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Lag Periods During the Self-Assembly of $\{Mo_{72}Fe_{30}\}$ Macroions: Connection to the Virus Capsid Formation Process

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Abstract: The kinetic properties of the self-assembly of hydrophilic Keplerate-type polyoxometalate (POM) $\{Mo_{72}Fe_{30}\}$ macroanions into single-layer, vesicle-like blackberry structures in solutions were monitored by the static and dynamic laser light scattering techniques. In the presence of additional electrolytes, an obvious lag period at the initial stage of self-assembly was observed, followed by a fast increase of the scattered intensity. The whole kinetic curve is sigmoidal with a lag phase. A two-step nucleation−growth mechanism is proposed to explain this lag phase: the $\{Mo_{72}Fe_{30}\}$ macroanions slowly associate into oligomers (mostly dimers), which are the thermodynamically unfavorable intermediates, at the initial stage; once the oligomers reach a critical concentration, the blackberry formation process is accelerated. Analytical ultracentrifugation (AUC) was used to confirm the oligomeric state in $\{Mo_{72}Fe_{30}\}$ solution during the lag period. The length of the lag period is dependent on temperature, ionic strength, and the valent states of the additional salts, as well as the solvent content. The kinetics (including the lag period) of the blackberry formation of the $\{Mo_{72}Fe_{30}\}$ macroanions show similarities to the self-assembly of virus capsid proteins (which are also soluble macroions) into spherical capsid shells, suggesting possible connections between the self-assembly behaviors of inorganic species and biological macromolecules.

1. Introduction

Giant polyoxometalate (POM) molecular clusters have been attractive owing to their unique properties and promising applications.[1] Some POMs are hydrophilic in nature and highly soluble in polar solvents. Beyond our expectation, those nanoscaled macroanions carrying a moderate amount of charges in solution behave differently from small inorganic ions, by self-assembling into more stable, single-layered, spherical vesicle-like "blackberry" supramolecular structures.[2−6] Those blackberry structures are quite different from vesicular structures formed by amphiphilic surfactants as the hydrophobic interaction does not play a role in the blackberry formation.[2,6a] We believe that the counterion-mediated attraction and hydrogen bonds are important for assembling the blackberry structures.[4−6]

How the fully hydrophilic components can spontaneously associate into hollow supramolecular structures in polar solvents is still a challenge to scientists. A close look at the thermodynamic and kinetic behavior during the self-assembly process would be very helpful. Our earlier thermodynamic studies were based on the blackberry formation of the 2.5-nm-size, hollow, spherical "Keplerate" clusters $\{Mo_{72}Fe_{30}\}$ (full formula $[Mo^{VI}_{72}Fe^{III}_{30}O_{252}(CH_3COO)_{12}\{Mo_2O_7(H_2O)\}_2\{H_2Mo_2O_8(H_2O)\}(H_2O)_{91}]\cdot{\sim}150H_2O$)[7] in aqueous solution by using static and dynamic light scattering techniques (SLS and DLS).[4]

$\{Mo_{72}Fe_{30}\}$ molecular clusters in aqueous solution behave as a weak acid due to the partial deprotonation of H_2O ligands coordinated to the 30 Fe^{III} centers.[5] $\{Mo_{72}Fe_{30}\}$ clusters exist as anions in solutions with a limited number of localized charges upon the release of protons ($\sim$7 H^+ per cluster at the concentration of 1.0 mg/mL, corresponding to a solution pH = 3.4).[5] The blackberry formation of $\{Mo_{72}Fe_{30}\}$ is unusually slow: it takes months to reach thermodynamic equilibrium at room temperature, and can be accelerated at an elevated temperature.[2] This is due to a high activation energy (E_a) of $\sim$115 kJ/mol (obtained from the Arrhenius Equation), suggesting that the single $\{Mo_{72}Fe_{30}\}$ anions have to overcome a very high energy barrier to form the blackberries.[4]

During the slow blackberry formation process, the scattered intensity from SLS studies continuously increases with time, indicating the continuous formation of large structures. However, DLS studies show that the average size of the supramolecular structures (in R_h) remains almost unchanged with time.[3a] On the basis of this observation, we proposed a possible two-step mechanism for the self-assembly: a rate-determining step for

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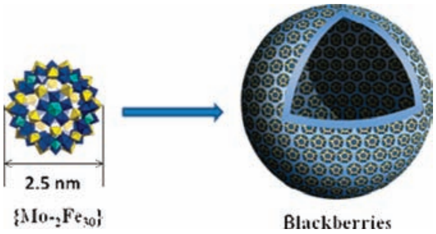
Scheme 1. Polyhedral Representation of the 2.5-nm Size Keplerate $\{Mo_{72}Fe_{30}\}$ Molecular Cluster and Their Self-Assembled Blackberry Structure



the oligomer formation, and a fast step of the blackberry formation.[6a] This proposed mechanism has not been well confirmed. Here we plan to change the external conditions of blackberry formation by introducing additional salts, which are expected to further stabilize the macroions and further slow down the formation process, in order to explore more details regarding the mechanism of the blackberry formation.

Furthermore, the POM macroionic solutions in the presence of additional salts are similar to the biological macromolecules in buffers. It is very interesting to notice that the single-layered, hollow, spherical blackberry structures mimic the virus shell structures formed by some capsid proteins; and such proteins can also be treated as macroions. To further explore the possible similarities and differences between these two types of assemblies, a close study on their formation kinetics could be very helpful. The kinetic properties, especially some unique characteristics of the self-assembly behavior can often review important information regarding the mechanism of the process. The self-assembly of both POM hydrophilic macroions and virus capsid proteins in dilute solutions can be very slow at room temperature. Consequently, the detailed kinetic properties can be explored easily by the time-resolved SLS and DLS measurements. The formation curves under the time-resolved SLS studies for the virus capsid protein shells usually present a characteristic "S" shape, which includes a significant lag period at the beginning.[8−11] This lag period denotes a slow formation of oligomers from unimers, which is very difficult. However, once enough oligomers are available, the assembly to final well-defined capsid shells is rapid. Naturally, it would be interesting to see whether the blackberry formation demonstrates similar features.

Analytic ultracentrifugation (AUC) is a powerful tool for exploring the hydrodynamic and thermodynamic properties of self-association of biomolecules.[12] Especially the sedimentation velocity (SV) can be very useful in the identification of the oligomeric state in solution.[13,14] In a typical sedimentation velocity experiment, raw data are displayed as absorbance traces obtained from time-dependent changes in solution absorbance as a function of radial position. The resulting family of curves can be quantified to deduce sedimentation coefficients (s) of sedimentating species and their corresponding sedimentation coefficient distribution $c(s)$. The s value is dependent on the molecular weight, shape, and density of particles, as well as the density and viscosity of the solvent. For slow self-associations, the continuous $c(s)$ distribution method is particularly sensitive to resolve sedimentation coefficients of monomers, dimers, and larger oligomers.[14,15]

Herein, we report a study on the kinetic property of the $\{Mo_{72}Fe_{30}\}$ blackberry formation, including the oligomer states, by using laser light scattering and AUC techniques. Special attention has been paid to the lag period at the beginning of the self-assembly, and how it changes with the presence of additional electrolytes (different ionic sizes and valent states), temperature, and the solvent content.

2. Experimental Section

2.1. Sample Preparation. The single crystals of $\{Mo_{72}Fe_{30}\}$ were synthesized based on a procedure in literature.[7] The crystals were dissolved in solvent (water or water/glycerol mixed solvents) at room temperature with stirring to make solutions at certain concentrations. The solutions were then freed of dust by filtering with Millipore 0.10 μm filters into LLS sample cells.

2.2. SLS and DLS. A commercial Brookhaven Instruments light scattering spectrometer[16] equipped with a Coherent Radiation 200 mW Diode-Pumped Solid-State (DPSS) laser operating at 532 nm and a BI9000 correlator was used for both SLS and DLS measurements. The sample chamber was thermostatted and could be controlled to within ± 0.05 °C.

The SLS data are analyzed based on the Rayleigh−Gans−Debye equation, valid for small, interacting particles in the form:[17]

$$HC/R_{90} = 1/M_w + 2A_2C \qquad (1)$$

where H is an optical parameter; M_w, weight-average molecular weight of the solutes; A_2, the second virial coefficient; and C, the solute concentration. For dilute solutions like those we are dealing with in this work, the $2A_2C$ term is negligible. Therefore, eq 1 can be written as follows

$$HC/R_{90} = 1/M_w \qquad (2)$$

or,

$$I \propto C \times M_w \qquad (3)$$

The use of SLS to monitor the slow blackberry formation and to obtain "reaction" parameters has been described earlier.[4] For $\{Mo_{72}Fe_{30}\}$ solutions, the continuous increase of I is mainly due to the increase of the number of blackberries while the mass of the blackberries only slightly increases. Discrete $\{Mo_{72}Fe_{30}\}$ anions have only negligible contribution to I.

The intensity−intensity time correlation functions were analyzed by the CONTIN method.[18] Unless otherwise specifically noted, the SLS and DLS measurements herein are monitored at a scattering

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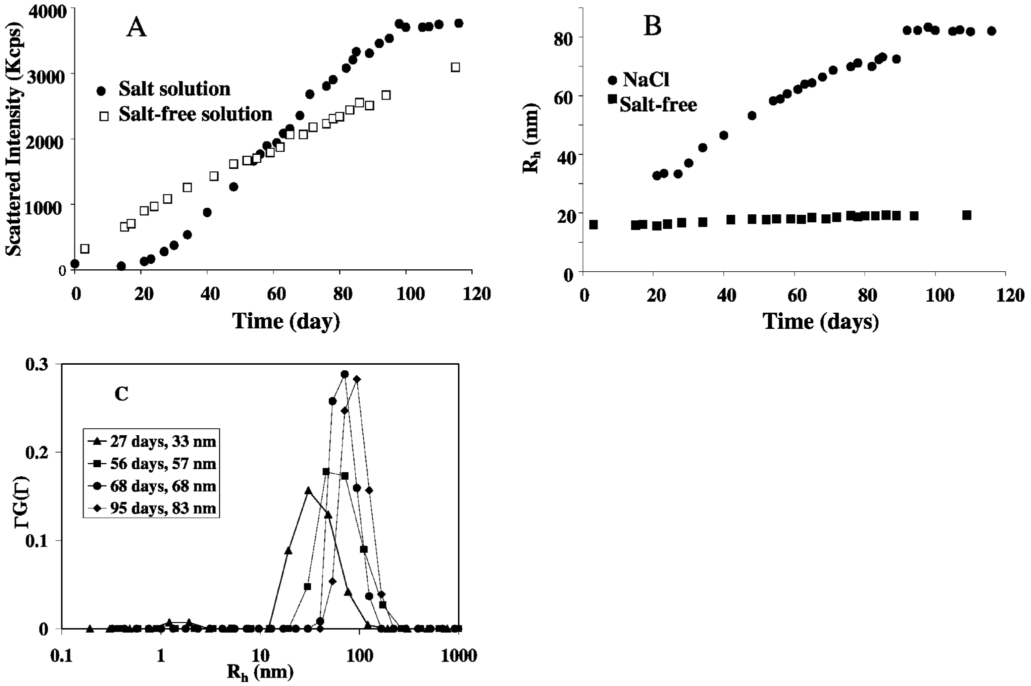

Figure 1. The comparison of scattered intensity increment (A) and R_h change (B) of two $\{Mo_{72}Fe_{30}\}$ samples along reaction time in 0.9 wt % NaCl and salt-free solutions. (C) CONTIN analysis of the DLS studies on the blackberry structures formed in 0.9 wt % NaCl solutions.

angle of 90°. During the entire SLS and DLS experiments, the experimental error in I and the apparent R_h are about ± 3%.

2.3. Sedimentation Velocity (SV). SV experiments were performed using a Beckman model XL-A analytic ultracentrifuge equipped with a photoelectric absorbance optical detection system. Samples (440 μL) and reference solutions (450 μL) were loaded into a conventional double-sector quartz cell and mounted in a Beckman An-60 Ti rotor. Experiments were carried out at 20 °C and a rotor speed of 30 000 rpm. Data were collected in continuous mode at a single wavelength of 450 nm and a step size of 0.005 cm. Multiple scans at different time points (~230 scans within 3 h) were fit to a continuous size distribution using the program SEDFIT.[19]

3. Results and Discussion

3.1. Lag Period in the Self-Assembly of $\{Mo_{72}Fe_{30}\}$ Macroions in the Presence of Considerable Amount of Additional Electrolytes. In aqueous solution containing no or a small amount of additional electrolytes (e.g., NaCl, NaBr, NaI, and Na_2SO_4 at concentrations of 0.017 mol/L), the $\{Mo_{72}Fe_{30}\}$ blackberry formation curve recorded by SLS studies is similar to that of a first-order reaction, i.e., the increment of the scattered light from $\{Mo_{72}Fe_{30}\}$ solution starts quickly once the solution is prepared.[4] However, a close look at the time-resolved SLS studies reveals that there is a very short lag period at the beginning (in minutes, see Figures 4, 8, and 10 in ref 4). This lag period could be related to an oligmer formation process because the formation of only oligomers does not significantly increase the scattered intensity from the solution. Therefore, the length of the lag period can be roughly treated as the time period needed for accumulating enough oligomers in solution for the blackberry formation.

The possibility of revealing more information regarding the kinetics of the blackberry formation from the detailed study of the lag period encourages us for further explorations. Especially, how the lag period responds to the additional electrolytes is intriguing. A typical study is shown in Figure 1, where NaCl (0.9 wt %, or 0.17 mol/L) was introduced into a 10.0 mg/mL $\{Mo_{72}Fe_{30}\}$ solution. Introducing such a large amount of NaCl into solution triggered some precipitations because the $\{Mo_{72}Fe_{30}\}$ macroions became less soluble due to the screening effect from the salts. After removing the precipitates, the remaining solution was a stable, saturated $\{Mo_{72}Fe_{30}\}$/NaCl solution (~7 mg/mL) at room temperature. The scattered intensity I of the solution at the scattering angle of 90° was recorded daily, as shown in Figure 1A. The initial value of I from this solution was very low (~78 kcps), not much higher than that of pure water (~20 kcps), suggesting that almost all of the $\{Mo_{72}Fe_{30}\}$ macroanions existed as discrete ions. I did not change for almost 20 days, then suddenly started to increase, indicating a slow formation of large structures. The increment of I then accelerated until it reached the level of over 4000 kcps within 3 months. Overall, the whole kinetic curve is sigmoidal with an extended lag phase. The 20-day-long lag phase is very unique and different from the $\{Mo_{72}Fe_{30}\}$ solution without additional salts. A typical time-resolved SLS study on the salt-free $\{Mo_{72}Fe_{30}\}$ aqueous solution is also presented in Figure 1A for comparison.

At the same time, the DLS technique was used to monitor the average hydrodynamic radius (R_h) change of the supramolecular structures in solution. As shown in Figure 1B, after the ~20-day lag period, DLS measurements revealed a new mode corresponding to large structures, which was coincident with the increase of I. The average size of the blackberries in NaCl solution also kept increasing with time: the initial average R_h of blackberries was ~30 nm, but the final R_h grew to over 80 nm when the solution reached the thermodynamic equilibrium. This is very different from that in salt-free solution, in which the average R_h of the blackberries kept constant during the whole process. The size distributions of the $\{Mo_{72}Fe_{30}\}$ blackberries

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(from CONTIN analysis) are relatively narrow, as shown in Figure 1C.

The sigmoidal curve for a self-assembly process has also been reported in other systems such as the self-assembly of virus capsid proteins,[8−11] ester hydrolysis,[20,21] vesicle formation,[22] and nanoparticle preparation.[23] In general, the sigmoidal curve is considered to be a typical feature of a two-step process: in the initial lag period, the "reaction" begins with the slow formation of oligomer nucleus; once the amount of the rate-limiting nucleus has reached a critical value, subsequent oligomers/monomers are quickly added to the growing assembly structures until it is complete.[24] This mechanism can nicely explain the blackberry formation process without additional salts. When additional salts are present, two major changes appear: first, the lag period from SLS study becomes much more significant; second, the R_h value of the large assemblies increases with time. Both observations could be explained by the fact that the blackberry formation becomes more difficult (in both steps), due to the stabilization capability of the additional electrolytes. Therefore, the existence of the extended lag period with additional electrolytes in $\{Mo_{72}Fe_{30}\}$ solution strongly confirms our previous speculation on the mechanism of the blackberry formation.[6a] A premise of the above discussion is based on the assumption that the lag period shown in SLS studies is not due to the limited sensitivity of the instrument, and the detailed analysis to confirm this is provided in the Supporting Information.

3.2. Identifying the Oligomer Formation during the Lag Phase. To confirm the above mechanism and identify the oligomeric state during the lag period, SV experiments were performed. The data were collected on the 18th day after the sample solution was prepared, corresponding to the final stage of the lag period in the kinetic curve (Figure 1A), where the oligomer concentration in solution should be close to the highest level. In a typical SV experiment, raw data points are displayed as absorbance traces obtained from time-dependent changes in solution absorbance as a function of the radial position (see Figure S2 of the Supporting Information). The experimental curves were fitted using the Lamm equation to deduce the sedimentation coefficients (s) of sedimentating species and their corresponding sedimentation coefficient distributions c(s). The s-values are commonly reported in Svedberg (S) units, which correspond to 10^{-13} sec.

The theoretical sedimentation coefficients for the monomer and different types of oligomers of $\{Mo_{72}Fe_{30}\}$ can be calculated by using eq 4:

$$s = \frac{M(1 - \bar{v}\rho)}{N_A f} \tag{4}$$

where M is the molecular weight (accounting for the loss of 150 water molecules) of $\{Mo_{72}Fe_{30}\}$ as 15944 g/mol, $\bar{v}$ is the partial specific volume as 0.37004 mL/g (see measurement of $\bar{v}$ value in Supporting Information), ρ is the density of the solvent as 1.0052 g/mL at 20 °C, N_A is Avogadro's number, and f is the frictional coefficient. The Stokes equation can be used to determine the f value for smooth, compact spherical particles:

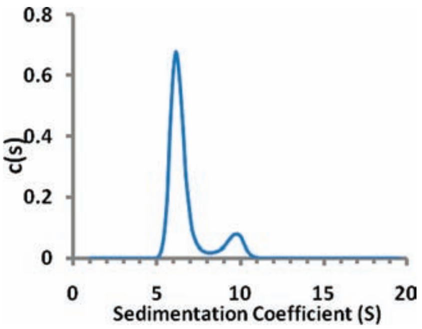

Figure 2. Continuous size distribution $c(s)$ analysis of $\{Mo_{72}Fe_{30}\}$ solution versus sedimentation coefficient, s. Experiments were performed at a $\{Mo_{72}Fe_{30}\}$ concentration of 10 mg/mL in 170 mM NaCl solution at 20 °C.

$$f_0 = 6\pi\eta R_0 \tag{5}$$

where f_0 is the frictional coefficient of the spherical particles, η is the viscosity of the solution as 1.0162 mPa·S, and R_0 is the radius of the sphere, which can be expressed as follows:

$$R_0 = \left(\frac{3M\bar{v}}{4\pi N_A}\right)^{1/3} \tag{6}$$

The s value for a sphere can be obtained by combining eq 4∼6:[25]

$$s_{\text{sphere}} = \frac{M^{2/3}(1 - \bar{v}\rho)}{\eta(162\pi^2 N_A^2 \bar{v})^{1/3}} \tag{7}$$

By using eq 7, the sedimentation velocity coefficients for smooth, compact spherical particles can be predicted. This s_{sphere} value is the maximum s value that can be obtained for a particle with a given molecular weight, because a compact sphere has the minimum surface area in contact with the solvent and consequently the particle would have a minimum frictional coefficient, f_0. For nonspherical particles, $f/f_0 > 1$.

As shown in Figure 2, the fitting results of the sedimentation coefficient distribution for $\{Mo_{72}Fe_{30}\}$ solution show the coexistence of two species: $s \approx 6.6$ S with the dominant abundance (56%) and $s \approx 9.5$ S (10%). On the basis of the calculation from eq 7, the theoretical sedimentation coefficients for $\{Mo_{72}Fe_{30}\}$ monomers and oligomers are as follows: 6.9 S for $\{Mo_{72}Fe_{30}\}$ monomers, 11.0 S for dimers and 14.5 S for trimers, assuming all of the species are spherical. Therefore, the species with $s \approx 6.6$ S corresponds to the monomers in solution. The species with $s \approx 9.5$ S is ascribed to be dimers. This value is lower than the theoretical value (11.0 S). A very possible reason is that the dimers are not spherical, therefore the geometrical factor $f/f_0 = s_{\text{theoretic}}/s_{\text{exp}} = 1.16$ should be applied. In addition, SV measurements also suggest the possible existence of small amount of larger oligomers (such as trimers) in solution. However, the very small amount is not sufficient to lead to reliable results from such studies.

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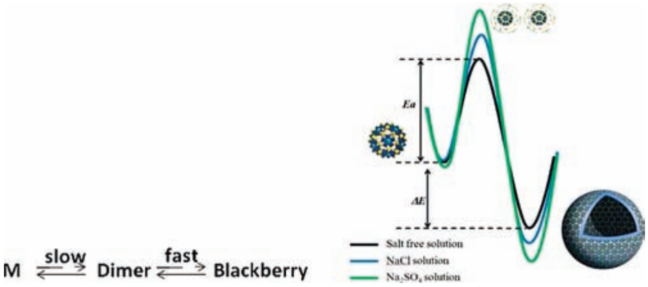
Scheme 2. Representative Scheme of Kinetic Models of the Blackberry Formation in the Salt Solution of $\{Mo_{72}Fe_{30}\}$ (left) and Proposed Free Energy Curve of $\{Mo_{72}Fe_{30}\}$ Giant Anions (right)[a]



[a] Two solute states are found: the first state represents the homogeneous distribution of single $\{Mo_{72}Fe_{30}\}$ anions, which is entropy favored. The second state is free-energy favored, with the $\{Mo_{72}Fe_{30}\}$ anions forming blackberry structures. A high energy barrier is needed for the blackberry formation, corresponding a transitional stage (mostly dimers).

On the basis of the above results, a reasonable model on a stepwise process of blackberry formation in salt solutions can be raised, as depicted in Scheme 2:

In the lag period, the discrete macroanions (M) slowly associate into the oligomers (mostly dimers), which is a thermodynamically unfavorable step. The associates grow by stepwise addition of monomers until the critical nucleus oligomer concentration is formed. During this period, no large blackberry is formed. Once the oligomers accumulate to a critical concentration, further association into blackberries becomes thermodynamically favorable because the blackberry state has a much lower total free energy. Therefore, the growth process from the dimers (or maybe also other oligomers) to the final blackberry structures in equilibrium state is much faster. Because the formation of each blackberry needs thousands of monomers, and the dimers are also stabilized with the help of additional salts, the growth rate for the blackberries also becomes very slow even though it is faster than the oligmer formation. The extended period for blackberry growth enables us to observe smaller blackberries with growing sizes (R_h from 30 to 80 nm) during the process. Accordingly, the increment of I should come from both the increment of the amount of blackberries (including the intermediates) and their masses. After the blackberries reached final thermodynamic structure ($R_h \approx 80$ nm), the process completes and no more increase in I or R_h was observed.

Without additional electrolytes, the only cations in $\{Mo_{72}Fe_{30}\}$ solution are the limited amount of protons deprotonated form $\{Mo_{72}Fe_{30}\}$'s water ligands.[5] On the basis of our model of oligomer formation, the collision of two adjacent $\{Mo_{72}Fe_{30}\}$ anions carrying a charge close to the collision site makes the counterions interact with each other, in which case macroanions associate into larger structures. Additional salts in the solution can help to stabilize the macroanions by increasing the concentration of counterions around macroions and introducing the co-ions. Overall, the enhanced screening effect will decrease the attractive force between the discrete macroanions.

Additional evidence of the effect of extra electrolytes on stabilizing macroanions can be found from the comparison of the self-assembly of $\{Mo_{72}Fe_{30}\}$ in salt and salt-free solutions (Figure 1A). The final I of the salt-free solution was 3095 kcps with an average $R_h = 19.3$ nm. In the presence of 0.9 wt % NaCl, the final I at 90° scattering angle was 3766 kcps and $R_h = 82.1$ nm (Figure 1). According to eq 3, $I \propto C \times M_w$, then $I \propto C \times R_h^2$, we can estimate the relative amount of blackberries in salt-free and salt-containing solutions: $C_{salt}/C_{salt\text{-}free} = (I_{salt}/R_{h,salt}^2)/(I_{salt\text{-}free}/R_{h,salt\text{-}free}^2) = 0.067$, i.e., much less amount of $\{Mo_{72}Fe_{30}\}$ have formed blackberries in the presence of NaCl than in pure aqueous solution, or there are a much larger amount of discrete macroanions left in the salt solution due to the enhanced stabilization capability provided by the additional salts. Considering that the larger particles show stronger angular dependence on the scattered intensity, if we extrapolate the scattered intensity to zero scattering angle, the $C_{salt}/C_{salt\text{-}free}$ ratio obtained above will be slightly higher.

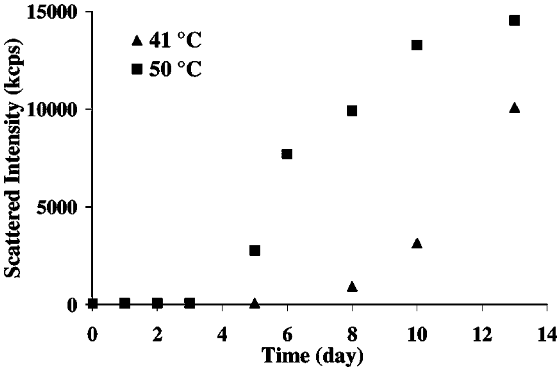


Figure 3. The increment of scattering intensity of a 0.5 mg/mL $\{Mo_{72}Fe_{30}\}$ sample in 0.17 mol/L NaI solutions at 41 and 50 °C.

The two-step mechanism can nicely explain the lag period in $\{Mo_{72}Fe_{30}\}$/salt solutions. The presence of external salts increases the activation energy of the blackberry formation and slows down the whole process. As a result, the initial process of the dimer formation from discrete macroanions is extended to a day-scaled time period. Furthermore, the additional salts also contribute to stabilize the dimers and make the second step of transition from dimers to blackberries more difficult. In addition, the final average blackberry size in the presence of 0.9 wt % NaCl is 3× larger than that in the salt-free solution, implying that each blackberry needs to be constituted of ~16× as the amount of macroanions as that in the salt-free solution. Therefore, this equilibrium structure factor, combined with a slow association rate, makes the process of the blackberry formation extensively prolonged, which corresponds to the occurrence of stepwise intermediates during the growth period.

3.3. Effect of Temperature. Our postulation on the mechanism of the blackberry formation can be further examined by changing external conditions. Results similar to those described in Section 3.1 were also observed in $\{Mo_{72}Fe_{30}\}$ solution when NaI was introduced. For an aqueous solution containing 0.17 mol/L NaI and 0.5 mg/mL $\{Mo_{72}Fe_{30}\}$, the initial I was very low. The solution was heated to 41 °C to accelerate the process. As shown in Figure 3, after a lag period of six days, the value of I started to grow and quickly reached >10 000 kcps on the 13th day. When the solution was kept at 50 °C, the lag period was further shortened to three days. This can be explained by the fact that a higher temperature can decrease the energy barrier for the oligomer formation, based on the Arrhenius Equation:

$$k = Ae^{-Ea/RT} \tag{8}$$

with A, E_a, R, and T being a frequency factor, the activation energy, the universal gas constant, and temperature, respectively. As the reaction rate k during the lag phase is inversely proportional to the delay time t, eq 8 can also be written as follows:

$$\ln(1/t) = \ln A - E_a/RT \tag{9}$$

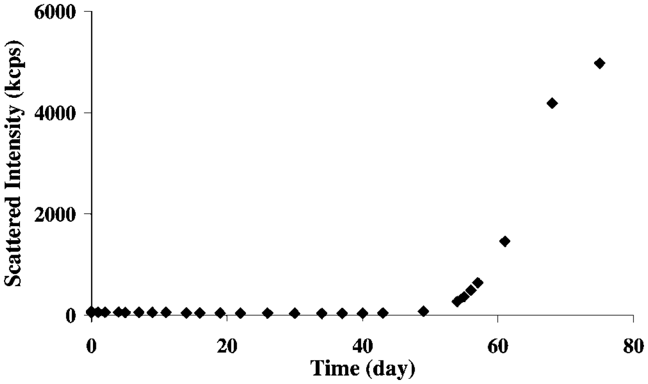


Figure 4. Increment of scattered intensity of a 0.5 mg/mL {$Mo_{72}Fe_{30}$} solution in the presence of 0.085 mol/L Na_2SO_4 solution at 50 °C.

From the SLS studies, $t_1 = 6$ days (41 °C) and $t_2 = 3$ days (50 °C), the E_a value of this nucleation process can be calculated as ~65 ± 5 kJ/mol. Such an energy barrier is lower than the E_a values for the entire of the blackberry formation in salt-free {$Mo_{72}Fe_{30}$} solution (117 ± 5 kJ/mol) or with a small amount of NaI (128 ± 5 kJ/mol at 0.017 mol/L).[4] However, considering that each blackberry contains >1600 single macroions, the oligomer formation process here should be considered as much more difficult than the overall process, i.e., the oligomer formation is the speed-limiting step. Furthermore, the lag period and its temperature dependence indicate that the macroions have been further stabilized so that the oligomer formation becomes more difficult than that in salt-free macroionic solutions. For comparison, we have previously shown that the very short lag periods in salt-free {$Mo_{72}Fe_{30}$} solutions did not show very obvious temperature dependence.[4]

3.4. Effect of the Valent State of Additional Anions. The experimental results on the {$Mo_{72}Fe_{30}$} solutions containing NaI and NaCl are similar since both types of anions are monovalent. However, for the {$Mo_{72}Fe_{30}$} solution containing Na_2SO_4 (with the same molarity of Na^+) the lag period is surprisingly long. As shown in Figure 4, for the {$Mo_{72}Fe_{30}$} solution containing 0.085 mol/L Na_2SO_4, the lag period lasted around 49 days. Considering that the Na^+ concentrations are the same for the above three solutions, this extremely long lag period should not be due to the effect of cations; instead, the role of anions might be important: Cl^- and I^- are both monovalent anions and have similar anionic structures, while SO_4^{2-} is a divalent anion and has a tetrahedral structure covered by oxygen atoms. A SO_4^{2-} anion contributes 4× to the ionic strengths and Coulomb electric interactions than a Cl^- or I^- ion does. As co-ions, they are better stabilizers for the macroanions than the monovalent anions. Moreover, the oxygen shell of SO_4^{2-} can form strong hydrogen bonds with solvent water molecules and give an extra water shell to the {$Mo_{72}Fe_{30}$} macroanions that are surrounded by SO_4^{2-} co-ions. This water shell can also stabilize the macroanions and slow down the oligomer formation. Overall, the multivalent anions make the discrete macroanions more stable and make the blackberry formation more difficult and less favorable in free energy.

3.5. Effect of Solvent Contents. The solvent content (often in dielectric constant) directly affects the behavior of macroionic solutions, e.g., the size of the blackberry structures is found to change with the acetone content in water/acetone mixed solvents.[6c] The kinetics of the blackberry formation was explored in {$Mo_{72}Fe_{30}$} aqueous solution when glycerol, a viscous and water-miscible solvent, was introduced. For the aqueous solution of 0.5 mg/mL {$Mo_{72}Fe_{30}$} containing 10 vol % glycerol, the blackberry formation curve from SLS studies is very similar to that in pure aqueous solution, and no obvious lag period was observed, as shown in Figure 5a. When the glycerol content was increased to 30 vol %, a lag period of at least one day was observed as the scattered intensity I showed almost no change during that period of time. The average R_h of the blackberries also increased with time. After the initial lag period, the average R_h value in 30 vol % glycerol/water mixed solvent kept increasing for three months (Figure 5b), indicating that the presence of a large amount of glycerol could slow down the whole process, for both oligomer formation and the consequent blackberry formation. In addition, similar results were obtained from the 0.5 mg/mL {$Mo_{72}Fe_{30}$} solution in methanol/water mixed solvent containing 25 vol % methanol where a two-day lag period was observed.

The above experiments were all performed in {$Mo_{72}Fe_{30}$} solutions without additional salts. Therefore, the observed lag periods are likely due to the solvent viscosity and polarity. The viscosities of 30 vol % glycerol/water and 25 vol % methanol/water mixed solvents are 2.0 mPa·s and 1.3 mPa·s, respectively, much higher than that of pure water (0.89 mPa·s). The high viscosity slows down the movement of all macroanions in solutions and thus could slow down the oligomer formation. Also, the deprotonation of {$Mo_{72}Fe_{30}$} macroanions become less favorable in the presence of methanol or glycerol, which will decrease the number of active sites on each {$Mo_{72}Fe_{30}$} macroion for effective collisions.

3.6. Comparison between the Self-Assembly Behaviors of Inorganic Macroanions and Virus Capsid Proteins. Interestingly, there are some obvious similarities between the self-assembly of the inorganic macroanions and natural biomacromolecules, such as virus capsid proteins. In general, the spherical (icosahedral) virus capsids are nanoscaled single-layer hollow shells similar to blackberries. A multiple of 60 protein subunits are dispersed equivalently on the surface of the shell, just like macroanions in blackberries. The weak interactions between protein subunits, such as hydrophobic interaction (as believed by many biochemists), play an important role in the capsid self-assembly.[26] In fact, there are only limited intersubunit interaction sites owing to the geometrical restriction and the specific interactions, such as chemical bonding, hydrogen bonding, and electrostatic interaction between subunits.[27,28]

More importantly, beyond the structural similarity between the blackberries and virus capsids, both types of self-assembly processes demonstrate similar kinetic features. For example, for human papillomavirus (HPV), its in vitro assembly process from protein subunits to icosahedral HPV capsid displays a sigmoidal kinetic curve in SLS studies, as shown in Figure 6. The lag period before the rapid growth of assemblies is dependent on the protein concentrations and the ionic strength. The theoretic nucleation-elongation model perfectly identifies the delay time and suggests that HPV assembly begins with the slow formation of a dimer of protein subunits.[11] Once the rate-limiting nucleus has formed, subsequent subunits quickly add to the growing polymer one at a time until it is complete. Similar results were

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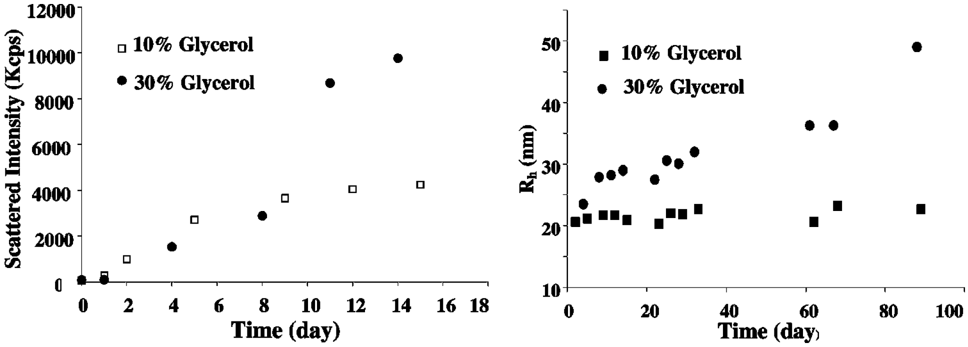

Figure 5. (a) Increment of the scattered intensity *I*, (b) change of the average R_h of the blackberry structures with time in 0.5 mg/mL $\{Mo_{72}Fe_{30}\}$ solutions in glycerol/water mixed solvents containing 10% and 30% glycerol, respectively, at 50 °C.

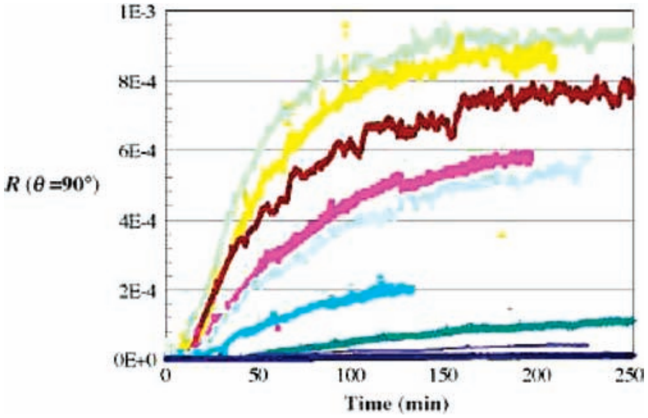

Figure 6. Light scattering study of the assembly of HPV capsid proteins at various HPV concentrations. The lag time, reaction slope, and extent of assembly were dependent upon the initial protein concentration. Although assembly is assumed to begin at $t = 0$, changes in scattered light are not observed until minutes later. (Reprinted from ref 11, copyright 2004, with permission from Elsevier).

also reported in the assembly process of in vitro hepatitis B virus (HBV) capsids[9,26] and cowpea chlorotic mottle virus capsids.[29]

In addition, the assembly process of virus capsid proteins is also sensitive to temperature and ionic strength, which is very similar to the blackberry formation of inorganic macroanions. As the temperature increases, the lag phase in the kinetic curve of the HBV capsid formation become shorter and the self-assembly process obviously accelerates.[10] The studies about the salt effect on the in vitro formation of virus capsids show that the self-assembly are usually initiated at high ionic concentration and/or low pH, because the small ions shield the ionic residue of capsid proteins and decrease the electrostatic repulsion between subunits.[10,30]

It is still worth mentioning that there are also some differences between self-assembly of inorganic macroanions and virus capsid proteins. Although in both systems, the kinetic properties are dependent on the ionic strength, the salt effect is different. As the salt concentration increases, the lag period of virus capsid proteins becomes shorter, whereas in the case of inorganic macroanions, it becomes longer. Additionally, the sizes of virus capsids generally keep constant within a wide range of salt concentration, while the blackberry size becomes sensitive to the salt concentration at relatively low salt concentrations[4,31] because the small ions tune the attractive interaction between neighboring macroanions, which changes the association numbers of macroanions in blackberries. Furthermore, the lag phase shortens with increasing the concentration of virus capsid proteins, while for the inorganic macroanions, there is no obvious dependence of the lag phase time period on the macroionic concentration.

On the basis of the comparison above, there are obvious similarities between formations of virus capsids and blackberries. Considering that both virus capsid proteins and POMs are nanoscaled, soluble macroions, and both self-assemble into single-layered spherical structures, it is reasonable to postulate that their self-assemblies might share similar driving forces and mechanisms. The hydrophobic interaction is widely thought of as the dominant driving force for the virus capsid formation. However, for the POMs, they do not contain any hydrophobic moieties and thus hydrophobic interaction does not play a role for the blackberry formation. Then a natural question is: if the macroions can assemble into single-layered hollow spherical structures without the assistance of hydrophobic force, is it possible that the electrostatic interaction might be underestimated in the virus shell formation? Is it possible that the counterion-mediated effect might also contribute to the attractive force in the assembly of virus capsid proteins? If the above questions have positive answers, i.e., various macroions follow similar types of assembly behaviors in solution, then ideally, the POM macroions might be useful as simple model systems to study the more complicated biomacromolecular systems.

4. Conclusions

In summary, we have explored the unique lag period at the beginning of the self-assembly of the hydrophilic Keplerate $\{Mo_{72}Fe_{30}\}$ macroions in dilute solutions. A two-step nucleation−growth mechanism is proposed to explain this lag phase, which is connected to the thermodynamically unfavorable step—the formation of oligomers, mostly dimers. Once the oligomers reach a critical concentration, the blackberry formation process is accelerated. Compared to salt-free or low-salt solutions, larger amounts of additional electrolytes can stabilize the macroanions and significantly slow down the whole process. This leads to an extended lag period. In addition, multiple factors such as temperature, the valent states of the small anions, and the solvent content also have impacts on the lag phase. The sigmoidal-

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shaped formation curves for the blackberry formation in the presence of additional salts are very similar to those for the formation of virus shells by capsid proteins. This observation, together with the fact that the blackberries and virus capsid shells are both assembled by macroions and structurally similar, raises the consideration regarding the role of electrostatic interaction on the self-assembly of virus capsid proteins.

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