

Interactions of Bovine Serum Albumin with Aluminum Polyoxocations



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(This application note is an excerpt taken from O. Deschaume, K.L. Shafran and C.C. Perry (2006) *Langmuir* 22, 10078-10088).

Introduction

In mildly acidic conditions at concentrations above the solubility limit of aluminum hydroxide, monomeric Al species, such as the hexaaquocation $[\text{Al}(\text{H}_2\text{O})_6]^{3+}$ and its hydrolysis products ($[\text{Al}(\text{H}_2\text{O})_5(\text{OH})]^{2+}$, $[\text{Al}(\text{H}_2\text{O})_4(\text{OH})_2]^+$), can undergo a succession of condensation reactions. These reactions can lead to the formation of small oligomeric Al species, such as Al dimers and trimers [1] and further transformation into large Keggin ions Al₁₃-mers [2] and the recently characterized Al₃₀-mer (figure 1). These have been employed in a number of applications including antiperspirant actives [3], preparation of Al₂O₃ nanoparticles [4] and composite materials [5].

Previous studies on protein-aluminum interactions have largely concentrated on elucidating bioavailability of this element and absorption/elimination pathways in living organisms [6]. Fewer studies have investigated interactions of Al species with proteins and other biopolymers with regard to materials chemistry applications. A significant effect of the surface charge of aluminum hydroxide on predominantly electrostatic interactions with BSA has been previously demonstrated [7,8]. The substantial impact of aluminum hydroxide as the adjuvant on antigen structure and function has also been reported [9].

A better understanding of the interactions of aluminum species with biomolecules is a necessary prerequisite for successful application of

a combined biomimetic-nanotectonic approach to advanced Al-containing materials. This application note describes the effect of a model protein, bovine serum albumin (BSA), on the generation and properties of hybrid Al-protein composite materials formed from various high-purity Al-containing aqueous nanosized precursors.

In this study, size characterization of the aluminum nanoparticles and Al-BSA samples was made using the technique of dynamic light scattering (DLS). Zeta potential measurements of the Al-BSA samples were made using the technique of laser Doppler electrophoresis. Further information on these techniques is contained in the technical notes "Dynamic Light Scattering: An Introduction in 30 Minutes" and "Zeta Potential: An Introduction in 30 Minutes" available from the Malvern® Instruments Ltd website.

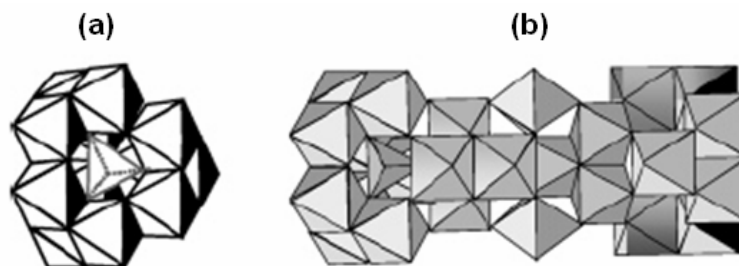


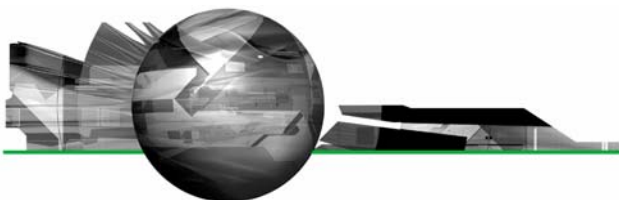
Figure 1: Structure of aluminum Keggin ions (a) Al₁₃-mers ($\text{AlO}_4\text{-Al}_{12}(\text{OH})_{24}(\text{H}_2\text{O})_{12}^{7+}$) and (b) Al₃₀-mers ($\text{Al}_{30}\text{O}_8(\text{OH})_{56}(\text{H}_2\text{O})_{24}^{18+}$)

Experimental

Details of the preparation of the Al₁₃- and Al₃₀-mers can be found in the literature [10]. The final Al concentration in all samples was 0.4M.

A series of Al-BSA solutions were prepared at room temperature by the addition of different amounts of fresh BSA stock solution to the appropriate model Al-containing system. All Al-BSA solutions were vigorously stirred during and after mixing of Al-containing and BSA solutions for 60 seconds using a vortex mixer and left aging at room temperature (25 ± 0.2 °C) for 24 hours. The final aluminum concentration in the Al-BSA solutions was 0.2M and the BSA concentration was varied from 0 to 25mg/mL in 2.5mg/mL.

Dynamic light scattering measurements of the Al₁₃-, Al₃₀-mers and Al-BSA complexes were made on



a Zetasizer® Nano ZS™ at a temperature of 25°C. This instrument contains a 4mW He-Ne laser operating at a wavelength of 633nm and an avalanche photodiode (APD) detector. The scattered light was detected at an angle of 173° and this novel optics arrangement maximizes the detection of scattered light while maintaining signal quality. This provides exceptional sensitivity that is required for measuring the size of nanoparticles, such as the Al clusters measured in this study.

Zeta potential measurements of the Al-BSA complexes were made on a Zetasizer Nano ZS using disposable capillary cells at a temperature of 25°C. The measured electrophoretic mobilities were converted into zeta potential values using the Smoluchowski approximation [11].

Results and Discussion

Figure 2 shows typical correlation functions obtained for the Al₁₃ and Al₃₀-mers. They exhibit two visible decay rates. The rapid decay rates seen in delay times up to 10 microseconds are interpreted as arising from the diffusion of the Al nanocluster particles. The slower decay rates seen near to the baseline of the correlation functions (at correlator delay times of between 10 and 1000 microseconds respectively) are probably due to aggregates.

Figure 3 shows the intensity size distributions for the Al₁₃ and Al₃₀-mers obtained from the analysis of the correlation functions with a non-negative least squares fit [12, 13]. Both samples have main peaks around 1nm in diameter. These intensity size distributions were converted into volume using Mie theory [14]. The volume size distributions obtained are shown in figure 4 and consist of single peaks. The intensity and volume peak means and modes are summarized in table 1.

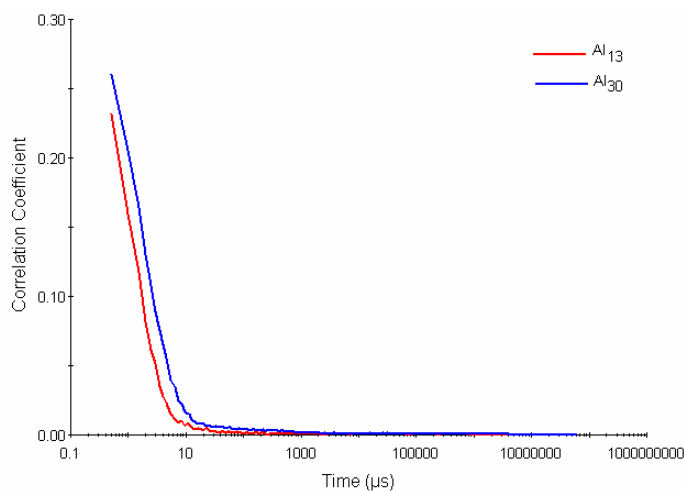


Figure 2: Typical correlation functions obtained for the Al₁₃ and Al₃₀-mers

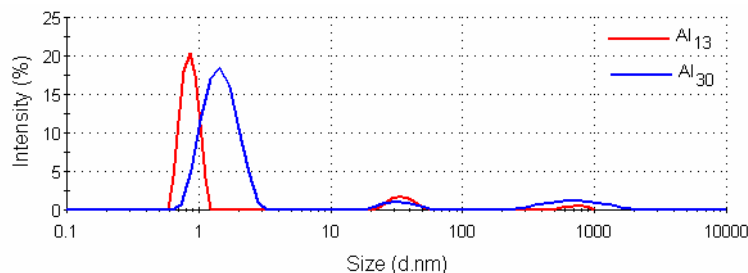


Figure 3: Intensity particle size distributions obtained for the Al₁₃ and Al₃₀-mers

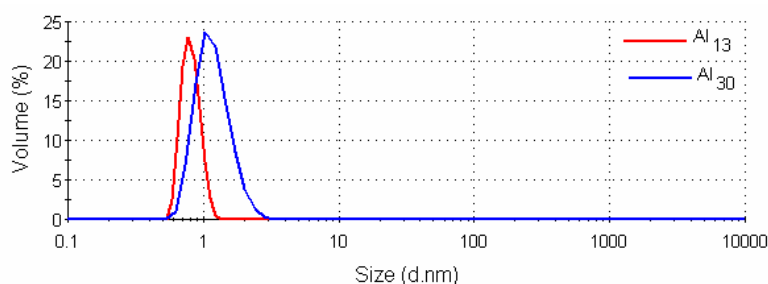
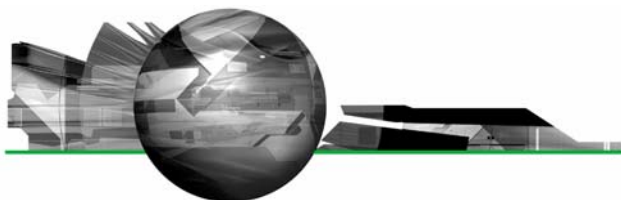


Figure 4: Volume particle size distributions obtained for the Al₁₃ and Al₃₀-mers



DLS measurements of the Al-BSA complexes are shown in Figure 5. Once BSA was added to the Al nanoparticles, the average particle size increased to $\approx 10.7 \pm 0.5$ nm. For the Al₁₃-mer-BSA samples, the mean size of the suspension did not change significantly with further increase of BSA concentration. However, in the case of Al₃₀-containing samples, the average particle size increased at BSA concentrations above 17.5 mg/ml. The mean diameter of BSA was measured to be ≈ 8 to 9 nm. These larger than expected mean particle sizes could arise from the adsorption of Al₁₃-mer clusters on the surface of BSA molecules that are negatively charged under mildly acidic conditions (pH < 5.0). These clusters would be expected to have a larger size than the BSA molecule.

This hypothesis is supported by zeta potential measurements of the Al-BSA complexes (figure 6). The zeta potential values for the Al polyoxocation-BSA complexes were positive at all BSA concentrations measured, with only small differences between the Al₁₃- and Al₃₀-containing samples being observed. The zeta potential of solutions of the Al₁₃ and Al₃₀ polyoxocations without BSA could not be measured as the size of these particles was too small to be detected.

Measurements of the zeta potential of BSA solutions of similar concentration gave negative zeta potential values of -8.6 ± 0.4 mV. The positive zeta potential values obtained for the Al-BSA complexes can therefore be explained by the adsorption of Al polyoxocations on the surface of the protein. With increasing concentration of BSA, the ratio of Al polyoxocations to protein falls resulting in a decrease in the zeta potential.

Table 1: Summary of the peak analysis of the intensity and volume size distributions

Sample	Intensity		Volume	
	Mean (nm)	Mode (nm)	Mean (nm)	Mode (nm)
Al ₁₃ -mer	0.86	0.85	0.81	0.77
Al ₃₀ -mer	1.49	1.44	1.20	1.03

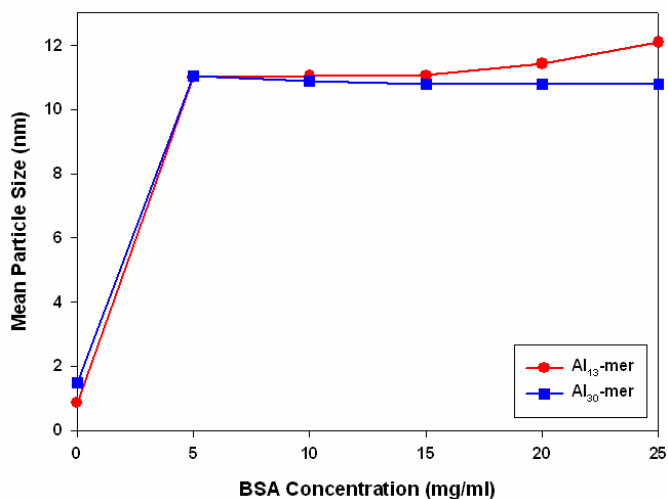


Figure 5: Increase in the mean particle size of Al-BSA complexes as a function of BSA concentration

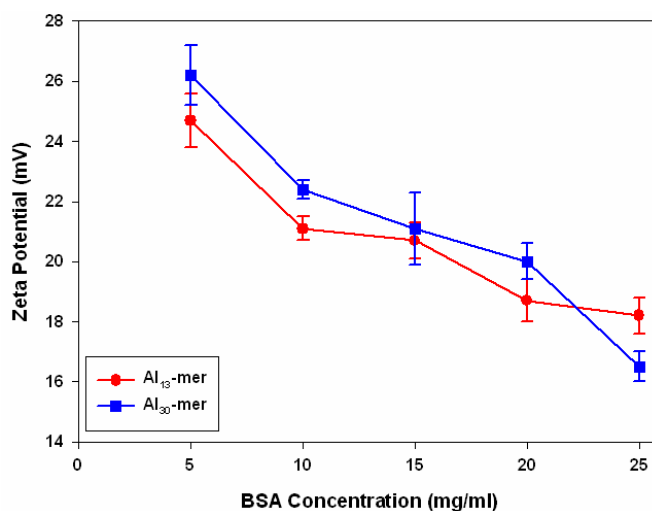
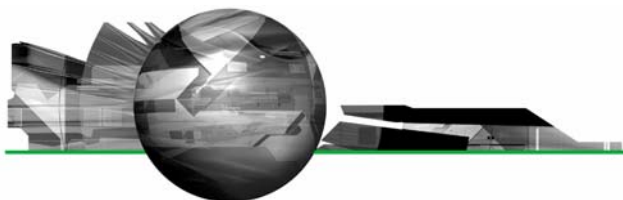


Figure 6: Zeta potential values for Al-BSA complexes as a function of BSA concentration



These zeta potential measurements provide supporting evidence for the predominantly electrostatic interactions between the Al containing species and BSA. Correlation of increasing BSA concentration with decreasing zeta potential indicates that the negative charge of BSA is being cancelled by the Al polyoxocations.

Conclusions

The Zetasizer Nano has been successfully used to determine the size of Al₁₃ and Al₃₀-mers and characterize the size of complexes formed between these aluminum polyoxocations and bovine serum albumin. The non-invasive backscatter (NIBS) optics of the Zetasizer Nano provides the exceptional sensitivity required to characterize particles around 1 nanometer in size such as the aluminum polyoxocations used in this study.

The measurement of the zeta potentials of Al-BSA mixtures has given an insight into the mechanisms of interaction involved in the formation of polyoxocation-protein complexes.

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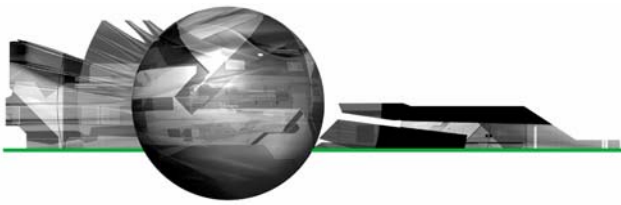
Zetasizer Nano

The Zetasizer Nano system from Malvern Instruments is the first commercial instrument to include the hardware and software for combined static, dynamic, and electrophoretic light scattering measurements. The wide range of sample properties available for measurement with the Nano system include, particle size, molecular weight, and zeta potential.

The Zetasizer Nano system was specifically designed to meet the low concentration and sample volume requirements typically associated with pharmaceutical and biomolecular applications, along with the high concentration requirements for colloidal applications. Satisfying this unique mix of requirements was

accomplished using a backscatter optical system and a novel cell chamber design. As a consequence of these features, the Zetasizer Nano specifications for sample size and concentration exceed those for any other commercially available dynamic light scattering instrument, with a size range of 0.6nm to 6µm, and a concentration range of 0.1ppm to 40% w/v.

These high sensitivity capabilities can also be applied to real time flow measurements, facilitating Absolute SEC and other HPLC measurements.



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