

EPR Investigation of the Adsorption of Dendrimers on Porous Surfaces

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Guest–host interactions of poly(amidoamine) dendrimers and porous surfaces, such as homoporous silica of different pore sizes, activated alumina (acidic, neutral, basic), and MCM-41 zeolite (Si/Al=30), were investigated by electron paramagnetic resonance (EPR) spectroscopy. Two generations of dendrimers were selected, G2 and G6, representing “early” and “late” generations, which were labeled with a nitroxide radical. It was found that the main parameters regulating the adsorption and interaction of the dendrimers and porous surfaces are (I) the size of the pores of the porous materials (host) relative to the size of the dendrimer (guest), (II) the total surface area of the host that is accessible to the guest dendrimers, and (III) the chemical composition of the host and guest surfaces that come into contact upon adsorption. If the pore size of the host surface is smaller than the guest dendrimer diameter, then adsorption of the dendrimer occurs exclusively on the external host surface. If the pore size of the host surface is bigger than the dendrimer diameter, then the internal surface area is accessible to the dendrimers and large amounts of dendrimers are adsorbed. The chemical composition of the host surface and guest surface determines the strength of the host–guest interaction. An increase in alumina content (silica < zeolite MCM-41 < alumina) of the host increased the electrostatic interaction of the dendrimer with the host surface. Increased protonation of the external dendrimer amino groups also increased the electrostatic interaction. For the large dendrimers (G6), which possess a closed surface structure, electrostatic interactions are the main forces of host–guest interaction. Therefore, the strongest interaction of G6 was observed with basic alumina. For small dendrimers (G2), which possess an open structure, hydrophobic interactions between the low polarity internal dendrimer structure and the low polarity Si–O–Si groups of the host surface provide an additional host–guest interaction. High protonation of the dendrimer surface and high alumina content of the host surface inhibits this hydrophobic type of interactions. The combination of low polar Si–O–Si groups and highly polar AlO[−] groups in the zeolite MCM-41 probably cause the strongest interaction of the guest G2 dendrimer with MCM-41 compared to the other investigated host surfaces.

Introduction

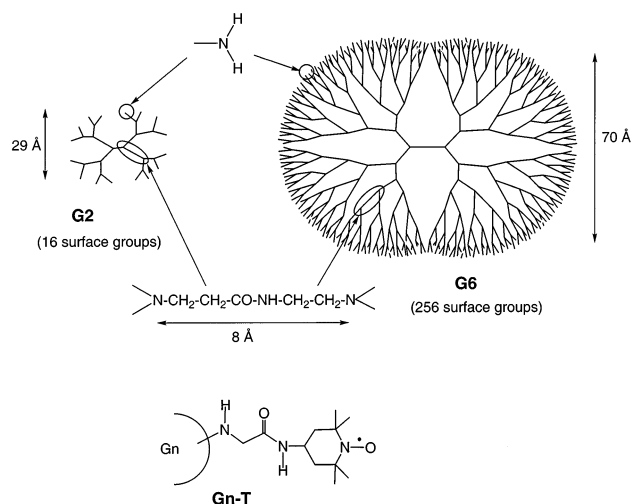
Dendrimers are a class of synthetic, highly branched, spherical molecules with well defined covalent connectivity and chemical functionality and a systematically variable set of surface groups.¹ Dendrimers have been identified as promising candidate materials for a variety of fields, including drug delivery,² gene therapy,³ chemical separation, nanoreactors, sensors,⁴ and catalysis.⁵ The interaction of dendrimers with solid surfaces and colloidal particles is of general interest and has been studied extensively.⁶ The analysis of dendrimer interactions with solid surfaces provides useful information, which can expand the possibilities of using dendrimers adsorbed on solids as new materials. For example, through adsorption, dendrimers may modify the properties of a solid surface and produce composite materials that are compatible with different kinds of environ-

ments, such as a biomedium, thus enhancing the range of application of dendrimer-surface supramolecular systems.

The dendrimers used in this work are poly(amidoamine)-dendrimers (PAMAM), which were synthesized by stepwise attachment of repeat unit layers or branches, termed generations (G, the dendrimers were henceforth termed G_n, where *n* indicates the generation), on an ethylenediamine central core and terminated with amino groups at the external surface (Scheme 1).⁷ In aqueous solutions, the external amino groups are partly protonated. The protonated sites of the dendrimers possess the characteristics of cationic polyelectrolytes and are expected to interact strongly with appropriate solid surfaces, such as those provided by particles of silica and alumina. Esumi and Gojino reported a study of the adsorption of poly(amidoamine)dendrimers on silica and alumina.^{6c} The authors found that the “earlier” generation dendrimers (*n* < 4) behave as ordinary electrolytes, whereas the later generations (*n* > 4) behave as surfactant or polyelectrolyte with respect to negatively charged

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SCHEME 1: Structures of the Dendrimers



silica particles, resulting in a dispersion-flocculation-redispersion sequence depending on the dendrimer concentration.

We report an EPR investigation of the adsorption and interactions of dendrimers with porous silica and alumina. The “effective porosity” of a solid surface (as a host) toward an adsorbed molecule (as a guest) is determined by the accessibility of the host surface to the guest and the interactions (bonding) of the guest to the accessible host surface. To a first approximation, the accessibility of a solid host surface (assumed to be rigid and inflexible) to a guest depends on geometric factors which are related to the relative size of the pores of the host compared to the size of the guest. Thus, if a guest has a molecular size that is sterically larger than the pore openings leading to the internal surface, adsorption and interactions can only occur with the external surface. If the size of the cages and pores of a host are comparable in size to those of the guest, and if the guest possesses sufficient flexibility, interactions with the surface are expected to be important, because significant contact of the guest molecular surface and the host solid surface will result in bonding because of dispersion interactions alone. On the other hand, if the host pore size is much larger than the molecular size of the guest, the contact between host and guest surface may be small, and significant attractive interactions with the solid surface will occur mainly through chemical and electrostatic interactions. Thus, the strongest adsorption and interactions are expected to occur when a proper combination of geometric and chemical factors occurs.

From these considerations, the extent and strength of adsorption of a guest onto a rigid solid surface is expected to depend on the following parameters: pore size of the host solid relative to the molecular size (generation) of the guest, the total surface area of the host that is accessible to the guest, the guest flexibility, the chemical composition of the accessible surface of the host, and the molecular surface of the guest. The chemical characteristics of both the solids and the dendrimers employed in this investigation are influenced by pH. In particular, because the surface of the PAMAM dendrimers investigated are terminated with amine groups, the extent of protonation of the dendrimer surface is an important parameter that can be varied and investigated systematically.

In this investigation, spin labeled dendrimers were synthesized so that dendrimer-solid surface interactions could be investigated by electron paramagnetic resonance (EPR). Previously, we have shown that the dendrimer spin labeling technique is very useful for the investigation of host–guest adsorption and interactions

TABLE 1: Relevant Structural Parameters of the Porous Materials and Dendrimers Used in This Study

silica gels (pore volume: 0.65 mL g ⁻¹ ; Fe, Cl ⁻ < 0.02%):		
silica	pore size (Å) ^a	surface area (m ² g ⁻¹) ^a
S40	40	600
S60	60	500
S100	100	400
S200	200	150
S500	500	50

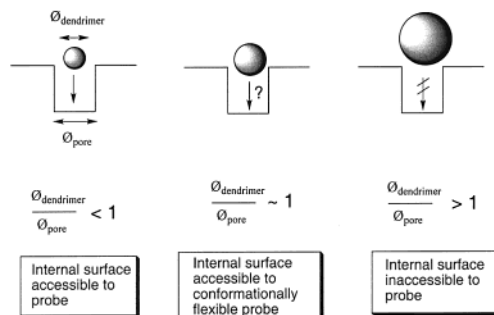
activated aluminas: Pore size:
58 Å; surface area: 150 m²g⁻¹ ± 10%
basic: pH_{aq, 5%} = 9.5 ± 0.3;
neutral: pH_{aq, 5%} = 7.5 ± 0.5;
acid: pH_{aq, 5%} = 4.5 ± 0.5
zeolite MCM-41: Si/Al=30;
pore size = 80 Å; surface area = 996 m²g⁻¹ ± 5%

dendrimer size, average area for surface group (A _M), and number of surface amino groups of G2 and G6 (ethylenediamine core)			
generation	size (Å) from SEC	A _M (Å ²)	surface groups
2	29	41.3	16
6	67	13.8	256

label size: between 7 and 10 Å, dynamic conformation

^a Accuracy 5–10%.

SCHEME 2: Geometric Features

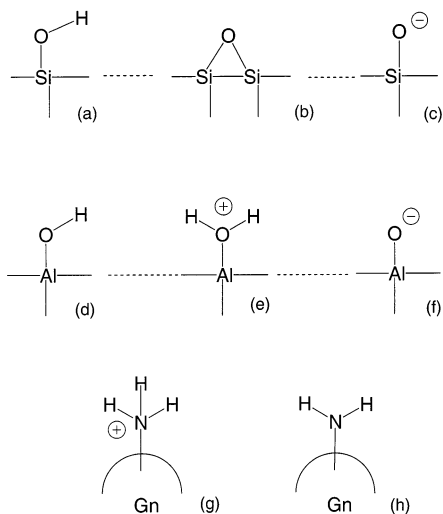


of dendrimers with DNA and vesicles.⁸ Two generations of PAMAM dendrimers were selected, G2 and G6, as representative of “early” and “late” generations (Scheme 1). Some of the relevant properties of the solids and dendrimers employed in this investigation are summarized in Table 1.

Size/shape characteristics are important factors in analyzing guest adsorption capacity and guest diffusion rates on a host system. For adsorption of dendrimers into the internal surface (pore) of the porous material (host), the pore size (\AA) and the diameter of the guest dendrimer (\AA) is of crucial importance. Because the internal surface area of the investigated porous materials is much larger than the external surface area, a much higher adsorption capacity for dendrimers is expected if the dendrimers have access to the internal surface. Three main binding possibilities can be distinguished and are summarized in Scheme 2: (a) the dendrimer diameter is smaller than the pore diameter (internal surface is accessible to the dendrimer), (b) the diameters of pore and dendrimer are similar (internal surface is accessible, but some conformational flexibility is required), and (c) the diameter of the dendrimer is bigger than the pore diameter (internal surface is inaccessible to the dendrimer; only adsorption at the external surface is possible).

Another important aspect for the discussion of our results is the chemical nature of the host and the guest surfaces.

SCHEME 3: Chemical Structure of Silica (a–c), Alumina (d–f), and Dendrimer (g,h) Surfaces at Different Protonation Levels



Electrostatic interactions between the silica or alumina surfaces with dendrimer surfaces are strongly influenced by the extent of protonation (pH). Scheme 3 illustrates the different chemical structure at different protonation levels of the host and guest surfaces. Depending on the pH, the surface of silica is composed of (a) Si–OH groups, which are hydrophilic, (b) Si–O–Si groups, which are hydrophobic, and (c) Si–O[−] groups.⁹ Depending on the pH, alumina surfaces are composed of (d) Al–OH, (e) Al–OH₂⁺, or (f) Al–O[−].¹⁰ Both acidic and basic activated aluminas were used in this work. Finally, a zeolite (mesoporous alumino-silicate MCM-41), which combines the surface properties of silica and alumina, was investigated.

Experimental Section

Poly(amidoamine) dendrimers (Scheme 1: G2, 16 surface amino groups; G6, 256 surface amino groups), where the generation refers to the number of covalently attached amidoamine layers, have been synthesized as described in previous papers.¹¹ G2 and G6, representative of the “earlier” and “later” generations, have been shown to demonstrate qualitatively different supramolecular binding characteristics.¹¹ Purification of the dendrimers was accomplished by ultrafiltration. The purity of the dendrimers has been determined by ¹³C NMR spectroscopy, by mass spectrometry, as well as by gel electrophoresis.¹² The labeling of the dendrimers (Gn-T, *n* = 2, 6; Scheme 1) was accomplished employing a modified method used by Pauly et al. for labeling of DNA-nucleosides¹³ and was described previously.¹⁴ The level of labeling was evaluated as 1 label for about 30 surface amino groups by quantitative EPR spectroscopy, employing a nitroxide standard.

Solutions of the labeled dendrimers in Millipore water were prepared at concentrations ranging from 5 to 50 mM. Unless otherwise specified, the concentration of the dendrimer solutions is in amino surface groups. The solutions were stored under nitrogen, to avoid oxidative degradation, and kept in the refrigerator when not in use.

Protonation of the surface amino groups was accomplished by adding controlled amounts of diluted HCl (0.05 M, Merck). The acid–base properties of the PAMAM dendrimers have been determined in a previous study.¹⁵ The natural pH of the aqueous dendrimer solution is about 8.5. Previous studies¹⁵ have demonstrated that at pH = 8.5 the dendrimer surface is partially

protonated and that complete protonation is observed at pH = 4.5. The pH of the dendrimer solution, corresponding to partial and complete protonation of the amino groups, was measured with a Sentron pH meter.

Homoporous silica gels (Merck) and activated alumina (Aldrich) were used as received. Table 1 reports the relevant properties of the used silica and alumina, including pore size and surface area.¹⁶ In addition, the table lists the size, the average area for surface, and the number of surface amino groups of 2G and 6G. MCM-41 was kindly provided by Prof. Di Renzo, CNRS Montpellier, France: its properties are also listed in Table 1.

The EPR spectra were recorded by means of a Bruker 200D or a EMX-Bruker spectrometer operating at X band (9.5 GHz), interfaced to a PC-IBM computer (Stelar software for 200D and Bruker software for EMX) for data acquisition and handling. The temperature was controlled with a Bruker ST 100/700 or a Bruker ST3000 variable-temperature accessory. Unless otherwise specified, all of the EPR spectra were recorded at 293 K. However, measurements at different temperatures were carried out to better differentiate the spectral components.

To determine the amount of dendrimers adsorbed on the solid particles, aqueous slurries (0.5 mL) of nitroxide labeled dendrimers and solid particles (200 mg) were stirred overnight. Afterward, the supernatant was injected into a flat quartz cell of the EPR spectrometer to determine intensity of the nitroxide signal. An experimental configuration, in which the flat quartz cell is fixed in the EPR spectrometer, was used to achieve constant experimental conditions. Standard nitroxide solutions (0.1–1 mM) were used as reference. The intensity measurements were considered valid only on condition of reproducibility in at least three independent experiments.

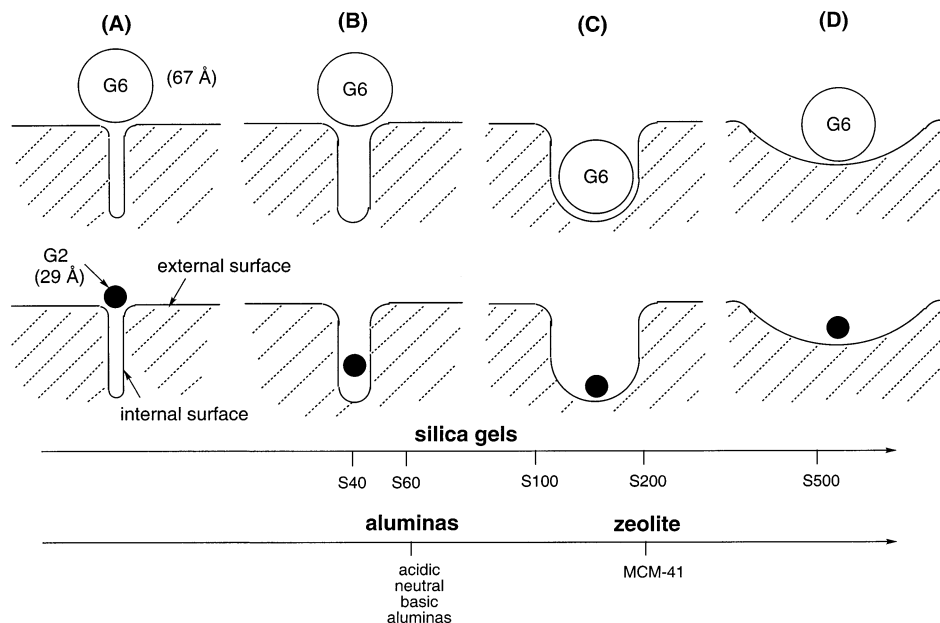
To investigate the interaction between the dendrimers and the solid particles, the supernatant of the above solutions was removed by filtration and the solids were gently dried on filter paper until the initial dry appearance was recovered. This “dry” powder, consisting of the solid particles with adsorbed dendrimers and intraporous water, was transferred into a 2 mm quartz tube and analyzed by EPR.

Results and Discussion

Adsorbed Amounts of Dendrimers at Solid Surfaces. Table 2 reports the fractions of adsorbed dendrimers from solutions at two representative dendrimer concentrations, 5 and 40 mM, onto silica, alumina, and the zeolite MCM-41. The fraction of adsorbed dendrimers on the porous surfaces was obtained from analysis of the EPR spectra of the supernatant solutions after the adsorption process, which was compared to the dendrimer solution before the adsorption process. From the analysis of the data in Table 2, the following conclusions are extracted in terms of the models described in Schemes 2 and 3:

Size/Shape Characteristics. Small dendrimers (G2, average diameter 29 Å) were adsorbed significantly on silicas, alumina, and MCM-41. On the other hand, larger dendrimers (G6, average diameter 67 Å) were adsorbed significantly only on surfaces with large pores, such as silicas S200 (average pore diameter 200 Å) and S500 (average pore diameter 500 Å), and the zeolite MCM-41 (average pore diameter 80 Å). Only very low adsorption of G6 was observed on silicas with small pores (S40, S60, S100, average pore diameter 40, 60, and 100 Å, respectively). The experimental results can be interpreted in terms of size/shape characteristics of the host and guest (Schemes 2 and 4). The small dendrimer G2 possesses a diameter ($\bar{\phi}_{\text{dendrimer}}$) of approximately 29 Å, which is smaller

SCHEME 4



than the average pore sizes ($\bar{\phi}_{\text{pore}}$) of all investigated surfaces (Table 1). Therefore, G2 can be adsorbed on the internal surface of all of the solids investigated. On the other hand, the larger dendrimer G6 possesses a diameter of approximately 67 Å, which is larger than the average pore size of S40 and S60 silicas. Consistent with the simple geometric model, negligible amounts of G6 were adsorbed on silica S40 and S60, and the adsorbed dendrimers were probably adsorbed on the external surface (Scheme 4B). The large-pore size silicas, S200 and S400, and MCM-41 possess pores that are larger than the diameter of G6; therefore, G6 can enter the internal surface of these porous solids (Scheme 4C,D). The silica S100 appears to be an intermediate case. The average pore diameter of S100 and the diameter of G6 are similar. It is known that the high generation dendrimers, such as G6, possess a densely packed relatively inflexible surface.¹¹ Probably because of the low flexibility of the G6 surface, only small amounts of G6 could enter the pores of S100. Possibly only the larger pores in the distribution are filled.

The average pore size (58 Å) of the alumina used in this study is considerably larger than the average diameter (29 Å) of G2. Therefore, one would expect efficient binding of G2 to all aluminas (acidic, neutral, and basic), if size/shape characteristics are the only determining factor for host–guest adsorption. However, efficient adsorption of G2 was observed on basic alumina, whereas negligible dendrimer adsorption was observed on acidic alumina (Table 2b). Therefore, in the case of adsorption of the dendrimers on alumina, other parameters beyond geometric factors, such as the chemical structure of the solid, evidently are important in determining the adsorption characteristics.

Chemical Structure of the Host and the Guest Surfaces. The chemical structure of the alumina surface is different at different protonation levels (Scheme 3d–f). This variation in structure should strongly influence electrostatic binding between the dendrimers and differently charged surfaces. The experimental results show that the fraction of adsorbed dendrimers on alumina increased in the order of acidic < neutral < basic alumina (Table 2b). In case of the acidic alumina, both the alumina surface (Scheme 3e) and the dendrimer surface (Scheme 3g) are positively charged. Therefore, electrostatic repulsion between equally charged systems minimizes the binding interactions

TABLE 2: Amount (%) of Adsorbed Dendrimers at the Silica Gel (a), Alumina (b), and MCM-41 (c) Surfaces

a. Silica Gel			
dendrimer	conc	silica	adsorbed dendr. (%) ^a
G2-T	0.04 M	S40	88
		S100	98
		S200	91
		S500	90
		S400	90
G2-T	0.005 M	S40	92
		S100	99
		S200	99
		S500	99
		S400	99
G6-T	0.04 M	S40	0
		S100	0
		S200	68
		S500	53
		S400	53
G6-T	0.005 M	S40	0
		S100	10
		S200	92
		S500	86
		S400	86
b. Alumina			
dendrimer	conc	alumina	adsorbed dendr. (%) ^a
G2-T	0.04 M	acid	0
		neutral	45
		basic	46
G2-T	0.005 M	acid	0
		neutral	96
		basic	98
G6-T	0.04 M	acid	0
		neutral	5
		basic	19
G6-T	0.005 M	acid	0
		neutral	16
		basic	76
c. MCM-41			
dendrimer	conc		adsorbed dendr. (%) ^a
G2-T	0.04 M		95
G2-T	0.005 M		~ 100
G6-T	0.04 M		96
G6-T	0.005 M		~ 100

^a Determined from analysis of the EPR spectra of the supernatant solutions after the adsorption process, which was compared to the dendrimer solution before the adsorption process; accuracy 5%.

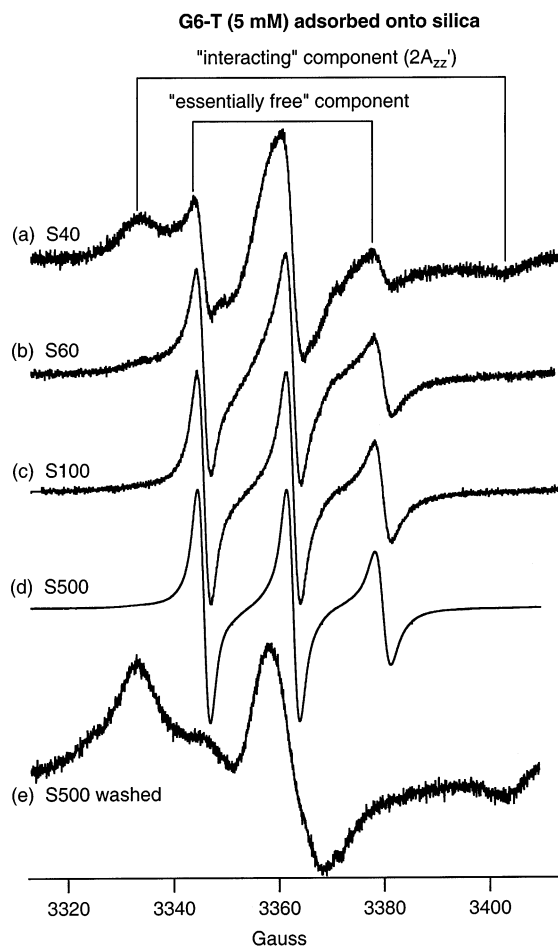


Figure 1. EPR experimental spectra (293 K) of G6-T (5 mM) adsorbed onto homoporous silica at various pore sizes (from 40 to 500 Å); the two main components (free and interacting) are indicated in the top spectrum: the distance between the high field and the low field absorptions for the interacting component corresponds to the $2A_{zz}'$ parameter. Spectrum e: G6-T adsorbed onto S500 (corresponding to spectrum d) was washed with water and then dried again on a filter paper.

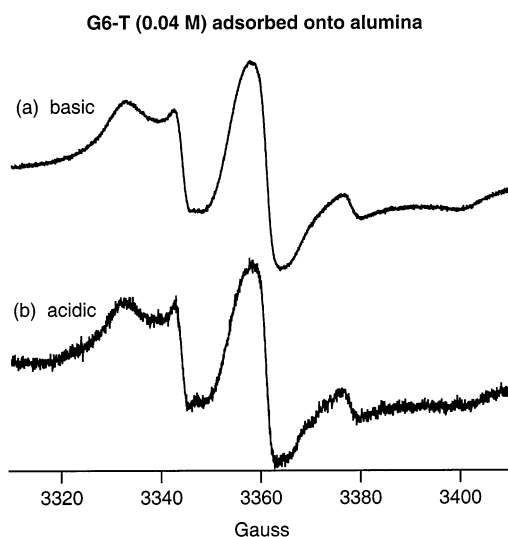


Figure 2. EPR experimental spectra (293 K) of G6-T (40 mM) adsorbed onto acid and basic alumina.

between the dendrimers and the solid surface, and little absorption is observed (negligible fraction of adsorbed dendrimers). On the other hand, in the case of the basic alumina, strong electrostatic binding between the negatively charged

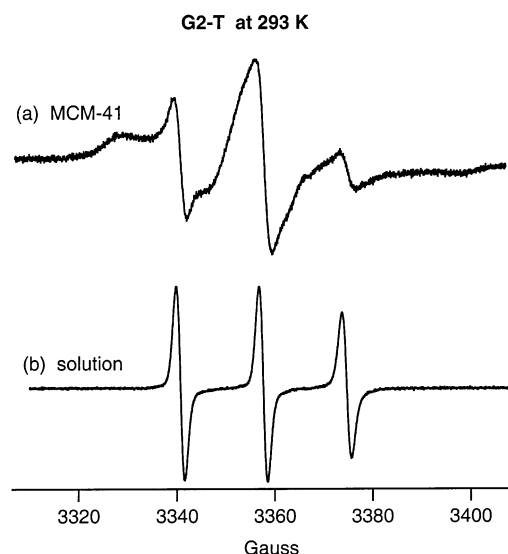


Figure 3. EPR spectra of G2-T (5 mM) adsorbed onto MCM-41 (a) and in aqueous solution (b) at 293 K.

alumina surface (Scheme 3f) and the partly protonated dendrimers (Scheme 3g) causes strong dendrimer adsorption. The interaction of dendrimers with neutral alumina is an intermediate case.

Surface Area. The combined surface area (internal and external surface) based on nitrogen adsorption varies dramatically for the porous surfaces investigated (50 to 1000 $\text{m}^2 \text{g}^{-1}$; Table 1). The available surface area for dendrimer adsorption, which is determined by the size/shape characteristics, limits the amount of dendrimer that can be adsorbed. The decrease in total surface area with the increase in pore size for the silica is probably responsible for the decreased amount of adsorbed dendrimers at S500 compared to S200 (Table 2a), because geometric constraints for adsorption are absent and the chemical nature of the surface is formally identical in both.

Concentration of Dendrimer. The adsorption of dendrimers on the solid surfaces was found to saturate at high dendrimer concentrations. The saturation value depends on the geometric and chemical properties of the solid surface. Table 1a–c shows the adsorbed amounts for two representative concentrations, one below saturation (0.005 M) and the other above saturation (0.04 M). The saturation conditions of the amino-surface dendrimers at alumina and silica surfaces have been already described by Esumi and Goïno.^{6c}

In summary, the amount of dendrimers adsorbed from a dendrimer solution on the investigated porous surfaces (mesoporous silica, alumina, and MCM-41) depends on the combination of size/shape characteristics, surface area, concentration, and chemical structure of the guest and the host.

Interaction of the Dendrimers with the Solid Surfaces. To study the interaction of the dendrimers with the solid surfaces in greater detail, aqueous slurries of the porous materials and dendrimers were filtered to remove the supernatant solution containing nonadsorbed dendrimers (termed “absolutely free” dendrimers). Then the solid particles were further dried on filter paper. This drying procedure does not remove adsorbed water molecules. Therefore, the adsorbed dendrimers can be studied under “hydration” of the porous surfaces.

Figures 1–3 show representative EPR experimental spectra (293 K) of complexes of the nitroxide labeled dendrimers adsorbed on the hydrated porous surfaces: Figure 1a–d: G6-T (5 mM) adsorbed onto silicas of varying pore sizes (from 40 to 500 Å); Figure 2: G6-T (40 mM) adsorbed onto acidic and

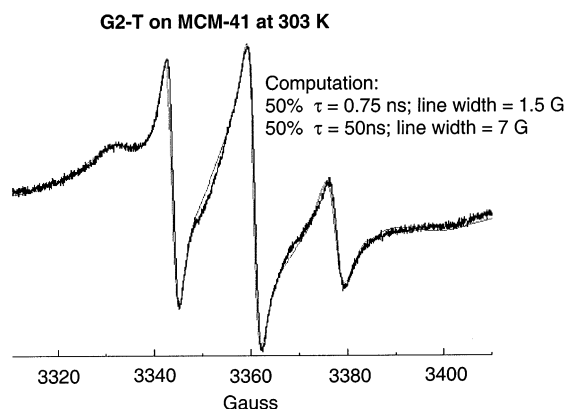


Figure 4. EPR spectrum of G2-T (5mM) at 303 K adsorbed onto MCM-41 (solid line); simulated spectrum (dashed line).

TABLE 3: Correlation Times for Labeled Dendrimers in Water Solution (Low and High Protonation; 0.005 M, 293 K)

dendrimer	$\tau_c (\times 10^{-10} \text{ s})^a$
G2-T	2.5
G6-T	4.0
G2-T ⁺	1.5
G6-T ⁺	3.0

^a Accuracy 5%.

basic alumina; Figure 3a: G2-T (5 mM) adsorbed onto MCM-41. For comparison, the EPR spectrum of free G2-T in water is shown in spectrum 3b.

The EPR spectra were analyzed by computer simulation of the spectral line shape by means of the well-established procedure by Schneider and Freed.¹⁷ The main parameters used for computation were the following: (a) the coupling tensor between the electron spin and the nuclear spin, **A**, mainly the A_{zz} component; (b) the correlation time for the rotational motion of the label. In most cases, the EPR spectra, based on extracted correlation times, consists of two components, one with low mobility and one with high mobility (Figure 1a). The component with low mobility was attributed to dendrimers which are in close contact with the solid surface sites as a consequence of the dendrimer-solid interaction (termed the “interacting” component). The highly mobile component was attributed to weakly interacting dendrimers (termed “essentially free”). The slower mobility (increased correlation time) of these “essentially free” dendrimers compared to nitroxide labeled dendrimers in water solution (“absolutely free” dendrimers, see Table 3 and Figure 3b) indicates an increased viscosity of the labels in the vicinity of the porous surfaces. Analysis of the EPR spectra requires a subtraction-addition procedure of the two components (“interacting” and “essentially free” component), which provides the relative percentages (by double integration of the correspondent signals) of the two components.

Figure 4 gives an example of this analysis for G2-T (5 mM) adsorbed onto MCM-41 at 303 K. We selected this temperature, which is higher than the temperature of the samples for Figure 3a, to show the expected effect of the increase in temperature: increased mobility of both components. The “interacting” component and the “essentially free” component were computed separately. For simplicity, we used the same **g** parameters ($g_{ii} = 2.0090, 2.0060, 2.0025$) and **A** parameters ($A_{ii} = 6.0, 7.0, 38.0 \text{ G}$) for both components. The two components were added to each other to reproduce the experimental line shape. The fitting allows the calculation of (a) the correlation time for motion of the “essentially free” component, τ_c , (b) the line

TABLE 4: a. Main Parameters Extracted from EPR Spectral Analysis of Complexes of Low (a) and High (b) Protonated Dendrimers (0.005 M) and Silica Gels after Filtration and Drying

dendrimer	silica	τ_c^a (s) essentially free labels	% ^a interact. labels	$A_{zz}'^a$ (G) interact. labels
a. Low Protonated Dendrimers				
G2-T	S 40	1.9×10^{-9}	65	33.3
	S 60	1.5×10^{-9}	55	33.3
	S 100	9.6×10^{-10}	40	n.m. ^b
	S 200	1.0×10^{-10}	37	n.m. ^b
	S 500	2.0×10^{-10}	20	n.m. ^b
G6-T	S 40	2.1×10^{-9}	73	34.5 G
	S 60	1.1×10^{-9}	32	n.m. ^b
	S 100	9.0×10^{-10}	0	n.m. ^b
	S 200	6.0×10^{-10}	0	n.m. ^b
	S 500	5.0×10^{-10}	0	n.m. ^b
b. High Protonated Dendrimers				
G2-T ⁺	S 40	1.6×10^{-9}	37	33.7
	S 60	1.3×10^{-9}	35	33.9
	S 100	1.0×10^{-9}	32	33.6
	S 200	5.0×10^{-10}	20	n.m. ^b
	S 500	3.9×10^{-10}	15	n.m. ^b
G6-T ⁺	S 40	1.6×10^{-9}	67	34.9
	S 60	1.1×10^{-9}	38	35.1
	S 100	7.6×10^{-10}	25	n.m. ^b
	S 200	7.1×10^{-10}	30	n.m. ^b
	S 500	5.6×10^{-10}	13	n.m. ^b

^a Accuracy 5%. ^b “Not measurable”, due to low resolution (low intensity) of the spectral components and/or the broadening of the interacting component.

TABLE 5: Main Parameters Extracted from EPR Spectral Analysis of Complexes of Low Protonated Dendrimers (0.005 M) and Aluminas after Filtration and Drying

dendrimer	[G-T]	alumina	$\tau_c (\times 10^{-10} \text{ s})^a$ essentially free labels	% ^a interact. labels	$A_{zz}'^a$ (G) ^a interact. labels
G2-T	0.04 M	acid	8.2	38	29.15 G
	0.04 M	neutral	12.0	60	30.9 G
	0.04 M	basic	12.2	62	30.4 G
G2-T	0.005 M	acid	5.5	n.m. ^b	n.m. ^b
	0.005 M	neutral	8.5	46	n.m.
	0.005 M	basic	8.7	42	n.m.
G6-T	0.04 M	acid	16.4	84	34.75 G
	0.04 M	neutral	17.2	87	33.6 G
	0.04 M	basic	17.2	88	33.65 G
G6-T	0.005 M	acid	10.0	n.m. ^b	n.m. ^b
	0.005 M	neutral	14.3	73	34.0 G
	0.005 M	basic	19.5	85	33.7 G

^a Accuracy 5%. ^b “Not measurable”, due to low resolution (low intensity) of the spectral components and/or the broadening of the interacting component.

widths, and (c) the relative percentages (by integrating the spectra) of the two components. For the “interacting” component, a simple parameter for evaluating the mobility variation in a series of similar samples (the same probe) is the A_{zz}' value, which is related to the z component of the hyperfine coupling described above for computation. The A_{zz}' value was directly obtained from the experimental spectra, as indicated in spectrum a of Figure 1. This parameter increases in a series of spectra from the same dendrimer because of a decreased mobility of the label. However, in several cases, A_{zz}' was not measurable (indicated in Tables 4 and 5 as: n.m.) because of the low relative intensity of the “interacting” component with respect to the “essentially free” component. The results are discussed in terms of the model described in Schemes 2 and 3.

Silica. Size/Shape Characteristics. Figure 1a–d shows the experimental EPR spectra of G6-T on silica. The largest fraction of the “interacting” dendrimers compared to the fraction of

“essentially free” dendrimers was observed for S40 (Table 4a). As discussed above (Table 2a), only small amounts of G6-T are adsorbed on S40 and S60 (probably adsorbed on the external surface), because the dendrimer diameter is larger than the pore diameter of S40 and S60 (Scheme 4B). As the pore diameter of the silica increases, the fraction of the “interacting” component decreases. In addition, the rotational correlation time (τ_c) of the “essentially free” component decreases and approaches the value for the “absolutely free” component. The high mobility of the dendrimers in the large pore size silica can be explained by relatively unrestricted motion in the hydration layers of the silica (Scheme 4D).

Chemical Structure of the Host and the Guest Surfaces. Table 4a shows a high percentage of “interacting” component for small dendrimers (G2-T), whereas for large dendrimers (G6-T), no “interacting” component was observed on silicas S100, S200, and S500, which possess pores larger than the dendrimer size. This suggests a stronger interaction of the small dendrimers with the silica surfaces. It is known that the earlier generation dendrimers possess a more flexible and open structure, whereas the later dendrimers possess a more closed and relatively nonflexible surface structure.¹¹ At the pH of the samples, silica surfaces consist of both polar silanol groups and low polarity Si—O—Si groups (Scheme 3a,b). Therefore, we propose that the dendrimers interact in the following way. The polar and partly protonated external amino groups could bind to the silanol groups (Scheme 3a). In addition, because of the open structure of G2-T the low polar internal dendrimer moiety (if sufficiently flexible and therefore accessible) or unprotonated amines could bind to the Si—O—Si sites (Scheme 3b). In the case of G6-T, the internal low polar moiety is not accessible to the Si—O—Si sites, because of the closed dendrimer structure. Therefore, only the external amino groups could interact with the surface. For S40 and S60, the total amount of adsorbed G6-T (“essentially free” + “interacting” component) is very low (Table 2a), because the pore sizes of S40 and S60 are smaller than the dendrimer size and only the external silica surface is accessible to G6-T. Interestingly, a large fraction of “interacting” component compared to the fraction of “essentially free” dendrimers was observed for S40 and S60 (Table 4a). The nature of this interaction on the external surface is not understood and could be caused by surface defects of the silica or specific interactions of the label with the silica surface.

Protonation of the dendrimer surface changes the dendrimer silica interaction (Table 4b). In the case of G6-T⁺, a significant “interacting” component was observed, indicating a stronger binding after dendrimer protonation. Conversely, the amount of the “interacting” component decreased for protonated G2-T⁺, indicating a decreased binding. Protonation of the dendrimer surface should increase the electrostatic interaction with silanol groups of silica. However, protonation of the dendrimers could also decrease the interaction of the low polar internal moiety with Si—O—Si groups. In the case of G6-T⁺, the increase of the interaction of the protonated amino surface groups of the dendrimers with the silanol groups could cause the stronger binding, whereas for G2-T with the open structure, the interaction decreasing effect of the internal low polar dendrimer groups could dominate.

To test if the dendrimers can be washed off from silica, a sample of G6-T adsorbed onto S500 corresponding to spectrum d of Figure 1 was washed with water and then dried again on a filter paper until the dry initial powder was recovered. The EPR spectrum of this washed sample is shown in Figure 1e. The spectrum represents only the “interacting” component,

TABLE 6: Main Parameters Extracted from EPR Spectral Analysis of Complexes of Low Protonated Dendrimers (0.005 M) and MCM-41 after Filtration and Drying

dendrimer	$\tau (\times 10^{-10} \text{ s})^a$ essentially free labels	% ^a interact. labels	$A_{zz}' (\text{G})^a$ interact. labels
G2-T	11.5	65	35.05
G6-T	8.5	35	34.9

^a accuracy 5%

indicating that the “essentially free” component was completely washed away. However, even several washing and drying procedures did not eliminate the “interacting” component, demonstrating that a fraction of dendrimers strongly binds to the solid surface. The importance of this experiment is also related to exclude the notion that the “essentially free” components correspond to labels on interacting dendrimers (surface bound dendrimers) but with labels positioned on the opposite side of the dendrimer-solid interface. Therefore, the “essentially free” labels belong to noninteracting dendrimers and can be washed out from the silica surface.

Alumina. The dendrimer size of G2-T is smaller than the pore diameter of the alumina, whereas the dendrimer size of G6-T is larger than the pore diameter of the alumina. As reported above, we concluded that G2-T is adsorbed mostly at the internal surface and G6-T is adsorbed on the external surface of the aluminas. Consistently, the total adsorbed amount of dendrimers is smaller for G6-T compared to G2-T (Table 2b), because the accessible surface area for G6-T (external alumina surface) is much smaller than for G2-T (external + internal alumina surface). Interestingly, a higher percentage of “interacting” component for G6-T adsorbed on neutral and basic alumina was observed compared to adsorbed G2-T (Table 5). The efficient binding of G6-T on the external surface of neutral and basic alumina could be caused by a strong electrostatic interaction between the external dendrimer amino groups and the Al—OH and Al—O[−] groups at the alumina surface (Scheme 3). Because G6-T possesses more amino surface groups (256) compared to G2-T (16 surface groups), G6-T can anchor more efficiently on the alumina than does G2-T through a larger number of relatively weak binding interactions. The other adsorbed dendrimer fraction, the “essentially free” component, shows a relatively low mobility on the aluminas (Table 5) compared to the “absolutely free” dendrimers in solution (Table 3). In addition, the mobility of the “essentially free” dendrimers increases with increasing basicity of the alumina (acidic < neutral < basic).

MCM-41. Small (G2-T) and large (G6-T) dendrimers are efficiently adsorbed on MCM-41 presumably on the internal surface (pores) (Table 2c). Interestingly, G2-T interacts stronger with the MCM-41 surface than does G6-T. This was concluded from the higher fraction of the “interacting” component and higher A_{zz}' value of G2-T compared to G6-T (Table 6). Furthermore, the mobility of the “essentially free” component is lower for G2-T than for G6-T, indicating that the smaller dendrimers are influenced more by the zeolite surface than are the bigger dendrimers. Zeolites, such as MCM-41, possess both silicon oxide and aluminum oxide surface groups, which combines the surface properties of silica and alumina. The polar aluminum oxide groups provide efficient binding sites for electrostatic binding with the external amino groups of G2-T and G6-T. The low polar Si—O—Si groups provide binding sites for the internal low polar dendrimer moiety. G2-T possesses an open structure, which makes the internal dendrimer moiety accessible, whereas G6-T possesses a closed structure. This

combination of polar interaction (amino surface groups of dendrimers with aluminum oxide groups of the zeolite) and low polar interaction (internal low polar moiety of dendrimers with Si–O–Si zeolite groups) probably causes the stronger binding of G2-T with MCM-41 compared to G6-T.

Conclusions

Computer-aided analysis of the EPR spectra obtained from nitroxide-labeled dendrimers provided information on the adsorption and interaction of dendrimers with various porous surfaces, such as homoporous silica of different pore sizes, activated alumina (acidic, neutral, and basic), and MCM-41 zeolite (Si/Al = 30). Two generations of poly(amidoamine)-dendrimers were selected, G2-T and G6-T, which are representative to “early” and “late” generations (Scheme 1).

By comparing the results obtained for the different systems, we extract the following conclusions, based on the model described in Schemes 2 and 3. The main parameters regulating the adsorption and interaction of the dendrimers and porous surfaces are (I) the pore size of the host (porous materials) relative to the dendrimer size (guest), (II) the total surface area of the host that is accessible to the dendrimers, and (III) the chemical composition of the host and guest surface.

If the pore size of the host surface is smaller than the dendrimer (guest) diameter, then adsorption of the dendrimer occurred exclusively on the external host surface (Scheme 4A). Therefore, the adsorbed amount of dendrimers is low, because of the low surface area of the external host surface. If the pore size of the host surface is bigger than the dendrimer diameter, then the internal surface area is accessible to the dendrimers and large amounts of dendrimers were adsorbed (Scheme 4C).

The chemical composition of the host surface and guest surface is an important parameter, which determines the strength of the host–guest binding. An increase in alumina content (silica < zeolite MCM-41 < alumina) of the host increased the electrostatic binding of the dendrimer with the host surface. Increased protonation of the external dendrimer amino groups also increased the electrostatic binding. For the large dendrimers (G6), which possess a closed surface structure, electrostatic interactions are the main forces of host–guest binding. Therefore, the strongest interaction of G6 was observed with basic alumina. For small dendrimers (G2), which possess an open structure, hydrophobic interactions between the low polar internal dendrimer moiety and low polar Si–O–Si groups of the host surface are an additional force of host–guest interaction. High protonation of the dendrimer surface and high alumina content of the host surface inhibits this type of interaction. The combination of low polar Si–O–Si groups and highly polar AlO[−] groups in the zeolite MCM-41 is probably causing the strongest binding of G2 on MCM-41 compared to the other investigated host surfaces.

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