

水溶液中の酸化カーボンナノホーン (CNHox) の凝集に及ぼすリゾチームの影響 Effect of Lysozyme on the Aggregation and Charging 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. We also investigated the aggregation-dispersion and charging behavior of CNHox with different dosages of lysozyme (LSZ). The shift of critical coagulation concentration (CCC) and clues of non-DLVO interactions were observed in the presence of LSZ.

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

1. Background

Aggregation-dispersion behavior is an important factor for the fate and transport of colloidal/nano particles in environment or organism and the application of colloidal/nano particles in industry and medicine [1]. Oxidized carbon nanohorn (CNHox) has gain extensive attention due to large specific surface area and their latent application prospect. Omija *et al.* [2] had proved that the salt-induced aggregation of CNHox follows the Derjaguin-Landau and Verwey-Overbeek (DLVO) theory, in which there are slow aggregation regime, fast aggregation regime, and a critical coagulation concentration (CCC) in terms of salt concentration of a colloidal system. In the meanwhile, there is a lack of research on the charging and aggregation-dispersion behaviors of CNHox with biological macromolecules such as proteins, which are ubiquitous in environment and bio-body. In this study, the effect of model protein lysozyme (LSZ) on the charging and aggregation-dispersion behaviors of CNHox is studied.

2. Materials and Methods

CNHox used in the research was produced by NEC Corporation (Japan). KCl (JIS special grade) from Wako Pure Chemical Industry was dissolved in the deionized water to prepare electrolyte solutions with different concentrations. Hen egg-white lysozyme (LSZ) from Sigma-Aldrich (L6876)

were used as a model protein.

Dynamic light scattering method was used to investigate the aggregation behavior of CNHox at different conditions. The relationship between hydrodynamic diameter d_h of aggregates and the particles' diffusion coefficient (D) was related by the Stokes-Einstein equation:

$$d_h = \frac{k_B T}{3\pi\eta D} \quad (1)$$

where k_B is the Boltzmann constant, T is the absolute temperature, η is the dynamic viscosity. From the relationship between d_h and time (t), the initial increased rate of the hydrodynamic diameter (dd_h/dt), which corresponds to apparent aggregation rate in this experiment, was obtained. All the measured values of (dd_h/dt) were normalized by the rate (dd_h/dt)^f at fast aggregation regime. The normalized rates can be related to the stability ratio W defined as:

$$W = \frac{(dd_h/dt)^f}{(dd_h/dt)} \quad (2).$$

We measured the electrophoretic mobility (EPM) of CNHox at different KCl and LSZ concentrations at pH 5.43 ± 0.27 . The condition that $\kappa a \gg 1$, where κ^{-1} is the thickness of the electric double layer and a is the radius of the colloid particle, for CNHox with or without LSZ was satisfied. Therefore, Smoluchowski's equation was used to calculate the zeta potential of CNHox:

$$\mu = \frac{\varepsilon_0 \varepsilon_r \zeta}{\eta} \quad (3)$$

μ is the EPM, ε_0 is permittivity of vacuum, ε_r is the relative dielectric constant of water.

3. Results and discussion

CNHox remains negatively charged while the KCl concentration ranges from 10 mM to 100 mM, though the magnitude of zeta potential is decreased due to the screening effect. This negative charge can be attributed to the deprotonation of carboxyl groups on CNHox.

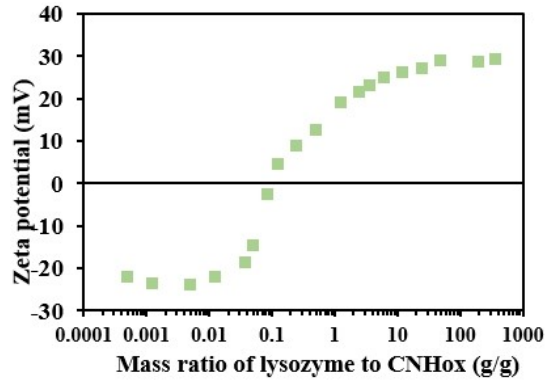


Figure 1. Zeta potential of LSZ-coated CNHox against the mass ratio of LSZ/CNHox with 10 mM KCl.

Fig. 1 shows the zeta potential of CNHox verse mass ratio of LSZ/CNHox. We notice that the charge reversal happened. This reversal is because the positively charged LSZ [3] neutralized the negative charge of CNHox as observed for silica particles neutralized by LSZ [4]. The hydrophobic interaction and the electrostatic force may contribute to the charge reversal. And then, different mass ratio of 0.01 g/g (low mass ratio), 0.0875 g/g (isoelectric point), and 50 g/g (high mass ratio) were set for further research of aggregation-dispersion behavior of CNHox with LSZ.

Fig. 2 depicts the inverse stability ratio ($1/W$) of CNHox at different values of mass ratio. The additional LSZ drastically changed the CCC at isoelectric point (IEP) and high mass ratio of 50 g/g. Van der Waals force is dominant interaction between the CNHox particles due to the neutralization caused by the LSZ.

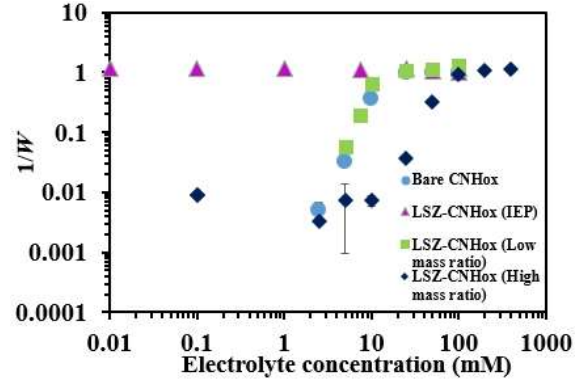


Figure 2. Reciprocal stability ratio $1/W$ against KCl concentration at several mass ratios.

There are clues of non-DLVO behaviors at high mass ratio in Fig. 2. The increment of $1/W$ at low KCl concentration may be attributed to heterogeneous charge on the particle's surface, say, the patch-charge attraction. What should also be concerned is the gradual slope at higher KCl concentration, because the slope of stability plots should be gradual for heterogeneous surfaces [5].

4. Conclusion

LSZ may neutralize and even reverse the net charge of CNHox. The presence of lysozyme induces or inhibits the aggregation of CNHox at IEP or high mass ratio, respectively. The outcomes of dynamic light scattering showed that the aggregation-dispersion behavior of CNHox in the presence of LSZ is in good agreement with the DLVO theory. In the presence of high mass ratio of LSZ, the existence of non-DLVO attraction and repulsion can effectively affect the aggregation of CNHox and show non-DLVO behaviors and even the shift of the CCC.

5. References

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