SELF-ASSEMBLING BEHAVIOR OF DENDRIMERS AND NANOCAGES IN SOLUTION DRIVEN BY COUNTER-ION MEDIATED INTERACTIONS

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SELF-ASSEMBLING BEHAVIOR OF DENDRIMERS AND NANOCAGES IN SOLUTION DRIVEN BY COUNTER-ION MEDIATED INTERACTIONS

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ABSTRACT

It has been demonstrated that polyoxometalates (POMs), which are large ions (macroions) sized between 1 to 10 nm, possess unique solution behaviors. POMs in solution can spontaneously self-assemble into hollow spherical structure, with the main driving force being counter-ion mediated attraction. Moreover, it’s been demonstrated that POMs with different charge densities would demonstrate a unique self-recognition behavior. Macroions other than POMs, if within the same size range and bearing similar charges, are expected to show similar behavior as similar counter-ions mediated interactions are present in the solution. The specific research target of this thesis would be the solution behavior of anionic dendrimers and nano-sized metal-organic nanocages, as they are also stable nano-sized macroions like POMs. Yet unlike POMs, the polydispersity and flexibility of those macroions will bring in more complexity. In this research, techniques including Dynamic Light Scattering (DLS), Static Light Scattering (SLS) and Transmission Electron Microscopy (TEM) will be employed to study solutions of nanocages and dendrimers. It’s discovered that the self-assembling and self-recognition behavior also exists in metal-organic nanocage solution. Similar hollow spherical structure also formed in dendrimer solution when the surface charges of dendrimer were adjusted. The effect of molecule flexibility in the self-assembling process of charged dendrimers will also be considered. The research would help establish better understandings for solution physics of macroions, eventually provide approaches to understand and predict the
behaviors of more sophisticated polyelectrolyte solution. It also might impact current applications of macroion in solutions, such as designing of drug-delivery system.
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CHAPTER I

INTRODUCTION

Most of chemistry in life and in the natural environment occurs in the solution state\(^1\). However, understanding the behavior of complex solution, where critical biological and environmental chemical reactions take place, has always been a challenging issue to physical scientists. The presently best understood solution system is the simple ionic solution. In those solutions, simple ions with size less than 1 nm could be treated as point charges with negligible volume. Adopting this assumption, Debyl-Huckel theory\(^2\) works very well in describing behavior of dilute simple ionic solution and could be extended to higher concentrations. On the other side of ion’s size scale, colloidal systems have been extensively studied as well. In colloidal systems, charged particles with size between 10 nm to 1000 nm are dispersed in the dispersion medium. Due to the intrinsic nature of the large colloidal particles they can no longer be treated as point charges, but approximations could be taken to analyze their behavior. For example, DLVO theory\(^3\) successfully predicts the force between charged surfaces interacting through a liquid medium and explains the aggregation of aqueous dispersions.

Despite the fact that simple ions solution and colloidal dispersions have been extensively studied, systems containing components with sizes between the two categories are not well studied in general. Macro-ions, which are ions with the size between 1 nm to 10 nm, were brought to interest as both Debyl-Huckel theory and
DLVO theory failed to explain their solution behaviors\textsuperscript{4}. The theory to describe macro-ion system is yet to be established, and it would require numerous experimental results to help building or testing a feasible model for the macro-ion system. Such model, if successfully constructed, would greatly enhance our understanding of complex solution system, and eventually help to tackle some of the basic questions in bio-chemistry and physical chemistry\textsuperscript{5-7}.

The purpose of this thesis is to provide experimental evidence of macro-ions’ solution behavior for future theory analysis. The thesis will mainly focus on two specific type macro-ions: nano-cages and charged dendrimers. The mentioned nanocages are giant complex molecules formed by noble metal ions and multi-dentate organic ligands\textsuperscript{8}. Their sizes were around 1 to 2 nm and would carry over 10 charges in aqueous environment. Dendrimers are repetitively branched macromolecules first developed by Newkome, Tomalia and Vogtle\textsuperscript{9}. They are highly symmetric and mono-dispersed polymer molecules. The dendrimers applied in this research were modified to be soluble and bear suitable charges in aqueous environment, and due to their relatively large size they should be categorized as macro-ions in solution. Both nanocages and charged dendrimers are emerging new functional materials and have been studied for various applications\textsuperscript{10,11}. However, their solution behaviors in liquid medium were less studied. So, they were selected as the study objects of this thesis not only because that their unique properties would shed insights in the future macro-ion solution behavior theory, but also because that the solution behavior study would aid researchers to develop future applications of these molecules in the dispersion state.

Previous studies on polyoxometalates (POMs), uranium clusters and several nanocages have demonstrated some of behaviors of macro-ions in solution\textsuperscript{12,13}. One
of the most notable phenomenon is that those macro-ions under certain circumstances tend to self-assembly into a large stable single-layer hollow spherical structure, called “Blackberry” by Liu et al.\textsuperscript{12} Detailed study on the self-assembling process showed that this process is charge-regulated, and the final assembled “Blackberry” structure is thermodynamically stable. The charge-regulated self-assembly is considered to be an important property of macro-ion systems, and would serve as an adequate hint to understand the macro-ions’ solution behavior. In this thesis, self-assembly process of nano-cages and charged dendrimers were also demonstrated under certain circumstances, and the conditions leading to the self-assembly and properties of the formed structure were studied and discussed.

Another noteworthy phenomenon reported in previous publications is the self-recognition of different micro-ions in the self-assembly process.\textsuperscript{14,15} In other words, as defined by Lehn, “mixtures of components yielding defined superstructures without interference or crossover”\textsuperscript{16}. Very few reports were published regarding the self-recognition resulted by long-range interactions like electrostatic interactions. For instance, Liu reported in 2011, that two different Keplerate-type POMs would form two different distinguishable “Blackberries” in aqueous solution.\textsuperscript{14} The reported self-recognition systems were composed of two macro-ions with relatively small difference in charge. In another report, chiral macro-ions with asymmetric parts were shown to self-recognize between two enantiomers. Despite the counterintuitive results presented, the question still remains that how small the difference would be to trigger a self-recognition in the self-assembly of macro-ions. In this thesis, nanocages of different compositions bearing same charges were examined in mixture and their self-recognition behavior were discussed to help answer the above question.

Most of experiments performed in this thesis were done with the aid of
Dynamic Light Scattering (DLS), Static Light Scattering (SLS), Phase Analysis Light Scattering (PALS) and Transmission Electron Microscopy (TEM). Those techniques combined could give information regarding radius, diffusion coefficient, effective charge, and morphology of nanometer sized species in solution.

Basic concepts and previous publications regarding macro-ions’ solution behavior are discussed in Chapter 2. It provides a brief insight of current understanding in this topic. At the end of Chapter 2, several previous studies on the solution behavior of nano-cages and charged dendrimers are presented to demonstrate the need for this study and the significance of discoveries in this thesis.

Chapter 3 contains information regarding sample preparation and instrumental set-ups in this study.

Chapter 4 presents the results and discussion. Section 4.1 discusses the self-assembly of nano-cages in solution state; Section 4.2 discusses the experiment done to testify the self-recognition possibility between two very similar nanocages; Section 4.3 focuses on the solution behavior of charged dendrimers in aqueous environment, the discrepancy with theory prediction and unsolved problems were also discussed in Chapter 4.

In Chapter 5, a summary of the major results of this research will be presented.
CHAPTER II
BACKGROUND

2.1 Introduction to Macro-ions and their solution behavior

Macro-ions are highly charged large ions with size range from 1 nm to 10 nm. Many important molecules or structures that are widely present in nature can be categorized as Macro-ions. For example, human serum albumin (HSA) has a size ranging from 2.5 to 3 nm at different pH, and it has a net charge around -20 in a buffer solution of pH 7. Many other proteins have similar charge and size in solution and could be treated as macro-ions when studying their solution behavior. Another example widely used in industry is cyclodextrins, which are a class of cyclic oligosaccharides. γ-cyclodextrin derivatives modified with phosphate has a size of 1.5 nm and could have around 8 charges when dissolved in deionized water, which is also larger than 1 nm and can be categorized as macro-ions. Some other common macro-ions are pictured and shown in Figure.2.1.
Despite the fact that macro-ions are common present in nature, their solution behavior is not understood very well. The size range of 1 nm to 10 nm contradicts with assumptions of common theories dealing with charges in liquid mediums. The Debye-Huckel theory, which works very well with simple ionic solutions, assumes the ions have negligible volume and can be treated as point charges. This assumption worked well with small ions less than 1 nm, but cannot be applied to macro-ions as macro-ions’ size would be too large to neglect. The DLVO theory quantitatively described the colloidal system with particle size ranging from 10 nm to 1000 nm, considering the competition between attractive van der Waals force and repulsive electrostatic interaction. However, the DLVO theory depicts a thermodynamically instable colloidal system, which is different from the experimental observed thermodynamically stable macro-ion solutions. What’s more, the mean-field assumption taken in both Debye-Huckel theory and DLVO theory might not be true as the anisotropic charge distribution on macro-ions has been shown to affect the solution behavior of macro-ions. In all, both Debye-Huckel and DLVO theory cannot satisfactorily describe the said macro-ion solution. And the size range of 1 nm to 10 nm remains unapproachable to current theory, thus a simple theory describing macro-ion’s solution behavior is yet to be established.
Various attempts have been conducted to study the solution behavior of macro-ions. Experimental evidences have been provided from studies on double-stranded DNA, microtubules and tobacco mosaic viruses. Most of these study targets were polyelectrolytes with complex structure, thus the coexistence of intermolecular and intramolecular interactions other than electrostatic interactions (e.g. hydrogen bonding, hydrophobic interaction and chain entanglement) would prevent these studies to give a clear physical explanation. Moreover, polyelectrolytes are poly-dispersed and cannot be considered as a single type of molecule in solution. Though the experimental results of these studies are intriguing and initiated questions like whether DLVO theory provides a complete description of macro-ion system.

2.2 Previous Studies on a POMs-based Macro-ion Solution System

In searching for an ideal macro-ion candidate of having “well-defined molecular structure (no intramolecular interaction), mass, shape (no polydispersity), charge density (even tunable in a certain range), and no additional salts”, Liu et al first reported the study on the solution behavior of polyoxometalates (POMs). The POMs are built with transition metal oxide polyhedrals connected together with sharing oxygen atoms at the corner of polyhedrals. The atoms in the POMs are connected by covalent bonds, generating a rigid and stable structure. These giant molecules are well-defined in structure, mass, shape and charge density. Lots of them are hydrophilic and can be dissolved in water. Some of typical POMs used in Liu’s study are shown in Figure.2.2 with their detailed formulas.
Figure 2.2 Information on the structure, charge density, and self-assembly behavior of macro-polyoxoanions in aqueous solutions.

When dissolving mentioned \{Mo154\} POMs in water, transmission electron microscopy (TEM) study found that many spherical structures with diameter around 90 nm existed even in very dilute solution. More detailed study revealed that those structures were hollow spherical assemblies of \{Mo154\}, which contained around 1150 \{Mo154\} molecules in a single layer. The distance between each POM molecule was estimated to be less than 1 nm. The structure obtained was named as “Blackberry structure” and is shown here in Figure 2.3. Later studies with controlled solvent conditions showed that the main driving force for this “Blackberry structure” formation was counter-ion mediated electrostatic interaction between POMs molecules, whereas hydrogen bonding, van der Waals force and hydrophobic interactions have very small impact on the \{Mo154\} self-assembly. This counterintuitive result demonstrated that the macro-ions in solution cannot be treated with DLVO theory even without interference of more complicated factors.
In order to theoretically understand this counterintuitive phenomenon, a simple mean-field model was built to show the interaction between macroions in a salt-free solution. The reduced interaction potential between the two macro-ions V(H) was defined as “the energy difference when their separation is decreased from infinity to the distance H, the shortest distance between the surfaces of the two spherical macroions with bound counterions” \(^{25,26}\). It’s demonstrated from the model that in a suitable medium with proper dielectric constant, counter-ion would induce electrostatic attractions between two macro-ions. Though the use of mean-field assumption in macro-ion systems is still debated, this preliminary model would be helpful to understand why micro-ions form said “Black-berry structure” in solvent and other micro-ions’ solution behaviors.
As the most ideal macro-ion candidate has been studied, the attempts to expand the observation to other, more complicated macro-ions have been conducted. One approach is to introduce hydrophobic interaction into the POMs-based system by covalently attach a hydrophobic carbon chain to the POMs. The resulted “giant surfactants” showed interesting properties different from normal ionic surfactants. The effect of hydrogen bonding has also been introduced into the POMs system and the self-assembly process presented an opposite relationship to temperature and solvent composition in the self-assembly process. Also POMs with an anisotropic charge density distribution was studied and the results showed that the charge distribution would have an impact on the final morphology of the self-assembled structure. In all, the self-assembly process served as an adequate probe process, allowing researchers to investigate relationship between macro-ion structure and solution behavior, and eventually contributing to a better understanding of macro-ions’ solution behavior.

2.3 Macro-ion Studies beyond POMs based system

As the studies on POMs-based system revealed more and more information regarding the macro-ion solution, studies on other macro-ions became more accessible thanks to the insights gained from POMs-based system. The effects of medium dielectric constant, solution ionic strength, etc. were first confirmed in POMs based system and later applied to other systems. Dong Li et al. were first to report a “Blackberry structure” formation in metal-organic nano-cage solution. The results
were shown in Figure 2.4. It’s demonstrated that the positively charged macro-cations also possess similar behavior to the negatively charged macro-ions, and that nano-cage can be used as a functional building block to build supramolecular structures.

Figure 2.4 The schematic display of metal-organic nano-cages and its formation into supramolecular structure.  

Another more challenging system investigated is the charged dendrimer solution. Dendrimers are repetitively branched macromolecules with highly symmetrical structures and highly monodispersed structure. When substituted with charged functional group, many dendrimers can be dissolved in aqueous solution as macro-ions. As dendrimers have much softer chains comparing to the rigid structure of POMs, their self-assembly as macro-ions remains an interesting topic. Ali et al. first reported in 2015 that Poly(Propylene imide) can form the same blackberry structure under certain conditions. More details about studies on dendrimer solution behavior are included in Section 2.4.
Other charged macromolecules were also reported to show similar self-assembly properties like the POMs system. Modified cyclodextrins\textsuperscript{34} and modified fullerenes\textsuperscript{35} have all been demonstrated to show self-assembly behavior in dilute solutions. Yet more or less, they were similar to POMs in terms of rigidity and charge uniformity, and their complexity does not exceed that of charged dendrimers.

2.4 Solution behavior studies of charged dendrimers

Dendrimers are well-defined highly branched polymers which possess a central core and a large number of terminal groups.\textsuperscript{36} The unique structure of dendrimers has triggered applications in different fields including catalysis, drug-delivery and molecular electronics.\textsuperscript{10,37,38} Many of the applications involve dendrimers in a solution state, and would assume dendrimers stay as individual molecules in complex solutions. On the contrary, dendrimers in solutions can show very interesting self-assembling behavior in various conditions. Previous studies have shown that dendrimer would self-assembly with the addition of dye molecules,\textsuperscript{39,40} transition metal ions\textsuperscript{41-43}, and si-RNAs\textsuperscript{44}. Different assembled structures were created including vesicles\textsuperscript{39,41}, ribbons\textsuperscript{43,45,46} and solid dendriplexes\textsuperscript{40,44}. The discovered structures could be potentially used in future supramolecular material designs. Moreover, these previous studies provided insights into how dendrimers would interact with each other in solution. In general, cooperative binding, $\pi-\pi$ interaction and electrostatic interactions have all been claimed to be forces involved in the self-assembling process of different dendrimers. To further illustrate the interactions of dendrimers in solution,
there is a need to investigate the mechanism of each driving force separately. And to the best knowledge of the author, such experiments haven’t been carried out till the present study.
CHAPTER III
EXPERIMENTAL

3.1 Sample Preparation

Different metal-organic hybrid nano-cage samples were purchased from Wako Chemical and used without further purification. The first one used was Pd₆L₄(NO₃)₁₂ \{Pd = ethylenediamine palladium(II), L = 2,4,6-tris(4-pyridyl)-triazine\}, later addressed as PdNC or Pd₆L₄. The second one used in the study was Pt₆L₄(NO₃)₁₂ \{Pt = ethylenediamine palladium(II), L = 2,4,6-tris(4-pyridyl)-triazine\}, later addressed as PtNC or Pt₆L₄. Both nano-cage have similar structure, with a diameter around 2 nm.⁴⁷

The dendrimers used in this study was provided by Dr. Newkome’s group at the University of Akron and was synthesized based on a procedure described in previous literature.⁴⁸ The received sample was a white solid which was soluble in water.

Other chemicals used in this study, unless specified, were purchased from Sigma-Aldrich and used without further purification.

3.2 Laser Light Scattering (LLS)

Both Dynamic Light Scattering (DLS) and Static Light Scattering (SLS) were applied as the major characterization techniques for the macro-ion solutions. A
Brookhaven Instrument Inc. Light Scattering Spectrometer equipped with a goniometer, a solid state laser (522 nm) and a BI-9000AT multi-channel correlator was used to carry out the Laser Light Scattering experiments.

The DLS measurement would typically set a correlation time range from 1 µs to \(1 \times 10^5\) µs. The correlation functions were analyzed by the constrained regularized (CONTIN) method, a widely accepted method to conduct the fit for the inverse-Laplace transfer required in the analysis. In each measurement, an average apparent translational diffusion coefficient \(D_{\text{app}}\) was calculated from the normalized distribution function of characteristic line width, which was one of the outcomes of the CONTIN analysis. The Stokes-Einstein equation was then applied to obtain an average hydrodynamic radius \(R_h\):

\[
R_h = \frac{k_B T}{6 \pi \eta D}
\]

where \(k_B\) is the Boltzmann constant, \(T\) is the temperature of measurement, \(\eta\) is the viscosity of the sample solution and \(D\) is diffusion coefficient, which in this case is equal to \(D_{\text{app}}\) obtained from DLS. Since all measurements were carried out in very dilute solution, the viscosity change introduced by the solute can be neglected, thus solvent viscosity was applied as the solution viscosity.

The SLS measurement was performed on the same instrument with a typical range of scattering angles from 30° to 90°, with a 5° interval. To eliminate the effect of bad optical alignment, some of the measurements for smaller aggregates were done in a range from 45° to 115°, with a 5° interval. A simplified Rayleigh-Gans-Debye equation was applied to treat the data to obtain the radius of gyration \(R_g\):

\[
\frac{Hc}{R_g} = \frac{1}{M_w}
\]

where \(H\) is the optical parameter, \(M_w\) is the weight-average molecular mass of
the solute. The concentration term in the original Rayleigh-Gans-Debye equation was neglected because the concentrations used in all experiments were all very low and thus had low impact on the optical parameter.

3.3 Transmission Electron Microscopy and Nuclear Magnetic Resonance

The Transmission Electron Microscopy (TEM) experiments were carried out on a JEM 1200XII model. A typical TEM sample was prepared by dropping around 5µL sample solution onto a carbon-coated TEM copper grid. The sample grid was then left under ambient condition to dry for a week. Bright-field TEM imaging was then taken with an accelerating voltage of 120 kV. The Nuclear Magnetic Resonance (NMR) experiments were done with a Varian Mercury 300 MHz NMR. Samples were dissolved in D$_2$O to make 1 mg/ml solutions for the NMR test.
CHAPTER IV
RESULTS AND DISCUSSION

4.1 The self-assembly behavior of Pt₆L₄(NO₃)₁₂ nano-cages

Before it was well demonstrated that the Pd₆L₄(NO₃)₁₂ nano-cages (noted as PdNC in later paragraphs) can self-assemble into “Blackberry” structures under certain circumstances. However, it remained unknown that if its Pt counterpart, Pt₆L₄(NO₃)₁₂ (PtNC), could show similar behavior when dissolved to make a dilute solution. Thus a series of PtNC dilute solutions were made with solvent being different composition mixtures of water and acetone. It was observed that PtNC was not soluble in pure acetone and could be soluble in pure water up to 2 mg/ml. Certain amounts of PtNC were weighed and then mixed with the pre-filtered pre-mixed binary solvent. For those 0.1 g/L solutions made in such a manner, it was observed that the sample can be dissolved until the volume percentage of the acetone in solvent reached 65%. All other solutions with acetone v% < 65% showed a clear and steady solution at the first day. The sample solutions were stocked in room temperature and were measured by SLS on a daily basis. The monitored scattering intensity versus time was plotted in Figure 4.1. As could be seen in Figure 4.1 The 60% acetone solution of PtNC was not stable and precipitated out in a week. And the ones with acetone content less than 50% remained stable over a period of 2 weeks and did not show any
intensity increase. Only the 55% acetone solution showed significant intensity increase and remained a stable and clear solution.

![Figure 4.1 Static Light Scattering intensities of the PtNC solutions versus time](image)

The scattering intensity from the SLS indicated the status of solution: higher scattering intensity might hint the formation of self-assembled structures, while a constant scattering intensity demonstrates that there is no large species forming in the solution. The intensity increasing curve from 55% acetone solution of PtNC showed a similar “lag phase” as was found in previous studies. This indicated that there could be similar self-assembly behavior taking place in the 55% acetone PtNC solution.

Further investigations were done with Dynamic Light Scattering (DLS). The 55% acetone PtNC solution was measured with DLS at different scattering angles and the obtained hydrodynamic radius ($R_h$) distribution curve was shown in Figure 4.2.
The sharp and mono-$R_h$ distribution at 90 degree showed that the formed structures were highly mono-dispersed structures. Also, obtained $R_h$ at different degrees were the same, indicating the sample would have formed spherical structures in the solution. The obtained $R_h$ at different angle was plotted against the scattering angle and extrapolated to 0 degree to obtain $R_{h0}$. And $R_{h0}$ was then compared with the radius of gyration $R_g$ obtained from SLS. The $R_{h0}$ obtained from extrapolation was calculated to be 116±5 nm, and the $R_g$ obtained from SLS was 126 ±3 nm. The ratio $R_g/R_{h0}$ was then calculated to be 1.09, a value close to 1.

Figure 4.2 The hydrodynamic radius distribution of PtNC 55% water/acetone solution at different scattering angles

Based on previous discussions on this ratio\textsuperscript{12}, a $R_g/R_{h0} = 1$ with a monodispersed system usually indicates that the species in the system possess a hollow spherical structure. It’s reasonable to assume the PtNCs followed the same rules as PdNCs and formed a hollow spherical structure, namely “Blackberry” structure, under this condition. Further evidence can be provided by TEM, as the
TEM images of the solution could be identified having spheres with radius around 100 nm. Two typical TEM images were provided in Figure 4.3.

![TEM images of PtNC's formed assemblies in dilute solution](image)

Figure 4.3 Transmission Electron Spectroscopy images of PtNC’s formed assemblies in dilute solution

Comparing the self-assembly behavior of both PtNC and PdNC, it’s clear that their solution behavior in acetone-water mixture possess lots of similarities. For instance, they were both soluble in water, and did not self-assemble in aqueous solutions. Their self-assembly behavior both appeared only in a range of acetone volume percentage, requiring a suitable acetone content. As suggested in previous literature, this was due to the electrostatic interactions between the macro-ions would require a suitable dielectric constant of the solvent to achieve self-assembly. However, the range of suitable acetone content was different for PtNC and PdNC, as for PdNC solutions, self-assembly could be triggered in a wide acetone volume range from 20% to 60%. This difference was particularly interesting considering the highly identical structure and same charge they bear in solution. It might be due to the atomic size difference of the Pt atoms and Pd atoms on the corner of the nano-cage, and
could demonstrate future possibility of electro-static self-recognition between the two nano-cages.

In general, experimental evidences confirmed the previous theoretical prediction that PtNC, being a counter-part of self-assembling PdNC, could also self-assembly into hollow spherical structures under certain circumstances. The similarity in the PtNC self-assembly behavior with the PdNC self-assembly behavior indicated that both nano-cages would undergo an electro-static force driven self-assembly in suitable water-acetone mixtures and form “blackberry” structures. The difference in their self-assembly behavior would require more investigations in the future. But it’s sufficient to demonstrate that the electrostatic interactions in the two systems were not identical and the two nano-cages could possibly self-recognize in solution. Further studies on the self-recognition behavior of the two were to be described in the next section.

4.2 The self-assembly behavior of Pt$_6$L$_4$(NO$_3$)$_{12}$ and Pd$_6$L$_4$(NO$_3$)$_{12}$ mixed solutions

Both the Pt$_6$L$_4$(NO$_3$)$_{12}$ (PtNC) and Pd$_6$L$_4$(NO$_3$)$_{12}$ (PdNC) possess 12 positive charges in their dilute aqueous solution. Their chemical structures were also similar, with the six corner atoms the only differences in the structure. If both of them were considered as point charges, they should show identical behavior in the solution. However, as shown in section 4.1, their behaviors were similar, but with remarkable differences, indicating that the electro-static interactions between them should be considered to be different. This again proved that macro-ions cannot be simply treated as single ions in solution. On the other hand, it’s an interesting topic whether the two
slightly different electro-static interactions could distinguish each other in solution, 
reproducing the self-recognition behavior as previously observed. This pair would 
better demonstrate the sensitivity of the electrostatic interaction induced self-
assemblies, as the difference between PtNC and PdNC would be even smaller than the 
previously reported \{MoFe\} and \{MoCr\} POMS.

To investigate the self-assembly behavior of the two nano-cages in their mixed 
solution, solutions were prepared in the following sets: (1) PtNC (0.1 mg/ml) in 
difference composition acetone/water mixtures; (2) PdNC (0.1 mg/ml) in difference 
composition acetone/water mixtures; (3) PtNC and PdNC (0.1 mg/ml each) in 
difference composition acetone/water mixtures; (4) PtNC (0.05 mg/ml) in difference 
composition acetone/water mixtures; (5) PdNC (0.05 mg/ml) in difference 
composition acetone/water mixtures; (6) PtNC and PdNC (0.05 mg/ml each) in 
difference composition acetone/water mixtures. The acetone volume percentages for 
all the six sets of solutions were all from 40% to 60%. The solutions were prepared as 
described in Section 4.1 and were all clear solutions in the first day. Then the 
solutions were monitored with SLS daily and measured with DLS when there is a 
significant scattering intensity increase. The measured hydrodynamic radiiuses were 
shown in the Table 4.1 below.

<table>
<thead>
<tr>
<th>No.</th>
<th>Content</th>
<th>40% Acetone</th>
<th>45% Acetone</th>
<th>50% Acetone</th>
<th>55% Acetone</th>
<th>60% Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PtNC 0.1 mg/ml</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>89nm</td>
<td>78.9nm</td>
</tr>
<tr>
<td></td>
<td>Sample</td>
<td>Scattering Intensity</td>
<td>R&lt;sub&gt;h&lt;/sub&gt; (nm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>--------</td>
<td>----------------------</td>
<td>------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>PdNC 0.1 mg/ml</td>
<td>Low</td>
<td>30.7nm, 48.7nm, 54.3nm, 75.3nm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Mixture 0.1 mg/ml</td>
<td>Precipitation</td>
<td>Precipitation, Precipitation, Precipitation, Precipitation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>PtNC 0.05 mg/ml</td>
<td>Low</td>
<td>Low, Low, Low, Low</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>PdNC 0.05 mg/ml</td>
<td>Low</td>
<td>Low, Low, Low, Low</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Mixture 0.05 mg/ml</td>
<td>Low</td>
<td>Low, Low, Low, Low</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: “Low” in the table indicates the scattering intensity was still too low (< 100 kcps at 90°) after two weeks. “Precipitation” indicates that the sample in the solution precipitated out in two weeks. R<sub>h</sub> of the self-assembled solution was measured at 90° scattering angle.

As shown in the table, the suitable concentration for nano-cages self-assembly would be around 0.1 mg/ml, and a concentration higher than that (experiment set 3) would trigger precipitation of the nano-cages. When the concentration is too low (experiment set 4, 5, 6), the scattering intensity would be too low to obtain a reliable DLS correlation function, thus a reliable R<sub>h</sub> cannot be obtained. However, the change in scattering intensities was still recorded and could be used as an evidence of self-assembly. The scattering intensities of the nanocages were plotted versus time in Figure 4.4.
Figure 4.4 The histogram of scattering intensities of different mixed nano-cage solutions. a PtNC solution, 0.05 g/L. b PdNC solution, 0.05 g/L. c PtNC and PdNC mixed solution, 0.05 g/L respectively.

As could be seen in Figure 4.4, the scattering intensities showed very different trends between the three different sets. The mixed nano-cages set 6 (noted as MixNC in Figure 4.4) was not a simple add-up of the PtNC (experiment set 4) and PdNC (experiment set 5). It could be seen that in Figure 4.4 a, 0.05 g/L PtNC solutions already showed an identifiable scattering intensity increase after 2 weeks, indicating a small amount of PtNCs in solution already started the self-assembly process mentioned in Section 4.1. On the contrary, the 0.05 g/L PdNC solutions under the same conditions did not show such self-assembly behavior. However, when a mixture solution was put under the same conditions, they behave like none of their single components. The scattering intensities were shown to grow over a timeframe of two weeks, but the speed of growing is much slower than that of PtNC alone. It’s evident that PtNC and PdNC in the mixture solution were interacting with each other, thus hindering the relatively fast self-assembly of PtNC. It was the first time that this
hindering effect was observed in macro-ion mixture solutions and it was different from what were observed in such self-recognition systems. The demonstrated interactions between PtNC and PdNC might prevent them from self-recognizing each other and forming different self-assembly structures.

In another effort to tweak the nano-cage system and achieve self-recognition, sodium adamantane carboxylic acid (NaC$_{11}$H$_{15}$O$_2$, AdCNa) was mixed with the two nano-cages. It was demonstrated by Fujita et al. that the AdCNa would be encapsulated by the nano-cages, thus reducing their charges in aqueous solution. The encapsulation was done by mixing AdCNa: PtNC at different ratio and dissolve them in water. NMR spectra were obtained to demonstrate the encapsulation, as shown in Figure.4.5.

![Figure.4.5 Nuclear Magnetic Resonance spectra for the PtNC/AdCNa mixed solution.](image)

- a PtNC: AdCNa =1:4 (mole ratio).
- b pure PtNC solution.
- c PtNC: AdCNa =1:2 (mole ratio)

The chemical shift of the hydrogen on 8.5 and 9.1 were from the original nano-cage organic linker. And after the AdCNa was encapsulated, the two peaks were further shifted to 8.8 and 9.3, respectively. It’s interesting that when the PtNC:
AdCNa ratio is 1:2, both kinds of peaks remained in the NMR spectrum, with no intermediate peaks were shown. It showed that the encapsulation of AdCNa was following a “all or nothing” rule, that each PtNC either capsulate 4 AdCNa molecules, or capsulate nothing at all. It could be explained that the first encapsulated AdCNa would further increase the hydrophobicity of the PtNC cavity, making the encapsulation of following AdCNa molecules easier.

As a result, when the PtNC:AdCNa = 1:2, two kinds of species were present in solution, one is the pure PtNC nano-cage, the other is the \{PtNC-4AdCNa\} complex. The formal species carries 12 positive charges in aqueous solution and the latter carries 8 positive charges. It’s reasonable to assume that these two species possess different electro-static interactions in solution and would possibly self-assemble into different structure. However, the solubility of the \{PtNC-4AdCNa\} complex in water/acetone mixture was relatively low and the solutions would quickly precipitate out in 1 or 2 days. Thus the attempt to tweak the charges of nano-cages with AdCNa had failed. However, there still remain possibilities of tweaking the charges of nano-cages with other small anions, which needs to be studied in the future.

In all, the PtNC-PdNC mixture solutions and their mixtures with AdCNa were monitored with light scattering to study their solution behavior. The concentrations of nano-cages were found to be critical to trigger the self-assembly of nano-cages. And in the low concentration experiments it was found that PtNC and PdNC had interactions in solution that hindered the self-assembly of PtNC. This was different from the previously reported POMs macro-ion solution and might lead to the fact that PtNC and PdNC cannot self-recognize each other in water/acetone mixture solution. The nano-cage and AdCNa were also studied and it was shown that this system might not be a good candidate for triggering self-recognition.
4.3 pH-controlled self-assembly of Newkome-type anionic dendrimer

Like Nano-cages, charged dendrimers are also an appealing category of macro-ions for their wide applications from catalysis to drug-delivery. Charged dendrimers are different from POMs and nano-cages as they were “soft” in nature. The absence of a rigid structure put a question mark to their self-assembly ability. Also, despite the claim that dendrimers are mono-dispersed “molecules”, they were often synthesized with defects to certain degree, thus making them slightly poly-dispersed “polymers” rather than “molecules”. All these special properties make dendrimers an interesting target as a sample system of complex macroion solutions.

In this thesis, the behavior of a generation 4 Newkome-type dendrimer 1 (as shown in Figure 4.6) in a simple aqueous solution at different pH was studied to show a self-assembly process mainly driving by electrostatic interactions. This is the first study in which the self-assembly behavior of dendrimers was observed without the presence of additional transition metal ions or complex agents. The dendrimer studied has 108 carboxyl acid groups on the external surface. In a typical 1 mg/ml solution of 1, the pH is 3.80, indicating that around 2 protons of the total 108 terminal groups were released into the solution. The solution was monitored with Laser Light Scattering (LLS) for several weeks and showed no obvious change in the scattered intensity, suggesting that the dendrimers are stable and exist as discrete molecules in solution (consistent with previous reports).
Figure 4.6 Structure of the Newkome-type dendrimer 1 and schematic representation of spherical structure formed with the addition of NaOH.

Large assembled structures of dendrimers were observed when NaOH was titrated into 2.0 mg/ml solution of dendrimer 1. Upon the addition of base, the protons were neutralized and the dendrimer carried more negative charges. The scattered intensity from a static light scattering (SLS) measurement showed a significant increase as the pH increased, and peaked at a pH of 12.64. Meanwhile the solutions at higher or lower pH did not show a significant increase in the scattered intensity (Figure 4.7). For a given solution, larger scattered intensity indicates that the scatterer in the solution has a larger volume. For all the solutions with pH values reported in Figure 4.7, the dendrimers remained stable and soluble in a clear and homogeneous solution upon the addition of NaOH. Thus, the increased intensity does not imply insoluble aggregates, but indicates the formation of larger soluble assemblies of the dendrimer.
Dynamic Light Scattering (DLS) experiment was conducted for the pH 12.64 and pH 12.99 solutions with dendrimer concentration at 2.0 mg/ml, and the size of the large species was determined by Constrained Regularization (CONTIN) analysis of the correlation function obtained from DLS. As shown in Figure 3, the DLS result indicate the existence of a dominant species with a narrow size distribution (Average around 100nm) in solution, which shall be regarded as assemblies of the dendrimers. The average hydrodynamic radius ($R_h$) of the assemblies at pH 12.64 was $108 \pm 2$ nm at 90° scattering angle. The $R_h$ measurement can be extrapolated to 0° to obtain $R_{h0}$, which is the actual hydrodynamic radius. The extrapolated $R_{h0}$ of the pH 12.64 solution was 144.8 nm. Static Light Scattering (SLS) experiments were also conducted to determine the radius of gyration ($R_g$) of the assemblies. The obtained $R_g$ and $R_{h0}$ values were compared in Table 4.2. The $R_g/R_{h0}$ ratio was close to 1, which indicated that the mass of the assembly was all distributed at the surface of the
assembly, suggesting that the assemblies possessed a hollow spherical structure as shown in Figure.4.8.

![Figure.4.8](image)

Figure.4.8 $R_h$ distribution of the assemblies in the pH 12.64 dendrimer solution

<table>
<thead>
<tr>
<th>pH</th>
<th>$R_g$ (nm)</th>
<th>$R_{h0}$ (nm)</th>
<th>$R_g/R_{h0}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.64</td>
<td>144.8</td>
<td>140.5</td>
<td>1.03</td>
</tr>
<tr>
<td>12.99</td>
<td>110.4</td>
<td>109.5</td>
<td>1.01</td>
</tr>
</tbody>
</table>

Prior to this work, most designed self-assembly processes of dendrimers involved various interactions, namely cooperative binding, $\pi-\pi$ interaction, hydrogen bonding, etc. In this work, all the interactions mentioned above were either absent or did not change when the pH was changed. As the pH increased, the carboxylic acid groups on the surface of dendrimer got deprotonated, and the charges on the dendrimer surface increased, triggering changes in electrostatic interactions. Meanwhile, the strength of hydrogen bonding and Van der Waals interactions
remained relatively unchanged. It is thus safe to claim that electrostatic interactions between charged dendrimers were the dominant driving force for self-assembly. Another fact to note is the different sizes of assemblies at different pH. The assemblies at pH 12.64 had an average $R_h$ of 140.5 nm, while the assemblies at pH 12.99 had an average $R_h$ of 109.5 nm, showing a smaller $R_h$ at a higher pH. The smaller curvature of larger assemblies was the consequence of less electrostatic interactions at lower pH. This phenomenon was in accordance with our previous results on polyoxometalates (POMs). Both POMs and dendrimers are macro-anions with similar size. As in the case of POMs, the radius of formed assemblies would decrease as the effective charges on the surface of POMs were increased. The stronger electrostatic interactions would not tolerate large curvature thermodynamically, thus leading to a smaller hollow spherical structure.

Another fact to note here is the sensitivity of this method. Among the broad tested range of pH, only a small range of pH would lead to obvious self-assembly of the dendrimers. It can be concluded that the electrostatic force driving self-assembly of dendrimers is very selective to the surface charges that the dendrimers bear. At lower pH, the carboxyl groups were not fully deprotonated, thus the surface charge density of dendrimers was not enough to trigger self-assembly. At pH higher than 13, the dendrimer started to dissociate and precipitates could be seen within a day. This pH responsiveness would have potential applications as a molecular sensor in extreme conditions.

To sum-up, Newkome-type dendrimer 1 behaves as weak acids in aqueous solutions that can deprotonate to different extent at different pHs. At certain pHs, the dendrimer bears suitable charge densities to self-assemble into hollow spherical supramolecular structure with radius around 100 nm. The size of formed assemblies
would decrease as the pH increases. The formation of the assemblies should be mostly
driven by electrostatic interactions. The self-assembly process showed the effect of
electrostatic interaction in dendrimer solutions, which is often overlooked when
studying complex solutions involving dendrimers. The pH sensitivity of this
assembling process may have potential for the design of novel molecular sensors.
CHAPTER V
CONCLUSION

In conclusion, the solution behaviors of certain nano-cages and charged dendrimers, especially their counter-ion mediated self-assembly behavior in dilute solutions, were studied in this thesis. The selected nano-cages and charged dendrimers were believed to possess some unique properties (i.e. encapsulation capability, soft structure) that were not studied in the known studies of macro-ion solution behaviors. Thus, they would act as a suitable intermediate to move from relatively simple POMs to the more complicated, biologically present polyelectrolytes.

It was found in the study that Pt$_6$L$_4$(NO$_3$)$_{12}$ nano-cages (PtNC) could also show similar self-assembly behavior as its Pd counterpart, Pd$_6$L$_4$(NO$_3$)$_{12}$ (PdNC). However, their behaviors in water/acetone mixture solution were not identical and the differences were attributed to the six different corner atoms leading to different charge densities of the macroions. The possible self-recognition between PtNC and PdNC in their mixed solution was also investigated. It was found that the PtNC and PdNC were both sensitive to concentrations and would only self-assembly at around 0.1 mg/ml in water/acetone mixture. Furthermore, it was identified that PtNC and PdNC were interacting with each other in their dilute solution, hindering PtNC’s self-assembly at given conditions. The interaction might be the reason that the self-recognition between PtNC and PdNC could not be observed. The interactions of nano-cages with organic anions were also studied. And it was found that the interaction of PtNC and
sodium adamantine carboxylic acid (AdCNa) followed an “all or nothing” pattern, that each PtNC is either fully filled or not filled. However, the complex of PtNC and AdCNa did not show a good solubility in the water acetone mixture.

Solution behaviors of a charged dendrimer (Newkome-type dendrimer, generation 4) were studied to show that the pH tuned charge density controlled the self-assembly process of the dendrimer in aqueous solution. It was found that at certain pHs, the dendrimer bears suitable charge densities to self-assemble into hollow spherical supramolecular structure with radius around 100 nm. The size of formed assemblies would decrease as the pH increases. The formation of the assemblies should be mostly driven by electrostatic interactions. It should be noted that soft dendrimers having similar solution behaviours as rigid POMs and nano-cages, thus giving more insights into more polydisperse and more soft polyelectrolytes.
BIBLIOGRAPHY


