Chapter 3

Dynamic Behavior/Evolution of Nanoscale Solution-state Species in Partially Reduced Polyoxomolybdate Solution

An unusual combination of analytical protocols has been used to study the solution-phase growth of a partially reduced Mo-POM. The evolution of Mo-POM nanoscopic features over the course of weeks was monitored by flow field-flow fractionation and corroborated by electron microscopy (Transmission and Scanning). Unusual, polydisperse size distributions of nanostructures were observed in the flow field-flow fractionation (FIFFF) and TEM measurements. Total Mo content in the solution and precipitate phases was followed off-line by inductively coupled plasma-optical emission spectroscopy (ICP-OES). During the self-assembling process of Mo-POM, we observed crystallization-driven formation of keplerate \( \{\text{Mo}_{132}\} \) and solution-phase-driven evolution of structurally related nanoscopic species (3 – 75 nm).

3.1 Introduction

The mystery of molybdenum blue solutions has attracted scientists’ attentions for over two centuries. Structural details of molybdenum blue solutions have been elucidated in only recent years by the solid state studies of Müller and co-workers.\(^1\)\(^2\) However, the solid state studies were limited to the nature of the
well-ordered crystals which were isolated from the solution; isolated species frequently have little to do with what are present in solution state. The characterization of complicated polydisperse Mo-POM is a challenging task; it is particularly difficult to determine which species are present and which species are more stable in molybdenum blue solutions. Ensemble analytical techniques such as dynamic light scattering (DLS) can measure the averages of size distributions whereas non-ensemble techniques such as electron microscopy can highlight the properties of individual structures such as particle morphology and composition. DLS techniques have been applied in studies of Mo-POM solution states; one study showed that in polar solvents, species in Mo-POM presented a distribution of aggregates instead of single anions. Similar results were obtained for a chemically related Mo-POM aqueous system. In that study, the structures of the uniformly large aggregates have been demonstrated to be vesicular. However, such techniques are not compatible with the Mo-POM under current study because its high absorbance precludes their use. Therefore a different analytical protocol is necessary to separate and characterize nanoscopic components in Mo-POM solution state. The versatile high-resolution separation technique, field-flow fractionation (FFF), is a good choice.

FFF is an elution-based, chromatography-like separation and sizing technique uniquely capable of separating materials within a wide size range (1 nm to 100 µm). The main difference between FFF and chromatography is that in FFF, separation is conducted in a thin, unpacked open channel instead of in a column filled with packing agents. Unlike the many kinds of chromatographies
that are based on exclusion or adsorption, FFF functions solely by physicochemical interactions with an external field perpendicular to the flow. The nature of the external field gives rise to different FFF sub-techniques. Currently, the common FFF sub-techniques are flow, sedimentation, thermal, electrical and magnetic FFF. Among the members of the FFF family, flow field-flow fractionation (FlFFF) is the most versatile technique tested so far. FlFFF has wide applications in biomedicine, environmental science, and industry.

However, the use of FFF to separate inorganic clusters has not been reported so far. Certainly no time-dependent chemistries have been elucidated with any FFF technique. These facts make the current work unique. In this study, we assessed the feasibility of FlFFF for the separation and characterization of partially reduced polyoxomolybdate species chemically related to keplerate \{Mo\textsubscript{132}\}. Material redissolved from the crystallization-driven preparation of keplerate \{Mo\textsubscript{132}\} and material from the mother liquor was used in the study.

3.2 Theory

3.2.1 Flow field-flow fractionation

Conventional FFF separation occurs in a thin, ribbon-like, open channel. The sample is introduced into the channel by a carrier fluid. The channel flow rate is controlled so that a parabolic flow profile is achieved in which the maximum flow velocity occurs at the center of the channel. In FlFFF, a cross-flow of fluid functions as the external field. This cross-flow fluid is applied
perpendicularly to the channel flow, driving the sample towards the accumulation wall while different diffusion rates of sample components move them away from the wall (Figure 3.1). Since each component has different diffusion coefficients and interacts differently with the external field, the components of the same size are retained at similar transverse positions across the channel and are eluted at different times. In the normal mode of FFF separation, d < 1 µm, small particles elute faster than larger particles. However, for particles with d > 1 µm, the steric/hyperlayer mode prevails in separation and larger particles elute faster.\textsuperscript{23} Since the polydisperse Mo-POM in this study had d < 1 µm, the normal mode of separation is active.

**Figure 3.1.** (A) Schematic representation of FIFFF channel. (B) Exploded view of channel (normal mode).
The theory of FFF has been detailed elsewhere; the basic principles are described briefly. In FFF measurements, the retention of a sample component is expressed as the retention ratio \( R = t^0/t_r = V^0/V_r \); \( t^0 \) is the retention time of void peak; \( t_r \) is the retention time of sample component; \( V^0 \) is the geometric volume of the channel while \( V_r \) is the elution volume of sample component.

The interaction of sample with the external field is best defined by the retention parameter \( \lambda \), which can be related to \( R \):

\[
R = 6\lambda \left( \coth \left( \frac{1}{2\lambda} \right) - 2\lambda \right)
\]

(3.1)

In FIFFF, \( \lambda \) is defined for each component by the following equation:

\[
\lambda = \frac{V^0 D}{V_c \cdot w^2}
\]

(3.2)

where \( w \) is the channel thickness, \( V_c \) is the volumetric cross-flow rate and \( D \) is the component’s diffusion coefficient. Under a given set of conditions, the parameters \( V^0 \), \( V_c \) and \( w \) are constant, \( t_r \) and \( V_r \) can be directly measured from experiments and thus \( \lambda \) can be calculated from \( R \). Therefore the diffusion coefficient \( D \) can be determined by eq. 3.2. From the Stokes–Einstein equation, we can derive the relation between the particle diameter \( d \) and the diffusion coefficient \( D \), which is expressed as:

\[
d = \frac{kT}{3\pi\eta D}
\]

(3.3)
here \( k \) is Boltzmann constant, \( T \) is the absolute temperature and \( \eta \) is viscosity. Diffusion coefficient and particle diameter are two parameters can be provided by FFF measurements.

### 3.2.2. Fractograms and particle size distributions

The raw data from FIFFF experiment is a plot of UV signal \textit{versus} elution time or elution volume, which is called as a fractogram. The equivalent spherical particle diameter at any given elution time or volume can be calculated as aforementioned. Provided that the UV detector response, which is designated UV\(_i\) at point \( i \) along the elution profile, is proportional to the particle mass concentration in the flow streamline \((dm^c/dV_i)\), a particle size distribution \((dm^c/dd_i)\) can be converted from the UV fractogram based on the following equation:\(^{21}\)

\[
\frac{dm^c_i}{dd_i} = \frac{dm^c_i}{dV_i} \cdot \frac{dV_i}{dd_i} \propto UV_i \cdot \frac{dV_i}{dd_i} \tag{3.4}
\]

where \( m^c_i \) is the cumulative mass of the sample eluted up to elution volume \( V_i \); \( d_i \) is the particle diameter fractionating at \( V_i \) and \( dd_i \) is the increment in \( d_i \) corresponding to \( dV_i \) increment in \( V \) at point \( i \) along the fractogram. The quantity \( dV_i / dd_i \) can be calculated with the FIFFF theory indicated above. The superscript \( c \) in the equation emphasizes the cumulative amount eluted up to point \( i \) on the fractogram.
3.2.3. Mo content distributions

If the FIFFF fractions are fed into an ICP-OES instrument, for element Mo, the mass concentration in the eluent \( (d m^c_{Mo}/dV_i) \) can be determined by calibration with standard solutions; thus a Mo-based particle size distribution can be determined by equation 3.5:\(^21\)

\[
\frac{d m^c_{Mo_i}}{d d_i} = \frac{d m^c_{Mo_i}}{d V_i} \cdot \frac{d V_i}{d d_i}
\]

where \( m^c_{Mo_i} \) is the cumulative mass of element Mo eluted up to point \( i \) on the fractogram; the Mo-based particle size distribution is obtained by plotting \( d m^c_{Mo_i}/d d_i \) against particle diameter \( d \).

3.2.4. Mo concentrations in particles

The distribution of element Mo mass per unit of particle mass at any point \( i \) along the elution time or volume axis is calculated as follows:\(^21\)

\[
\frac{d m^c_{Mo_i}}{d m^c_{i}} = \left[ \frac{d m^c_{Mo_i}}{d V_i} \cdot \frac{d V_i}{d d_i} \right] \propto \frac{d m^c_{Mo_i}}{d V_i} / UV_i \tag{3.6}
\]

here \( d m^c_{Mo_i}/d V_i \) is the Mo concentration in the eluent and is determined by ICP analysis; \( UV_i \) is the UV response at elution volume \( V_i \). This calculation is based on the assumption that the mass concentration of particles in the eluent is proportional to the UV signal.
3.2.5 Surface density distributions of Mo

The amount of Mo on the particle surface can be described as surface density distribution, which is a plot of the amount of Mo per unit particle surface area \( \frac{dm_{Mo}^c}{dA^c_i} \) as a function of particle size. The surface density of Mo can be determined as follows based on the assumption that a constant spherical shape (single-layer) and density present for the particles:

\[
\frac{dm_{Mo}^c}{dA^c_i} = \left( \frac{dm_{Mo}^c}{dm_i^c} \right) \times \left( \frac{\delta m_i^c}{\delta A^c_i} \right) \propto \left( \frac{dm_{Mo}^c}{dV_i} \right) / UV_i \times d_i \quad (3.7)
\]

3.3 Particle sizing and characterization of redissolved keplerate \{Mo\_{132}\} solution by FIFFF

Chapter 1 describes the development of a protocol for the kinetic precipitation of Mo-POM with de novo chelating agents and makes the argument that with tripodal 1.1 the distributions of particle sizes and the particle morphologies revealed by TEM generated repeatable snap shots of dynamic equilibrium of Mo-POM in solution. To apply such techniques, the phase transition needs to occur faster than structural rearrangement in solution. Although definitive evidences for kinetic precipitation has been shown in chapter 1, elucidation of the nature of keplerate \{Mo\_{132}\} solution structures could benefit from a time-dependent assay. Here we applied time-dependent FIFFF technique to fractionate and characterize the keplerate \{Mo\_{132}\} background solution. The aqueous keplerate \{Mo\_{132}\} solution was prepared exactly the same as that used in the precipitation experiments by dissolving 3 mg keplerate \{Mo\_{132}\} solid in 2 ml
deionized water. Since Mo\textsuperscript{VI}/Mo\textsuperscript{V} mixed valence has a characteristic absorption at 455 nm, the particle sizing of Mo-POM nanoscopic components was monitored at 455 nm.

**Figure 3.2A** shows the FIFFF fractograms of homogeneous keplerate \{Mo\textsubscript{132}\} solution as a function of time on the scale of days. Surprisingly, freshly prepared keplerate \{Mo\textsubscript{132}\} solution fractionated into a trimodal distribution of sizes instead of eluting as one uniform peak. Provided that the UV response is proportional to the particle mass concentration in the FIFFF stream, the size distribution of each fraction can be calculated from the UV fractogram based on the equation 3.4. As shown in **Figure 3.2B**, the aqueous keplerate \{Mo\textsubscript{132}\} has three distinct size distributions.

In **Figure 3.2B**, the particles with average diameter of approximately 3 nm corresponded clearly to the single keplerate molecules. At this scale material with strong absorbance at 455 nm and UV-transparent material were observed. The second and third size distributions, with average diameter of 8 and 18 nm respectively, could have contributed to self-assembled aggregates from keplerate building blocks. The FIFFF measurements on the same solution after different days indicated significant changes on the contribution from each fraction.

A time-dependent decrease in the population of single keplerate molecules with concomitant formation of larger aggregates was observed. More interestingly, the maximum particle size of the largest aggregate increased by ~2-3 nm per day over the period of monitoring.
Figure 3.2. (A) FIFFF fractograms of redissolved keplerate \{Mo_{132}\} aqueous solution. (B) Particle size distributions of redissolved keplerate \{Mo_{132}\} solution (Step D in page 2: Figure 1.1). FIFFF experimental conditions: cross-flow rate = 3.00 mL/min; channel flow rate = 0.5 mL/min.
In order to gain more information about the unusual solution-state behavior of redissolved aqueous keplerate \( \text{Mo}_{132} \), the FIFFF cross-flow rate was decreased; similar results were obtained as showed in Figure 3.3. The slow assembly process observed in this study is in accordance with the results of chemically related Mo-POMs reported by Müller and Liu respectively.\(^4,5\)

![Figure 3.3. Particle size distributions of keplerate \( \text{Mo}_{132} \) aqueous solution over the time (Step D in page 2: Figure 1.1). FIFFF experimental conditions: cross-flow rate = 0.77 mL/min; channel flow rate = 0.5 mL/min. Calculation based on equation 3.4.](image)

FIFFF measurements applied to the keplerate \( \text{Mo}_{132} \) solution state gave similar results as kinetic precipitation with tripodal 1.1 described in Chapter 1. The size regime early in the dissolution of keplerate \( \text{Mo}_{132} \) found in the FIFFF distributions approximated the features present in the transmission electron micrographs of the coprecipitate. These experiments lead to the conclusion that
Mo-POM solution-state structure is not at equilibrium upon dissolution of keplerate \(\text{Mo}_{132}\). Evolution toward nanoscopic species requires days. These facts directly support the hypothesis that tripodal 1.1 kinetically trapped polydisperse aqueous Mo-POM before any structural deviations from the solution state occurred.

3.4 Separation and characterization of Mo-POM mother liquor by FIFFF

By FIFFF, solution phase Mo-POM anionic species slowly and continuously self-assembled into large aggregates \((r \sim 3-35 \text{ nm})\). The aforementioned crystalline keplerate \(\text{Mo}_{132}\) is only the species isolated from partially reduced Mo-POM. The formation of this material is likely driven by favorable interactions in the cubic crystal lattice. The dynamic behavior of the solution state species is more complicated. To unveil the long-term mystery of the aqueous behavior of polymeric polyoxomolybdate in more details, we focused on the Mo-POM mother liquor of the preparation of keplerate \(\text{Mo}_{132}\).

The Mo-POM mother liquor was prepared as published procedure.¹ To allow the system to come to a stable state, we did not make any attempts to filter solids from the solution. Samples from the mother liquor were subjected to FIFFF and TEM measurements at different reaction times. Figure 3.4 shows the UV fractograms of Mo-POM mother liquor. Apparently three fractions can be observed in the fractograms. Since the intensity of UV/Vis signal is proportional to the particle mass concentration in the eluent, it is reasonable to assume that the intensity of UV/Vis signal reflects the mass content from each fraction. Time-
dependent studies of these fractograms indicated that structural evolution of Mo-POM species presented in solution.

Figure 3.4. FIFFF fractograms of Mo-POM mother liquor with various reaction times (Step F in page 2: Figure 1.1).
The signal intensity from the solution phase of the published preparation (about 10 minutes after the initiation of the reaction) was very weak, indicating no Mo$^V$/Mo$^{VI}$ mixed valent material and therefore no keplerate $\{\text{Mo}_{132}\}$. Upon standing, the solution phase of the keplerate $\{\text{Mo}_{132}\}$ preparation darkened to opaque brown. The UV/Vis signal also increased with time. After ~8 h, the UV/Vis signal was most intense; subsequently the UV/Vis absorbance decreased over two days presumably due to the precipitation of keplerate $\{\text{Mo}_{132}\}$. After two days, the UV/Vis response continued to decrease but obviously at a slower rate. In the fractogram, after initiation of the reaction, the fraction assigned to keplerate $\{\text{Mo}_{132}\}$ decreased within several hours followed by concomitant evolution of larger particles at $d > 10$ nm; see Figure 3.4. The rates of both particle formation and crystallization slowed down after two days.

Under the given FlFFF experimental conditions (see experimental section), particles with diameter of ~3 to 75 nm were detected and polydisperse size distributions were observed (Figure 3.5). The maximum for each distribution migrated a little with a narrow range: $3.2 \pm 0.3$ nm, $11.5 \pm 1.2$ nm, and $25.0 \pm 3.0$ nm respectively. Although the relative mass of the first peak in the size distribution was very low, the corresponding peak appeared in the fractogram consistently over the period of monitoring. One important thing should be mentioned is that the size of the three populations in the mother liquor of the preparation of keplerate $\{\text{Mo}_{132}\}$ did not change significantly over 7 days. This was the major difference from the redissolved dilute keplerate $\{\text{Mo}_{132}\}$ solution. The results indicate that the nanoscopic species are thermodynamically stable.
after they form in aqueous solution even when they are continuously washed by both channel-flow and cross-flow fluids during an FIFFF run.

**Figure 3.5.** Particle mass-based size distributions of Mo-POM mother liquor with various reaction times (Step F in page 2: Figure 1.1). The corresponding size distribution of each fraction was determined directly from UV fractograms.
The Mo concentration of the mother liquor was monitored by inductively coupled plasma-optical emission spectroscopy (ICP-OES). The results showed that the total Mo(aq) dramatically decreased within the first two days followed by a period of slow decrease after day two, indicating a continuous formation of crystalline keplerate \{Mo_{132}\} (Figure 3.6A). After day 30, no detectable change can be determined.

**Figure 3.6.** (A) Time-dependent Mo-concentrations in Mo-POM mother liquor; (B) Time-dependent Mo concentrations in eluting fractions (Step G in page 2; Figure 1.1).
Furthermore, from monitoring the UV/Vis absorption and total Mo content in the solution simultaneously, the UV/Vis detector signals change were coincident with the total Mo by ICP analyses. For some samples, the fractions eluting from FFF were collected and Mo compositions were determined off-line by ICP-OES. As shown in Figure 3.6B, it is apparent that Mo concentrations in the eluent also decreased continuously over the course of monitoring. Figure 3.7 presents a clearer picture of the relationship between the ICP Mo distribution and particle mass distribution. The result showed that the Mo mass distribution was not in accordance with the particle mass distribution; higher Mo density was observed for small particles whereas the Mo density decreased for large particles. This indicated that the Mo contents in the particles were not uniform.

![Figure 3.7.](image)

**Figure 3.7.** Particle mass-based and Mo-based size distributions of Mo-POM mother liquor (Step G in page 2: Figure 1.1). Calculation of relative Mo mass based on equation 3.5.
There is a possibility that some Mo mass was in the form of large particles too large to be detected by current FIFFF experimental conditions. The hypothesis was tested by slowing down the FIFFF cross-flow rate \( (V_c) \) to optimize for measurements of large particles (100 nm to up). Under the FIFFF conditions which generally can sort particles up to 500 nm, no significant contribution from large particles (100 nm and up) was observed in UV fractograms.

Another hypothesis suggests that some large particles may decompose and convert back to UV/Vis–undetectable solution state. This hypothesis was tested by monitoring the UV/Vis absorption and total Mo content in the solution simultaneously (Figure 3.6A). The results showed that the UV/Vis response reflected the ICP Mo concentration throughout the reaction. Therefore the particles did not revert to UV-transparent material. Adhesion to the FIFFF membrane during elution could account for “missing Mo.” To evaluate how much Mo was recovered after elution, the total Mo of the whole eluted solvent of a fractogram was determined by ICP-OES and compared to the total Mo introduced at the initiation of the FIFFF run. The absolute recovery of the sample was ~80%. This is a typically recovery for an FIFFF run.

To obtain more information about the distribution of Mo in the particles, the Mo concentration was plotted per unit particle mass against the particle diameter (Figure 3.8). Based on equation 3.6, the ratio of the Mo concentration in the eluent to the UV detector is directly related to the composition of Mo in the particles. It is reasonable to assume that Mo per unit of particle mass should be same over the particle size range if the particles are solid.
Figure 3.8 also shows the corresponding surface density distribution of Mo, calculated by assuming the particles are spherical and composed of only a single layer at the surface. The Mo surface density distribution should remain constant versus the particle size distribution if the particles are hollow and single layer is contained.

![Graph showing Mo content distributions in the particles](image)

**Figure 3.8.** Mo content distributions in the particles (Step G in page 2: Figure 1.1). Calculation of Mo mass per unit particle mass based on equation 3.6; Determination of Mo mass per unit particle surface area based on equation 3.7.

It is noteworthy that the Mo concentration maxima of both curves in Figure 3.8, coincided in the smallest particles with diameter of about 3 nm. This observation strongly supported the speculation that the smallest particles were denser than larger particles. Initially Mo content substantially decreased as particle size increased from 3 to 25 nm. More remarkably, the minimum of both
curves was observed at diameter of approximately 25 nm, suggesting that denser aggregates of keplerate building blocks were formed below 25 nm and that particles probably organize into thin vesicles or less dense particles at around 25 nm. Between particle diameters 25-52 nm, Mo mass per unit particle mass increased slightly.

The Mo mass per unit of particle surface area increased dramatically within the particle diameter range of 25-52 nm. The non-constant distribution of Mo mass per unit particle surface area suggested that the particles in the mother liquor were not simply aggregated in a single layer of Mo-POM spheres. Particles of diameter 25 nm were most probably vesicular.

3.5 TEM study of keplerate \{Mo_{132}\} mother liquor

The TEM results were consistent with FIFF results (Figure 3.9). Typically the particles sizes with diameters of approximately 7 to 75 nm were imaged in the micrographs. The individual keplerate molecules with diameters of \(~3\) nm were invisible; probably they merged with the granularity of the micrographs and disappeared in the background. The morphology of the Mo-POM is continuous with morphologies of keplerate \{Mo_{132}\} and ppt1.1. More remarkably some micrographs showed nanoscopic features (Figure 3.9 day 2) diffracted in a homologous manner throughout the entirety of the features and were probably representative of the formation of a smooth, continuous shell of molybdate at the molecular level. These patterns are called moiré patterns which are caused by overlapping lattices.
Figure 3.9. High-resolution TEM micrographs of Mo-POM mother liquor with various times (Step H in page 2: Figure 1.1). White arrows indicate features contain moiré pattern.

In the preparation of TEM samples, the mother liquor was placed on a lacey carbon-support, copper grid and was dried at room temperature and atmospheric pressure prior to introduction into the high-resolution TEM instrument. The nanostructures in the mother liquor probably deformed or
collapsed during the drying process as a result of loss of internal solvent molecules. Larger, less symmetrical features than observed with ppt1.1 were found in these TEM images (Figure 3.10A). We also observed ordered microcrystalline packing across small areas which produced an ordered diffraction pattern (Figure 3.10B), presumably due to lattice structures in the solid state.

Figure 3.10. (A) represents deformed nanoscopic species caught in the TEM images. (B) a image of ordered packing over small areas (Step H in page 2: Figure 1.1). The inserted figure is a diffraction pattern of the area in the image.

3.6 SEM study of the precipitates formed in keplerate {Mo$_{132}$} preparation

Solids from the mother liquor of the keplerate preparation were assayed by scanning electron microscopy (SEM). Two representative SEM micrographs were presented in Figure 3.11. It is fascinating that two distinct morphologies of precipitates are observed. The well-ordered octahedral crystals corresponded to
keplerate \{\mathrm{Mo}_{132}\}, determined by indexing these crystals. Some spherical, non-crystalline solids were also present in the material that precipitated over days from the mother liquor of the preparation of keplerate \{\mathrm{Mo}_{132}\}. These were observed to disappear over time (compare Figure 3.11A and B) to leave only the crystalline material.

Figure 3.11. SEM micrographs of precipitates formed in the keplerate preparation (Step I in page 2: Figure 1.1). (A): after day 7; (B): after day 36.
More remarkably, structural deviation in the spherical species may be caught in some SE micrographs. SEM analysis suggested that the merger of small spherical structures with larger structures. Because spherical structures disappeared with time in a concentrated solution to leave only the octahedral crystals, the material in the spherical structures must have converted to the octahedral structures by either a direct solid-state process or by a re-dissolution/crystallization process. Like the TEM studies reported in the previous pages, this SEM study supported the notion that spherical nanoscopic solids continued to grow in the Mo-POM mother liquor; the species observed in the SEM study had comparatively large dimensions (100-1500 nm). These dwarfed the structures observed by TEM that adsorbed from the mother liquor onto the lacy carbon copper grids (10-100 nm). It is logical that the nanoscale, spherical material that precipitated would have been bigger than the nanoscale material that was stable in solution. Large polymeric species tend to be less soluble than smaller species.

Based on the results from FIFFF, TEM and SEM studies, two hypotheses were proposed to elucidate the structural evolution of solution-phase Mo-POM nanoscopic species. In both hypotheses the synthesis of keplerate \( \{ \text{Mo}_{132} \} \) is driven forward by an energetically favored crystal-lattice. The two hypotheses differ however in regard to the nature of the solution-state material. In the first hypothesis, single keplerate building blocks function as ‘seeds’ for the self-aggregation process. At the initiation of the growth, single keplerate units aggregate into small amorphous clumps. The clumps undergo a morphological change to become into vesicles, hollow species at \( d \sim 25 \) nm. In a third stage of
growth lamellar accretion increases the vesicle size above 25 nm. In this hypothesis, vesicular growth occurs by adding more layers rather than expanding diameter of a single layer. The vesicles become denser when they attain diameters greater than 25 nm until they are too large to remain in solution. This paradigm is a modification of Liu's single-layer vesicle model for a chemically related Mo-POM. Figure 3.12 is a schematic representation of Mo-POM growth model (hypothesis 1) related to keplerate \{\text{Mo}_{132}\}.

Figure 3.12. Mo-POM growth model (hypothesis 1) related to keplerate \{\text{Mo}_{132}\}.

In the second hypothesis (Figure 3.13), the self-aggregation process also starts from keplerate \{\text{Mo}_{132}\} building blocks. When keplerate molecules come close enough, they merge into ellipsoidal intermediates. Kinetically unstable, these non-spherical species rearrange to spherical species. Stepwise growth of intermediates results in thin vesicles at d~25 nm. Further growth occurs by the aggregation of vesicular structures.

Figure 3.13. Mo-POM growth model (hypothesis 2) related to keplerate \{\text{Mo}_{132}\}.
The merits of both hypotheses need to be evaluated in light of the experimental evidence. The first hypothesis is a modified version of one found in the literature, that was used to explain data from dynamic light scattering. In that work, Liu deduced that most of the mass that was on the surface of nanoscopic species was related to the UV-transparent keplerate \( \{\text{Mo}_{72}\text{Fe}_{30}\} \). He hypothesized that keplerate \( \{\text{Mo}_{72}\text{Fe}_{30}\} \) is a solution-stable species and that the larger nanoscopic species evolve from these smaller units via an \textit{aufbau} process: the building up process. Liu’s dynamic light scattering studies and the FIFFF data in the current work can not adequately differentiate the first from the second hypothesis.

However, the first hypothesis does not fare as well in light of the TEM data. By the first hypothesis species of d~7-9 nm should be composed of 6-9 keplerate units that are closed packed in some fashion. However by TEM features in this range of sizes are smoothly constructed. They do not appear to be the result of small species composed of closely packed spheres of keplerate \( \{\text{Mo}_{132}\} \).

The second hypothesis is favored by the observation of ellipsoidal features in the transmission electron micrographs and the dynamic nature of these polyoxomolybdate structures. When these species were trapped by kinetic precipitation, the elliptical species had one axis twice as long as the other. Asymmetric, smoothly constructed species were also observed in TEM samples of polyoxomolybdate in the mother liquors of keplerate preparations. The nondescript aggregation in hypothesis two is supported by the TE and SE
micrographs obtained in the current study. Preliminary time-dependent, kinetic precipitation experiments with $1.1$ indicate that aggregated species dominate the TEM field after the dissolved keplerate is left to stand for more than one day. Individual nanoscopic species do not stand out in these micrographs.

### 3.7 Conclusion

In this study, an unusual combination of analytical techniques (time-dependent FFF, SEM and TEM) has been used to clarify the dynamic nature of partially reduced Mo-POM solutions and to offer information about the long-standing mystery of partially reduced molybdenum oxide aqueous solutions; such as what species are really present in the molybdenum blue solutions.

The current work has demonstrated for the first time that nanoscopic components in partially reduced Mo-POM solutions can be fractionated by using flow field-flow fractionation. Material redissolved from the crystalline keplerate {Mo$_{132}$} and material from the mother liquor was used in this study. Distinct polydisperse size distributions have been observed not only for keplerate {Mo$_{132}$} aqueous solution but also for the Mo-POM mother liquor related to keplerate {Mo$_{132}$}. While the particle size changed over the course of monitoring in keplerate {Mo$_{132}$} aqueous solution, the Mo-POM mother liquor presented a different story. The particle sizes in the mother liquor were almost the constant although their population decreased dramatically at the first two days and then reached a steady state over a longer time. The difference between the dynamic behaviors of these two materials must have been simply because keplerate
{Mo$_{132}$} upon dissolution was further from thermodynamic equilibrium than the nanoscopic material evolving in the mother liquor of the preparation of keplerate {Mo$_{132}$}. The most stable state for the polyoxomolybdate in the crystal is keplerate {Mo$_{132}$}. The most stable state for the polyoxomolybdate in aqueous solution is a distribution of nanoscopic species.

The calculated molybdenum distribution in unit particle mass as well as molybdenum distribution in unit particle surface area presented a clearer picture for understanding Mo distributions in particles. Within the particle size range of 3 to 25 nm, Mo concentrations generally decreased with the increase of particle sizes. After the point of d~25 nm, both Mo mass concentrations started to increase according to particle sizes. These results suggest that the aggregates are not all single-layer vesicles buildup of {Mo$_{132}$} building blocks but the thickness of the vesicles varies with particle sizes. This conclusion is different from Liu’s light scattering studies of a chemically related keplerate {Fe$_{30}$Mo$_{72}$}. In that study, the author concludes that the large Mo-POM aggregates are single-layer vesicles.$^5$

Further TEM results were in accordance with FIFFF results for the particle size distributions. The results indicate that the formed nanostructures are thermodynamically stable over the course of monitoring. SEM study of the precipitates formed in the keplerate preparation showed two distinct nanoscopic components in the solids. Among them, the spherically nanoscaled objects were not stable and they slowly converted to more crystalline octahedral species over the course of weeks.
3.8 Experimental Section

Mo-POM solutions analyzed were derived from the published preparation of the keplerate \( \{\text{Mo}_{132}\} \).\(^1\) N\(_2\)H\(_4\)·H\(_2\)SO\(_4\) (0.08 g, 0.61 mmol) was added to a 25 mL solution of (NH\(_4\))\(_6\)Mo\(_7\)O\(_{24}\)·4H\(_2\)O (0.56 g, 0.45 mmol) and ammonium acetate (1.25 g, 16.2 mmol) and stirred for 10 min. Aqueous acetic acid (50% vol, 8.3 mL) was subsequently added and the reaction solution was stored in an open flask at 20 °C without further stirring.

**FIFFF Carrier Liquid and Standards:**

The FIFFF carrier solution was pure, deionized Milli-Q water (Millipore) containing 0.007\% (w/v) Triton X-100 surfactant and 0.02\% (w/v) sodium azide (NaN\(_3\)) bactericide. The carrier fluid was passed through a Millipore HPLC inlet solvent filter with a pore size of 10 µm in the channel and cross-flow delivery lines. In order to monitor the performance of the FIFFF instrument and to calibrate the size distributions of the particular particles, standard polystyrene beads of 20 and 50 nm in diameter (Duke Scientific, Palo Alto, CA, USA) were used. One drop of the original concentrated suspension was dispersed in 5 mL of FIFFF carrier solution to obtain an individual standard solution for the FIFFF injection.

1. Samples

**Redissolved Keplerate \( \{\text{Mo}_{132}\} \):** 10 µL solution was directly taken for FIFFF measurement each time. **Mother liquor:** 10 µL suspension in the flask was taken for FIFFF measurement each time. Upon sampling, the suspension was diluted
by 30 µL deionized Milli-Q water. The diluted suspension (5 µL) was then directly injected into the FIFFF channel.

2. FIFFF Instrumentation

FIFFF separations were conducted by using a standard instrument (FFFractionation LLC, Utah, USA, Universal Fractionator model F1000). The dimensions of the channel were 29.4 cm in tip-to-tip length, 0.0197 cm in thickness and 2.0 cm in breadth. The geometrical void volume was 1.09 cm$^3$. A regenerated cellulose membrane with a 10,000 Dalton molecular weight (about 3 nm) cut-off was used. Sample of about 5 µL was injected into the channel through a Rheodyne sample injection port. Two Intelligent Pump Model 301 HPLC pumps were used to deliver carrier liquid in the channel and non-recirculating cross-flows. A channel flow of 0.5 mL·min$^{-1}$, a cross-flow of 3.00 mL·min$^{-1}$ and an equilibrium time of 0.72 min were used. During the equilibrium time, the cross-flow establishes a steady-state distribution of the particles in the channel prior to initiation of the channel flow. Fractograms were obtained by monitoring the absorbance of the eluate at 455 nm by using a Linear Instruments Model 200 UV/Visible detector.

Samples for SEM analysis:

A small amount of precipitates was suction-filtered through an alumina filter membrane (Whatman Anodisc 13, pore size 20 nm) and was dried at room temperature. A piece of a carbon conductive tab was adhered onto a copper plate of the SEM specimen holder. The membrane was placed onto the carbon
conductive tab and was finally coated with Au (Emscope, model SC 400) prior to SEM measurement. The materials were examined using a Hitachi S900 field-emission scanning electron microscopy.

**Samples for TEM analysis:**

About 10 µL reaction solution was daily sampled for TEM measurement. Upon sampling, about 10 µL was placed on a lacey carbon copper grid (Lacey Carbon Type-A, Ted Pella, Inc.). After soaking the grid for 2 min, the excess solution was removed by filter paper and the grid was allowed to air dried at room temperature. The materials were examined using a high-resolution Transmission Electron Microscope (JEOL JEM-2010F).

**Samples for ICP analysis:**

1: 20 µL of Mo-POM mother liquor was diluted into 10 mL using CH₃COOH/CH₃COONH₄ buffer. Half of this solution was subjected to UV/VIS measurements (UV 3101PC, Shimizu); the left solution was fed directly into the ICP torch of an ICP-OES (VISTA-PRO, Varian) to determined the total Mo content in the mother liquor. 2: The eluent from the FIFFF was collected every four minutes and fed directly into the ICP torch to determine the Mo content in the FIFFF fractions. Calibration of instrument was achieved using a standard solution containing 1000 ppm Mo. This standard solution was diluted 100-, 200-, and 2000-fold to obtain calibration curve for Mo element.
References:


