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Interaction between Vesicular Stomatitis Virus Sample and Electromagnetic Waves

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Abstract: ***Interaction between vesicular stomatitis virus sample and electromagnetic waves has been studied. Interdisciplinary methods were applied to study of the virus sample. The optical absorption of viral sample has been measured for the wavelength range from 400 to 750 nm. The dispersion curves for the dielectric constant real and imaginary parts have been constructed. Obtained data can be used for calculation of interaction between the virus and various nanoobjects and for development of new methods of study viruses samples..***

Keywords: vesicular stomatitis virus, nanoparticles, dielectric constant, extinction coefficient, refractive index

1. Introduction

Viruses have been a relevant object of research for a long time. At first, scientists tried to develop different methods of dealing with viruses. Afterwards, viruses began to be used for various proposes, e.g. for drug delivery [1-2] or for fighting bacteria [3-4] and even other viruses [5]. Nanophysics also has opened new opportunities for antiviral therapy.

There are a lot of experimental data on interaction between viruses and surfaces, e.g. Refs. [6-7]. Furthermore, there are a lot of potential applications of this interaction. For example, the properties of this interaction lie at the origin of the new method of purification of biological fluids.

Hence, one needs to know the properties of the interaction between a virus and a nanostructured surface. It is easy to understand, that study of the interaction between viruses and nanostructured surfaces can cause the appearance of new techniques of antiviral therapy and develop new uses of viruses in nanotechnologies. However, there is no theory that could explain this interaction and allow one to calculate its parameters.

As it was shown in Refs. [8-10], virus can be considered as a nanoparticle. Consequently, the theory of interaction between a virus and a nanostructured surface can be based on the theory of interaction between two non-point-like nanoparticles and the theory of interaction between a nanoparticle and a flat solid surface, which have been constructed by authors in Refs. [11,9]. These theories exploit the concept of the effective susceptibility [12], so they use dielectric function of interacting objects. Also, these theories take into account the viruses shapes and dimensions. Consequently, one needs to know them too.

Besides, recently the interaction between viruses and nanoparticles has become more and more relevant problem in modern science. This problem is studied by the scientists all over the world both experimentally and theoretically. Antiviral effect has been shown in experiments by silver [13], gold [14], silica [15] and other nanopaticles. Theoretical bases of the idea of antiviral action of nanoparticles have been discussed in Ref. [16]. The authors of Ref. [10] have suggested two sets of mechanisms of the antiviral activity inhibition. Theoretical model of the virus-nanoparticle interaction also can be based on the theory of the interaction between the two nonpoint nanoparticles [11].

As it was shown above, it is important to know physical properties of viruses for modelling various physical processes involving them. Mechanical parameters of all known viruses have been studied already; scientists decode viral DNA and RNA and obtain information about chemical formulas of viral proteins. However, while studying the interaction of viruses with nanoscale objects and electromagnetic radiation, it is necessary to have information about virus optical parameters. There are some papers that consist of information on adsorption spectra of viruses, e.g. Refs. [17-18].

Let one consider vesicular stomatitis virus. This virus is an interesting object of the research because of the ease of its obtaining and reproduction rate. Its mechanical properties are well-known. It has a bullet shape, its length is about 170 nm and width is about 70 nm. It is covered with the glycoprotein shell, and there is a negative-strand RNA inside it. There are a lot of research papers devoted to study of this virus. They are aimed at its modelling [19], study of the structure of its RNA [19] and its proteins [21-22], mechanism of encapsidation [23-24], etc.

Because of the fact that there is no widespread information on electrodynamic properties of the vesicular stomatitis virus, the goal of this work is to study them. Furthermore, study of the absorption spectra of virus samples and of virus samples with nanoparticles may be one of the methods of study of the effect of nanoparticles on viruses. Thus, this work is devoted to experimental study of parameters of interaction between vesicular stomatitis virus sample and electromagnetic waves in the visible range and to construction of the dispersion curves of the refractive index and the extinction coefficient.

2. Materials and Methods

2.1 Experimental Study

For the experiments we have used a sample of vesicular

stomatitis viruses, a buffer, where the viruses were dissolved, and a growth medium, where the viruses have been grown. The samples were irradiated with halogen lamp through the lens, and transmitted light led to photoelectric multiplier. The gained signal was processed with computer program. Absorption was calculated as difference between the light intensity transmitted through the cuvette with the buffer and the one transmitted through the cuvette with the sample. All the experiments were repeated for five times during one day with the period in one hour in order to eliminate the possibility of virus destruction in the sample due to the effect of light. All the absorption spectra in a series were statistically equal.

Absorption spectra were measured for the samples of pure viruses with different virus concentration, for the samples of viruses with nanoparticles, for the samples of viruses in one week after their preparation and after one-day illumination with direct sunlight, for the samples of buffer and growth medium. All the samples were kindly supplied by scientists of the D. Zabolontyi Institute of Microbiology and Virology, NAS of Ukraine, Kyiv, Ukraine (N. Zholobak, O. Shydlovska). VSV was prepared on ST culture (testis from Sus scrofa, pig) from culture museum of Institute of Veterinary Medicine UAAS. The activity of the virus was determined by the cytopathic effect (CPE) of VSV. Cytopathic effect of VSV on ST-line is destruction of monolayer. Virus titer of the sample was 5lg. It was determined by 10-fold dilutions. Virus titer is equal to the logarithm of the last dilution in which there is cytopathic effect of virus. The nanoparticles were the CeO2 nanoparticles with dameter of 2-3 nm stabilized with citrate.
It should be mentioned, that the sample consists of not only viruses, but proteins, glycoproteins and other chemicals being in virus in small concentrations. In order to eliminate the influence of these compounds and of inactive viruses in the sample the absorption spectra for the samples of viruses in one week after their preparation and after one-day illumination with direct sunlight and heating to 50oC for half an hour was measured. The influence of high temperature and direct sunlight leads to the inactivation of virus sample. Also, in order to study the possibility of the evaluation of parameters of virus sample by measuring its absorption, the samples of viruses with CeO2 nanoparticles were studied.

2.2 Theoretical Study

As a result of series of experiments, we have obtained the absorption spectra of vesicular stomatitis virus sample with taking into account the absorption of the buffer and growth medium in the visible optical range and in the ultraviolet one. Because of the sample is supposed to be transparent in the wave range under consideration, omitting the light scattering causes the relative error about 5% [25]. Furthermore, for proteins and other biochemical objects this effect is smaller, and the relative error is about 1% in the visible and ultraviolet ranges [26]. Therefore in our work we studied only absorption spectra of the sample.

In the work we didn`t take into account the change of the buffer components concentrations because of their smallness (the biggest concentration value is about 50 ng/ml). Therefore, the extinction coefficient of the sample has been obtained using the Lambert-Bouguer law.

In order to obtain the dielectric function, the representation of dielectric constant dispersion in the form of combination of a finite number of oscillators and the Kramers-Kronig relations were used as in Refs. [27,28]. We assumed that dielectric function of the sample is described by formula [28]:

$$\varepsilon(\omega) = \varepsilon_\infty + \sum_{i=1}^{N} \frac{f_i - i f_{R,i} E}{E_i^2 - E^2 - i\gamma_i E}, \quad (1)$$

where γ_i, f_i is the damping and the intensity of the *i*-th oscillator, respectively, E_i – the energy of photon with angular frequency ω_i, ω_i – the natural frequency of the i-th oscillator, and $f_{R,i}$ – the coefficient that characterize relaxation of the i-th oscillator.

The natural frequency of oscillator, its damping and intensity was defined by the presentation of absorption spectra as a sum of absorption curves of each oscillator. The coefficient that characterize relaxation of the i-th oscillator was chosen thus that by least squares method the difference between experimental and simulated extinction coefficient is the lowest.

Hence, the real and imaginary parts of dielectric function are [28]:

$$\varepsilon'(\omega) = \varepsilon_\infty + \sum_{i=1}^{N} \frac{f_i (E_i^2 - E^2) + \gamma_i f_{R,i} E^2}{(E_i^2 - E^2)^2 + \gamma_i^2 E^2}, \quad (2)$$

$$\varepsilon''(\omega) = \sum_{i=1}^{N} \frac{E(\gamma_i f_i - f_{R,i}(E_i^2 - E^2)}{(E_i^2 - E^2)^2 + \gamma_i^2 E^2}. \quad (3)$$

Refraction index and extinction coefficient have been calculated by the formulae [29]:

$$n = \sqrt{\frac{\sqrt{\varepsilon'^2 + \varepsilon''^2} + \varepsilon'}{2}}, \quad (4)$$

$$k = \sqrt{\frac{\sqrt{\varepsilon'^2 + \varepsilon''^2} - \varepsilon'}{2}}. \quad (5)$$

3. Results and Discussions

3.1 Absorption Spectra

Obtained absorption spectra for different samples are presented in Figs. 1-3. Virus samples after the action of direct sunlight and high temperature demonstrate negligible absorption in the studied wavelength range. As vesicular stomatitis virus is very unstable the virus should inactivate under direct sunlight and high temperature. This can indicate accordance of these absorption lines to infective virus.

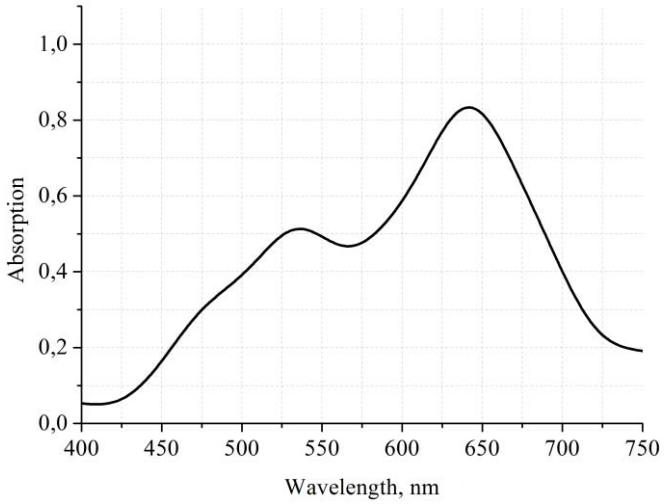

Figure 1: Absorption spectrum of vesicular stomatitis samples.

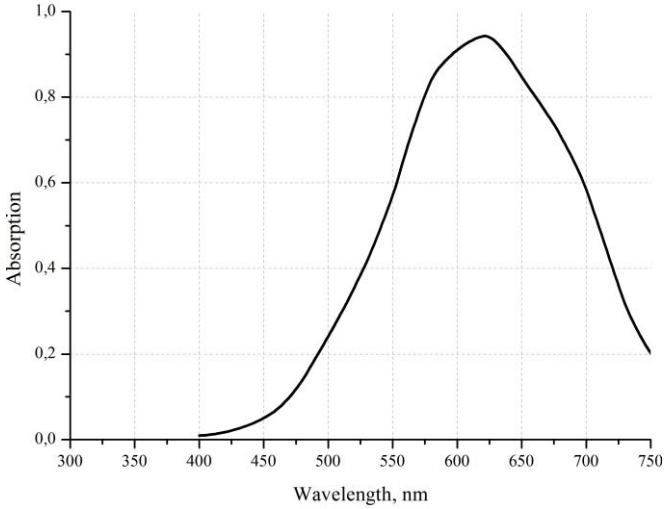

Figure 2: Absorption spectrum of the CeO_2 nanoparticles sample

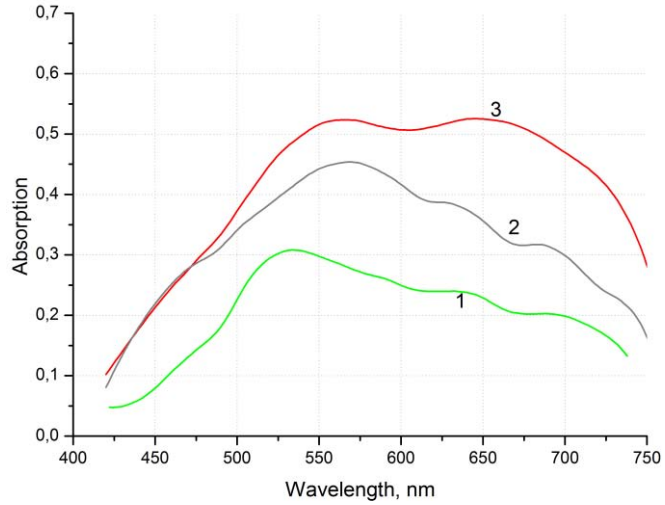

Figure 3: Absorption spectra of vesicular stomatitis virus sample with CeO_2 nanoparticles:
1 - n_{np}=0.1 mmol; 2 - n_{np}=1 μmol; 2 - n_{np}=0.1 μmol.

Obtained It was established that the absorption spectrum of the pure viral sample consists of three oscillators in the visible range. When the nanoparticles are added to the sample, the intensities of two oscillators decrease a little, while the intensity of the other one oscillator decreases drastically. Furthermore, the higher the nanoparticles concentration is the more is the decrease in the oscillators intensities.

It is well known, that nanoparticles samples have antiviral effect [8,10,30-32]. Furthermore, performed studies of antiviral action of various nanoparticles show that the higher the nanoparticle concentration is the higher their antiviral effect is [8,10,30-32]. Thus, our results are in good agreement with the results of biomedical experiments. Hence, the shape and the intensity of absorption spectrum of the virus sample can give information on its content and virus infectivity.

3.2 Optical parameters

Having analyzed the absorption spectrum of vesicular stomatitis virus sample in optical range, three oscillators have been determined and using presentation of absorption spectra as a sum of absorption curves of each oscillator (Fig. 4) their parameters have been calculated. Likewise, the same work has been done for the case of the ultraviolet range. The results are presented in table 1.

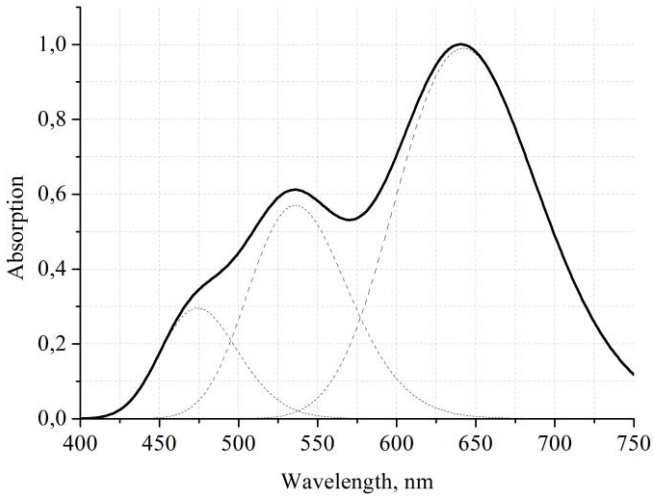

Figure 4: Presentation of the absorption spectrum as the sum of oscillators

Table 1: Oscillators parameters of vesicular stomatitis virus sample, obtained from the absorption spectrum in optical and ultraviolet ranges

i	λ_i, nm	E_i, eV	γ_i, eV	f_i, eV^2	$f_{R,i}, eV$
1	468	2.654	0.318	0.27	0.01
2	541	2.296	0.318	1.02	0.35
3	644	1.929	0.318	4.79	0.08

Real and imaginary parts of the complex refractive index of vesicular stomatitis virus sample in the visible optical range are shown in Fig. 5. Besides, in the current work dielectric function of the sample has been simulated. The dispersion curves are presented in Fig. 6.

One can see, that dielectric constant value in these range is about 2-5. Also, it has been established, that $\varepsilon_\infty = 3.2$.

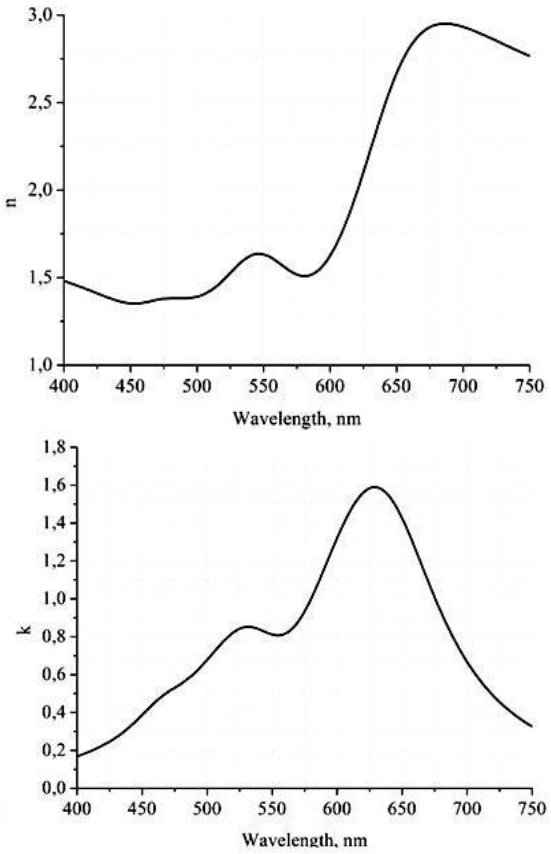

Figure 5: Real part *n* (refraction index) and imaginary part *k* (extinction coefficient) of the complex refractive index of vesicular stomatitis virus sample in the visible range

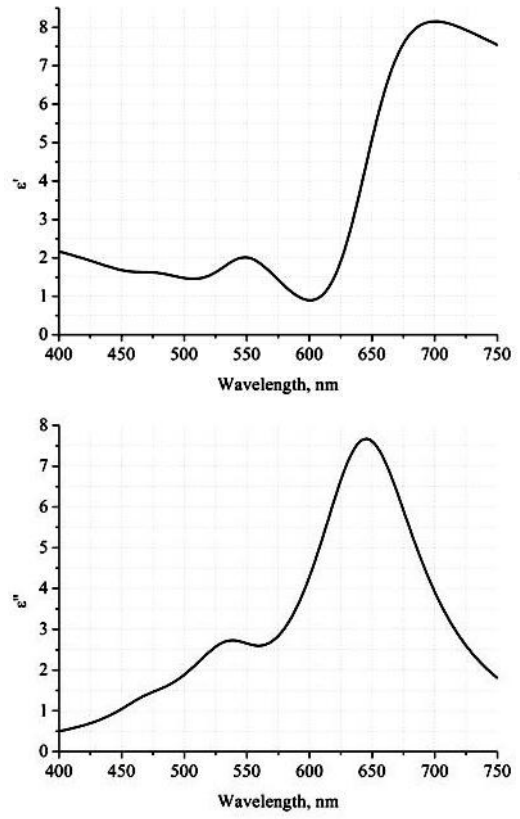

Figure 6: Real ε′ and imaginary ε″ parts of the dielectric constant of vesicular stomatitis virus sample in the visible range

4. Conclusions

Optical parameters of vesicular stomatitis virus sample have been studied. The dispersion curves of the refraction index and the extinction coefficient of vesicular stomatitis virus sample in the visible and ultraviolet optical ranges have been obtained by simulation and agree with results of our experiments.

Dielectric constants of viruses obtained in the work can be used for study of their structures and nature. The absorption spectra of the sample can be used for study of the interactions between the sample and nanoparticles. This may open promising prospects for use physical and interdisciplinary methods in biomedical applications, such as detection, identification of virus, decreasing of their infectious activity, dealing with virus diseases, etc.

Optical measurements performed in visible range show that the loss of the virus infectivity is due to change of their structure (or structure of molecules ion its surface, which is supposed). Furthermore, the type and degree of these changes can give information on the sample content (pure virus or virus with nanoparticles) and its infectivity.

5. Outlooks

Based on the results of the work the method of measuring of absorption spectra of the virus sample can be recommended to use for further studies of mechanism of interaction between the virus and nanoobjects. Accordance between the changes in light absorption and virus infectivity should be studied in more detail to develop new possible method of evaluation of virus infecivity.

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