Solution Behavior of Cascade Polymers.

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SOLUTION BEHAVIOR OF CASCADE POLYMERS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and Agricultural and
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Doctor of Philosophy

in

The Department of Chemistry

by

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December, 1995
To my Mother, Okjoo Kim Han,

and My Family, Soo-Chang, Jin, and Jeemin Yu
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LIST OF SYMBOLS

a distance between particles
A_i amplitude of species i
A_{\text{max}} maximum amplitude
B baseline of the autocorrelation function
B_r fitted baseline of autocorrelation function
B_t theoretical baseline of autocorrelation function
c concentration of solution
d particle diameter
d_{\text{app}} apparent diameter
D_c diffusion coefficient of complex
D_F diffusion coefficient of free dye
D_m mutual diffusion coefficient
D_s self diffusion coefficient
D_r fractal dimension
E(t) electric field
f_m mutual friction coefficient
f(A) instrument parameter in autocorrelation function
f(A)_{\text{max}} maximum value of f(A)
G^{(2)}(t) intensity autocorrelation function
\( G^{(1)}(t) \) electric field autocorrelation function

\( g^{(1)}(t) \) normalized electric field autocorrelation function

\( I \) intensity of scattered light

\( K \) spatial frequency in fluorescence photobleaching recovery

\( K_{eq} \) equilibrium constant

\( k \) Boltzmann constant

\( L \) striped pattern spacing in fluorescence photobleaching recovery

\( l \) characteristic length

\( M \) molecular weight

\( N_a \) Avogadro's number

\( n \) refractive index

\( q \) scattering angle

\( R_g \) radius of gyration

\( R_c \) cross section radius of gyration

\( T \) temperature

\( T_1 \) transition temperature determined from the intersection between upper baseline and slope

\( T_2 \) transition temperature determined from the middle point between upper and lower baselines
$T_3$ transition temperature determined from the intersection between lower baseline and slope

t characteristic time
t$_b$ bleaching duration
t$_w$ time window
x$_c$ molar fraction of complex
x$_F$ molar fraction of free species (LPAMAM-5)
z number of binding sites
$\Gamma$ decay time
$\Gamma_{avg}$ average decay time
$\Gamma_f$ decay time of fast mode
$\Gamma_s$ decay time of slow mode
$\Delta H$ enthalpy
$\lambda_o$ laser wavelength *in vacuo*
$\sigma_B$ baseline uncertainty
$\eta$ viscosity of solution
$\pi$ osmotic pressure
$\theta$ angle between the incident and scattered light
$\tau$ time delay (lag time) in dynamic light scattering

$(d\pi/dc)_{T,P}$ osmotic compressibility
**LIST OF ABBREVIATIONS**

<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AOM</td>
<td>acoustooptic modulator</td>
</tr>
<tr>
<td>AC(t)</td>
<td>magnitude of ac signal</td>
</tr>
<tr>
<td>CONTIN</td>
<td>Laplace inversion algorithms</td>
</tr>
<tr>
<td>CUMU</td>
<td>cumulant analysis</td>
</tr>
<tr>
<td>DSC</td>
<td>differential scanning calorimeter</td>
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<tr>
<td>DLS</td>
<td>dynamic light scattering</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>EDA</td>
<td>ethylenediamine</td>
</tr>
<tr>
<td>EXSAMP</td>
<td>Laplace inversion algorithms (a smoothed exponential sampling algorithm)</td>
</tr>
<tr>
<td>FFEM</td>
<td>freeze fracture electron microscope</td>
</tr>
<tr>
<td>FITC</td>
<td>fluorescein isothiocyanate</td>
</tr>
<tr>
<td>FPR</td>
<td>fluorescence photobleaching recovery</td>
</tr>
<tr>
<td>LJFM</td>
<td>labeled jeffamine</td>
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<tr>
<td>LNaPSS</td>
<td>sodium poly(styrene sulfonate)</td>
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<tr>
<td>LPAMAM</td>
<td>labeled poly(amidoamine) cascade polymers</td>
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<tr>
<td>LS</td>
<td>light scattering</td>
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<tr>
<td>LSU</td>
<td>Louisiana State University</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>NaPSS</td>
<td>sodium polystyrene sulfonate</td>
</tr>
<tr>
<td>NEXP</td>
<td>multiexponential analysis</td>
</tr>
<tr>
<td>PDMDAAC</td>
<td>poly(dimethyldiammoniumchloride)</td>
</tr>
<tr>
<td>PLPAMAM</td>
<td>purchased labeled poly(amidoamine) cascade polymers</td>
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<tr>
<td>PMT</td>
<td>photomultiplier</td>
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<td>SALS</td>
<td>small angle light scattering</td>
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<td>SAXS</td>
<td>small angle x-ray scattering</td>
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<tr>
<td>SEC</td>
<td>size exclusion chromatography</td>
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<td>THF</td>
<td>tetrahydrofuran</td>
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ABSTRACT

This thesis consists of four chapters. In the first, a general introduction is presented. Chapter 2 deals with the characterization of polyamidoamine (PAMAM) cascade polymers. Diffusion coefficients of four different generations (generation 3, 5, 7, and 9) of PAMAMs were measured in aqueous solutions by dynamic light scattering. Generation 3 and 5 were characterized again, after labeling with fluorescein isothiocyanate, by fluorescence photobleaching recovery. The dynamic light scattering results depended on the concentration of added salt in the low salt limit, while the fluorescence values were almost independent of salt concentration. The polyelectrolyte effects, or solution nonidealities, strongly affected light scattering, and were observed in the lower generations of PAMAMs at zero added salt. Nevertheless, PAMAMs should make suitable markers and diffusion probes unless they are studied at low salt with dynamic light scattering. In Chapter 3, the interaction between positively charged PAMAM and negatively charged Sodium Polystyrene Sulfonate (NaPSS) is considered. The complex formation between oppositely charged polyelectrolytes was confirmed, and its dependence upon added salt and pH values was studied. At low ionic strength, all PAMAM molecules were bound to NaPSS, and only one diffusion mode corresponding to LPAMAM-5/NaPSS complex was observed. However, two diffusion modes, slow and fast,
appeared as the ionic strength was increased. The slow one is interpreted as the diffusion of LPAMAM-5/NaPSS complex while the fast one is not explained yet. At low pH, LPAMAM-5/NaPSS complex made a precipitate, while no complex was formed at high pH. Two diffusion modes were observed at pH 9.

Chapter 4 concerns the gelation of arborols, another family of cascade polymers that makes aggregation in appropriate solvent. Several two directional dumbbell shaped arborols with hydrophilic terminal hydroxy groups and lipophilic hydrocarbon skeleton were investigated. Gelation was first confirmed with tilting test and intensity measurement by light scattering. The transition point from gel to fluid was measured by Small Angle Light Scattering and Differential Scanning Calorimeter. The cross section radius of gyration and average distance between neighboring particles were determined with Small Angle X-ray Scattering. The structure of arborols was pictured by Freeze Fracture Electron Microscope.
CHAPTER 1

INTRODUCTION
1.1 CASCADE POLYMERS

Recently developed new classes of highly branched polymers have important and different properties from linear polymers in that branches can give many terminal functional groups. These terminal functional groups allow the remarkable synthetic control of the size, and may be modified chemically to provide a variety of functionalities in shape, flexibility, surface chemistry, and solubility of polymers. These materials thus mimic the characteristics of supermolecular assemblies (1.1-1.3) and micelles (1.4, 1.5). There are several different families synthesized and named by different ways such as cascade polymers (1.6, 1.7), starburst dendrimers (1.8-1.10), silvanols (1.11), and arborols (1.12, 1.13). Most of their names originated from different languages. For example, dendrimers are coined from Greek for tree and arborols from Latin for tree. However, all of them have the same meaning in that they reflect the tree-like molecular topologies. Here we prefer the name "cascade polymers". Cascade polymers are grown from various small molecules called initiator cores into higher molecular weight polymers through radially branched layers, termed generations, by a reiterative, stepwise synthetic method. Like a tree, a cascade polymer has many spaces between branches which gives rise to lots of applications in areas such as drug delivery (1.14-1.16). The size or terminal functional groups are controlled by the nature of the initiator core and
the branches, and their many applications include photocatalysis and structural materials (1.17-1.19). For their synthesis, both divergent (1.20, 1.21) and convergent (1.22, 1.23) synthetic approaches have been employed. The number of functional groups or molecular weight can be easily calculated based on the initiator multiplicity and the branch juncture multiplicity (1.24).

1.2 PROBE DIFFUSION STUDIES

Probe diffusion in polymer solutions, gels, and other porous media has received considerable experimental and theoretical attention in recent years (1.25-1.27). For example, the diffusion of proteins in DNA solutions plays a large role in biological processes such as gene regulation (1.28). In probe diffusion studies (1.29), a gel or other complex polymer solution is characterized by monitoring the motion of well defined probe particles.

There are several reasonable choices for big probes. Especially popular are latex particles that are easily measured by Dynamic Light Scattering (DLS) because the scattering of the complex solution is usually small compared to that of a large latex. In principle, smaller probe particles are more useful for characterizing materials having narrow pores, such as aerogel precursors and other concentrated polymer solutions. Two problems are encountered in using small probes. First, they tend to be polydisperse. Second, dynamic light scattering becomes ineffective except in rare situations where the solution under
study is weakly scattering. Therefore, the first challenge in probe diffusion is to find an experimentally viable system that can provide general physical insight without complications due to aggregation, polyelectrolyte effects, or specific interactions between the polymer matrix, the probe and/or solvent. In contrast, the interaction between probe particles and matrix polymer solutions also can give important information about the properties of matrix. In particular, charged particles have various applications in probe diffusion studies as long as the chemistry of the particles is not changed by the interaction with oppositely charged matrix. Therefore, it is clear that once charged probe particles are confirmed as good probes, their practical applications in biological systems would be many.

1.3 POLYELECTROLYTE EFFECT

Polyelectrolytes are defined as macromolecules possessing a large number of charged or chargeable groups when dissolved in a suitable polar solvent, generally water (1.30). Several terms are used to denote different ions in polyelectrolytes such as polyions (polymers with charge), counter ions (small, oppositely charged ions hanging around the polyions), and coions (added salt). Most biological macromolecules, such as proteins, nucleic acids, the hydrophobic lipid components of membranes, and polysaccharides, are polyelectrolytes. Solutions of polyelectrolytes exhibit a considerably different
behavior from those of uncharged macromolecules due to the existence of charges. This can lead to intra- and inter- macromolecular interactions that influence the characteristic properties of polyelectrolytes, such as conformation, intrinsic viscosity, and electrical properties.

Several theoretical approaches, such as scaling (1.31, 1.32) and counterion condensation (1.33), have been proposed to understand the behavior of polyelectrolytes.

In dynamic light scattering experiments, the unique behavior of polyelectrolytes, especially at low salt or without added salt, has been observed first by Lee and Schurr (1.34). These authors reported an ordinary to extraordinary transition "(O-E transition)" which takes the form of a very sudden decrease in the apparent diffusion coefficients over a small range of salt concentration. Similar transitions also have been observed in other systems (1.35-1.41). Sometimes, one diffusion mode at high salt content is split into two diffusion modes, one very fast and one several decades slower, at low salt. These and other experimental observations are not clearly understood yet. There still remain many complicated phenomena unsolved, and there is need for more theoretical development, even though lots of efforts have been devoted to polyelectrolytes already.
1.4 RELATION OF CASCADE POLYMERS TO GELATION

Gelation has been studied for many years because gels exhibit distinct properties from other forms of matter. Gels were defined as the thermodynamically stable amorphous solids or a matter with finite shear modulus or rigidity (1.42). Hecht et al. additionally thought of gels as being equivalent to polymer solutions of infinite molecular weight (1.43). Most gels are made by physical (such as heating and cooling) or chemical reactions, and there are two different gel types, reversible and irreversible gels. All these terms are fully described in ref. 1.44. Several theoretical investigations were reported (1.45-1.48), and Stockmayer suggested that chains with high degree of branching could make more stable gels than unbranched chains.

Highly branched molecules, such as cascade polymers, become important not only in probe diffusion studies but also in gelation processes. Cascade polymers with small size and non-aggregating properties appear to be good probe particles to reveal the structures of complex solutions and gels. In contrast, cascade polymers that have aggregation can serve good examples for gelation.

One of cascade families, the arborols, has hydrophilic terminal hydroxyl groups and hydrophobic carbon skeletons which cause aggregation leading to a
gel. The self assembly of these small molecules into a macroscopic network is another facet of cascade polymers and one that has scarcely been explored.

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CHAPTER 2

CHARACTERIZATION OF POLYAMIDOAMINE CASCADE POLYMERS
2.1 INTRODUCTION

Cascade polymers (2.1-2.3) continue to receive a great deal of attention. Starting from a multifunctional initiator, the layer-by-layer growth of such "precision macromolecules" is carried out in a recursive, stepwise fashion. A given preparation may be referred to by the number of "generations" synthesized. There are several families of cascade polymers (2.4-2.13). One of the more widely studied types is the polyamidoamine (PAMAM) polymers developed by Tomalia and coworkers (2.7). PAMAM's have been characterized by size exclusion chromatography (SEC). Hydrodynamic diameters were obtained using the relationship of Hester and Mitchell (2.14) and compared to intrinsic viscosity data and geometric dimensions from molecular models (2.2, 2.7, 2.8). In principle, PAMAM's are well suited for calibration standards, filter challenges, membrane integrity tests and other analytical uses (2.15-2.17). With the attachment of a fluorescent dye, they also hold promise as probes and markers in complex solutions and living cells, and in a variety of other physical measurements. Their combination of relatively small and yet uniform size is quite special and appealing. But compared to other particles that might reasonably be used (dextrans, latex particles, silica spheres and proteins) cascade polymers have virtually no demonstrated track record. Given the complicated synthesis and chemically reactive nature of the
PAMAM's, one may legitimately question whether they may be used confidently over a wide range of pH and salt, and what the effects of dye attachment might be. In this chapter, several different generations of PAMAMs, 3, 5, 7, and 9, are characterized in detail. Small generations of PAMAMs, generation 3 and 5, have been labeled with fluorescein isothiocyanate and measured again. The hydrodynamic diameters of PAMAMs have been determined in three different conditions: in pure water, salt, and at different pH values. The possible polyelectrolyte effects which exist in the charged polymers in low or zero added salt are discussed. The employed techniques are dynamic light scattering, DLS, and fluorescence photobleaching recovery, FPR.

2.2 BACKGROUND

2.2.1 DYNAMIC LIGHT SCATTERING

2.2.1.1 PRINCIPLES

Dynamic Light Scattering (DLS) has been used for a long time to obtain information about the molecular dynamics by monitoring the time dependence of the intensity of light scattered by molecules. DLS has many advantages in that it can applied to particles with a broad range of hydrodynamic diameters (0.001 < d < 2 µm) and polymers in difficult or corrosive solvents (2.18) without seriously perturbing the sample, and without the need for calibration. From DLS, the mutual diffusion coefficient based on relaxation of a concentration
gradient is determined. The theoretical principles of DLS are introduced elsewhere (2.19-2.21). In brief, when light hits a solution it scatters, and the intensity of scattered light fluctuates about its average value at a rate that depends on the speed with which the scatterers move in solution. In other words, the intensity fluctuations reflect alternating constructive and destructive interferences as the molecules in the detected volume undergo diffusive motion. On a very long time scale, these fluctuations appear random. However, they are not totally random; if two intensities separated by a very short time interval are compared, they are found to be similar, i.e., correlated. Thus, the intensity fluctuations have a finite lifetime that is inversely related to the polymer diffusion coefficient. The intensity of scattered light mirrors the instantaneous random diffraction from the random motion of scatters or the random arrangement of scatterers. This randomly fluctuating quantity can be characterized by its autocorrelation function, and the time autocorrelation function of electric field of the scattered light, \( G^{(1)}(\tau) \), is introduced.

\[
G^{(1)}(\tau) = \langle E(0)E(t) \rangle = \lim_{T \to \infty} \frac{1}{2T} \int_{-T}^{T} E(t) E(t+\tau) \, dt \tag{2.1}
\]

The dynamic light scattering experiment does not measure the autocorrelation function of the electric field directly. It measures the autocorrelation function of the intensity which is defined as:
\[ G^{(2)}(\tau) = \langle I(0)I(t) \rangle = \langle |E(0)|^2 |E(t)|^2 \rangle = \lim_{T \to \infty} \int_T^T I(t) I(t+\tau) \, dt \quad (2.2) \]

The parenthesized superscript 2 indicates a second order correlation function involving intensities. Superscript 1 indicates a correlation function of the electric field. When \( \tau \) approaches zero, \( I(t) = I(t+\tau) \), and the above integral clearly represents the average of the squared intensity, \( \langle I^2 \rangle \). As \( \tau \) becomes very large, \( I(t) \) no longer has any relation to \( I(t+\tau) \), so the above integral yields \( \langle I \rangle^2 \), which must be less than \( \langle I^2 \rangle \). Thus the correlation decays, usually exponentially. The field correlation function can be obtained from the relationship between field and intensity. If the scattered field is a random Gaussian process (2.19, 2.20), one measures the second order (intensity) autocorrelation function:

\[ G^{(2)}(x) = B (1+ f \| \langle g^{(1)}(\tau) \|^2 ) \quad (2.3) \]

Here, \( \tau \) is the lag time, which in a linear correlator is a multiple of the single channel time, \( B \) is the baseline and \( f \) is an instrumental parameter (0 < \( f < 1 \)) depending mostly on the number of coherence areas detected (2.19, 2.20), but also on the scattering power of solvent relative to the solution. The normalized first order autocorrelation function \( g^{(1)}(\tau) \) contains the useful information. For monodisperse sample, \( g^{(1)}(\tau) = \exp(-q^2 D \tau) \) where \( D \) is the mutual diffusion coefficient and \( q \) is the magnitude of the scattering vector (\( q=4\pi n \sin(\theta/2)/\lambda_0 \)).
where $n$ is the solution refractive index, $\theta$ is the scattering angle, and $\lambda_0$ is the laser wavelength in vacuo. From the mutual diffusion coefficient the particle size (hydrodynamic diameter, $d$, ) is determined by the Stokes-Einstein relationship,

$$d = \frac{kT}{3\pi\eta D} \quad (2.4)$$

The numerator, $kT$, is the thermal energy per molecule and $\eta$ is the solvent viscosity. In a polydisperse system, $g^{(1)}(\tau)$ is a weighted sum of discrete exponential that can be closely approximated by a continuous distribution:

$$g^{(1)}(\tau) = \sum_{j} A_j \exp\left(-\Gamma_j \tau\right) = \int_{0}^{\infty} A(\Gamma) \exp\left(-\Gamma \tau\right) d\Gamma \quad (2.5)$$

The subscript $i$ will be used to denote an individual scatterer. Modern algorithms for Laplace inversion of equation (2.5) yield a set $\{A\}$ of scattering amplitudes for a discrete set $\{\Gamma\}$ of decay rates. In typical sizing applications, an amplitude $A_i$ is proportional to the concentration, $c_i$, and molecular weight, $M_i$, of a scattering component, modified by the particle scattering form factor, $P(qR_g)$, which is a function of radius of gyration, $R_g$: $A_i = c_iM_iP(qR_g)$. The constant of proportionality is not important. A decay rate $\Gamma_i$ is inversely proportional to $d_i$. If it is known how $d$ scales with $M$, it is also possible to convert to molecular weight.
In the case of polyelectrolytes, such as the PAMAM's, the decay spectrum $A(\Gamma)$ is the subject of much debate. In particular, slow decay modes possibly signal the existence of "domains", "temporal aggregates", or "clusters" of macroions at low ionic strength (2.22-2.25). Light scattering is exquisitely sensitive to such large structures but, unfortunately, unable to distinguish them from less interesting particles, such as dust or aggregates. Scattering from solutions is also fundamentally a thermodynamic process, strongly affected by solution nonidealities (2.26-2.27). As a result, the intensity of scattered light usually decreases precisely under those conditions which favor the strong intermolecular interactions. These complexities are discussed further below.

2.2.1.2 EXPERIMENTAL METHOD

**Instrument**

A dynamic light scattering instrument consists of several components: the light source (laser), an optical system, detector, signal analyzer, sample holder, and temperature controller. Our DLS apparatus is shown in Figure 2.1. The instrument used a Lexel Model 95-2 Argon ion laser operated in constant current mode. Up to 1.4 watts were available at 5145Å. Electronics consisted of a Pacific Precision Model 126 photon counting system, Hamamatsu R928P photomultiplier, 272 channel Langley Ford Model 1096 digital autocorrelator operated in the linear mode, and IBM compatible computer.
Figure 2.1 Dynamic light scattering apparatus.
As a sample holder, a specially designed toluene bath whose refractive index is closely matched with that of glass test tube used for DLS cell is used. The toluene is cleaned automatically by circulation with a pump through an attached 0.2 μm Gelman filter. A calibrated water bath, Model Neslab RTE-110, is used to control the temperature.

**Alignment**

Instrument alignment is usually carried out with strong scatterers, polystyrene latex particles, with a known diameter. First, the light beams through the sample holder with subsequent collision on the wall with the target. Second, the beam splitter is inserted into sample holder to divide the beam in half. Third, the detector is adjusted at zero angle and the beam is focused by moving the lens and microscope adjustment. Finally, the multiangle measurements of the standard polystyrene latex particles at scattering angles, 30, 45, 60, 90, and 120 degrees, are performed, and the highest and lowest \( \Gamma \) values are obtained from third-cumulants (3CUMU) and exponential analyses (1EXP G2), respectively. By plotting \( \Gamma \) vs \( q^2 \), the diffusion coefficient with error estimates providing the information about diameter by Equation 2.4 is extracted. The determined diameter is then compared to that of the advertised one. If their values are close to each other with the zero intercept within error and correlation coefficient close to 1 in the plot of \( \Gamma \) vs \( q^2 \), the instrument is
ready to use for a real measurement. Once it is aligned, it stays stable and no effort, such as calibration, for every single measurement is required unless a part of the instrument is moved.

**Light Scattering acquisition procedure and data analysis**

Data were acquired as ≈10 short runs, which were ranked by intensity. Short runs without anomalously high intensities (and, therefore, no obvious dust contamination) were individually analyzed by a second-order cumulants (2.28) fit. Quality parameters included decay rate, weighted residuals, channel-to-channel correlation of residuals, and "polydispersity parameter" (ratio of the second cumulant to the square of the first). Most short runs were summed together and reanalyzed by first- to third- order cumulants (1CUMU - 3CUMU). Screen and hardcopy graphics programs were used to decide which correlation functions were candidates for nonlinear least squares, multiexponential analysis (NEXP where N is a small integer). Laplace inversions were performed using Provencher's CONTIN (2.29, 2.30) program, and also by a smoothed exponential sampling algorithm, EXSAMP (2.31, 2.32). First, the NEXP fits are performed to identify the approximate range in decay rate space and the number of exponentials required for a good fit. Then, programs EXSAMP and CONTIN are used to approximate the distributions. Both programs fit a sum of exponentially decaying functions to the data while constrained to positive
amplitudes and "regularized" (2.29, 2.30, 2.33) to prevent unrealistically sharp peaks in the distribution caused by noise in the correlation function. Both programs place the exponentially decaying functions uniformly in a logarithmic decay rate space. Program EXSAMP is generally used prior to CONTIN in order to interactively establish the range of decay rate space and number of "grid points" (i.e., the number of decay functions) used to approximate the distribution. CONTIN produces several fits with different degrees of regularization; all these fits are inspected routinely, but the results here are for the particular fit chosen automatically by CONTIN. An interactive program can convert the A\{Γ\} distribution to more conventional representations, such as relative concentration vs. diameter, using the relationship \(A_i \propto c_i M_i P(qR_g)\), but corrections are virtually negligible for the PAMAM polymers.

The smaller PAMAM's are not strong scatterers. Solvent scattering was significant enough that low values of \(f\) (e.g., \(f(A) = 0.004\) and \(f(A)_{\text{max}} = 0.0268\)) were sometimes observed even for detector settings that produce high \(f\) values for strong scatterers. As a result of the diminished optical coherence, measurements were considerably more dependent on baseline uncertainty than usual. The theoretical baseline is \(B_i = P(P-O)/N\) where: \(P\) is the total number of photopulses detected (typically \(5\times10^7 - 1\times10^9\)); \(O\) is the number of correlator shift register overflows (typically \(0\)); and, \(N\) is the total acquisition time divided
by the channel time (the ideal of $N = P$ was difficult to achieve for the smaller, fast-diffusing samples; total acquisition times were 1200 - 2800 s). All cumulant fits use $B_t$; the user is warned if this baseline is significantly different from the correlator's delayed channels. The baselines for 2EXP, EXSAMP, and CONTIN fits are all specified manually, guided by baselines fitted to $G^{(2)}(\tau)$. Typically, baselines $B_{t,1EXP}$ and $B_{t,3EXP}$ were obtained from 1- and 3-exponential fits to $G^{(2)}(\tau)$, and compared to $B_t$. The difference, $B_t - B_{t,NEXP}$, normalized by the baseline uncertainty $\sigma_B = B_t^{1/2}$, is called the "liftoff" because a fit with floating baseline attempts to mask dust or other slow variations in signal as increased baseline. Typical liftoff for 1EXP fits were 3.1 (only 0.045 % of baseline but fully 11 % of coherent signal) for generation 3, decreasing to 1.3 (0.009 % of baseline, 0.03 % of coherent signal) for generation 9 where a very high precision correlation function was obtained. For the larger PAMAM's, the effects of baseline uncertainty were not big enough to alter the conclusion in any significant way.

2.2.2 FLUORESCENCE PHOTobleaching Recovery

2.2.2.1 PRINCIPLES

Fluorescence Photobleaching Recovery (FPR) technique has been developed (2.34), and is already known as a tool to measure the mobility of
specific components in complex biological systems and gels. The tracer (or self) diffusion coefficients defining the diffusion of a particle without considering concentration gradient, can be determined from FPR, while the mutual diffusion coefficients are usually obtained by DLS. The principle of FPR measurement is introduced elsewhere (2.35) and it is simple. The first requirement of a FPR experiment is that sample should be labeled by fluorescent dye (fluorophore) through a covalent attachment. The specific pattern (or spot) on the sample is then bleached by a brief pulse of high intensity light (called photobleaching), and an inequivalence, bleached and unbleached region, is made for the labeled molecules. The bleached region will eventually disappear and an equivalence will be recovered if the particles move freely. The disappearance rate, $\Gamma$, (or decay rate) actually depends on the size of the bleached area and the rate of diffusion of the labeled molecules:

$$\Gamma = D_s K^2$$

where $D_s$ is the self diffusion coefficient of the labeled species and $K$ is the circular spatial frequency of the grating ($K = 2\pi / L$ where $L$ is the distance between stripes in the sample). The modulation ac envelope decays exponentially with decay rate $\Gamma$ by the equation: $ac(t) = ac(0) \exp (-\Gamma t)$. The $\Gamma$ is usually extracted by nonlinear least squares algorithm to one or more exponentials. The diffusion coefficient and associated error are derived from the slope of a linear least squares fit of $\Gamma$ vs $K^2$ plot.
2.2.2.2 EXPERIMENTAL METHOD

Instrument

The major component of an FPR is a microscope, intense light source (laser), and the means of quantifying the extent of the photobleaching and the time scale of recovery. The typical apparatus used in our lab is shown in Figure 2.2. The instrument consists of an Olympus BH2 epifluorescence microscope and Excel Model 3000 Argon ion laser with a power output of up to 2 watt at 4880Å (blue) or 5145Å (green). Intensity shifting is accomplished with a Newport Research 35085 acoustooptic modulator (AOM). The AOM is set to produce the strongest intensity at the first order of the diffracted beam and is used for photobleaching. The contrast of ratio of bleaching (when AOM is turned on) to reading (when AOM is turned off) is between 1000 and 2000. The efficiency of the diffraction in laser output is about 85%. A coarse diffraction grating, or commercial Ronchi Ruling (RR) with a striped pattern of period L, is held in the rear focal plane of a microscope objective lens. Twenty different grating constants, $K=2\pi/L$, are available by combination of four different objectives (4 x, 7 x, 10 x, and 18 x) and five different gratings (50, 100, 150, 200, and 300 lines per inch). The fluorescent signal is detected by an RCA 7265 Photomultiplier (PMT) which is protected by an electronic shutter.
Figure 2.2 Fluorescence photobleaching recovery apparatus.
(Newport 846HP) during the photobleaching pulse. The detected signal is then sent to amplifier via low-noise preamplifier (Stanford SR 560) that is connected to oscilloscope. The amplified ac signal and dc are transformed into digital data with IBM data acquisition board attached to computer.

Like the usual instrument with laser, FPR instrument should be aligned and tuned before performing experiment. The procedure of alignment is fully described elsewhere (2.36). Tuning is accomplished by adjusting motor speed to maximize the output of the tuned amplifier circuit after a fluorescently tagged gelatin has been bleached. Once the FPR instrument is stable after aligning and tuning, measurement can be performed by keeping two conditions in mind:

1. $t_b < t / 10$, where $t_b$ is the bleach duration and $t$ is the characteristic fluorescence recovery time which is equal to inverse decay rate ($1/\Gamma$);

2. $t_w = 10 \ t$ where $t_w$ is time window. A typical $t_b$ is 0.01s for a standard sodium fluorescein (NaF) at objective 10 x. This value is increased when a small objective (objective 4 x or 7 x) is used.

All data are saved on floppy disk and backed up when analysis is needed. Data analysis is based on the Marquardt nonlinear least squares algorithm. The reproducibility of ac signal and stability of the instrument are described in Appendix.
2.3 PREPARATION OF SAMPLES

2.3.1 SAMPLES FOR DYNAMIC LIGHT SCATTERING

Water system

Water for rinsing and dilution was supplied by a Barnstead Nanopure 4-stage purifier, equipped with spiral-wound ultrafilter (ca. 0.005 μm) and final 0.2 μm filter to catch any particles shed by the ultrafilter. The water source for this device is a Millipore R/Q purifier (reverse osmosis/deionization/0.45 μm filter). In tandem, these devices routinely supply many gallons per day of absolutely dust-free, deionized water.

Cells

Cells are 13 mm O.D. Pyrex test tubes, either rinsed with clean water only or silanated with chlorotrimethylsilane to deactivate the surface and to simplify rinsing. There are always concerns about "dust" contamination in DLS. Thus, not only samples but also cells must be clean, and all this cleaning of cells needs lots of labor and time. During cleaning, the disposable vinyl gloves with no powder should be worn to prevent any possibility of getting dust or dirty residues from hand into cell. For making silanated cells, 13 mm O.D. Pyrex test tubes are soaked in concentrated HNO₃ for 5 minutes. After rinsing the soaked cells with water, cells are soaked in concentrated HCl for another 5 minutes. The soaked cells are rinsed with water first and CH₃OH later. The
rinsed cells are wrapped with aluminum foil and dried in an oven. The dried cells are soaked in silane solution made by mixing 200 ml of toluene and 5 to 10 ml of chlorotrimethyl silane, SiCl(CH₃)₃, for 1 hour while the cells are hot. As a posttreatment, cells taken from silane solution are rinsed with anhydrous CH₃OH first to react excess silane followed by rinsing with super water or filtered ultimate solvent, and dried in the oven. The silanated cells are distinguished from unsilanated regular ones by labeling "s" on the upper part of the cell with a diamond pencil. All cleaned cells are sealed with polytetrafluoroethylene lined screwcap lids. Each cell is tested for the absence of dust by filling with water and inserting into a custom light scattering apparatus (Figure 2.1) which features the ability to see the focused laser beam traversing the sample cell at about 100 x magnification. The precise volume to be detected can be observed, but for cleanliness testing, a much larger volume is viewed. The scattering of light by pure water is easily seen, and "dust" down to sizes < 0.2 μm is readily evident when present.

**Samples in water**

The synthetic procedures of PAMAMs are introduced in ref. 2.8. In brief, the PAMAMs begin with ammonia initiator core which has three reacting sites. Methyl acrylate is then added and triester is made by Michael addition (step A in Figure 2.3). The subsequent triester intermediate reacts with
large excess of ethylenediamine (EDA) at room temperature (step B in Figure 2.3) to produce terminal triamine core cell which is called generation 0. Repeating the sequence of step A and Step B leads, via a hexaester (generation = 0.5), to a hexaamine (generation = 1.0). Continuing the sequence of step A and step B produces denser higher generations with either a carboxylated (half generation) or amine (full generation) terminated groups. During the synthesis, isolating intermediate and testing each step are required to ensure that unwanted reactions do not render a given generation unreactive. The full generation is easily distinguished from half generation by reaction with CuSO₄; full generation makes deep purple colored solution while half generation has deep blue colored solution (2.7).

Four different PAMAM's were obtained from Polysciences. They are distinguished by their generation number—e.g., PAMAM-9 for generation 9. (PAMAM-3: Cat. #. 21099; Lot #. 96555; PAMAM-5: Cat. #. 21152; Lot #. 403611 and #. 425292, for the first and second arrival, respectively; PAMAM-7: Cat. #. 21154; Lot #. 403110; PAMAM-9: Cat. #. 21156; Lot #. 403572). The diameter for PAMAM-3 was not supplied. A value d = 31 Å has been reported (2.2, 2.8). The advertised diameters for generations 5, 7 and 9 were 54 Å, 84 Å, and 107 Å, respectively. These diameters are not in complete agreement with those reported in ref. 2.2 and 2.8.
The theoretical molecular weights for generations 3, 5, 7 and 9 are, respectively: 5147, 21563, 87222, and 349883. According to Polysciences, all PAMAMs arrived dissolved in pure, deionized water. Conductivities measured on PAMAM-3 and PAMAM-5 before and after dialysis confirm this claim. The reported concentrations were 0.7 %, 0.4 %, and 0.2 % for the 3rd, 5th, and 9th generation PAMAM's respectively. The concentration for PAMAM-7, 0.3 %, was determined gravimetrically in our laboratory. PAMAM's were filtered through 0.02 μm Anotop inorganic filters. These filters exhibit some variability in effectiveness, not only in water but also in other solvents, so careful preselection is necessary. Solutions exhibiting dust were centrifuged directly in the measuring cells at 1800g for three hours by Sorval superspeed RC 2-B automatic refrigerated centrifuge.

Samples in salt

PAMAM-3, 5 (Cat. #. 21152; Lot #. 425292), and 7 were made in different salt concentrations. Stock salt solution at 1M was prepared with GR grade NaCl, filtered through a 0.02 μm Anotop inorganic filter, and centrifuged over night. For PAMAM-3, six different salt conditions were prepared by keep adding clean 1M NaCl: \(2 \times 10^2\), \(2 \times 10^{-1}\), 10, 30, 80, and 150 mM solutions. For PAMAM-5, eight different salt conditions were made by adding stock 1M NaCl solution: 0.1, 0.4, 16, 66, 150, 310, 760, and 1500 mM. For PAMAM-7, only
Figure 2.3 The synthetic method of polyamidoamine cascade polymers (ref. 2.8).
one salt condition, 1000 mM NaCl, was considered.

**Samples at different pH values**

PAMAM-5 was considered at several different pH values. Stock solvents of 1M NaCl, 0.1M NaOH, and 0.1M HCl were prepared by using clean dust free water. Both 1M NaCl and 0.1M HCl solvents were filtered through 0.02 μm Anotop filters, and 0.1M NaOH was filtered through a 0.2 μm Gelman filter because of the variability in effectiveness of Anotop filter for different solvents. PAMAM-5 (Cat. #. 21152; Lot #. 425292) at pH 9 was made first by adding 6 drops of clean 1M NaCl to filtered 1.5 ml of PAMAM-5. A sample at pH 7 was made from PAMAM-5 at pH 9 by adding 4 drops of clean 0.1M HCl. PAMAM-5 at pH 3 was made by further addition of couple of drops of 0.1M HCl to PAMAM-5 at pH 7. For a sample at pH 11, 2-3 drops of clean 0.1M NaOH were added to the PAMAM-5 at pH 9. A calibrated Orion SA 520 pH meter with an electrode was used for the pH measurement of PAMAM-5 at pH 9. The pH meter needs at least 3ml of sample to conduct the measurement. However, the small amount of PAMAM samples did not permit the use of the pH meter. In addition, the concern of dust contamination in DLS measurement restricted the use of pH meter. Therefore pH indicator (or pH paper), yellow strip, was used after confirmation of accuracy. The pH values of most samples
were determined by pH papers. All measurements in DLS were performed at 25 ± 0.1 °C.

2.3.2 SAMPLES FOR FLUORESCENCE PHOTobleaching RECOVERY

Labeling

Labeling the sample is the first step in FPR measurement. PAMAM-5 (Cat. #. 21152; Lot #. 425292) and PAMAM-3 (Cat. #. 21099; Lot #. 96555) were labeled with fluorescein isothiocyanate (FITC) to produce LPAMAM-5 and LPAMAM-3, respectively. FITC (0.02425 g) was predissolved in 5 ml of acetone and subsequently added to regular unlabeled PAMAM solutions, which were then allowed to stand overnight at room temperature with occasional stirring. After removing the acetone carrier solvent by sparging with air, the labeled PAMAM solutions were extensively dialyzed against pure water through sterilized and rinsed membrane tubings (Spectrapore type CE, MWCO 5,000 and 3,500 for PAMAM-5 and PAMAM-3, respectively). Carbon dioxide was not excluded during dialysis. After dialysis, both of the LPAMAM-5 and LPAMAM-3 solutions were filtered through 0.2 μm Gelman polytetrafluoroethylene filters (pre-wetted with alcohol, then rinsed with water). The total amount of dye added was adjusted to label just one amino group per PAMAM molecule, assuming perfect reaction efficiency. Additionally, an
FITC-labeled fifth generation polymer, to be referred to as PLPAMAM-5, was purchased from Polysciences (Cat. #. 21752; Lot #. 420559). Based on its absorption spectrum, we believe this material to be very heavily labeled. However, according to the vendor, only about twice as much FITC was added as in our own labeling scheme (PLPAMAM-5 was labeled by adding 40 mg of FITC to per gram of PAMAM-5 while 17.72 mg of FITC was added to per gram of PAMAM-5 in our lab). It may be that our labeling scheme resulted in the attachment of substantially less than one dye moiety per PAMAM molecule. For example, pH determines the nucleophilicity of the PAMAM amino groups towards the isothiocyanate dye, and it was not controlled during labeling.

**Samples at different salt concentrations**

Samples with different salt concentrations were prepared by adding different concentrations of salt solutions to LPAMAM-3, LPAMAM-5, and PLPAMAM-5. A stock salt solution at 1M NaCl was prepared with GR grade NaCl, and filtered through a 0.02 µm Anotop filter. Several different concentrations of salt solutions were made by diluting the stock solution. For LPAMAM-3, 4.5x10^{-1}, 5, 17, 60, 100, and 150 mM salt concentrations were made. For LPAMAM-5, 5x10^{-1}, 1, 10, 40, 80, 100, and 150 mM salt conditions were considered. Eight different salt concentrations were made for PLPAMAM-5: 5x10^{-1}, 1, 50, 100, 200, 400, 500, and 1000 mM NaCl. The
polymer concentrations in different salt solutions at each labeled PAMAMs were kept almost constant at the values of 0.4, 0.2, and 0.4 % for the LPAMAM-3, LPAMAM-5, and PLPAMAM-5, respectively by adding the same amount of different salt solutions to the same amount of each labeled PAMAMs.

**Samples with different pH values**

Labeled samples in different pH conditions were prepared by adding 0.1M NaOH or 0.1M HCl to LPAMAM-5 in 0.1M NaCl solution. Four different pH conditions (pH3, pH7, pH9, and pH11) were considered. All pH values were determined by pH papers. The reason for the use of pH papers instead of pH meter is explained in sampling procedure for DLS measurement.

**Cells**

Cells for FPR measurement were rectangular capillary tubes (Vitrodynamics) of path length 0.2 mm and width 4 mm. All cells were flame sealed and held at 25 ± 0.1 °C during measurement.

**2.4 RESULTS AND DISCUSSION**

**2.4.1 PAMAMs IN WATER**

This section concerns the measurements of the as-received materials. The salt and pH conditions are not controlled. Conductometric measurements place the salt concentration (as NaCl) at < 20 mM--and some of this
conductivity arises from the PAMAM's themselves. After establishing the absence of anomalous angle-dependent phenomena, dynamic light scattering measurements were performed at $\theta = 45^\circ$. Most samples were sufficiently clean for automatic measurement data collection. However, the correlator was operated manually while watching a ratemeter, discarding data when deflection due to the very occasional dust particle appeared. A typical correlation function for PAMAM-3 under these conditions appears in Figure 2.4. There was very little coherent scattering during this measurement, but the acquisition time (2800s) was sufficient to produce usable data. The straight semilogarithmic plot, computed with the baseline $B_{\text{L,EXP}}$, indicates a relatively monodisperse polymer. When baseline $B_i$ was used instead, the semilog plot was similarly linear in the initial part of the decay, but suddenly leveled out at lag time $\tau \approx 0.1$ ms due to baseline error. Such leveling is easily distinguished from polydispersity, which results in a gradual change of slope, not sudden leveling. Figure 2.5 is analogous to Figure 2.4 but for the largest PAMAM, PAMAM-9. As this molecule is a fairly strong scatterer, the coherence parameter, $f$, is much larger ($f \approx 0.3$). These data are very quiet; although computed with the theoretical baseline, there is no sudden leveling. Yet it is clear that the correlation function is not a single exponential. Figures 2.6 and 2.7 are also
Figure 2.4 Semilogarithmic plot for PAMAM-3 in water.
Figure 2.5 Semilogarithmic plot for PAMAM-9 in water.
Figure 2.6 Semilogarithmic plot for PAMAM-5 in water.
Figure 2.7 Semilogarithmic plot for PAMAM-7 in water.
correlation functions for intermediate PAMAMs, PAMAM-5 and PAMAM-7, respectively. The f values from both PAMAM-5 and PAMAM-7 are very small even though detector settings that produce high f values for strong scatterers were used. The f values from both PAMAM-5 and PAMAM-7 are very small even though detector settings that produce high f values for strong scatterers were used. Semilog plots exhibit intermediate levels of multiexponential. There is really no cause to subject the data of Figure 2.4. to Laplace inversion, except to distinguish this monodisperse sample from the rest. However, much effort has been devoted to the analysis of data. Figure 2.8(a)-(c) shows a correlation function and distribution of decay rate for PAMAM-3. Figure 2.8 (c) is actual amplitude of the distribution of decay rate, in which the amplitude from CONTIN is divided by square root of baseline, while Figure 2.8(b) shows the normalized amplitude shown by conventional practice where the maximum amplitude is defined as 1. The single peak in Figure 2.8(b) obtained by CONTIN is in good agreement with EXSAMP. Figure 2.9(a)-(c) is the analogous plot for PAMAM-9. As shown in Figure 2.9(b), three peaks are evident. One of these two smaller peaks lies at 97 Å, close to the SEC value (105 Å) while the other lies at 33 Å---very near to the PAMAM-3 value. The broad, large-diameter peak corresponds to the long-time decay in Figure 2.5; it is temporarily attributed to aggregation, though other effects that produce long
Figure 2.8(a) Correlation function for PAMAM-3 in water. (b) CONTIN results and normalized first order correlation function. (c) Actual amplitude of distribution of decay rate.
Figure 2.9(a) Correlation function for PAMAM-9 in water. (b) CONTIN results and normalized first order correlation function. (c) Actual amplitude of distribution of decay rate.
time DLS decays are well known (if poorly understood) in polyelectrolyte solutions at low salt (2.22-2.25). The diameter of the putative aggregates will exceed the filter pore size, so they must form after filtration. Aggregation that was reversible at high shear rates (as can be generated during filtration) was reported (2.2) in PAMAM-7 after surface exposure to n-butanol. Aggregation was also observed in sodium carboxylate PAMAM salts (the half-integer generation PAMAM's) but it was at least temporarily reversible by addition of a hydrogen bond competitor. Figure 2.10(a)-(c) is also the correlation function and distribution of decay rate which resulted from the Laplace inversion algorithms of Figures 2.6. PAMAM-5 has two peaks with one dominant broad peak at a diameter close to generation 3 and another peak (a shoulder, really) bigger than the size reported for PAMAM-5. As stated before, the small \( f \) values in this system made analysis of the data difficult, and a minor baseline adjustment removed the shoulder. Specifically, 0.04% of baseline adjustment caused the disappearance of shoulder (theoretical baseline, \( B_t \), is \( 0.5808 \times 10^9 \) and \( B_f \) for the removement of shoulder is \( 0.58102 \times 10^9 \)). This fact is clearly shown in Figure 2.11. For generation 5, a second sample which has different Lot numbers has been purchased and measured under the same conditions as for the first-arrived sample. The second sample also has very low \( f \) values, and the correlation function and the CONTIN results with normalized \( g^{(1)}(\tau) \) are
Figure 2.10(a) Correlation function for PAMAM-5 in water. (b) CONTIN results and normalized first order correlation function. (c) Actual amplitude of distribution of decay rate.
shown in Figure 2.12(b)-(d). The correlation function for the strong scatterer (polystyrene latex sphere) is represented in Figure 2.12(a) as a reference. The maximum f value, 0.2, was produced under the same instrument settings as used for PAMAM-5. Like the first arrival, the second one also shows a main peak and two additional small amplitudes of peaks which are, in principle, responsible for the slow (about 6% of the total amplitude) and fast decay modes (about 13% of the total amplitude). The slow decay mode is removed by the minor adjustment of baseline (0.05% of baseline) while that for the fast decay mode stays (14% of the total amplitude). This disappearance of slow decay in Figure 2.11 and 2.12 may be an artifact of the data analysis. However, raising baseline may mask the presence of slow decay which is in the range of low frequency spectrum and is very sensitive to baseline adjustment. Therefore, it is not easy to state the presence of slow decay mode in PAMAM-5. The peak for the fast decay mode corresponds to a diameter of 5Å. This peak also may be caused from either artifact or plasmon mode which is controversial. In the author's point of view this small peak is from artifact because the measurement of that small diffuser of diameter less than 5Å is out of range of our correlator. Based on Figures for PAMAM-5, the determined size close to that for PAMAM-3 is pronounced and is reliable, but the other small or large size shown is not clear to determine as real size. The pronounced peak in the first
Figure 2.11 Distribution of decay rate for PAMAM-5 in water. (a) $B_r=0.58100 \times 10^9$ is used. (b) $B_r=0.58102 \times 10^9$ is used.
Figure 2.12 (a) Correlation function for standard polystyrene latex sphere in water. (b) Correlation function for PAMAM-5 in water. (c) CONTIN results when $B_f = 0.22954 \times 10^8$ is used. (d) Normalized correlation function with CONTIN results when $B_f = 0.22960 \times 10^8$ is used.
arrival PAMAM-5 coincides with that of second sample. Figure 2.13(a)-(c) represent the correlation function and CONTIN analysis for PAMAM-7. The representation at the top shows how very little signal is actually present above the baseline, and this may cause the same baseline uncertainty problem as in PAMAM-5. Therefore, it is very hard to state with certainty that a real slow mode exists in this sample. actually present above the baseline, and this may cause the same baseline uncertainty problem as in PAMAM-5. Therefore, it is very hard to state with certainty that a real slow mode exists in this sample.

In DLS measurements on a weakly scattering system, some compromise between an acceptably large signal and high optical coherence is necessary. Most of the measurements above were made using instrument settings with small f values, which can frequently cause the baseline uncertainty. To avoid the baseline uncertainty problem, specially designed detection optics using a 5 x microscope objective instead of normal focusing lens (ca. 20 cm), was used for the measurement of PAMAM-3. With this modification of the instrument, we could produce f(A) ≈ 0.1. The determined size from system with higher f(A) values is the same as that from old settings with small f values. The rest of the PAMAMs were not re-measured by this modified instrument because of lack of sample supply.

Based on all figures for PAMAMs in water, lower generation, PAMAM-3, shows a definite monodispersity while the higher generations seem
Figure 2.13(a) Correlation function for PAMAM-7 in water. (b) CONTIN results and normalized first order correlation function. (c) Actual amplitude of distribution of decay rate.
to show polydispersity based on non single exponential decay in correlation function and possible slow decay mode. According to Tomalia (2.2), it becomes progressively more difficult to prepare generations higher than 4-5, although improved methodology, from which the present samples do not benefit, may lead successfully to generation 10. Our data are consistent with some failure leading to stunted growth beginning after generation 3 for the present samples. Low-mass impurities were also noted in a recent size exclusion chromatography study of the half-integer, carboxylated dendrimers (2.15). Although our results are consistent with bimodal character, there is another possible explanation. For a long time, it has been known that solutions of monodisperse polyelectrolytes can, under conditions of low salt, give rise to bimodal $g(\tau)$ (2.40-2.47). In addition, the measurement of polyelectrolytes with weak scattering in water is not enough to explain their possible complicated polyelectrolyte behavior. The measurement should have been also performed in salt because the terminal amine functional groups may acquire positive charge and repel each other in water and cause bimodal correlation function while amine end groups are pretty stable in salt due to the screening of the charges by counter ions. In conclusion, it will become clear whether PAMAMs aggregate in water or possible polyelectrolyte effect pretends the
aggregation, if the experiment is performed by varying salt concentration or adopting an alternate technique, such as Fluorescence Photobleaching Recovery (FPR). Further full discussion will follow in the next section.

2.4.2 SALT EFFECT ON PAMAMs

Lower generation PAMAMs, PAMAM 3, PAMAM-5, and PAMAM-7 were selected for additional study. Most of them were very weak scatterers and have small value of \( f \) providing difficulty in explanation of the results from Laplace inversion algorithm in DLS measurements. None exhibited the serious "aggregation" type of phenomena of PAMAM-9 which has a big broad peak at a size exceeding the filter pore size.

For PAMAM-5, eight different salt concentrations ranging from 0.1 to 150 mM were considered. The correlation functions were pretty similar to each other and the typical correlation function in 150 mM NaCl is selected and shown in Figure 2.14(a)-(c) with the distribution of decay rate. In Figure 2.14(b) one sharp peak close to the size advertised for PAMAM-5 is evident and well compared to the broad distribution of PAMAM-5 in water, Figure 2.12(c). The actual amplitude of the decay distribution (Figure 2.14(b)) (The amplitude from CONTIN is divided into squared root of base line) is compared to that of normalized one made from conventional practice, and one peak is still evident while an almost flat distribution was observed for PAMAM-5 in water
(see Figure 2.10(c)). The increase in intensity with salt concentration is shown in Figure 2.15. The diameters determined from the slope of $\Gamma$ vs $q^2$ plot are presented with EXSAMP and CONTIN results in Figure 2.16. Their diameters are virtually identical. The remarkable feature of Figure 2.15 and Figure 2.16 is that both diameter and intensity increase sensitively with added salt, before leveling out at $[\text{NaCl}] > 50\text{mM}$. Light scattering, either static or dynamic, is often interpreted in terms of single particle behavior. In fact, both methods reflect the behavior of many particles, and a general thermodynamic theory is required except at infinitely low concentrations and, for polyelectrolytes, high salt to screen any electrostatic repulsion. The increase in both intensity and apparent diameter with added salt are sensibly interpreted as Coulombic screening of the strong electrostatic repulsion among the PAMAM-5 molecules that exists in pure water. The scattered intensity is inversely proportional to the osmotic modulus $d\pi/dc_{\tau,p}$; thus, at high salt, the concentration fluctuations that can arise spontaneously at a given temperature are larger than they are at low salt, leading to greater intensity. The apparent diameter is derived from the diffusion coefficient which, at finite concentrations, is given by:

$$D_m = \left(\frac{M}{N_a}\right)(d\pi/dc)_{\tau,p}/f_m$$

(2.6)

where $N_a$ is Avogadro's number, $M$ is the molecular weight, and $f_m$ is the mutual friction coefficient. Equation 2.6 neglects a volume factor correction
Figure 2.14(a) Correlation function for PAMAM-5 in 150 mM NaCl. (b) CONTIN results with normalized first order correlation function. (c) Actual amplitude of distribution of decay rate (The amplitudes from CONTIN are divided into the squared root of baseline).
Figure 2.15 Intensity changes with added salt concentrations for PAMAM-5.
Figure 2.16 Apparent diameters for PAMAM-5 from EXSAMP, CONTIN, and the average of 3CUMU and 1EXP analyses.
term, which has been variously estimated (2.21, 2.27, 2.37). The volume fraction correction is not important at the low concentrations of this study. The mutual diffusion coefficient for osmotically stiff solutions (low-salt solutions with high $(d\pi/dc)_{TP}$) exceeds the infinite dilution limit, and this results in a too-small value for the apparent hydrodynamic diameter; see equation 2.6. Obtaining the correct hydrodynamic diameter by extrapolating to zero concentration would be time-consuming and technically difficult, especially for small PAMAM's.

Instead of relying on very small thermally driven concentration fluctuations, as DLS does, the FPR experiment deliberately creates large fluctuations—not in the overall polymer concentration, but only in the distribution of bleached and photobleached molecules. As diffusers with photobleached dye moieties are not often dramatically different chemical species from diffusers with their dye labels still intact, the chemical potential gradients associated with the photobleached/unbleached distribution are small. Even if the unbleached molecules were to differ greatly from the bleached molecules, such that a chemical potential might exist, the low concentrations accessible to FPR should prevent serious problems. Thus, diffusion coefficients from the FPR technique are not ordinarily susceptible to thermodynamic interaction and are thought to very closely approximate the self diffusion
coefficient of the tagged species. If the dye label is much smaller than the
molecule to which it is attached, and causes no structural perturbations, then the
tracer self diffusion coefficient approaches the self diffusion coefficient of the
unlabeled molecule of interest.

Figure 2.17(a) shows a typical FPR recovery trace for LPAMAM-5. The
inset represents the linear dependence of recovery rate, Γ, upon the squared
spatial frequency, K². Concurrently, the zero intercept indicates a purely
diffusive recovery mechanism. Chemical recovery of the bleached dye is
absent. The linear semilog trace, Figure 2.17(b), indicates good sample
uniformity. Figure 2.18 shows the determined sizes for labeled and unlabeled
PAMAM-5 by means of DLS and FPR, respectively. The purchased, labeled
PAMAM-5, PLPAMAM-5, was measured by FPR and also is shown in Figure
2.18. PLPAMAM-5 measurements were made at a concentration of about
4 mg/mL similar to those used for PAMAM-5 in the DLS measurements. As
expected from the previous paragraph, the diameters obtained by FPR do not
decrease at low salt. Indeed, there is a small increase which probably arises
from PAMAM expansion or counterion drag in the electrically unscreened low-
salt solutions. Due to the presence of the dye, one might have expected the FPR
diameters to slightly exceed the high-salt DLS values. However, they are
smaller—and there is some difference between the sample labeled by
Polysciences and that prepared by us. The absorption peak for the Polysciences-labeled PLPAMAM was blue shifted from the normal FITC maximum (near 4900Å). The LPAMAM-5 labeled in our own lab gave a normal absorption spectrum and somewhat smaller hydrodynamic diameters. We believe the PLPAMAM is a heavily labeled material which may be exhibiting a kind of self-quenching. Even assuming 100 % reactivity of fluorescein isothiocyanate to PAMAM, our LPAMAM-5 was lightly labeled. There is about one dye unit per PAMAM molecule. With one dye unit per molecule, LPAMAM-3 is also lightly labeled. The small diameter for our LPAMAM-5 suggests that the dye moiety causes PAMAM to undergo some contraction—perhaps to help protect the hydrophobic portions of the fluorescein conjugated ring system. The Polysciences labeled material is also smaller than the unlabeled PAMAM-5, but larger than our LPAMAM-5, possibly due to the heavier labeling. Fluorescently tagged PAMAM's used as probes or markers should, therefore, be measured carefully to assess the structural perturbation caused by the dye moiety. Also, there is a mild expansion of the labeled PAMAM structure in the absence of salt, according to these FPR results. It seems likely that this expansion would also occur in unlabeled PAMAM's, though the appropriate DLS experiments to find it would be tedious, as already mentioned.
Figure 2.17(a) FPR trace for LPAMAM-5. Inset shows diffusive recovery behavior. (b) Semilog plot of LPAMAM-5.
Figure 2.18 The diameters of unlabeled and labeled PAMAM-5 with added salt (PAMAM-5/DLS, unlabeled PAMAM-5 measured by DLS; LPAMAM-5/FPR, self labeled PAMAM-5 measured by FPR; PLPAMAM-5/FPR, purchased labeled PAMAM-5 measured by FPR).
Figure 2.19 shows the intensity measurement of PAMAM-3 at various salt concentrations. The intensity is fairly constant. The diameters were also determined from both 3CUMU and Laplace inversion algorithms, EXSAMP and CONTIN, and presented in Figure 2.20. All CONTIN results are from chosen CONTIN that the program selects, and are in good agreement with EXSAMP. The values from 3CUMU are somewhat smaller than those from EXSAMP or CONTIN, but agree within typical error. PAMAM-3 has been labeled with FITC fluorescent dye in our lab. The self diffusion was measured by FPR, and the diameters were determined. Figure 2.21(a)-(b) shows the typical FPR trace for LPAMAM-3. The linear increase in the inset in Figure 2.21(a) and the linear semilogarithmic trace in Figure 2.22(b) indicate the absence of chemical recovery and sample uniformity, respectively. Figure 2.22 shows the results for PAMAM 3 and LPAMAM-3, in which the apparent diameter increases modestly with added salt while the FPR diameter does not change. Based on all Figures for PAMAM-3 the effect of thermodynamic nonidealities and chain expansion at low salt seem less for this smaller polymer, which has fewer terminal amine functional groups. The determined small diameter for LPAMAM-3 is also caused from the contraction of dye attached.

Figure 2.23(a)-(c) shows the correlation function $G^{(2)}$ and CONTIN results overlayed with the normalized $g^{(1)}$ for PAMAM-7 at 100 mM NaCl.
Figure 2.19 Intensity measurement for PAMAM-3 with added salt.
Figure 2.20 Apparent diameters for PAMAM-3 from EXSAMP, CONTIN, and the average of 3CUMU and 1EXP analyses.
Figure 2.21(a) FPR trace for LPAMAM-3. Inset shows diffusive recovery behavior. (b) Semilog plot of LPAMAM-3.
Figure 2.22 FPR and DLS data combined for PAMAM-3 with added salt.
Figure 2.23 (a) Correlation function for PAMAM-7 in 100 mM NaCl. (b) CONTIN results with normalized first order correlation function. (c) Actual amplitude of distribution of decay rate.
Like other PAMAMs at high salt, PAMAM-7 has one decay mode, and the actual amplitude of decay rate is also pronounced compared to that from PAMAM-7 in water (Figure 2.13). As explained before, the charges on polymer are screened by counter ions, and any solution nonidealities are not seen under this high salt condition. We did not measure the intensity or diameter with increasing salt concentration for PAMAM-7. The measurement was done at only one high salt condition, but we are sure that both intensity and diameter are increased and leveled out at some point of salt concentrations like PAMAM-5 in salt. The salt concentration, 100 mM, seems to be enough to have plateau in both intensity and diameter for PAMAM-7 because PAMAM-5 shows the plateau at [NaCl] > 50 mM. Until now we have considered the salt effect on PAMAMs without changing pH. However, the positively charged PAMAMs are sensitive to pH changes as observed in any charged macromolecules. The pH effect is studied in the next section.

2.4.3 pH EFFECT ON PAMAMs

The pH effect is considered only on PAMAM-5, due to the sample supply. The diameters for PAMAM-5 at different pH conditions were measured by both DLS and FPR, and are shown in Table 2.1.

The effect of pH is mild in the range 3 < pH < 11 compared to the salt effect discussed in the previous chapter. However, the size at low pH is
increased about 20% compared to that at basic condition (pH 11), which is not negligible. The size at pH 3 in water is 10% bigger than that at pH 3 in 100 mM NaCl. The same trends of the increased diameter at low pH condition also have been reported for a different type of cascade polymer (2.38, 2.39).

Table 2.1. Hydrodynamic diameters for PAMAM-5 as functions of pH and salt by DLS and FPR.

<table>
<thead>
<tr>
<th>Solution Type</th>
<th>pH</th>
<th>[NaCl]/mM</th>
<th>d/Å</th>
<th>d/Å</th>
<th>d/Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base/Salt</td>
<td>11</td>
<td>100</td>
<td>47</td>
<td>50</td>
<td>41</td>
</tr>
<tr>
<td>Water/Salt</td>
<td>9</td>
<td>100</td>
<td>49</td>
<td>54</td>
<td>43</td>
</tr>
<tr>
<td>Acid/Salt</td>
<td>7</td>
<td>100</td>
<td>51</td>
<td>53</td>
<td>44</td>
</tr>
<tr>
<td>Acid/Salt</td>
<td>3</td>
<td>100</td>
<td>57</td>
<td>59</td>
<td>49</td>
</tr>
<tr>
<td>Water</td>
<td>9</td>
<td>0</td>
<td>26</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>Water/Acid</td>
<td>3</td>
<td>0</td>
<td></td>
<td></td>
<td>54</td>
</tr>
</tbody>
</table>

a\textsuperscript{3}CUMU fits use theoretical baseline, B\textsubscript{t}
b\textsuperscript{1}EXP fits use a floating baseline, B\textsubscript{t,1EXP}

The increased diameter at low pH is presumably due to unscreened charge-charge repulsion among the protonated amine terminal groups. The lower pH condition makes more protonated amine terminal groups, and leads to more chain expansion providing bigger size. The added salt would restrict this
expansion because of screening of charges (as shown in Table 2.1). Thus the maximum expansion can be expected from low pH condition with low or zero added salt.

2.5 SUMMARY

Several different generations of PAMAMs have been characterized under three different conditions: in water only, salt, and at different pH values. Lower generation PAMAM, PAMAM-3, is very stable in both water and salt. The measured intensities by DLS do not vary with added salt. In addition, the diameters of unlabeled and labeled PAMAM-3 measured by DLS and FPR are almost constant and less sensitive to added salt than PAMAM-5. The smaller size of labeled PAMAM-3 compared to the unlabeled one may be due to contraction of dye attached. The small number of terminal amine functional groups in PAMAM-3, the charged amine groups, (the number of terminal groups = 24) seems insufficient to produce any serious polyelectrolyte effect. Based on our experimental results, PAMAM-3 can be applied to probe diffusion studies as small probe either in water or salt. Both DLS and FPR techniques would be useful for this small PAMAM.

In contrast, one of intermediate size PAMAMs, PAMAM-5, has shown polyelectrolyte effects especially at low or zero added salt conditions. The measured intensity and diameters in DLS are very sensitive to the added salt,
except above 50 mM salt concentration, and are well explained by the osmotic
susceptibility caused from the charge-charge repulsion. The determined smaller
diameters of labeled PAMAM-5 than those of unlabeled one are due to the
contraction of dyes attached. The pH effect is not significant compared to salt
effect. However, the greater size of approximated 20% at low pH than that at
high pH has been observed. This can be explained by chain expansion in acidic
condition. The chain expansion would be maximum at conditions of low pH
and low or zero added salt.

PAMAM-7 in water also has shown the same solution nonidealities as
PAMAM-5.

The observed experimental results indicate that the intermediate size
PAMAMs, PAMAM-5 and PAMAM-7, would be used as probes unless they
are in low pH or without added salt. FPR technique is useful for them both in
salt and low/without added salt. However, great attention should be paid when
DLS is applied for PAMAMs at low or without added salt because the measured
mutual diffusion coefficient in DLS is directly related to the osmotic
susceptibility.

The larger generation PAMAM, PAMAM-9, has different phenomena
from small or intermediate size PAMAMs because PAMAM-9 in water has
shown not only non single exponential correlation function but also three well
separated peaks in the distribution of decay rate. Moreover, the pronounced largest size is bigger than the filter pore size used. Unfortunately PAMAM-9 has not been characterized under added salt conditions due to the depletion of sample supply, and it is very hard to tell at this moment that whether PAMAM-9 has aggregation or polyelectrolyte effect pretends the aggregation. A recent study of a half-integer (generation 7.5) carboxylated PAMAM by Valachovic et al. (2.48) found three decay modes under some conditions of charge and salt (especially, at significant degrees of charge ionization and yet moderately low total salt). Our experimental results might be explained by three different diffusion modes as referred by them. The point is that slow decay modes really can exist in high-generation PAMAM polyelectrolyte solutions at low salt. However, the clear answer will be given after the salt effect study on PAMAM-9 is performed.

2.6 REFERENCES


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CHAPTER 3

INTERACTION BETWEEN POSITIVELY CHARGED LABELED PAMAM AND NEGATIVELY CHARGED SODIUM POLYSTYRENE SULFONATE
3.1 INTRODUCTION

The interaction between oppositely charged polyelectrolytes has been an interesting subject not only for theoreticians but also for experimentalists because this interaction leads to the complex formation which plays an important role in both natural and commercial processes. In commercial applications, synthetic polyelectrolytes usually make a complex, and have been used for the flocculation of colloids in water clarification (3.1), coatings, and microencapsulation (3.2). For the natural systems, most biological macromolecules are polyelectrolytes, and the interaction of protein with nucleic acid is the beginning of the transcription process (3.3). Besides, the enzymatically active insulin or catalase can be immobilized or stabilized by complex formation (3.4). The purification or precipitation of proteins also could serve as examples of related processes (3.5).

In addition to these applications, a vast number of theoretical investigations have been made to assist the interpretation of experimental studies. For example, in 1980, Odijk studied the binding of a long flexible polycation to a rodlike polyanion, and predicted that charged flexible chains could collapse on oppositely charged rigid rods as the ionic strength is reduced below some critical value (3.6). Muthukumar and von Goeler considered the adsorption of a polyelectrolyte onto oppositely charged spherical and cylindrical
surfaces in 1994, and obtained explicit formulae for the adsorption characteristics by changing temperature, the Debye screening length, polyelectrolyte charge density, molecular weight, and curvature (3.7). These theoretical treatments have been well supported by several experimental observations (3.13, 3.15, 3.18, 3.23). The fact is that the interaction between oppositely charged macromolecules strongly depends on several variables, such as the ionic strength, the charge per polymer repeat unit, and the particle charge density. For instance, complexes can easily be formed in the condition of no added salt (low ionic strength), and this complex is very stable if the polyelectrolytes have high charge density. And the magnitude of electrostatic interaction between particles increases as the charge per polymer repeat unit and particle charge density are raised. For all of these interesting phenomena numerous techniques have been employed to follow the course of complex formation: i) scattering techniques (3.9, 3.13, 3.15, 3.17, 3.18, 3.22-3.26); ii) titration methods (3.12-3.16); iii) nuclear magnetic resonance spectroscopy (3.21); iv) fluorescence (3.27, 3.28); and v) fluorescence photobleaching recovery (3.29).

In this Chapter, the interaction between positively charged labeled polyamidoamine cascade polymers (LPAMAM) and negatively charged sodium polystyrenesulfonate (NaPSS) is considered. To be specific, the fifth generation
PAMAM, PAMAM-5, has been used after labeling with fluorescein isothiocyanate (FITC) dye, which is referred to be LPAMAM-5. PAMAMs and labeled PAMAMs have been extensively characterized in our lab and fully described in Chapter 2. As described there, PAMAM-5 and LPAMAM-5 have been proved to be useful probes for the general probe diffusion studies unless they are used at low salt or very low pH and studied by dynamic light scattering.

NaPSS is one of the typical strong polyelectrolytes and has attracted much attention due to the curious behavior called the ordinary/extra-ordinary transition: an apparent diffusion coefficient is suddenly decreased over a small range of salt concentration. The ordinary behavior is observed in high salt and is well understandable. Instead, the extra-ordinary behavior which has been observed at low or zero added salt is still poorly understood. Several interpretations have been applied (3.30-3.37). In general, polyelectrolytes at high salt are treated like neutral polymers because the charges on the polymer are screened by added salt. However, polyelectrolytes at low or zero added salt should be treated very carefully because the unscreened charges on polymers repel each other and may cause different phenomena that are not observed in neutral polymers (some people say it "polyelectrolyte effect"). Lots of works have been reported on NaPSS at several different conditions by several groups (3.33, 3.34, 3.38-3.47). However, little is known about the self diffusion in salt
free solutions. To the author's knowledge, no direct measurement of self diffusion coefficient by fluorescence photobleaching recovery has been reported.

Thus, in the first part of this Chapter, NaPSS with molecular weight 100,000 was characterized prior to the study of complex formation, and self diffusion coefficients have been determined as functions of polymer concentration and added salt. Additionally, the mutual diffusion coefficient has been obtained by dynamic light scattering, and its value at zero polymer concentration is compared to the self diffusion coefficient from FPR. Mutual diffusion was measured as a function of polymer concentration in high salt solution because it is very difficult to read the diffusion coefficient at salt free solution due to the two diffusion modes (extraordinary behavior).

The second part of this Chapter contains the study of complex formation between LPAMAM-5 and NaPSS. Polyelectrolyte complexes can be formed via electrostatic interactions between polymers with opposite charges, and the complex formation usually occurs rapidly on mixing. Dubin et al. have studied the polyelectrolyte complex formation between poly(dimethyl diammonium chloride), PDMDAAC, and anionic micelles with several techniques (3.14). He also has paid attention to the binding between PDMDAAC and carboxylated cascade polymers (3.53). Similar works were performed on carboxylated
cascade polymers, that is the half generation analogs of the materials studied here (3.54, 3.55). To the author's knowledge, the interaction between positively charged full generation cascade polymers and negatively charged polymers has not been studied yet. Here we consider the complexes formed between positively charged labeled full generation PAMAM-5 and negatively charged NaPSS. Self diffusion coefficients of complexes with several variables, such as ionic strength, pH, and molar ratio of polyelectrolytes were determined by FPR. The ionic strength was controlled by changing NaCl concentration. The pH was adjusted by adding acid or base to the solution. The molar ratio of NaPSS to LPAMAM-5 was varied by diluting either NaPSS or LPAMAM-5 concentrations while either LPAMAM-5 or NaPSS concentration was held constant.

The most widely used techniques for the characterization of NaPSS and LNaPSS were DLS and FPR, respectively. For the study of complex formation between NaPSS and LPAMAM-5, only FPR, which is appropriate for the measurement of self diffusion coefficient of labeled molecules, was employed. Dubin et al. have used several techniques (3.8, 3.10-3.20, 3.22-3.23, 3.25) for the study of complex formation. We have adapted FPR, which has been reported as a reliable technique for the measurement of translational diffusion coefficients of small ions and other species in solution (3.48, 3.49). FPR has
some advantages: i) it does not need a calibration; ii) sampling is very easy and a small amount of sample (less than 0.05 ml) is required; iii) experiment takes only a few minutes; iv) broad range of applications (it can be applied not only to small ions but also to polymer solutions only if the molecules of interest are labeled); v) solution nonidealities that are usually present in polyelectrolytes do not affect FPR measurement.

3.2 SAMPLE PREPARATION AND EXPERIMENTAL TECHNIQUES

Samples for NaPSS and LNaPSS characterization

Sodium poly(styrenesulfonate), NaPSS (Cat#, 16253, Lot#, 430478), purchased from Polysciences, Inc, had a molecular weight of 100,000 g/mol with Mw/Mn < 1.10 according to the manufacturer. NaPSS was initially dissolved in 0.1M NaCl solution which was prepared with GR grade NaCl and centrifuged overnight after filtering through a 0.1 Millipore VV filter. The predissolved NaPSS was then fully dialyzed against 0.1M NaCl salt solution with the pretreated Spectrapore membrane tubing (MWCO = 12,000-14,000) for two days with periodic changes of the salt solution. The pretreatment of the membrane tubing was done by rinsing several times with clean water after sterilization by boiling. The dialyzed NaPSS was filtered through a 0.2 μm Gelman PTFE filter. The final concentration of NaPSS was about 1%. This solution was stored in the refrigerator as a stock solution. For the measurement
of the diffusion coefficient of NaPSS, four different concentrations of NaPSS solutions were made by diluting the stock solution with the dust free 0.1 M NaCl: 0.5, 0.1, 0.25, and 0.05 %. Solutions exhibiting dust were centrifuged extensively.

NaPSS was labeled with fluorescent dye to measure the self diffusion. The labeling was performed by M. L. Mclaughlin’s group in the Chemistry Department at LSU. The labeling procedure consisted of two steps, the substitution of sodium sulfonate into sulfonyl chloride (Figure 3.1(a)) and the reaction of sulfonyl chloride with amine containing fluorophore (Figure 3.1(b)). The labeling procedure is as follows. Three grams of NaPSS were suspended in 30 ml of phosphorous oxychloride, POCl₃, in a 100 ml round bottom flask. The mixture was stirred and refluxed for 2 days, then cooled to room temperature. Water was added to neutralize the remaining POCl₃. The resulting solid was filtered and washed with H₂O until a neutral pH was obtained. The product was placed in an oven at 80°C over 3 days, after which 1.90 g of a beige solid was obtained. Decomposition temperature ranges of both the starting material and the product were checked during the each step (starting material 160 (white) -200 °C (white), product 160 (brown)-200 °C (black)). In a 100 ml Schlenk flask, 1.16 g of the product of the first step were suspended in 20 ml of dry tetrahydrofuran, THF. The polymer suspension was
Figure 3.1 The labeling procedure for LNaPSS. (a) The substitution of sodium sulfonate into sulfonyl chloride. (b) The reaction of sulfonyl chloride with amine containing fluorophore.
stirred at room temperature. Fluoresceinamine (0.01 g, 0.03 mM) and triethylamine (0.87 g, 8.64 mM) were dissolved in 5 ml of dry THF. The dye-THF solution was heated to reflux to ensure complete dissolution and subsequently added dropwise to the product of the first step. Color changes signaled instantaneous reaction. The THF was removed at reduced pressure. The yellow residue (labeled poly(styrenesulfonyl chloride), LPSSCl) was dissolved in 10 ml of 10% sodium hydroxide and refluxed for 20 min to hydrolyze unreacted sulfonyl chlorides. Then, 3M HCl was added until the pH was neutral. The resulting bright yellow gel was dried in an oven at 80 °C for 24 h. The yellow colored LNaPSS powder was dissolved in 0.1M NaCl solution and dialyzed against 0.1M NaCl with a sterilized and rinsed Spectrapore membrane tubing having MWCO 12,000 - 14,000. The extensively dialyzed LNaPSS was filtered through a 0.2 μm Gelman PTFE filter. The final concentration of LNaPSS was about 4.8%. Seven different concentrations of LNaPSS solutions were prepared by adding the clean 0.1M NaCl: 2.4, 2.1, 1.6, 0.96, 0.7, 0.48, and 0.24%.

Samples for the complex formation between LPAMAM-5 and NaPSS

First, the fifth generation of polyamidoamine cascade polymer (PAMAM), PAMAM-5 (Cat. #. 21152; Lot #. 425292) was purchased from Polysciences, Inc. Its advertised diameter is 54 Å and the molecular weight is
21,563 by computation (3.52). The purchased PAMAM-5 was then labeled with fluorescein isothiocyanate (FITC) dye, to be referred to LPAMAM-5. The labeling procedure is described in detail in chapter 2. In brief, the predissolved FITC in acetone was added to the regular unlabeled PAMAM-5 solution, which were then allowed to stand overnight at room temperature with occasional stirring. The solution was dialyzed against pure water through a pretreated Spectrapore membrane tubing (MWCO 5,000) after removing acetone carrier solvent by sparging with air. After dialysis, LPAMAM-5 solution was filtered through a 0.2 μm Gelman PTFE filter (pre-wetted with alcohol, then rinsed with water). The final concentration was about 0.4 %.

A stock solution of NaPSS was prepared by dissolving a white powdered NaPSS (Cat. #. 16253; Lot #. 430478) with molecular weight 100,000 g/mol and Mw/Mn < 1.10 in pure water. The dissolved NaPSS solution was then dialyzed against pure water with a sterilized and rinsed Spectrapore membrane tubing with MWCO 12,000 - 14,000 for two days. The supplied water was very carefully deionized and filtered by a Barnstead Nanopure 4-stage purifier, equipped with spiral-wound ultrafilter (ca. 0.005 μm) and final 0.2 μm filter. The fully dialyzed NaPSS was filtered through a 0.2 μm Gelman PTFE filter. The concentration of stock solution of NaPSS was 4.1 %.
Samples for salt effect of complex between LPAMAM-5 and NaPSS

Ten different concentrations of salt solutions were prepared with GR grade NaCl. Aliquots of 0.01 ml of each salt solution was added to 0.03 ml of NaPSS solution and left for 15 mins. Later, 0.03ml of LPAMAM-5 solution was then added to each of NaPSS solution in salt. Ten different complex solutions with different salt concentrations ranging from 2.9 mM to 430 mM NaCl were prepared. The complex solutions were allowed to stand for about 30 before the experimental measurement was performed. The polymer concentrations of NaPSS and LPAMAM-5 remained constant for all the complex solutions in salt even though the salt concentrations differed.

Samples for pH effect of complex between LPAMAM-5 and NaPSS

Samples were prepared by adding acid or base to the complex solutions with and without salt. One complex solution in water and two complex solutions with different salt concentrations, 140 and 360 mM NaCl, were first selected. All complex solutions without added acid or base showed pH values at 9. Thus, samples at pH 3 and pH 11 were made by adding a couple of drops of 0.1M HCl and 0.1M NaOH solutions to the complex solution at pH 9, respectively. The pH measurement was carried out with pH papers. A small amount of the sample prevented the use of the pH meter; at least 3 ml is needed
to soak the electrode unless a special probe were to be purchased. The accuracy of pH papers was confirmed in Chapter 2. The polymer concentrations of all complex solutions were almost constant at various pH values.

**Samples for the critical molar ratio of NaPSS to LPAMAM-5 to make complex**

Stock solutions of NaPSS and LPAMAM-5 in water were used. To make different molar fraction of NaPSS to LPAMAM-5, either NaPSS or LPAMAM-5 solution was diluted. Eight different ratios of NaPSS to LPAMAM-5 have been considered: 3.3, 2.2, 0.73, 0.22, 0.11, 0.02, 0.06, and 0.002.

**Experimental Methods**

For the characterization of NaPSS and LNaPSS two techniques, DLS and FPR, were used. Only FPR was employed for the complex formation between LPAMAM-5 and NaPSS. DLS was used for the measurement of mutual diffusion of NaPSS while FPR was performed to determine the self diffusion coefficient (or tracer translational diffusion coefficient) of fluorescently tagged NaPSS. The DLS instrument and data analysis were fully described in Chapter 2. In brief, a laser beam with wavelength 5145Å impinged the sample in a silanated glass cell. The scattered light is then detected at specific angles and sent to a digital correlator, yielding a correlation function. Ten runs are carried out and those with anomalously high intensity are removed. The sum of
remaining runs is analyzed by first- to third order cumulants (1CUMU-3CUMU). Multiexponential analysis is then used to decide which correlation functions should be analyzed by nonlinear least squares algorithms.

The measurements were performed at five different angles, 30, 45, 60, 90, and 120 degrees, for each concentration of NaPSS. The diffusion coefficient was determined from the slope of the plot of $\Gamma$ vs $q^2$ where $\Gamma$ is the decay rate and $q$ is the scattering vector ($q = 4\pi n \sin (\theta/2)/\lambda_0$ where $n$ is the solution refractive index, $\theta$ is the scattering angle, and $\lambda_0$ is the laser wavelength in vacuo).

FPR was developed by Peters et al. in 1974 for the measurement of the translational motion in the biological membranes (3.50). The principles and experimental details of FPR are introduced in Chapter 2. To summarize briefly, a periodic photobleaching pattern is produced in the labeled particles to make a bleached and unbleached region by a brief, intense illumination of light through a grating. The grating is then allowed to move back and forth to monitor the recovery of the bleached or unbleached region. The recovery is due to the diffusion of the labeled particles, and the recovery rate is extracted by the nonlinear least squares fit to one or more exponentials. The diffusion coefficient is derived from the slope of a linear least squares fit of $\Gamma$ vs $K^2$ plot, in which $\Gamma$ is the recovery rate and $K$ is the circular spatial frequency of the
grating (K = 2π / L where L is the distance between stripes in the sample). All FPR experiments were conducted at three different K values. In other words, three different microscope objectives, 4 x, 7 x, and 10 x, were used. The striped pattern (Ronchi Ruling, RR) was 50 lines per inch for all objectives. Several runs were repeated for each objective, and runs showing good exponential decays were selected. All samples were loaded in the Vitro dynamics capillary tubes and flame sealed. For high photobleaching efficiency, oxygen was blown into a capillary tube before the sample was loaded. All measurements were made at 25 °C.

3.3 RESULTS AND DISCUSSION

3.3.1 CHARACTERIZATION OF NaPSS AND LNaPSS

Figure 3.2 shows the mutual diffusion coefficient, D_m, determined at various polymer concentrations by DLS. As expected for polyelectrolytes in high salt, only one mode was found. A straight semilogarithmic plot (which is not shown here) was observed. D_m reduced as polymer concentration decreased, and the diffusion coefficient extrapolated to zero polymer concentration, 3.3 x 10^{-7} cm²/sec was determined. The self diffusion coefficients of LNaPSS at several different polymer concentrations were also determined by FPR, and are presented in Figure 3.2. In general, the self diffusion coefficient decreases with polymer concentration. However, the self
diffusion coefficient of LNaPSS is constant with polymer concentration. The estimated average value of self diffusion coefficient is \((4.5 \pm 0.26) \times 10^{-7}\) cm\(^2\)/sec. Compared to the value of \(D_m\) at zero polymer concentration in DLS, the self diffusion coefficient of LNaPSS is greater. This may be caused from the contraction of dye attached. However, the experiment to prove this issue has not been performed.

The self diffusion of LNaPSS was measured at various salt concentrations and appears in Figure 3.3. The self diffusion coefficient increased with salt concentration. Indeed, this is the typical phenomenon for polyelectrolytes and easily understood by adopting the screening effect of added salt. For example, in water, the like charges on polymers repel each other. This permits the polymer chain to stretch (or expand) and thus slow diffusion is observed. But, in salt solutions, the added salt can screen the charges on polymer and reduce the repulsive forces reducing the chain expansion and increasing diffusion coefficient. As stated in the introduction, NaPSS has shown two different decay modes at low or zero added salt in DLS. However, here in FPR, only one decay mode is seen even at zero added salt. This is because the solution nonidealities present are not sensed by FPR. Other methods can detect some of the nonidealities. It turns out that the diffusion coefficients of polyelectrolytes at low or zero added salt should be determined.
Figure 3.2 The mutual and self diffusion coefficients of NaPSS and LNaPSS by DLS and FPR, respectively ( [NaCl]=0.1M ).
Figure 3.3 Diffusion coefficient of LNaPSS with added salt ($c_{\text{LNaPSS}}=2\%$)
by FPR or a competing self diffusion method such as pulsed field gradient NMR if one wants to know the diffusion of polyions in salt free solutions.

3.3.2 COMPLEX FORMATION BETWEEN LPAMAM-5 AND NaPSS

3.3.2.1 BINDING OF LPAMAM-5 TO NaPSS

The binding of LPAMAM-5 to NaPSS can be considered as a type of chemical reaction. Thus, the binding process is described through an equilibrium constant:

\[
\begin{align*}
  \text{f} \text{F} + \text{p} \text{P} & \rightleftharpoons \text{c} \text{C} \\
  K_{eq} = \frac{[C]^c}{[F]^f[P]^p}
\end{align*}
\]  

(3.1)  

(3.2)

where F stands for free LPAMAM-5, C represents LPMAMAM-5/NaPSS complex, and P is for NaPSS. In general, there are two different binding processes, rapidly interchanging binding and slowly interchanging binding. In the case of the rapidly interchanging binding, the free and bound species (free and bound LPAMAM-5) interchange very quickly (the time period of interchange is shorter than that of FPR measurement), and only one diffusion mode is usually observed. In contrast, two diffusion modes, slow and fast, appear simultaneously in the slowly interchanging process. For the rapidly interchanging binding, a Langmuir-type adsorption equilibrium expression was adopted in ref. 3.49:
\[ K_{eq} = (1-x_F) \frac{[F]}{[F]} \left( \frac{Z}{[P]} - x_F \right) / x_F \]  

(3.3)

where \( z \) is the number of binding sites and \( x_F \) is the mole fraction of bound species (bound LPAMAM-5). The equilibrium constant is calculated by finding \( x_c \) in the measurement of the average diffusion coefficient:

\[ D_{avg} = (1-x_F) D_F + x_F D_c \]  

(3.4)

where \( D_F \) is the diffusion coefficient of free fluorescein and \( D_c \) is the diffusion coefficient of complex (LPAMAM-5/NaPSS complex). For the slowly interchanging binding, there is no expression for the equilibrium constant reported yet. The binding of LPAMAM-5 to NaPSS appeared to be an example of slowly interchanging binding because the binding seemed to be strong and stable. Therefore, the equilibrium constant can not be determined for this case.

### 3.3.2.2 SALT EFFECT ON COMPLEX BETWEEN LPAMAM-5 AND NaPSS

As expected (3.6, 3.7), the electrostatic interaction between positively charged LPAMAM-5 and negatively charged NaPSS was significant. The formation of complex was confirmed first by measuring the self diffusion of LPAMAM-5 both before and after adding NaPSS. Figure 3.4(a) shows the typical decay mode of labeled PAMAM-5 in water. The inset is a linear increase of decay rate, \( \Gamma \), vs \( K^2 \) with zero intercept. The linear plot of semilogarithmic representation in Figure 3.4(b) demonstrates a single
exponential decay. The determined self diffusion coefficient of LPAMAM-5 in water is $(1.1 \pm 0.44) \times 10^{-6} \text{ cm}^2/\text{s}$. Figure 3.5(a) shows the decay mode of LPAMAM-5 in water after NaPSS is added (0.03 ml of 4.1 % NaPSS was added to 0.03 ml of 0.4 % LPAMAM-5). A single exponential decay is again evident. The inset is the linear plot of $\Gamma$ vs $K^2$ with zero intercept. The determined self diffusion coefficient is $(4.8 \pm 0.08) \times 10^{-7} \text{ cm}^2/\text{s}$. The linear increase of $\Gamma$ vs $K^2$ in semilogarithmic plots for both LPAMAM-5 only and LPAMAM-5/NaPSS complex indicates that the chemical recovery of dye attached or convection is absent. The determined slower diffusion coefficient of the complex in Figure 3.5(a) compared to that of free LPAMAM-5 in Figure 3.4(a) clearly reveals that LPAMAM-5 binds to NaPSS.

The above complex was made in pure water, and it was formed rapidly upon the addition of NaPSS solution to LPAMAM-5 solution. What if salt is added to this complex? The salt effect on the complex has been considered by varying the salt concentrations. Figure 3.6(a) is the decay mode of complex in 360 mM NaCl. Nonlinear semilog plot in Figure 3.6(b) indicates two decay modes, slow and fast. To see the salt effect on complex formation in detail, the FPR traces for LPAMAM-5/NaPSS complex at different salt concentrations are given in Figure 3.7. The FPR trace of LPAMAM-5 in water is also represented in Figure 3.7. At zero added salt, the semilog plot is almost straight, indicating
Figure 3.4 (a) FPR trace for LPAMAM-5 in water (c_{LPAMAM-5} = 0.2 %). Inset shows the diffusive recovery behavior. (b) Semilog plot of LPAMAM-5 in water (Objective=4 x, RR=50 lines/inch).
Figure 3.5(a) FPR trace for LPAMAM-5/NaPSS in water (0.03 ml of 4.1 % NaPSS was added to 0.03 ml of 0.2 % LPAMAM-5). Inset shows diffusive recovery behavior. (b) Semilog plot of LPAMAM-5/NaPSS in water (Objective=4 x, RR=50 lines/inch).
Figure 3.6 (a) FPR trace for LPAMAM-5 / NaPSS complex in 360 mM NaCl. Inset shows diffusive recovery behavior. (b) Semilog plot of LPAMAM-5/NaPSS complex in 360 mM NaCl (Objective=4 x, RR=50 lines/inch).
Figure 3.7 FPR traces for LPAMAM-5/NaPSS complex formation at different salt concentrations (Objective=7 x, RR=50 lines/inch).
Figure 3.8 The determined diffusion coefficients of the slow and fast modes with added salt (D_s is determined from the slope of Φ vs Φ^2 plot).
one exponential decay. As the salt concentration increases the semilog plot
becomes curved, and two exponential decay is clearly evident at high salt.

From these FPR traces, the diffusion coefficient at each salt solution is
determined and presented in Figure 3.8. The two modes, slow and fast, are
evident. Slow mode seems to be constant while the fast one is very sensitive to
the added salt. The slow mode is from the complex. The fast mode appears to
be from the free LPAMAM-5 which does not bind to NaPSS due to the
interruption of the added salt. However, the value of the fast diffusion
coefficient at high salt is higher than that of PAMAM-5, and close to that of
free FITC dye, while the number at salt concentrations less than 114 mM agrees
with that of LPAMAM-5. The fast mode at low salt (less than 114 mM) can be
interpreted as LPAMAM-5 unbound, but the fast mode at high salt can not be
interpreted yet. There are three possibilities for the fast mode at high salt: (1)
free FITC dye which was not removed completely on dialysis, (2) unbound
LPAMAM-5 which was compressed by NaPSS on mixing and replaced later,
(3) breakage of LPAMAM-5 or NaPSS by salt. Figure 3.8 was obtained by
applying a two exponential fitting even though some of data did not show a
distinct two exponential decay. The averaged apparent diffusion coefficient,
$D_{app \text{avg}}$ is shown in Figure 3.9.

$$D_{app \text{avg}} = \frac{A_s D_s + A_f D_f}{A_s + A_f} \quad (3.5)$$
Figure 3.9 The averaged apparent diffusion coefficient as a function of salt concentration (Objective=7 x, RR=50 lines/inch).
Figure 3.10 (a) The normalized amplitude of slow mode with added salt. (b) The normalized amplitude of fast mode with added salt (Objective=7 x, RR=50 lines/inch).
where $D_s$ and $D_f$ are the apparent diffusion coefficients of slow and fast modes, respectively. $A_s$ and $A_f$ are amplitudes of slow and fast modes, respectively.

All diffusion coefficients in Figure 3.9 are extracted from data using the same objective at 4 x and Ronchi Ruking at 50 lines per inch. The averaged diffusion coefficient increases with salt concentration. The salt effect is again evident.

Figure 3.10 shows the amplitudes of slow and fast modes. The amplitude of slow mode increases with added salt indicating fast mode becomes pronounced. The above salt effect on complexes was considered at a constant pH value of 9. However, different pH conditions may give interesting results on complexes because at low pH values, LPAMAM-5 has more positive charges due to more $H^+$, and attenuates the electrostatic interactions with NaPSS. At high pH values, LPAMAM-5 has less positive charges and less or no complex formation can be anticipated. At low pH values, LPAMAM-5 has more positive charges due to more $H^+$, and attenuates the electrostatic interactions with NaPSS. At high pH values, LPAMAM-5 has less positive charges and less or no complex formation can be anticipated.

3.3.2.3 pH EFFECT ON COMPLEX BETWEEN LPAMAM-5 AND NaPSS

The diffusion of LPAMAM-5/NaPSS complex at different pH values has been considered and the diffusion coefficients appear in Table 3.1.
Three different solution types were made at three different pH values.

First, the solutions with no salt and different pH values were considered.

Solution at pH 9 has shown a single exponential decay in the FPR trace. The determined diffusion coefficient is $4.2 \times 10^{-7} \text{ cm}^2/\text{sec}$, and it represents the LPAMAM-5/NaPSS complex. For solution at low pH value, pH 3, the diffusion could not be measured because white precipitates form instantaneously upon mixing NaPSS with LPAMAM-5. For solution in basic condition, pH 11, a single exponential decay is evident in the FPR trace, and the measured diffusion coefficient is $1.5 \times 10^{-6} \text{ cm}^2/\text{sec}$.

<table>
<thead>
<tr>
<th>Solution Type</th>
<th>pH 3</th>
<th>pH 9</th>
<th>pH 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>No salt/acid or base</td>
<td>white precipitate</td>
<td>4.16±0.12 (1)</td>
<td>15.22±0.13 (1)</td>
</tr>
<tr>
<td>140 mM NaCl / acid or base</td>
<td>2.37±0.11 (0.70) $^a$</td>
<td>1.73±0.12 (0.34)$^a$</td>
<td>9.24±0.37 (1)</td>
</tr>
<tr>
<td></td>
<td>13.90±0.94 (0.30)$^b$</td>
<td>32.90±1.98 (0.66)$^b$</td>
<td></td>
</tr>
<tr>
<td>360 mM NaCl / acid or base</td>
<td>1.72±0.18 (0.5)$^a$</td>
<td>2.45±0.17 (0.2)$^a$</td>
<td>13.56±0.12 (1)</td>
</tr>
<tr>
<td></td>
<td>24.20±1.95 (0.5)$^b$</td>
<td>39.6±0.68 (0.8)$^b$</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ numbers in parenthesis show mode amplitude of slow mode

$^b$ numbers in parenthesis show mode amplitude of fast mode
It appears that LPAMAM-5 does not complex with NaPSS at this high pH condition. Secondly, solutions with salt and different pH values, pH 7 and pH 3, have shown two exponential decay modes as introduced in the previous section, slow and fast. In both solutions at 140 mM NaCl and 360 mM NaCl, the diffusion coefficients of fast mode are decreased as pH values are decreased from pH 7 to pH 3 while the diffusion coefficients of slow mode are nearly unchanged. Thirdly, solutions in salt and pH 11 have exhibited only one exponential decay in the FPR trace indicating no interaction between NaPSS and LPAMAM-5.

Here we have observed pH effect on complex formation and recognized that pH is a very important variable like salt, and appropriate pH adjustment may be required to make the needed complexes.

3.3.2.4 THE CRITICAL MOLAR RATIO OF NaPSS TO LPAMAM-5 TO MAKE COMPLEX

In the above experiments, the complex formation between oppositely charged polyelectrolytes has been confirmed and the observed results indicate that the electrostatic interaction is relatively sensitive to ionic strength and pH adjustment. In addition, the soluble or insoluble (precipitate) complexes have been made by adjusting two variables, salt and pH. In general, there are two kinds of polyelectrolyte complexes, stoichiometric and nonstoichiometric (3.51, 3.25). Stoichiometric complexes are called "coacervates" or "precipitates", and
tend to be insoluble in the medium in which they were formed. In contrast, nonstoichiometric polyelectrolyte complexes are soluble and remain ionized even when the charges are completely neutralized in stoichiometric complexes. Here we have focused on nonstoichiometric complexes, and the critical fraction of polyelectrolyte concentration which may need to make this soluble complex is considered. Eight different fractions of polyelectrolyte concentration are made by varying the concentrations of either NaPSS or LPAMAM-5.

Figure 3.11 shows the diffusion coefficients of complex and/or LPAMAM-5 versus the molar ratio of NaPSS to LPAMAM-5. This experiment has been made at salt free solutions. At the low ratio of NaPSS to LPAMAM-5 less than 0.02, the determined diffusion coefficient is very close to that of free LPAMAM-5 indicating no complex formation. When the ratio is increased to 0.06, two diffusion modes begin to appear. This indicates that the complexation becomes prevalent when the ratio of [NaPSS] to [LPAMAM-5] is higher than 0.06. Two modes are continued until NaPSS concentration is increased to the ratio of 0.22. It is clear that two modes, slow and fast, are evident at the ratio between 0.06 and 0.22; the fast mode is from unbound LPAMAM-5 and the slow one is from complex formed. This suggests that more NaPSS molecules are required to complex with the remaining unbound LPAMAM-5
Figure 3.11 The diffusion coefficients of complex and LPAMAM-5 with polymer concentration in water (the numbers in parenthesis represent the normalized amplitudes obtained at one K value).
remained (without remaining unreacted LPAMAM-5). In the higher concentrations of NaPSS, at ratios higher than 0.73, only one diffusion mode is observed and its value is close to, but somewhat larger than, that of complex with the ratio between 0.06 and 0.22. The reason is that when NaPSS concentration is high enough, all LPAMAM-5 can bind to NaPSS and no free LPAMAM-5 remains providing one diffusion mode of complex. When the concentration of NaPSS is lowered, some of LPAMAM-5 is then remained unbound and complex with less particles of LPAMAM-5 shows relatively faster diffusion than that with more LPAMAM-5 molecules. The minimum and maximum fraction of NaPSS to LPAMAM-5 to initiate complex formation are 0.6 and 7.3 to 10, respectively. These minimum or maximum numbers may be changed if the complex is made in salt solution. It would be another interesting research project if the critical ratio of NaPSS to LPAMAM-5 is studied under salt solutions.

3.4 SUMMARY

The interaction between oppositely charged particles in different salt, pH, and polymer concentration has been considered. The experimental results can be summarized.

(1) the negatively charged NaPSS interacts with positively charged LPAMAM-5, and this interaction leads to soluble or insoluble complexes.
(2) the complex formation is sensitive to the changes of both ionic strength and pH.

(3) the slow diffusion mode at all salt concentrations is due to LPAMAM-5/NaPSS complex.

(4) the fast diffusion mode at low salt comes from LPAMAM-5 unbound.

(5) the fast diffusion mode at high salt can not be fully accounted for, but possibilities are: i) free FITC dye unremoved on dialysis, ii) the broken pieces of LPAMAM-5 or NaPSS, iii) compressed unbound LPAMAM-5.

(6) complex formation is dependent on pH, and at high pH, the electrostatic interaction is too low to make complex and no complex is formed both in water and salt.

(7) at low pH, LPAMAM-5 has H⁺ ions on the surface which can increase the electrostatic forces, and the white precipitates is made at pH 3 in water.

(8) the complex formation requires the minimum fraction of NaPSS to LPAMAM-5.

3.5 RECOMMENDATIONS FOR FUTURE STUDY

The salt effect on complex formation was evident, but the fast mode at high salt limit could not be interpreted. To find its explanation, several
experiments are suggested: 1) Measurement of diffusion coefficient of FITC dye with added salt, 2) Measurement of the diffusion coefficient of complex between labeled NaPSS and unlabeled PAMAM-5 instead of labeled PAMAM-5 and NaPSS.

3.5 REFERENCES

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3.53 Private communication with P. L. Dubin.


CHAPTER 4

GELATION OF ARBOROL CASCADE POLYMERS
4.1 INTRODUCTION

Nature likes to make its replication generation by generation. Two synthetic groups, Tomalia's and Newkome's, have followed nature, and made the highly branched polymers, called cascade polymers and arborols, respectively (4.1, 4.2). These macromolecules have attracted much attention because of their potential applications, and several different types of cascade polymers have been made (4.3-4.9). In addition, the U.S.Army has recently focused on these cascade polymers for their numerous potential military applications, such as coatings of clothes for chemical and biological agent resistance, adhesives, chemical and biological agent sensors, and strong body armor for corrosion prevention (4.10). All these possible applications are due to the unique properties of cascade polymers, such as, precise and controllable molecular dimensions, high surface reactivity, and functionalities of interior and exterior. Synthetic methods and details of applications are introduced in Chapter 2. In general, all of these "cascade" macromolecules are synthesized according to the same principles, and have the same tree-like topology. As stated above, lots of efforts have been devoted to these cascade polymers. However, this area still needs more considerations in investigating the physical characteristics of cascade polymers.
In this chapter, arborols cascades, prepared by Newkome's groups are studied. These polymers strongly aggregate, and may also make gels.

Newkome et al. first reported one directional arborols which had only one branched arm attached to the central core (Figure 4.1). Later, the application of improved synthetic techniques allowed them to replace two (4.11, 4.12), three (4.13), and four (4.14-4.17, 4.19) cascade spheres (Figures 4.2, 4.3, and 4.4). They also have tried to vary the core from alkane to biphenyl, spirane (4.18), benzene (4.13) and adamantane (4.19, 4.20). According to them, the physical and chemical properties of arborols can be easily manipulated by the variation of core moiety, the length of bridging backbone, and the terminal groups. For example, the greater size of the spirane core and the large steric demand of the biphenyl do not allow the units to get close enough to make aggregation leading to a gel. Therefore, it is required that each of the arborols be studied precisely. Here, we have investigated several different two directional dumbbell shaped arborols in which two hydrophilic terminal groups are attached to a lipophilic hydrocarbon skeleton. They are abbreviated as \([m]-n-[m]\) arborols, where \(m\) represents the number of terminal hydroxyl groups for each sphere and \(n\) denotes the size of the hydrocarbon bridge. Here we have focused on one of physical properties, gelation, whose utility has been demonstrated for various applications (4.21). The topology of this molecules is well studied by computer molecular modeling (4.12) and looks like the tree
Figure 4.1 The structure of one directional arborol (ref. 4.12).
Figure 4.2 The structure of two directional arborol (ref. 4.12).
Figure 4.3 The structure of three directional arborol (ref. 4.13).
Figure 4.4 The structure of four directional arborol (ref. 4.19).
having symmetry with its root. Some of the physical properties of these
dumbbell shaped arborols have been discovered previously in our group by
means of light scattering, viscometry, and optical microscopy (4.11, 4.12). The
thermally reversible aqueous gel formation of [9]-n-[9] and [6]-n-[6] arborols
in which n is between 10 and 13 and 8 and 13, respectively, has been observed.
Much stiffer gels have been made by MeOH and water mixed solvent. This
gelation was also visualized by the electron microscope, and the long fibrous
rod structure was reported. They suggested that this gelation occurred through
aggregation and was constructed by either orthogonal criss-cross or
nonorthogonal helical or scissors-like stacking. Based on the previous
observations and suggestions, this study considers the phase transition point
from gel to fluid of three different arborols, [6]-13-[6], [9]-13-[9], and [9]-12-
[9] Light Scattering (LS) and Differential Scanning Calorimeter (DSC). Small
Angle X-ray Scattering (SAXS) was used to determine the cross section radius
of gyration and the average distance between particles for [6]-10-[6], [6]-11-
[6], [6]-13-[6], and [9]-13-[9] arborols. The structure of [9]-12-[9] arborol
gels was visualized by Freeze Fracture Electron Microscope (FFEM).

4.2 SAMPLE PREPARATION

[9]-n-[9] arborols

Arborols were provided to us by professor George R. Newkome of the
Department of Chemistry at the University of South Florida. They were
synthesized through two steps: nucleophilic substitution and amidation. An
electrophilic substitution was first proceeded by adding the appropriate α, ω-
dibromoalkane to triethyl sodiomethanetricaboxylate, NaC(CO₂Et)₃, in C₆H₆
and dimethylformamide, DMF, solvent mixture (1:1) at 90 °C for 24h to
produce hexaethylic ester. The reaction of hexaethyl ester with tris-
(hydroxymethyl)aminomethane (Tris) in Me₂SO at 70 °C followed, and
anhydrous K₂CO₃ was then added to produce two directional [9]-n-[9] arborols
via amidation.

[6]-n-[6] arborols

The tetraester, instead of hexaester for [9]-13-[9], were first made by the
reaction of a α,ω-dibromoalkane with an excess of dimethyl malonate in DMF
with K₂CO₃. The same amidation procedure as for [9]-13-[9] arborols followed
by adding Tris in Me₂SO and anhydrous K₂CO₃ to tetraester.

Making arborol gels

White powdered arborols were vacuum dried for 12 hours at 40 °C
before use. An appropriate amount of clean methanol, which was filtered
through a 0.1 μm VV type Millipore filter and centrifuged overnight, was added
to the dried arborols. The same volume ratio of clean, deionized water was then
added to the above arborol solutions. This sample was made in an unsilanated
light scattering cell because the silanated cell might prohibit adhesion to the wall of tube. The test tube containing the sample was inserted into water bath where a thermometer and a stirring bar were placed. The sample was slowly heated until all arborols dissolved to make a homogeneous solution. All of the arborols dissolved below 80 °C. The completely homogeneous solutions were then taken out from the hot water bath, and allowed to cool at room temperature to make gels. Four different concentrations of [6]-13-[6] arborol gels were prepared for SALS measurement: 3.5 %, 5.6 %, 8.4 %, and 10.2 %. Three different concentrations of [9]-12-[9] arborol gels were also made for SALS measurement: 3.2 %, 5.3 %, and 7.4 %. For LS measurement 5.2 % of [9]-13-[9] and 2.5 % of [6]-13-[6] arborol solutions were prepared. SAXS was conducted on 2 % freeze-dried samples of [6]-n-[6] (n = 10, 11, 13) and [9]-13-[9] arborols. For DSC [6]-13-[6] arborol gel with 5.6 % of concentration was considered. Most of gels were made in 5 or 10 minutes after cooled down to room temperature. The gels are destroyed by heating, and may reform upon cooling. However, after several heating and cooling cycles, gel formation is not assured.

4.3 EXPERIMENTAL METHODS

**Differential Scanning Calorimeter**

Differential Scanning Calorimeter (DSC) has been widely used to identify the polymer melting (4.26, 4.29). The transition point from gel to fluid
was determined by DSC (Seiko instrument II DSC 120). DSC was first
calibrated using Indium (In) and Lead (Pb) standards prior to the measurements.
A small portion of pre-made gel (about 15mg) was loaded on Aluminum DSC
pans (AL15, P/N 560-002), and covered with aluminum DSC pan cover
followed by crimping with the sample sealer. The safely sealed DSC pan was
placed on the sample holder and methanol and water mixture solvent on the
reference holder in the DSC module. The temperature range considered was
from 25 °C to 100 °C with 2 °C/ min heating rate. The transition temperature
was determined by taking the intersection of baseline and peak slopes. Enthalpy
measurement (ΔH (mJ/mg)) is also available from the peak area.

**Small Angle Light Scattering**

Small Angle Light Scattering (SALS) has been used for the melting and
crystallization of polymer (4.22, 4.23, 4.25). In this study, the kinetics of phase
transition is pursued by means of SALS. The apparatus used is shown in Figure
4.5. The scattering set up consisted of a red (670 nm) laser light source, a
Mettler hot stage (FP 82) that was controlled by computer and was able to heat
up to 300 °C, Mettler Central Processor (FP80), and diode detector with a filter
attached. Both hot stage and diode detector were connected to an IBM
compatible computer through a Central Processor to measure the scattered light
Figure 4.5 Small angle light scattering apparatus.
quantitatively. The experimental principle and procedures are as follows.

Briefly, a red laser beam hits the sample in hot stage and the scattered light is captured by the detector. The scattered light from the sample is quantitatively measured and shown on computer screen as the sample is heated or cooled. The ramping rate used was 10 °C/min and the temperature ranges were from 25 °C to 90 °C for the most of samples. The premade gel was placed on a piece of microscope slide, covered with Corning cover glass and sealed with silicone grease. Lower concentration gel samples that were too fluid were loaded into prewarmed rectangular cells (Vitro Dynamics) with a pathlength of 0.1 mm, and flame sealed. The slides or cells containing samples were inserted into a hot stage. The heating or cooling process was controlled by computer. The measurement was made with the room lights off. A 670 nm bandpass filter before the detector reduced the residual stray light. The scattering angle, 10 degrees, was determined by the scale shown in Figure 4.5. The transition point was determined by meeting of the extrapolation of baseline and slope in scattered intensity vs temperature plot.

**Light Scattering**

The Light Scattering (LS) apparatus shown in Figure 2.1 was used. Details about the instrument were described in Chapter 2. A sample was made in a regular clean unsilanated light scattering cell, and filtered directly into a
clean preheated cell through a 0.2 μm Gelman filter while the sample was hot. The scattering cell containing the hot sample was placed in the LS instrument connected to water bath keeping temperature at 25 °C. The scattered intensity of sample was measured as a function of time at a scattering angle of 90 degrees.

**Freeze Fracture Electron Microscopy**

Freeze Fracture Electron Microscopy (FFEM) is an electron microscope technique which allows replication of the fractured surfaces of frozen specimens. FFEM has provided three dimensional information in the study of internal aspects of the lipid bilayers of membranes. The general principles and experimental set up are described in detail in ref. 4.24. The measurements of FFEM for the [9]-12-[9] arborol gels (2.2 % and 3.2 %) were performed by Ms. Laura Younger in Professor W.G. Henk's group at the LSU School of Veterinary Medicine. The following describes the experimental procedures used. The arborol gels were plunge frozen in liquid nitrogen at -196 °C. Frozen samples were fractured in a Baltzers BAF 400 freeze fracture apparatus by passing a cold moveable knife (razor blade) across the surface of the frozen samples. Etching followed for 4 minutes at sublimation conditions (-100 °C at 10⁻⁷ torr) and platinum/carbon was deposited on the fractal surface of the specimen. The replica (the thin platinum/carbon cast of the fractured specimen
surface) was digested using warm methanol/water (50/50) mixture and washed with distilled water. The specimen-free replica was then placed on a copper grid and allowed to dry. The dried replica was examined using a Philips 410 transmission electron microscope.

**Small Angle X-ray Scattering**

Small Angle X-ray Scattering (SAXS) provides valuable information about sizes and structures, and it is also applied for wide ranges of states of materials, such as, liquid, solid, and crystalline (4.33). The principles and instruments are introduced elsewhere (4.30, 4.34). Gelation processes have been investigated with SAXS (4.35, 4.36, 4.37). The general scattering regime is plotted in Figure 4.6. In the very low angle regime, the radius of gyration, \( R_g \), (or correlation length for concentrated solution, \( \xi \)) is determined from the slope of \( \log I(q) \) vs \( q^2 \), which is called Guinier plot. From the moderate angle regime, the cross section radius of gyration, \( R_c \), is obtained from the linear slope of \( \log [qI(q)] \) vs \( q^2 \) plot. A bump at high angle regime gives the information about the distance between particles, \( a \).

For the gelation of arborol gels, SAXS was performed at Oak Ridge National Laboratory with a pinhole camera using a 64 x 64 2D area detector. The Cu-K\( \alpha \) line was used (\( I = 1.54 \text{ Å} \)). The scattering wavevector covered was from 0.005 to 0.5 Å\(^{-1}\). The samples were packed in 0.5 mm path length cell or a
slice of uniform fixed thickness. The volume and weight and thickness of the samples were carefully measured. Absolute intensity is calibrated by using a standard "Lupolen" sample from Oak Ridge and converted to scattered cross section per unit volume.

4.4 RESULTS AND DISCUSSION

Several different types of two directional dumbbell shaped arborols have been considered: [6]-10-[6], [6]-11-[6], [6]-13-[6], [9]-12-[9], and [9]-13-[9]. Most of them have formed gels at concentrations as low as 2 wt % in MeOH/H2O mixtures (50/50 volume ratio). The gelation was initially checked by a simple conventional tilting test, in which the sample container (usually a test tube) was turned upside down. Gelation was indicated when no visual flow was occurred within ten seconds. The scattered intensities of [9]-13-[9] and [6]-13-[6] arborols prepared at 80 °C and placed directly into the LS instrument at 25 °C were measured. The scattered intensity increased as a function of time (Figures 4.7 and 4.8) indicating aggregation. This aggregation was regarded as the initial step of gelation (4. 11). Both observations of no visual flow of sample in tilting test and the increased scattered intensity in LS measurement clearly confirm that arborol makes a gel through aggregation.

The aggregates can be described by fractal dimension with scattering techniques. The fractal concept has been used to understand nonequilibrium
Figure 4.6 Schematic diagram of general scattering regime. Low angle regime gives the information about the radius of gyration, $R_g$; moderate regime is for the cross section radius of gyration, $R_c$; high angle regime provides the distance between neighboring particles.
growth and aggregation processes by defining the relationship between mass and length scale through the exponent called the fractal dimension (4.27, 4.28):

\[ M = L^{D_f} \]

where \( M \) is mass, \( L \) is a characteristic length, and \( D_f \) is the fractal dimension. In general, the scattered intensity is measured at several different scattering angles, and the slope of the plot of scattered intensity versus scattering angles represents the fractal dimension:

\[ \log I = -D_f \log q \tag{4.1} \]

where \( I \) is the scattered intensity and \( q \) is the scattering vector (\( q = 4\pi \sin(\theta/2)/\lambda_o \)), where \( \theta \) is the scattering angle and \( \lambda_o \) is the wavelength of incident light).

The scattered intensity of \([9]-12-[9]\) arborols was measured at 22 different scattering angles ranging from 15 to 120 degrees at \( \lambda_o = 514.5 \) nm. The solvent scattering was subtracted from solution scattering. The fractal dimension, 1.9, was determined from the slope of plot \( \log I \) vs \( \log q \) in Figure 4.9.

The transition point of sample from gel to fluid was measured by small angle light scattering (SALS). Figure 4.10 through Figure 4.16 show how the scattered intensities of arborol gels change as temperature is increased. The transition point is determined by three ways: (1) the point at which the extrapolation of the upper baseline and the slope meet (\( T_1 \)); (2) the middle point between the extrapolation of the lower plateau (the baseline) and the upper
Figure 4.7 Scattered intensity of [9]-13-[9] arborols (5.2 %) as a function of time.
Figure 4.8 Scattered intensity of [6]-13-[6] arborols (2.5 %) as a function of time.
Figure 4.9 Scattered intensity vs scattering angle for [9]-13-[9] arborols (5.2 %). Fractal dimension, $D_f$, is obtained from the slope of this plot.

Slope $= - (1.9 \pm 0.07)$
plateau ($T_2$); (3) the point at which the extrapolation of the lower baseline and the slope meet ($T_3$). $T_1$ and $T_2$ may be considered as the starting and ending point of melt, respectively. $T_3$ is the intermediate melting point. The baselines in Figures 4.7 and 4.12 are not completely flat but fluctuating. The fluctuation of the scattered intensity above 80 °C is due to the refluxing solvent (the boiling point of solvent is 80 -85 °C measured by Siwoloboff method in General Chemistry). Therefore the nonflat baselines above the solvent boiling point shown do not affect the reading of transition point. The determined transition points of [6]-13-[6] and [9]-12-[9] arborols at several different concentrations are summarized in Table 4.1. The transition point from gel to fluid does not depend systematically on the polymer concentration. The phase transition temperature from gel to fluid was also determined by DSC for [6]-13-[6] arborols (5.6 % ), and is shown in Figure 4.17. The starting temperature was ambient (about 25 °C) because there was no cooling accessory attached to DSC.

Figure 4.17 shows a big sharp main peak and little one. The little one is not from the transition. Originally it should be flat as a baseline, but it isn’t because the starting point is very close to the transition temperature. If the measurement starts from -20 °C, for example, this will be flat. The determined transition temperature is 53 °C and is close $T_2$ for [6]-13-[6] arborols (5.6 %) in SALS measurement. The enthalpy of melting, 16.1 mJ / mg
(or 13KJ / mol), was obtained from the area of the main peak.

Table 4.1 The transition points of [9]-12-[9] and [6]-12-[6] arborol gels by SALS.

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Transition point (°C) of [9]-12-[9] arborol gel</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_1^a$</td>
<td>$T_2^b$</td>
<td>$T_3^c$</td>
<td></td>
</tr>
<tr>
<td>3.2</td>
<td>43</td>
<td>52</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>5.3</td>
<td>36</td>
<td>53</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>7.4</td>
<td>38</td>
<td>54</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Concentration (%)</td>
<td>Transition point (°C) of [6]-13-[6] arborol gel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$T_1^a$</td>
<td>$T_2^b$</td>
<td>$T_3^c$</td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>36</td>
<td>49</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>5.6</td>
<td>36</td>
<td>54</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>8.4</td>
<td>35</td>
<td>49</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>10.2</td>
<td>33</td>
<td>51</td>
<td>65</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Transition point determined from the intersection between upper baseline and slope.  
$^b$ Transition point determined from the middle point between upper and lower baseline.  
$^c$ Transition point determined from the intersection between lower baseline and slope.

SAXS results are shown in Figure 4.18 in which the scattering patterns from dried arborol gels at high angles are overlayed. The presence of a bump in the scattering curve is related to the average distance, $a$, between neighboring
Figure 4.10 Scattered intensity with increasing temperature for [6]-13-[6] arborol (3.5%).
Figure 4.11 Scattered intensity with increasing temperature for [6]-13-[6] arborol (5.6%).
Figure 4.12 Scattered intensity with increasing temperature for [6]-13-[6] arborol (8.4%).
Figure 4.13 Scattered intensity with increasing temperature for [6]-13-[6] arborol (10.2 %).
Figure 4.14 Scattered intensity with increasing temperature for [9]-12-[9] arborol (3.2%).
Figure 4.15 Scatterred intensity with increasing temperature for [9]-12-[9] arborol (5.3%).
Figure 4.16 Scattered intensity with increasing temperature for [9]-12-[9] arborol (7.4%).
Figure 4.17 Transition point from gel to fluid in DSC measurement for [6]-13-[6] arborol (5.6%).
particles (4.30). To determine "a" for the each sample, all scattering curves in
Figure 4.18 are presented separately in Figure 4.19 through Figure 4.22. The
value of "a" is calculated from the maximum q, q_{\text{max}}, at which the bump has the
maximum point:

\[ a = 2\pi / q_{\text{max}} \]  \hspace{1cm} (4.2)

To determine the exact value of "a", the highest and next highest points of q
were selected and their average was calculated with error estimates.

The scattering pattern at low q is shown in Figure 4.23. The apparent
radius of gyration, R_g, (or correlation length in concentrated solution) was
estimated from the slope of the curve of log I vs q^2. In principle, R_g is
calculated from the initial slope by the approximate law of Guinier (4.30):

\[ \log I(q) = -(R_g^2 / 3) q^2 \log_{10} e + \beta q^4 + \ldots + \text{constant} \]  \hspace{1cm} (4.3)

at q = 0, equation (4.3) can be written as

\[ \log I(q) = -(R_g^2 / 3) q^2 \log_{10} e \]  \hspace{1cm} (4.4)

The inset in Figure 4.23 shows the magnification of the initial part of the
slope in which R_g is calculated. As can be seen, the real linear regime for R_g is
is not shown at this low angle measurement. Thus the exact value of R_g is not
available.

The cross section radius of gyration, R_c, is obtained from the moderate q
measurement using Guinier approximation as introduced by Cohen's paper
(4.31): \[ I(q) = \frac{A}{q} \exp\left(-\frac{R_c^2}{2}\right) q^2 \] (4.5)

where \( A \) is a constant. \( R_c \) is calculated from the slope of log [\( qI(q) \)] vs \( q^2 \) plot (Figure 4.24). Several appropriate intermediate regimes were selected to take linear slope, and the average values with estimated errors were obtained. The determined values of \( R_c \), and "a" are summarized in Table 4.2.

The cross section radius of gyration seems to increase with the number of carbon bridge atoms. However, their values are within the noise almost. Table 4.2 shows that the longer carbon bridge (\( n=13 \)) has the larger value of "a" than the shorter one (\( n=10 \) or 11). This is because the large size of arborol gels made with aggregation of arborol molecules with \( n=13 \) does not allow the neighbor gels to come close due to its steric effect. Therefore, the small value of "a" for arborol gels with \( n=10 \) or 11 is due to less steric hindrance.

The morphologies of [9]-12-[9] arborol gels (2.2 % and 3.3 %) were visualized with FFEM. This technique has some advantage over conventional electron microscope in that it can picture not only the surface but also deep inside of gels.

Figures 4.25(A)-(D) clearly show a fibrous rod like structure. The same fibrous rod like structure has been observed for [9]-10-[9] arborols with
electron microscope (4.11, 4.12). The rods in Figure 4.25 have uniform
diameter, and the size, 30 Å, is close to the end to end distance of each [9]-12-[9] arborols molecule.

Table 4.2 The determined values of \( R_c \), and "a" of [6]-n-[6] (n = 10, 11, 13) and [9]-13-[9] arborols.

<table>
<thead>
<tr>
<th>Arborol</th>
<th>( R_c ) (Å)</th>
<th>a (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[6]-10-[6]</td>
<td>30±1.2</td>
<td>18±0.4</td>
</tr>
<tr>
<td>[6]-11-[6]</td>
<td>33±1.1</td>
<td>19±0.1</td>
</tr>
<tr>
<td>[6]-13-[6]</td>
<td>34±1.1</td>
<td>26±0.4</td>
</tr>
<tr>
<td>[9]-13-[9]</td>
<td>37±0.5</td>
<td>23±0.4</td>
</tr>
</tbody>
</table>

4.5 SUMMARY

Several of two directional dumbbell shaped arborols ([9]-n-[9] and [6]-n-[6], n = 10-13) were studied. Gelation was confirmed through the tilting test and the intensity measurement in LS. Aggregation appeared to occur prior to gelation. The transition point from gel to fluid is between 35 to 70 °C, and it does not depend on the concentration of gels. The cross section radius of gyration was constant. The distance between neighboring gels increased with the number of carbon bridge due to steric effect. The radius of gyration is usually obtained from low angle SAXS regime. This study is not able to give
Figure 4.18 Scattered intensity vs scattering vector at high angles in SAXS.
Figure 4.19 Scattered intensity vs scattering vector for [6]-10-[6] arborol (2 %) at high angles in SAXS.
Figure 4.20 Scattered intensity vs scattering vector at high angles in SAXS for [6]-11-[6] arborol (2 %).
Figure 4.21 Scattered intensity vs scattering vector at high angles in SAXS for [6]-13-[6] arborol (2%).
Figure 4.22 Scattered intensity vs scattering vector at high angles in SAXS for [9]-13-[9] arborol (2%).
Figure 4.23 Scattered intensity patterns for [6]-n-[6] (n=10, 11, and 13) and [9]-13-[9] arborols at low angles in SAXS. Inset shows the magnification of the slope where the $R_g$ is obtained. The real linear regime is not shown so that the true $R_g$ is not available from this plot.
Figure 4.24 Scattered intensity multiplied by $q$ vs scattering vector. The slope gives the value of $R_c$. 
Figure 4.25 The structures of [9]-12-[9] arborol gels from FFEM ((A) 2.2 % at 21,500 x, (B) 2.2 % at 112,000 x, (C) 3.2 % at 180,000 x, (D) 3.2 % at 112,000 x).
Figure 4.26(a)-(c) DLS measurement for [9]-12-[9] arborol gels (2\%) with increasing temperature.
Figure 4.26(d)-(e) DLS measurement for [9]-12-[9] arborol gels (2 %) with increasing temperature.
the answer for $R_g$, because no real linear Guinier regime was found. The FFEM pictures show the fibrous rod-like structure of arborol gels.

4.6 RECOMMENDATIONS FOR FUTURE STUDIES

The gelation of arborols has been investigated with several techniques. The transition point from gel to fluid was determined by intensity measurement of light scattering and DSC experiment. However, this gel point may be also determined by another technique, dynamic light scattering (DLS). There have been several results for gelation by DLS (4.38-4.40). Lang and Burchard observed the power law behavior at gel point with DLS. During the course of these studies, the gelation of arborol gels with increasing temperature has been performed by DLS. Figure 4.26(a)-(e) show DLS results, and they are promising a future study. In addition, neutron scattering experiment is recommended for the determination of radius of gyration of arborol gels that was not available from SAXS.

4.7 REFERENCES


CHAPTER 5

CONCLUSION
Polyamidoamine cascade polymers (PAMAMs) have been characterized. Their small size, water soluble, and spherical structure appear to be appropriate for probe diffusion studies as probes. Their behavior, after the attachment of fluorescent dye, also attracts much attention. Lower generation (Generation 3) shows monodispersity both in water and in salt. The size determined from CONTIN is close to the advertised one. Possible solution nonidealities and chain expansion at low or zero added salt are not pronounced. Therefore, the small generation PAMAM can be used as the probe in any conditions. However, the intermediate size PAMAMs have shown the solution nonidealities at low or zero added salt. Their increased size and scattered intensity with added salt proved the above phenomena. The chain expansion was observed at low pH and salt. Fluorescence Photobleaching Recovery (FPR) technique is recommended for the measurement of diffusion coefficient of interesting species that show the solution nonidealities. Dynamic Light Scattering (DLS) can be used unless samples are studied in low or zero added salt. The attachment of dye causes a chain contraction providing smaller size but still holds the promise as probes.

The fully characterized PAMAM-5 labeled with fluorescent dye (LPAMAM-5) was used again for the study of complex formation as a probe. The slow diffusion coefficient of LPAMAM-5, after adding sodium polystyrene
sulfonate (NaPSS), indicates the formation of complex between positively charged LPAMAM-5 and negatively charged NaPSS. This complex formation was sensitive to added salt: two diffusion modes, slow and fast, were evident, and at low salt, the fast diffusion coefficient is close to that of LPAMAM-5 while the values at high salt is close to dye attached. The slow modes caused from complex at all concentrations were almost constant. Different pH values affected the complex formation. Low pH condition makes more protonated terminal amine groups on LPAMAM-5 and accentuate the electrostatic interactions that allow all LPAMAM-5 to bind to NaPSS. In contrast, high-pH values disturb the interaction and less LPAMAM-5 is bound to NaPSS or no complex formed.

Cascade polymers that make aggregation are also important in gelation process. Two directional dumbbell shaped arborol cascade polymers were studied, and the transition point from gel to fluid was measured by differential scanning calorimeter and small angle light scattering. The transition point was nor dependent on the gel concentration. The cross section radius of gyration was close to each other for arborols with different numbers of carbon bridges. The average distance of neighboring particles were reduced for arborols with less carbon bridges. The freeze fracture electron microscope pictures show the fibrous rod-like structure for arborol gels.
APPENDIX

THE STABILITY OF FLUORESCENCE PHOTOBLEACHING RECOVERY INSTRUMENT
The stability of the fluorescence photobleaching recovery (FPR) instrument was checked by measuring ac signals. For the measurements, a labeled Jeffamine, referred to be LJFM, with molecular weight 20,000 was used. This jeffamine was labeled with fluorescein isothiocianate dye (FITC) under the same labeling procedure for LPAMAMs introduced in Chapter 2. The free dye around was completely removed through a home made Sephadex G-75 column (labeled jeffamine was clearly separated from free dye in column). LJFM was loaded in the Vitro dynamics capillary tubes and flame sealed.

First, the reproducibility of FPR instrument was considered under the same experimental conditions such as the same sample, bleaching time, intensity, objective, and RonchiRuling. Four runs were repeated, and the ac(0) signals are represented in Figure A.1, indicating that the machine is well reproducible. Next the variance of ac(0) was measured at different bleaching time but with the same experimental conditions (Figure A.2). The initial ac(0) is very sensitive to bleaching time, and increases with that. Several more data points seem to be required at short bleaching time, but an exposure shorter than 0.3 sec did not bleach LJFM sample. Figure A.3 shows the ac(0) with increased intensity of light. Ac(0) is dependent on the intensity. Several data points appear to be missed again at lower intensity. However, the sample was not
Figure A.1 The reproducibility of FPR instrument (Objective=7, RR=50 lines/inch, Intensity of light=1W, Bleaching duration=0.6 s).
Figure A.2 Ac(0) at different bleaching duration (Objective=7, RR=50 lines/inch, Intensity of light=1W). Ac(0) = (Maximum value of Ac - fitted baseline).
Figure A.3 Ac(0) at different intensity of light (Objective=7, RR=50 lines/inch, Bleaching duration=0.6 s).
bleached at extremely low intensity of light, such as 0.5 W or less. In principle, ac(0) at 0 w should be zero. Therefore the Figure A.2 may have curvature at near zero intensity. Based on Figures A.1, A.2, and A.3, it turns out that FPR instrument is reasonably reproducible, and this leads to confidence of the amplitude measurements of Chapter 2 and Chapter 3.
Keunok Han Yu was born in the southern part of South Korea, Chonbuk, on Dec. 27, 1961. She received a B.A. degree in Chemistry from Chonbuk National University in 1984, and her M.S. degree in Chemistry from Chonbuk National University in 1986. She entered Louisiana State University as a doctoral student in Summer of 1990. Her interested field is a Macromolecular in Chemistry, and her graduate adviser is Dr. Paul S. Russo.
Candidate: Keunok Han Yu
Major Field: Chemistry
Title of Dissertation: Solution Behavior of Cascade Polymers

Approved:

[Signatures]

Major Professor and Chairman
Dean of the Graduate School

Examinining Committee:

[Signatures]

Date of Examination:

September 27, 1995