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# Coagulation of tobacco mosaic virus in alcohol–water–LiCl solutions

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ABSTRACT

The coagulation and colloidal stability of tobacco mosaic virus (TMV) in alcohol–water–LiCl solutions were studied. Without the addition of LiCl salt, the coagulation was promoted by the increase of hydrophobicity of the alcohols that is proportional to their alkyl chain length and concentration. Addition of the LiCl salt reduced the electrostatic repulsion between TMV particles resulting in coagulation in methanol–water and ethanol–water solutions. In water–alcohol–LiCl mixture, the coagulation of TMV was driven by both the hydrophobic interaction of the solution and the screening effect of the salt simultaneously. To understand the particle–particle interaction during the coagulation, the interaction energy was calculated using DLVO theory. Considering the electrostatic repulsive energy, van der Waals attractive energy, and hydrophobic interaction energy, the total energy profiles were obtained. The experiment and model calculation results indicated that the increase of alcohol concentration would increase hydrophobic attraction energy so that the coagulation is promoted. These results provide the fundamental understanding on the coagulation of biomolecular macromolecules.



## 1. Introduction

The aggregation of biomolecules in various solutions has been investigated for a long time in order to separate biomolecules effectively from the crude juice [1,2]. The most common process of biomolecular separation exploiting the aggregation of biomolecules is the protein precipitation known as the salting out process driven by the solubility change of protein [3–6]. Recent progress in bio-nanotechnology exploiting biomolecules pays attention to the aggregation of biomolecules because the controlled aggregation of biomolecules would provide methods to prepare a well-dispersed bio-nanomaterial system and to construct complex mesostructures. Since the protein solubility is known to be changed by many factors like particle size, surface charge, solvent polarity, and the ionic strength of the solution, it is required to obtain informative data on the aggregation behavior of the suspending biomolecules in various solutions for the applications of biomolecules in bio-nanotechnology.

Among many biomolecules, tobacco mosaic virus (TMV) is a widely used viral biomolecule as a template in synthesizing inorganic nanotubes and nanowires due to its solid cylindrical structure [7–10]. However, the coagulation of TMV as a template was not addressed in fabricating TMV-based materials, and most of the studies are only focused on the synthesis itself. In general, many biomolecule-based processes are carried out in colloidal biomolecular suspensions involving a combination of inorganic salt, non-polar co-solvents, and aqueous solvents. In these suspensions, the salts and co-solvents are adjusted to induce coagulation of the biomolecular templates [9,11,12]. Thus, to insure a well-controlled fabrication of nanotubes or nanowires from TMV molecules, the study on the colloidal stability of macroscopic biomolecules, such as TMV, is imperative.

In this report, experimental and theoretical studies on the coagulation of the colloidal wild-type TMV in alcohol–water–LiCl solution was carried out. This system is chosen as a model suspension similar to the fabrication system commonly used in preparing TMV-based nanotubes. Monohydric alcohols miscible with water were chosen in order to inspect the effect of alkyl chain length. The coagulation of TMV particles were monitored with the variation of the properties of the solution using light scattering and electron microscopy techniques. In addition, the colloidal interaction energy based on DLVO theory was calculated from the TMV model. Specifically, the electrical repulsion, van der Waals attraction, and hydrophobic interaction energies were considered to describe TMV aggregation. The considerations on the long and short range interactions on the cylindrical viral particle has not developed yet to our knowledge. This study provides both the qualitative origin of coagulation and the experimental data providing the colloidal stability criteria. In addition, the hydrophobic interaction parameters could be estimated from the colloidal interaction

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model. These results are useful in optimizing experimental conditions for the TMV template with various inorganic sources and suspended in aqueous-alcoholic liquids.

## 2. Materials and methods

### 2.1. Tobacco mosaic virus isolation

The virus particles were isolated and concentrated from infected plants as previously described [2]. Extracted virions were further purified using a 10–40% continuous sucrose density gradient and centrifugation for 2 h at approximately 20,000$g$.

### 2.2. Sample preparation

The wild type TMV concentrate was dispersed in water–alcohol–LiCl solutions to the concentration of 1.0 mg/ml. This particle concentration was known to be the upper limit that was free from particle interaction in measuring the electrophoretic mobility of the TMV particles [13]. In addition, this concentration has an isotropic phase even above 70 mM of ionic concentration [14]. Thus, the possibility of liquid crystalline phase formation could be ignored. Deionized water (conductivity = 2.6 μS/cm) and HPLC grade (Aldrich, 99+%) alcohols were used for solution preparation without additional purification. Two types of alcohols were applied; methyl and ethyl alcohols. Lithium chloride (LiCl, Mallinckrodt, 99%), which is soluble to both water and alcohol, was added to prepare solutions with the desired salt concentration. The pH of all the samples was pH $6.3 \pm 0.4$ where TMV is negatively charged. The samples were stored in the refrigerator for 24 h in order to give enough time for the sample to reach the final aggregation state.

### 2.3. Apparatus

All the particle size measurements were carried out with a conventional dynamic light scattering apparatus equipped with a 20 mW He–Ne laser source at room temperature. A photon correlator (UNICOR-SP, US) and software (Photo-Cor, US) were used for the calculation of the correlation function and data processing. The hydrodynamic radius was calculated from the data using the Stokes–Einstein equation. Physical constants required in calculation were obtained and estimated from physical data handbooks [15,16]. Zeta potential and orientation-averaged electrophoretic mobilities were measured with a commercialized laser Doppler capillary electrophoresis equipment (DELSA, Coulter, US) at 25 °C. Shapes of the coagulated virus particles were observed using transmission electron microscopy (TEM, ZEISS EM10 CA, German). In preparing the TEM sample, a drop of TMV solution was positioned on the carbon-coated copper grid and stained with a 2 wt% uranyl acetate solution.

### 2.4. Interaction energy calculation

The interaction energy between two model TMV particles was calculated to analyze the coagulation behavior. Specifically, the long-range colloidal interaction was calculated based on the DLVO theory. Firstly, the electrostatic repulsive energy and van der Waals attractive energy were considered in the configuration of two parallel cylindrical particles. The parallel configuration was chosen as a model system because the parallel configuration is known to have a maximum repulsive energy among the many possible configurations of two cylindrical particles [17,18]. The cylindrical particle–particle interaction was modeled under the assumption of the Derjaguin approximation. The interaction energy was calculated by solving the nonlinear Poisson–Boltzmann (PB) equation using the Galerkin finite element method (G/FEM). The details on the modeling work are described elsewhere [18].

**Table 1**
Relative permittivity and refractive indices of alcohol–water mixture

| Alcohol (wt%) | $\varepsilon_r$ | $n_m$ |
|---|---|---|
| Methyl alcohol | | |
| 20% | 69.994 | 1.3381 |
| 30% | 65.581 | 1.3407 |
| 50% | 56.283 | 1.3431 |
| Ethyl alcohol | | |
| 20% | 67.199 | 1.3469 |
| 30% | 61.167 | 1.3535 |
| 50% | 48.943 | 1.3616 |

The electrostatic repulsive energy is derived from calculation of the osmotic pressure difference that is expressed as a middle-point potential between two surfaces. The electrostatic repulsive energy ($V_r$) of two colloidal surfaces suspending in an 1:1 electrolyte solution is given as

$$V_r = \int_H^\infty 2C_\infty RT\left\{\cosh(\tilde{\psi}_m) - 1\right\} dx, \tag{1}$$

where $\tilde{\psi}_m$ is dimensionless potential at the middle point that defined as

$$\tilde{\psi}_m = \frac{F\psi_m}{RT}. \tag{2}$$

$H$ is shortest separation distance between surfaces, $C_\infty$ is concentration of LiCl, $R$ is the gas constant, $T$ is absolute temperature, $\psi_m$ is potential at middle point, and $F$ is Faraday constant [19]. For the calculation, the surface potential and Debye screening length are required as parameters. The surface potential of the TMV particles was assumed as the value of zeta potential measure by capillary electrophoresis, and the Debye screening length ($\lambda_D$) was determined from the following equation:

$$\frac{1}{\lambda_D^2} = \frac{2F^2C_\infty}{\varepsilon_0\varepsilon_r RT}. \tag{3}$$

Here, $\varepsilon_0$ is the vacuum permittivity, and $\varepsilon_r$ is the relative permittivity.

As the relative permittivity changes with the variance of the medium composition, these values are estimated from physicochemical databases [15,16]. The relative permittivity is summarized in Table 1.

The attractive van der Waals energy between two parallel cylindrical particles ($V_a$) is also considered. The attractive energy is represented as the following equation [20]:

$$V_a = -\frac{A_H L\sqrt{a}}{24H^{3/2}}, \tag{4}$$

where $A_H$ is Hamaker constant, $L$ is length of cylinder, $a$ is radius of cylinder, and $H$ is the shortest distance of separation. The Hamaker constant changes with the variance of the medium composition. Therefore, the Hamaker constant is estimated using Lifshitz theory that can be simplified in the system of interest as follows [20];

$$A_H = \frac{3}{4}kT\left(\frac{\varepsilon_T - \varepsilon_m}{\varepsilon_T + \varepsilon_m}\right) + \frac{3h\nu_e}{16\sqrt{2}}\left\{\frac{(n_T^2 - n_m^2)^2}{(n_T^2 + n_m^2)^{3/2}}\right\}, \tag{5}$$

where $\varepsilon_T$ is relative dielectric constant of TMV ($\varepsilon_T = 55.0$), $\varepsilon_m$ is relative dielectric constant of suspending medium, $n_T$ is refractive index of TMV ($n_T = 1.57$), $n_m$ is refractive index of suspending medium, and $\nu_e$ is main electronic absorption frequency ($\nu_e = 3 \times 10^{15}\ s^{-1}$) in the UV region, respectively. The dielectric