The influence of the type of charged particles on the parameters of zigzag patterns in BSA films

Dmitriy Glibitskiy[1], Olga Gorobchenko[2], Oleg Nikolov[2], Tatyana Dzhimieva[2], Inna Zaitseva[2,3], Alexander Roshal[4], Mihail Semenov[1], Gennadiy Glibitskiy[1]

[1]O. Ya. Usikov Institute for Radiophysics and Electronics, NAS of Ukraine, 12 Academician Proskura Str., Kharkiv 61085, Ukraine;
[2]V. N. Karazin Kharkiv National University, 4 Svobody Sq., Kharkiv 61022, Ukraine;
[3]O. M. Beketov National University of Urban Economy in Kharkiv, 17 Marshal Bazhanov Str., Kharkiv 61002, Ukraine;
[4]Institute for Chemistry, V. N. Karazin Kharkiv National University, 4 Svobody Sq., Kharkiv 61022, Ukraine
e-mail: dima.glib@gmail.com

In the previous research [1], it was observed that a saline solution of BSA (bovine serum albumin), when prepared and dried under certain conditions, produces "zigzag" crystallization patterns on the surface of the resulting film (Fig. 1), with the amount and properties of such patterns being affected by the state of the protein in the solution and the type of charged particles (ions). Besides the visual analysis of the films, the state of BSA and its environment in solutions themselves was also investigated, using the methods of microwave dielectrometry, pH-metry, ultraviolet spectroscopy of absorption and fluorescence, and dynamic light scattering.

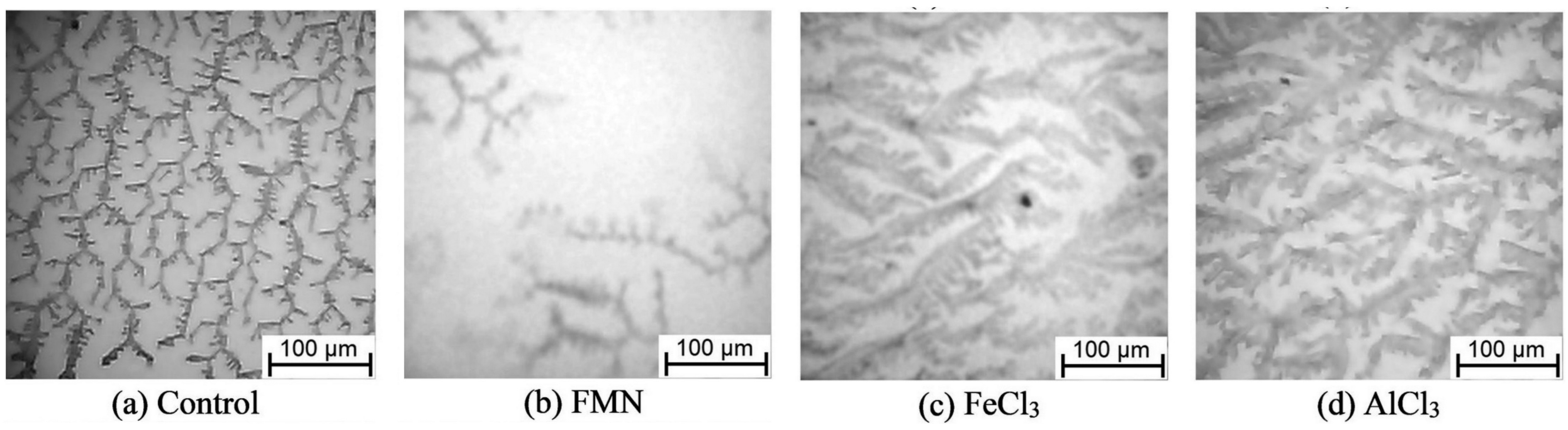

Fig. 1. Typical examples of zigzag patterns on films obtained from 7.5 µM BSA + 20 mM NaCl solutions, with the corresponding substances being added.
(a) Control BSA + NaCl, (b) addition of 0.03 mM FMN, (c) addition of 0.2 mM $FeCl_3$, (d) addition of 0.4 mM $AlCl_3$.

The prior studies have shown [1] that, in the presence of organic (flavin mononucleotide) and inorganic ($FeCl_3$, $AlCl_3$) additives, the conditions for zigzag pattern formation are increasingly disturbed with the rising concentration of the additive (Fig. 2, Fig. 3). Specifically, that (1) the zigzag segment count and specific length drop from the range of control values to almost zero when the relative concentration of FMN to BSA goes from 1:1 to 4:1; (2) increasing concentrations of $AlCl_3$ result in the gradual decline in zigzag parameters as the zeta potential of BSA grows neutral and then positive (Fig. 4); (3) adding $FeCl_3$ affects zeta potential less (Fig. 4), but also results in the formation of large colloidal particles of iron hydroxide, and zigzag parameters begin reducing at $FeCl_3$:BSA concentration ratios of 25:1 or above.

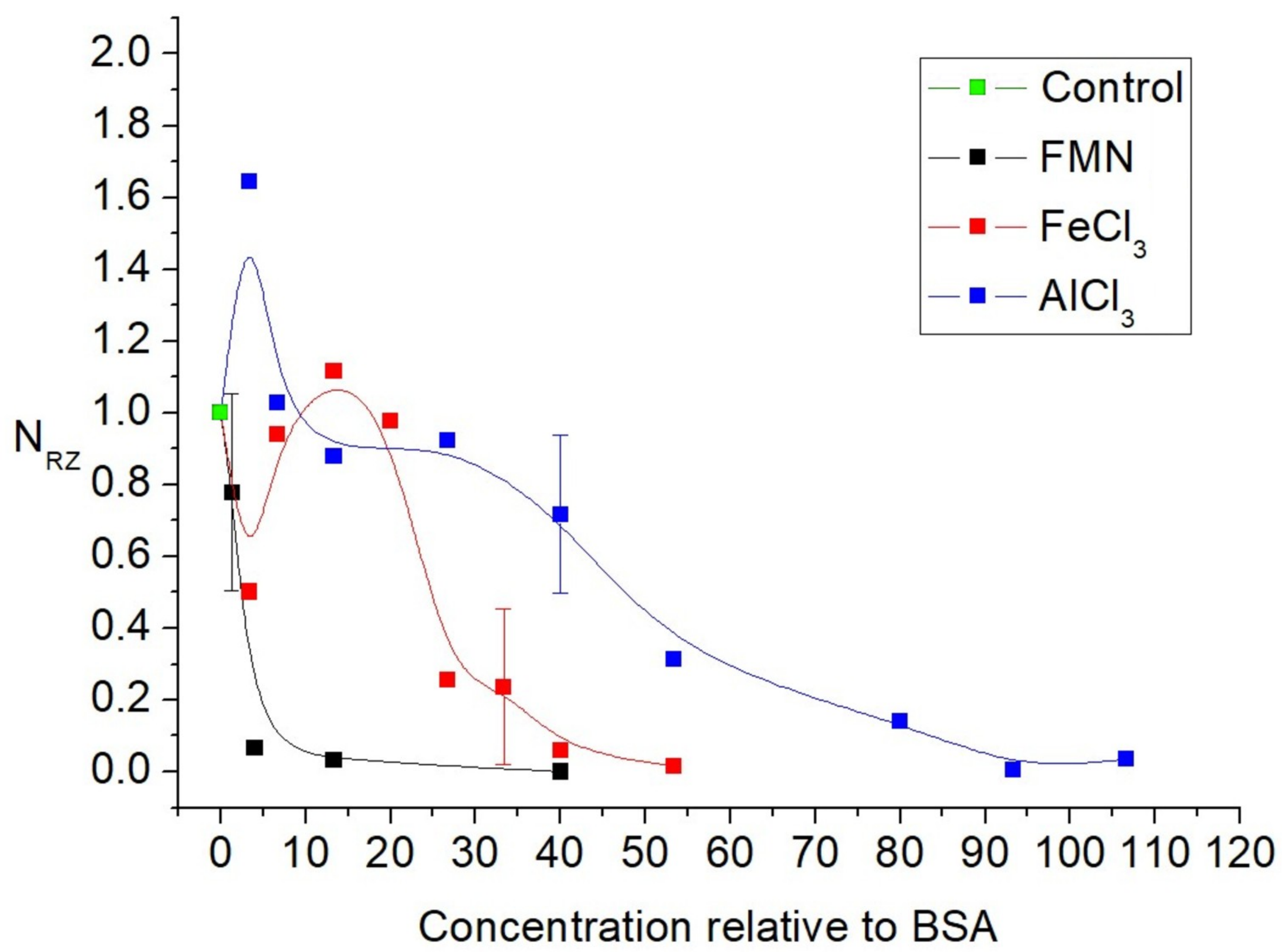

Fig. 2. Relative zigzag segment count N_{RZ} for the addition of FMN, $FeCl_3$ or $AlCl_3$.

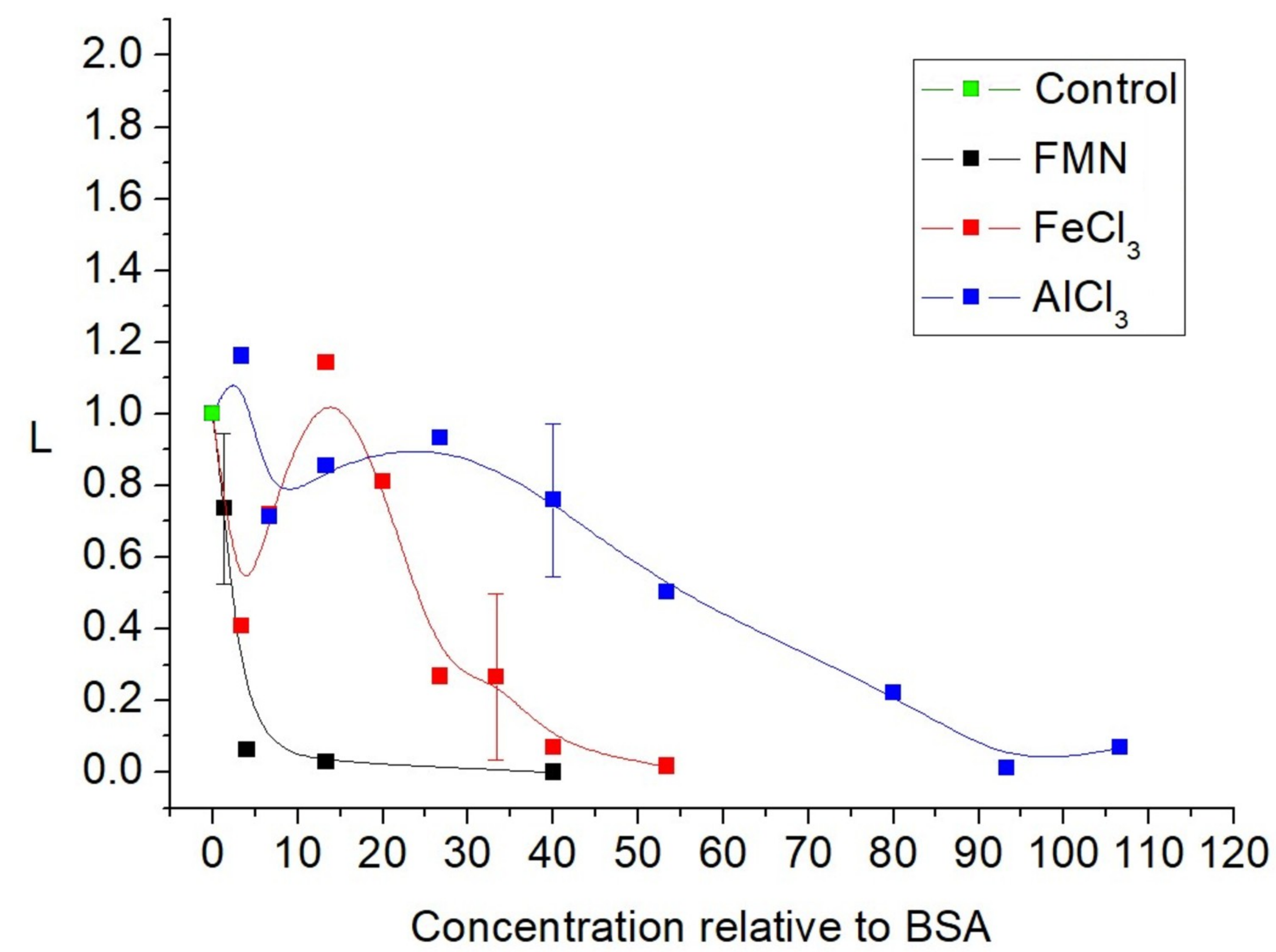

Fig. 3. Relative specific length L of zigzag segments for the addition of FMN, $FeCl_3$ or $AlCl_3$.

Previously, we have attributed such effects to the presence of colloidal particles, aggregates, and negatively charged associates of FMN, as well as to a decrease in the surface potential and hydration of negatively charged BSA particles in solution. However, even though BSA appears to aggregate at $AlCl_3$:BSA concentration ratios of 1:10–1:50, according to the light scattering data (Fig. 4, Fig. 5), the ability to form zigzag patterns is, evidently, not affected. Traditionally, one would expect BSA to be stable when having either positive or negative potential, and precipitate when its potential is neutralized by Al^{3+}, but a minimum of zigzag parameters at those concentrations is not observed.

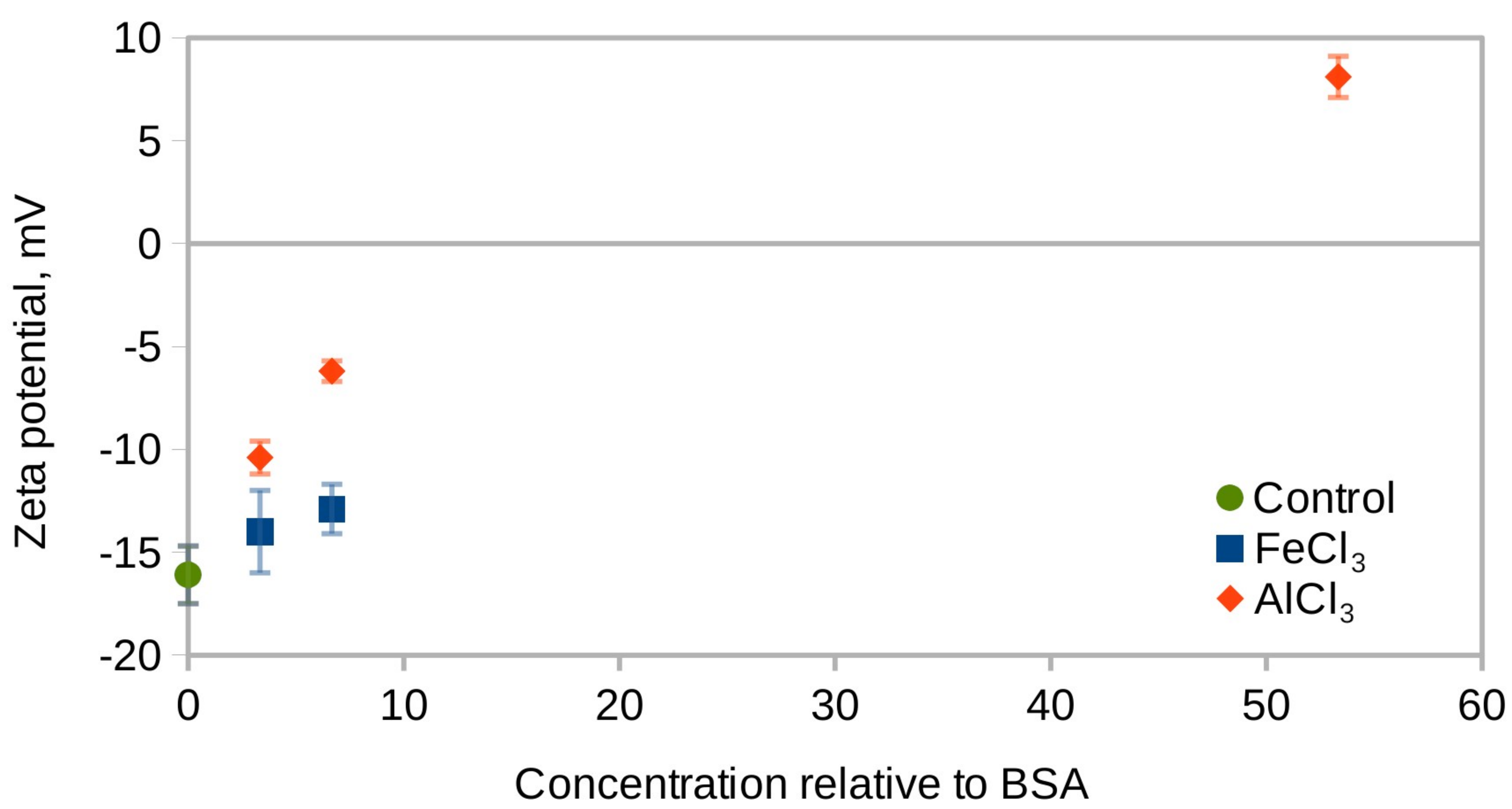

Fig. 4. Zeta potential of BSA when adding $FeCl_3$ or $AlCl_3$.
In solutions with an Al^{3+} concentration of 0.1–0.3 mM, signs of BSA aggregation and sedimentation were observed. In this range of Al^{3+} concentrations, the potential of BSA particles is close to zero, which promotes the aggregation of protein molecules.

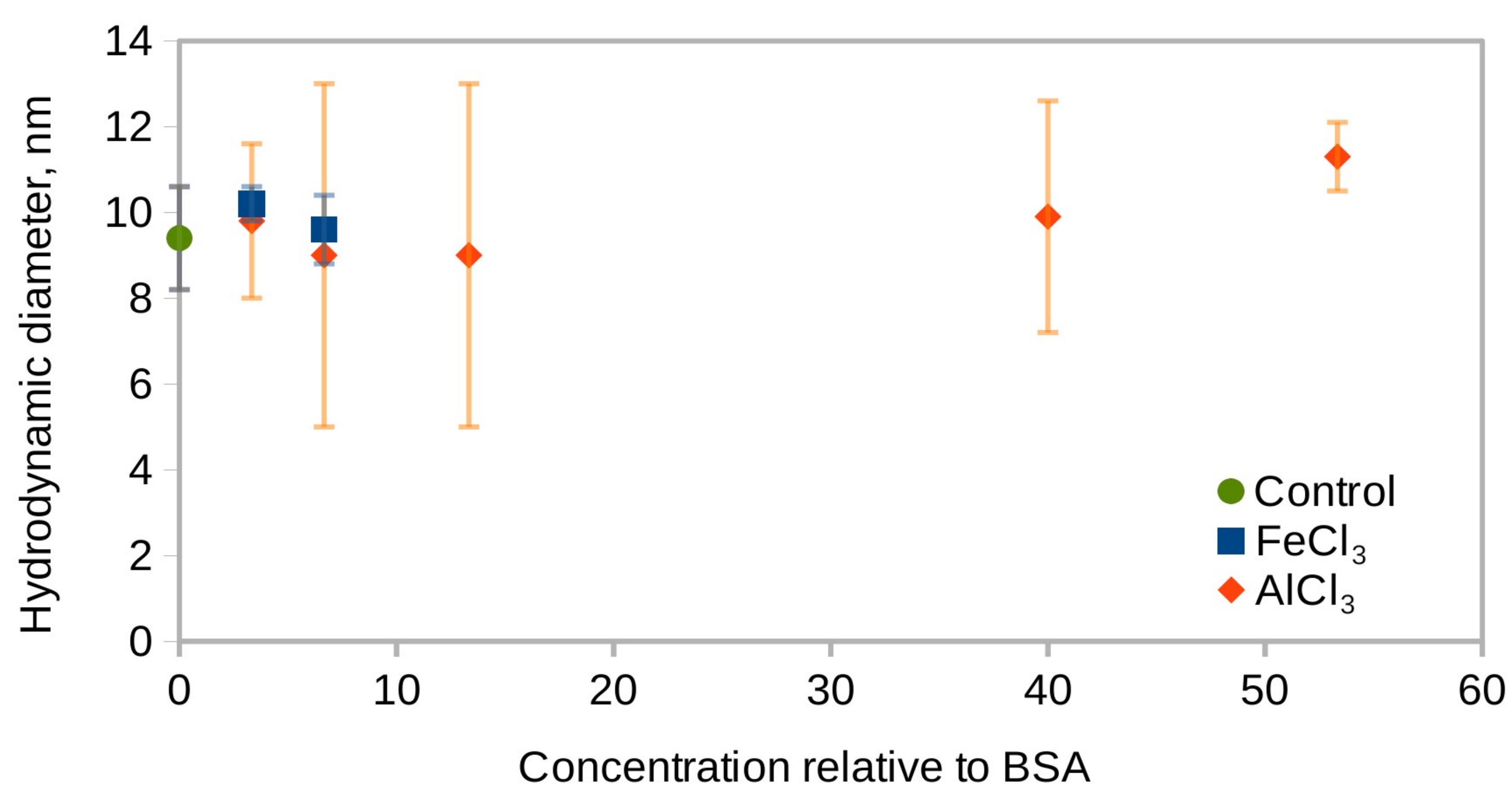

Fig. 5. Hydrodynamic diameter of BSA when adding $FeCl_3$ or $AlCl_3$.
The measurement at 0.1 mM $FeCl_3$ and higher was not possible due to the presence of colloidal particles larger than 100 nm in diameter (most likely formed by iron hydroxide).

In light of a recent study [2] that brings attention to the mutually attracting and clustering behavior of negatively charged particles in aqueous solutions, it is worth considering the possibility that such phenomena may contribute to the above observations. Specifically, clusters can be formed even from dissimilar particles, so long as the sign of their zeta potential is the same, and negatively charged biomolecules can gather into droplet-like condensates even in the presence of salt ions [2]. Since both FMN and BSA have a negative potential, they would likely be located nearby in those droplets, and upon drying, this might quickly violate the crystallization conditions. In case of Al^{3+}, the parameters' gradual decline appears to correlate with the potential becoming more positive, which in the study [2] is shown to reduce the tendency to form clusters and cause the particles to be more uniformly distributed throughout the solution. Unlike the negatively charged FMN, the colloidal aggregates of $Fe(OH)_3$ are neutral and, therefore, will not tend to form droplets with BSA, but instead precipitate; as such, it might take a greater amount of this "random debris" to interfere with the crystallization to the same extent as the FMN particles located right beside BSA.

[1] O. A. Gorobchenko, D. M. Glibitskiy, O. T. Nikolov, T. A. Cheipesh, T. N. Dzhimieva, I. S. Zaitseva, A. D. Roshal, M. A. Semenov and G. M. Glibitskiy, Low Temp. Phys. 50, 48 (2024). https://doi.org/10.1063/10.0023892
[2] S. Wang, R. Walker-Gibbons, B. Watkins, M. Flynn and M. Krishnan, Nat. Nanotechnol. (2024). https://doi.org/10.1038/s41565-024-01621-5