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Frequency Shift of Acoustic Oscillations of the Tobacco Mosaic Virus with Varying Suspension Parameters

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Abstract—When low-frequency stimulated Raman scattering (SRS) is excited in the tobacco mosaic virus (TMV) suspension, an anomaly in the scattering line frequency shift is detected, i.e., the dependence on the concentrations of TMV and tris-buffer as the TMV concentration increases. An explanation of the phenomenon, based on the idea of the formation of the hydration shell of the virus capsid is proposed.

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Introduction. Despite the great number of experimental data, a convincing physical mechanism describing the nonthermal effect of microwaves on biological objects has not yet been proposed [1–3]. One of the features of experiments in this field is the poor reproducibility of results. At the same time, the appearance of significant factors can be very noticeable in some cases [1]. In recent work [4], we detected a resonant effect of TMV RNA modification in the case of coincident electromagnetic radiation frequency and the eigenfrequency of longitudinal acoustic oscillations of the virus in the range of 6–9 GHz at a relatively low (excluding heating) intensity. We note that eigenfrequencies of acoustic oscillations of such objects as aqueous solutions of viruses can be excited and measured by optical methods, in particular, when observing stimulated Raman scattering (SRS) [5].

The objective of this work is to study the effect of the ion concentration (ionic strength) in a tris-buffer suspension on the resonant frequency of radial oscillations of the tobacco mosaic virus (∼60 GHz), which appears upon excitation of low-frequency stimulated Raman scattering (LFSRS) [4, 9].

Experimental. As an object to be studied, we used an aqueous suspension of TMV with added tris-HCl buffer (tris-hydroxymethyl aminomethane chloride) which provides $pH \sim 7.5$ at an initial virus concentration of $\sim 0.5 \cdot 10^{12}$ cm^{-3} (sample "a"). The suspension was poured into in a cell with an effective length of ∼20 mm. The cell with suspension was exposed to the second harmonic of a pulsed single-frequency YAG:Nd laser (the emission wavelength $\lambda = 0.532$ nm, the emission duration is $t \sim 10$ ns, and the pulse energy $E_{\rm pul} - 40$ mJ). Laser radiation was focused on the middle of the cell by a lens with a focal length of 30 mm. SRS spectra were measured using Fabry–Perot interferometers (free spectral range is $\Delta t = 2.5$ cm^{-1}). The experimental scheme and measurement technique are described in detail in [6]. The suspension concentration was gradually increased by evaporating a solution fraction to $\sim 1.0 \cdot 10^{12}$ cm^{-3} (sample "b") and to $\sim 2.0 \cdot 10^{12}$ cm^{-3} (sample "c") in the virus concentration. Accordingly, the tris-buffer ion concentration in the solution was changed.

Results and discussion. As the laser energy was increased from ∼1 mJ to ∼30 mJ, the spectrum of radiation scattered by sample "a" contained only lines of stimulated Brillouin backscattering (SBS), the Stokes shift was $\Delta\nu \sim 0.255$ cm^{-1} (Fig. 1).

In the spectrum of radiation scattered by sample "b" at a laser pulse energy of ∼20 mJ, the low-frequency (both "forward" and "backward") SRS lines with the shift $\Delta\nu \sim 1.47$ cm^{-1} were observed,

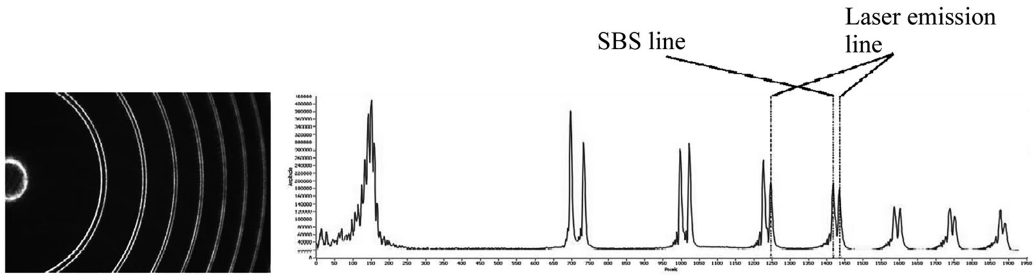

Fig. 1. Interference pattern of the laser emission and ("backward") SBS signal lines in TMV suspension (sample "a"); the Stokes frequency shift of scattered radiation is $\Delta\nu \sim 0.255$ cm^{-1}.

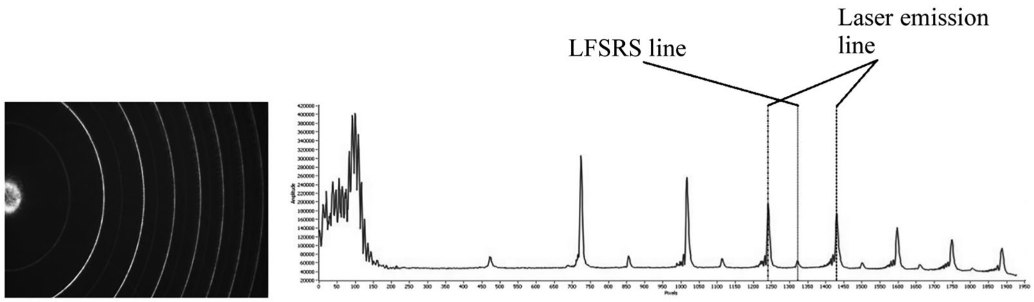

Fig. 2. Interference pattern of the laser emission and LFSRS signal lines in the "backward" direction in TMV suspension (sample "b"); the Stokes frequency shift of scattered radiation is $\Delta\nu \sim 1.47$ cm^{-1}.

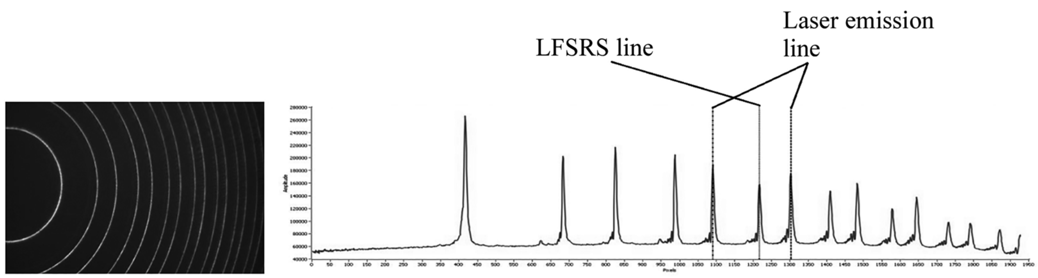

Fig. 3. Interference pattern of the laser emission and LFSRS signal lines in the "backward" direction in TMV suspension (sample "c"); the Stokes frequency shift of scattered radiation is $\Delta\nu \sim 1.046$ cm^{-1}.

which corresponds to the oscillation frequency of ∼44.1 GHz (Fig. 2). In this case, the SBS line was absent. Thus, it was detected for the first time that an increase in the nanoparticle (TMV) suspension concentration to $\sim 1.0 \cdot 10^{12}$ cm^{-3} is accompanied by SBS suppression.

In sample "c", at a laser energy of ∼20–30 mJ, the (both "forward" and "back") LFSRS line with the shift $\Delta\nu \sim 1.046$ cm^{-1} or ∼31.38 GHz was detected in the spectrum. In this case, SBS lines were also absent (Fig. 3).

We note that it is unlikely that the observed strong decrease in the resonant frequency is related to the appearance of the interaction between individual virus particles, since the characteristic distances between viruses at indicated concentrations are 0.8–1.2 μm. It is difficult to assume any interaction at such long distances. However, as follows from the data obtained, this distance (the tris-buffer layer thickness in suspension) is insufficient to develop SBS. It would be assumed that the observed effect is caused, e.g., by increasing the solution viscosity with the tris-buffer concentration. However, estimations show that the buffer solution viscosity changes in this case from 1.001 tc 1.0037 cP. Most likely, this increase cannot cause such a significant frequency change.

The explanation proposed below seems more probable. In the case at hand, during evaporation, the tris-buffer ion concentration increases twofold. However, as is known, with respect to the hydration shell structure, ions are classified into kosmotropic (increasing molecular order around themselves) and chaotropic (increasing "chaotic" packing) ones [7, 8]. For kosmotropic ions, hydration shell thickening with ion concentration is observed [7, 8]. Hydroxyl ions (OH^-) in the tris-buffer provide an alkalescent medium (pH ∼7.5 in the initial concentration) and belong to kosmotropic ions. This means that an increase in the ion concentration can lead to virus hydration shell thickening, thus a decrease in the acoustic oscillation eigenfrequency with increasing sample geometrical sizes.

The applicability of this hypothesis can be tested by direct calculations (the format of brief communications restricts the description of calculation details which will be a subject of further publication). To this end, eigenfrequencies of radial oscillations of the cylindrical rod (virus) in an aqueous medium were calculated in the classical approach approximation, taking into account the icelike hydration shell. Here the general solution to the wave equation for the acoustic potential (velocity potential), as well as for the acoustic pressure, is a linear combination of the zero-order Bessel functions of the first kind; for the acoustic velocity, it is the linear combination of the first-order Bessel functions. Rejecting the functions diverging at coordinate zero and using boundary conditions on the "protein–ice" and "ice–water" hydration surfaces (namely, the continuity condition, i.e., the equality of acoustic oscillation velocities and the equality of acoustic pressures), we obtain a system of algebraic equations for determining the acoustic wave amplitudes in each medium. The existence condition of the solution to this system is a corresponding equal-zero determinant formed by coefficients at amplitudes. This is exactly the eigenfrequency ω equation; furthermore, the imaginary part of the complex solution to this equation $\mathrm{Im}(\omega)$ defines the Q-factor of such an oscillator. This transcendental equation containing Bessel functions was solved numerically by the tangent method. The solution was performed using the following parameters: the speed of sound in the capsid (cylinder), in ice (shell), and in water is 2000, 3950, and 1450 m/s; the cylinder diameter is 18 nm, the shell thickness is $dL = 0.8$ nm.

It was found that the characteristic frequency of radial oscillations of the cylinder in the absence of shell ($dL = 0$) is $\nu = 55.8$ GHz ($\mathrm{Im}(\omega) = 0$) is in good agreement with the TMV SRS frequency measured in previous experiments (∼60 GHz) [4, 5]. As the hydration shell grows from 0 to 8.0 nm, the eigenfrequency almost linearly decreases to $\nu = 32.5$ GHz. It should be noted that the solution was obtained on the assumption of high oscillator Q-factor in all cases ($\mathrm{Im}(\omega) = 0$).

Thus, the classical calculation within the described model (cylinder–shell) yields the frequencies close to the experimental ones, although, show slightly overestimated thicknesses of the icelike hydration shell of the virus (∼8 nm). This minor disagreement (in general, the shell thickness does not exceed 1–2 nm) can be caused by inaccurate speeds of sound used in the calculation.

Thus, in the case of a twofold increase in the suspension concentration, a significant decrease (∼25%) in the frequency of radial acoustic oscillations of the TMV was detected by the LFSRS line shift. The model of the detected phenomenon based on the formation mechanism of the multilayer hydration shell of the virus capsid with an icelike structure was proposed.

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