in both CV and RDE experiments.

For redox molecules that undergo facile electron-transfer events, Dahms and Ruff have considered the effect that self-exchange electron transfer has on the diffusion coefficient.^{48–50} An experimentally observed diffusion coefficient (D_{exptl}) consists of two contributions:

$$D_{\text{exptl}} = D_0 + (\pi/6)\delta^2 k_{\text{ex}} C \quad (5)$$

where k_{ex} is the self-exchange electron-transfer rate constant, C is the concentration, δ is the distance between molecules when the electron transfer takes place, and D_0 is the diffusion coefficient without the effects of self-exchange. It is possible that there is a contribution to the experimentally measured diffusion coefficient from self-exchange, however such contributions are usually noted in polymer films and are on the order of 10^{-10} – 10^{-11} cm²/s.⁵¹ They are always additive, making the observed diffusion coefficient larger than expected. In our electrochemical experiments, the observed diffusion coefficients are smaller than expected, leading to the (anticipated) conclusion that self-exchange does not play an important role in explaining the data we have presented.

The previous discussion has addressed the potential role that solvent and counterions as well as self-exchange, might play in giving rise to the unreasonable diffusion coefficients determined electrochemically, and all indications are that the explanation for this phenomenon lies elsewhere. For a molecule with multiple noninteracting redox centers, such as the dendrimers that we are investigating, it is possible, if not likely, that only a fraction of those centers can be oxidized or reduced at a given time. If the measured current is not derived from the oxidation or reduction of all of the redox centers present, that

effect will be reflected in the diffusion coefficient calculated from that electrochemical measurement. If only a fraction of the redox centers on a given dendrimer are sampled, i.e., oxidized or reduced, in a given event, the diffusion coefficient *calculated* for that molecule will be smaller than the *actual* diffusion coefficient for the molecule.

To undergo electron transfer, a molecule must be in contact with or in close proximity to the electrode surface, and for large globular molecules with pendant redox moieties, it is not possible for all of the redox-active centers to simultaneously be in contact with or in close proximity to the electrode surface. To successfully oxidize or reduce all of the metal centers on a large dendrimer, the molecule must rotate to allow each center access to the electrode. The rate of rotation then becomes very important since the molecule is in close proximity to the electrode surface for a finite time period. If that time is shorter than the time necessary to allow all redox sites to be sampled by the electrode, the observed current will reflect the fact that only a fraction of the redox centers on the dendrimer were able to be oxidized or reduced.

The rotational diffusion of a spherical molecule has been characterized by Einstein and others and leads to an expression for the rotational diffusion coefficient

$$D_R = kT/8\pi\eta R^3 \quad (6)$$

which has units of radians/s.⁵² Using this expression as well as the expression for translational diffusion (eq 4) allows a calculation of the extent of rotation that a spherical molecule undergoes as a function of the distance that it diffuses. The result of such a calculation is that for N radians of rotation, a spherical molecule travels $(4N/3)^{1/2}R$ where R is the radius of the molecule. For a large molecule where all of the redox centers cannot be in contact with the surface at once, it is possible to estimate the minimum number of complete rotations of the molecule that are necessary to bring all redox centers into contact with the electrode surface. Using the Dend-64 as an example, if the radius of the molecule is estimated at 50 Å and its “footprint” as it sits on a surface is a disk 30 Å in diameter, it would take a minimum of 3.3 complete rotations of the molecule to bring the entire surface of the sphere into contact with or in close proximity to the electrode. This indicates that, in the time necessary for those rotations, the molecule would diffuse a distance approximately equal to $5R$ or 250 Å. However, not only is the figure of 3.3 rotations the minimum number of rotations necessary, but also this estimation assumes that the molecule rotates freely when it is very near to the surface, which is not necessarily the case. In fact, we have previously shown

that adsorbed dendrimers have very limited mobilities.⁵³ The actual distance that the molecule would diffuse in the time that it would take to make all redox sites accessible to the electrode surface could be considerably greater than $5R$.

The larger and more globular the dendrimer, the more pronounced this effect would be. A smaller dendrimer, with a radius of 25 Å and a "footprint" of 15 Å, would still have to rotate at least 3.3 times to allow all its redox sites to be sampled by the surface. However, it would diffuse only a distance of 125 Å in that time, compared to the larger dendrimer that would move 250 Å. When compared to those on the larger dendrimer, the redox centers on the smaller dendrimer will be much closer to the electrode surface during the molecular rotations. Also, the smaller dendrimers, which are less globular than their larger relatives, are more easily able to deform and allow all redox sites access to the surface. From the previous analysis, it can be seen that the rotational time scale of large dendrimers could lead to the sampling, by electrochemical techniques, of only a fraction of the redox centers present. The diminution in the current due to this effect could lead to an incorrect determination of diffusion coefficients for such large molecules.

One might observe that the redox sites of a such a dendrimer are quite close together and perhaps are able to transfer electrons efficiently by self-exchange. If this is the case it might not be necessary for the entire surface of the molecule to contact the electrode in order for all of the redox sites to be oxidized or reduced. However, a closer analysis of the situation indicates that it is not feasible to completely oxidize or reduce all of the redox centers of a large dendrimer in this fashion.⁵⁴

We have previously noted that there are significant differences between the diffusion coefficients determined by CV and those determined by RDE. One might be tempted to use the model proposed above to account for those differences by theorizing that the stirring that occurs in the RDE experiment somehow causes an increase in the rotational motion of the molecules. However, upon closer examination, it becomes clear that the amount of rotational energy transferred by the rotating disk to a dendrimer molecule, i.e., the rotation rate of a sphere in a sheared solution, is dwarfed by the rotation due to kT . Even if the stirring of the solution were to lead to a dramatically increased rate of molecular rotation, there is a stagnant layer (again, the Levich layer) of solution in proximity to the electrode through which all material must pass. It takes much more time for a molecule to cross that stagnant layer of solution and reach the electrode than it does for the rotation rate of the molecule to relax to its thermally determined value.⁵⁵ Thus, it does not appear physically reasonable, at least for the model proposed above, to theorize that stirring the solution causes the diffusion coefficients determined by CV and by RDE to be so different from each other.

However, if the consequences of molecular rotation are considered in a slightly different fashion, it is possible to form a hypothesis regarding the difference between the CV and the RDE results. In electrochemical experiments, it is generally assumed that once a molecule comes to the electrode surface and reacts, it diffuses away never to return.⁵⁶ Even if it did return to the electrode, there would be no effect on the measured current because the molecule had already reacted. However, this assumption might not be valid for the large dendrimers with multiple redox centers under investigation. Both theory⁵⁷ and simulation⁵⁸ of random-walk diffusional processes show that a molecule can revisit a particular point in space, i.e., an electrode surface, many times before finally departing. For large dendrimers with many pendant redox-active moieties, as in the

present case, each collision with the electrode exposes only a fraction of the redox sites to the electrode surface. Before the molecule can collide with the electrode a second time, rotational diffusion will randomize the orientation of the molecule so that, during the second collision, a fraction of the redox sites that contact the electrode will have reacted in the previous collision. The net result is that each subsequent collision of the molecule will result in less electron transfer than for the previous collision. This process of diminishing returns per collision continues until the molecule leaves the region near the electrode surface for good.

Unlike CV, the use of a RDE involves the stirring of the solution, which constantly replaces material in the vicinity of the electrode with "fresh" (completely unreacted) material. The more readily the partially reacted molecules are removed from the vicinity of the electrode and replaced with "fresh" ones, the higher the observed current will be, since the "fresh" molecules will transfer more electrons per collision than molecules that have partially reacted. It is possible that a mechanism such as this could be responsible for the difference between the diffusion coefficients determined by CV and those determined by RDE. When a partially reacted molecule diffuses away from a RDE, at a certain distance it will enter the region of moving fluid and will be irrevocably swept away from the electrode surface. When a partially reacted molecule has moved a similar distance from an electrode in an unstirred solution, there is the possibility that it will return to the electrode and engage in an "inefficient" electron-transfer event. This would lead to the observed current in CV experiments being smaller than expected, relative to that observed in RDE experiments (i.e., the diffusion coefficients from RDE being larger than those from CV), which is the result seen from our experiments.

Moreover, when RDE and CV experiments were done under experimental conditions where the thickness of the diffusion layer was comparable (by controlling rotation rate and sweep rate for RDE and CV experiments, respectively), the above-mentioned differences although less pronounced, were still evident. This, again, is consistent with the concept of incomplete sampling. The hypothesis about incomplete sampling of all redox sites is currently under investigation, so the above analyses should be regarded only as a suggested explanation for the observed phenomena. However, results of Takada, et al., from a study of surface adsorbed cobalt terpyridyl pendant dendrimers have shown that the adsorbed molecules do not rotate freely on the time scale of a cyclic voltammetry experiment.⁵³ While those data are for adsorbed species and this work deals with dendrimers in solution, it demonstrates that molecular motion can be an important factor in the electrochemical response of large molecules with multiple redox centers. Experiments are currently underway to determine, in an unambiguous fashion, whether the molecular motion of macromolecules is relevant to their electrochemical response and the extent of such an effect.

Conclusion

We have shown that the electrochemical determination of the diffusion coefficients of large molecules with multiple redox centers is fraught with complications. For the families of ruthenium polypyridyl pendant PAMAM dendrimers studied, we have found that the diffusion coefficients determined by cyclic voltammetry are smaller than those determined by rotating disk electrode techniques, which in turn are smaller than those determined by pulsed-field-gradient spin-echo NMR. The Stokes-Einstein equation allows the size of a molecule to be calculated from an experimentally measured diffusion coef-

ficient. The sizes of the dendrimers determined by the Stokes–Einstein equation from the electrochemical measurements of diffusion coefficients are much larger than is physically realistic, indicating that the electrochemically determined diffusion coefficients are much too small. On the other hand, the molecular sizes calculated from the Stokes–Einstein equation for diffusion coefficients measured by PFGSE-NMR are physically very reasonable. We have addressed the potential effects of solvent and supporting electrolyte on the measurement of diffusion coefficients and have found them unsatisfactory for explaining the large differences seen among the results from the three techniques employed. A hypothesis has been proposed which attributes the variety of results to incomplete sampling of the redox centers. If a dendrimer is rotating at a rate such that, in the interval of time when it is in close proximity to the electrode surface, only a fraction of the redox centers are able to be oxidized or reduced, the current measured and, hence, the diffusion coefficient calculated in the electrochemical experiment will be diminished. Because it does not rely on redox chemistry, the PFGSE-NMR technique is not affected by an incomplete sampling of dendrimer redox sites, and it offers the most reasonable values for the diffusion coefficients of all of the dendrimers studied.

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