水溶液中の酸化カーボンナノホーン（CNHox）の帯電と凝集におよぼすタンパク質の影響

Effect of Proteins on the Charging and Aggregation of Oxidized Carbon Nanohorn (CNHox) in Aqueous Solution

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Abstract

The dependence of aggregation and charging behavior of oxidized carbon nanohorn (CNHox) on salt concentration was revisited in the presence of proteins, lysozyme (LSZ) or bovine serum albumin (BSA). The shift of critical coagulation concentration (CCC) and clues of non-DLVO interactions were observed in the presence of LSZ or BSA.

Key Words: Dynamic light scattering, Electrophoretic mobility, Non-DLVO interaction

1. Background

The aggregation-dispersion properties of nanoparticles are critical in controlling and predicting the transport and fate of the particles within natural ecosystems. Carbon nanohorn (CNH) is a type of carbon nanomaterials which have gained significant attention in recent years due to their unique structure and properties. Several functional groups can be introduced onto their surfaces by oxidation to promote their dispersibility in different kinds of solvents. The oxidized carbon nanohorn (CNHox) is dispersed in water after the introduction of carboxyl groups. The aggregation-dispersion behaviors of CNHox [1] in the presence of cations of various valences follow the Derjaguin-Landau and Verwey-Overbeek (DLVO) theory, which explains the aggregation-dispersion in colloidal dispersion by combining Van der Waals attraction and electric double layer repulsion. A slow aggregation region, a fast aggregation region, and a critical coagulation concentration between them can be found in a DLVO-like colloidal system. However, the aggregation-dispersion of CNHox in the presence of proteins, which are abundant in the environment, bio-body, and municipal sewage, has not been studied yet. In this study, the aggregation-dispersion behaviors of CNHox in the presence of lysozyme (LSZ) and bovine serum albumin (BSA) were studied.

2. Materials and Methods

CNHox used in the whole study was manufactured by NEC Corporation (Japan). KCl (JIS special grade) was from FUJIFILM Wako Pure Chemical Corporation. Hen egg white lysozyme (LSZ) from Sigma-Aldrich (L6876) (isoelectric point IEP 11.35) and BSA from Sigma-Aldrich (A6003) (IEP 4.9) were used as model proteins. All chemicals were prepared using de-ionized water treated by Elix purification system.

Electrophoretic light scattering (ELS) method was used to measure the electrophoretic mobility (EPM) of bare and protein-coated CNHox at different KCl concentrations. Since $\kappa a \gg 1$ for bare and protein-coated CNHox particles is satisfied, Smoluchowski's equation was used to calculate the zeta potential (ZP) of CNHox:

$$\mu = \frac{\varepsilon_0 \varepsilon_r \zeta}{\eta} \quad (1).$$

where κ^{-1} is the thickness of the electric double layer, a is the radius of colloidal particles, μ is the EPM, ε_0 is permittivity of vacuum, ε_r is the relative dielectric constant of water. The average pH of all experiments without pH adjustment was 5.57 $\pm$ 0.32.

Aggregation behavior of CNHox was investigated by using dynamic light scattering method. The Stokes-Einstein equation describes the relationship between hydrodynamic diameter d_h of aggregates and the diffusion coefficient *D*:

$$d_{\mathrm{h}} = \frac{k_{\mathrm{B}}T}{3\pi\eta D} \quad (2).$$

where k_{B} is the Boltzmann constant, T is the absolute temperature, η is the dynamic viscosity. By analyzing the relationship between d_{h} and time *t*, the initial increase rate of hydrodynamic diameter $(\mathrm{d}d_{\mathrm{h}}/\mathrm{d}t)_{t\to 0}$, reflecting the apparent aggregation rate in this experiment, was derived. Each measured value of $(\mathrm{d}d_{\mathrm{h}}/\mathrm{d}t)_{t\to 0}$ was subsequently normalized by the rate $(\mathrm{d}d_{\mathrm{h}}/\mathrm{d}t)_{t\to 0}{}^{\mathrm{f}}$, representing the rate in the fast aggregation regime. These normalized rates can be correlated to the stability ratio *W*, which is defined as:

$$W = \frac{(\mathrm{d}d_{\mathrm{h}}/\mathrm{d}t)_{t\to 0}{}^{\mathrm{f}}}{(\mathrm{d}d_{\mathrm{h}}/\mathrm{d}t)_{t\to 0}} \quad (3).$$

3. Results and discussion

It is known that CNHox remains negatively charged while the KCl concentration ranges from 10 mM to 100 mM, though the magnitude of ZP is decreased due to the screening effect. This negative charge can be attributed to the deprotonation of carboxyl groups on CNHox [1].

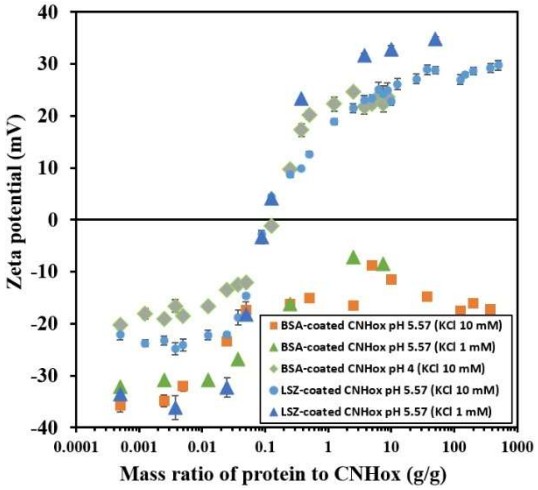

Figure 1. Zeta potential of LSZ-coated and BSA-coated CNHox against the mass ratio of protein/CNHox with 10 mM KCl.

Fig. 1 shows the ZP of CNHox versus mass ratio of protein/CNHox. We notice that the charge reversal happened in the presence of LSZ at pH 5.57 or BSA at pH 4. These reversals are because the positively charged LSZ [2] or BSA at pH 4 neutralized the negative charge of CNHox as observed for silica particles neutralized by LSZ [3]. In the case of BSA at pH 5.57, however, the magnitude of ZP decreased and reached to a negative plateau. Although both BSA and CNHox are negatively charged at pH 5.57, the adsorption still happened due to the existence of positively charged lysine groups on BSA [4]. Furthermore, the magnitude of ZP of LSZ-coated CNHox decreased at higher electrolyte concentration due to the screening effect. Things are different for the BSA-coated CNHox which need further experiment.

Fig. 2 depicts the inverse stability ratios of protein-coated CNHox. While the LSZ-coated CNHox shows a DLVO like aggregation behavior when the ZP is around -25 mV according to Figure 1, BSA coated CNHox does not show fast aggregation at all even though ZP is around -20 mV.

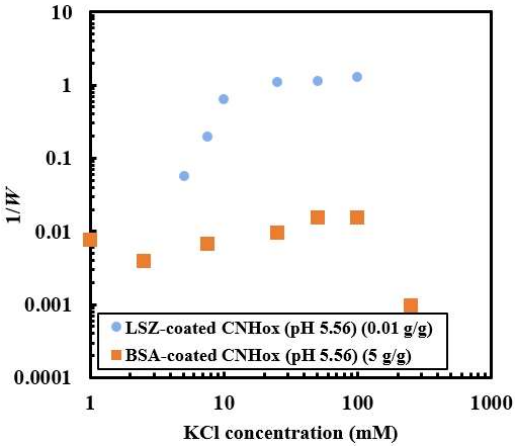

Figure 2. Inverse stability ratio ($1/W$) of protein-coated CNHox in various conditions.

This non-DLVO like behavior may be due to the existence of non-DLVO repulsion. The effect of steric repulsion can drastically inhibit the aggregation, which is also proved by Saleh *et al.* [5].

4. Conclusion

LSZ may neutralize and even reverse the net charge of CNHox. The presence of BSA inhibits the aggregation of CNHox at 5 g/g due to the non-DLVO repulsion. The outcomes of electrophoresis show the adsorption of BSA can happen even when CNHox and BSA are negatively charged due to positively charged groups on BSA.

5. References

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