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Dielectric spectroscopy of Tobacco Mosaic Virus

I. Ermolina[a], H. Morgan[a,*], N.G. Green[a], J.J. Milner[b], Yu. Feldman[c]

[a] *Bioelectronics Research Centre, Department of Electronics and Electrical Engineering, University of Glasgow, Rankine Building, Glasgow G12 8LT, Scotland, UK*
[b] *Plant Molecular Science Group, Institute of Biological and Life Sciences, University of Glasgow, Glasgow G12 8LT, Scotland, UK*
[c] *School of Applied Science and Technology, The Hebrew University of Jerusalem, Givat Ram, Jerusalem 91904, Israel*

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Abstract

The dielectric properties of the Tobacco Mosaic Virus (TMV) have been measured using time domain dielectric spectroscopy (TDDS) in the temperature range from 1 to 40 °C. A single dielectric dispersion is observed in the MHz range. The activation energy of the process is found to be in the range 1–2 kcal/mol. The experimental data could not be completely accounted for by current theoretical models, but evidence indicates that the dielectric loss arises from polarisation of charge on and around the virus.

Keywords: Dielectric spectroscopy; Tobacco Mosaic Virus; Counterion; Polarisation

1. Introduction

Dielectric spectroscopy has been used as a tool to investigate the biological properties of cells and organelles for over 75 years. In 1925, Fricke [1,2] derived the capacitance and thickness of the erythrocyte membrane from impedance measurements. Following on from the pioneering work of Debye [3] on polar molecules, Oncley [4], Onsager [5] and Kirkwood [6] investigated the dielectric properties of proteins. In the 1960s, Schwan amongst others [7,8], laid the foundations for the understanding of the dispersion of a biological cell suspended in an electrolyte according to the theories of Maxwell [9] and Wagner [10,11]. Since then, the dielectric properties of cells and bacteria have been extensively studied by a number of workers [12–18] and the observed dielectric data generally interpreted as arising from interfacial polarisation effects at high frequencies and from relaxation of the double layer at much lower frequencies.

The dielectric properties of protein molecules has also been the subject of investigation by a number of groups [3,14,19,20] and the data interpreted in terms of the relaxation of a fixed dipole, in combination with surface conductance effects. However, investigations of the dielectric properties of viral particles has received scant attention and to the best of our knowledge there is only one publication reporting the dielectric properties of the un-enveloped Alfalfa Mosaic Virus [21]. The authors treated the virus as a rod-like molecule and compared their experimental data with theoretical predictions concerning the polarisation of a counterion atmosphere surrounding a rod-shaped polyelectrolyte. In this paper, we report measurements of the dielectric properties of Tobacco Mosaic Virus (TMV). Dielectric data was collected using time domain dielectric spectroscopy (TDDS) covering a wide frequency range from 100 kHz to 1 GHz. In an attempt to ascertain the nature of the process responsible for the observed dielectric dispersion, the activation energy of the relaxation was also measured.

2. Experimental

2.1. Virus

TMV is a rod-shaped virus approximately 280 nm in length and 18 nm in diameter. The coat protein surrounds an RNA core with 6240 nucleotides and an average of 2.13 nucleotides per angstrom length. The molecular weight of the sodium salt of the TMV particle is estimated to be

* Corresponding author. Tel.: +44-141-330-5237; fax: +44-141-330-4907.
E-mail address: h.morgan@elec.gla.ac.uk (H. Morgan).

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4×10^7, and its density is 1.325 g cm^{-3}. (For further details of the virus structure, see, for example, Ref. [22]).

The TMV used in our work was strain U1, raised in *Nicotiana tabacum* cv. Petite Havana SR1, and purified by differential centrifugation [22]. The original inoculums were kindly donated by Prof. T.M.A. Wilson (Scottish Crop Research Institute Invergowrie, Scotland). TMV were purified by differential centrifugation, using a Beckman J2-21 centrifuge at $7000 \times g$ for 10 min and a Sorval T-865 ultracentrifuge at $200{,}000 \times g$ for 45 min at 4 °C. Both centrifugations were repeated two times to wash and resuspend virus in an appropriate buffer. Solutions of TMV were prepared in KCl and in HEPES buffer, pH 7.0, at a range of conductivities or molarities. Virus concentration was estimated spectrophotometrically using the published extinction coefficient for a 1 mg ml^{-1} solution of $E_{280} = 3.0$ [23]. Volume fractions of the different concentrations of TMV solutions were estimated using the density quoted above.

2.2. Dielectric spectroscopy

Time domain dielectric measurements were performed with a time domain dielectric spectrometer (Dipole TDS Ltd., Jerusalem) and a 0.19 pF sample holder. The recording system used a nonuniform time scale up to 5 μs (corresponding frequency range from 100 kHz to 1 GHz) [24]. The setup utilises the difference method of measurement, with a unique registration of primary signals on a nonuniform time scale. Evaluation of the sample dc conductivity and electrode polarisation correction were performed in the time domain and are described in detail elsewhere [24,25]. The dielectric measurements were performed over the temperature range 1–40 °C using a thermostatically controlled sample cell. For these measurements, the virus was suspended in KCl or HEPES buffer with a conductivity which varied between 3.5 and 5 mS m^{-1} depending on the volume fraction.

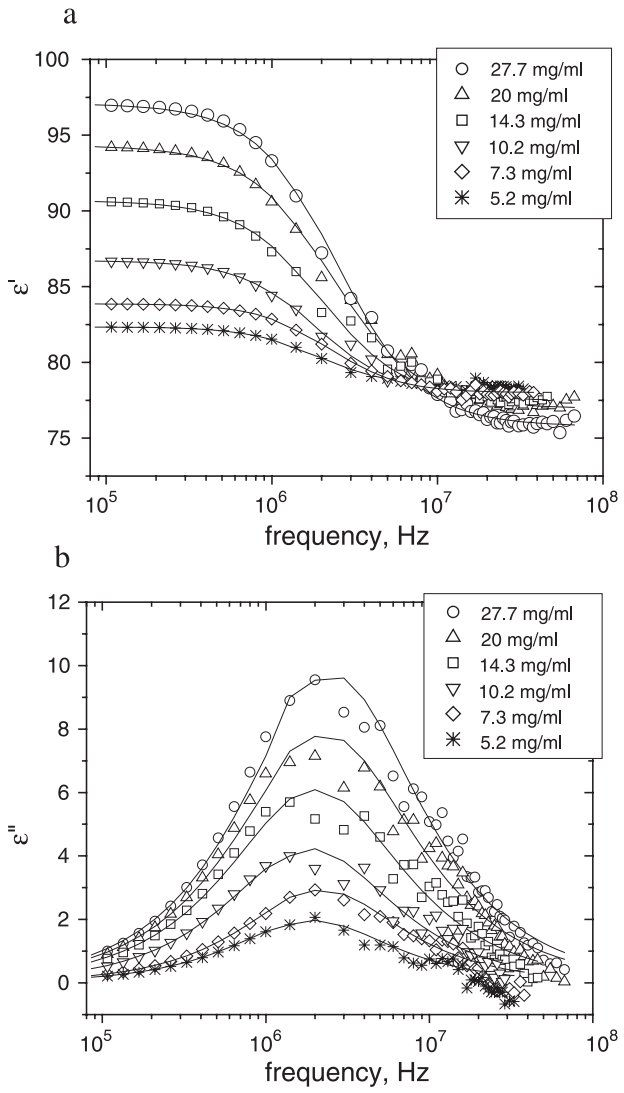

Fig. 1. The dielectric permittivity (a), and loss (b) for a solution of TMV in KCl (conductivity 3 mS m^{-1}) for different virus concentrations. The solid line is a best fit to the data using the Cole–Cole equation (see text for details).

3. Results

The dielectric loss data for a suspension of TMV at different concentrations (measured at 25 °C) are presented in Fig. 1 for the frequency range 100 kHz–100 MHz. This figure does not include dielectric data for frequencies greater than 100 MHz because the signal level is low and noisy. The magnitude of the dielectric dispersions are plotted after subtracting the dc conductivity in the time domain. Analysis of the spectra has shown that the best fit can be obtained by using the Cole–Cole empirical function:

$$\varepsilon^*(\omega) = \varepsilon_\infty + \frac{\Delta\varepsilon}{1 + (j\omega\tau)^{1-\alpha}} + \frac{\sigma}{j\omega\varepsilon_o} \qquad (1)$$

where $\Delta\varepsilon$ is the magnitude of the dielectric dispersion, τ is the relaxation time, α is the Cole–Cole distribution parameter, ε_∞ is the high-frequency limit of the dielectric permittivity and σ is the dc conductivity of the suspension evaluated directly from the TDDS experiment in the time domain [24]. The solid lines in Fig. 1 are the best fits to Eq. (1). Since the parameter α in Eq. (1) is less than 0.1 for all the experimental spectra, subsequent analysis of the data was performed assuming that the data conformed to a single Debye process and α was set = 0.

Fig. 2(a),(b) shows the magnitude of the dielectric dispersion $\Delta\varepsilon$ and the relaxation time τ plotted as a function of virus concentration. This figure shows that $\Delta\varepsilon$ varies linearly with concentration of virus up to 20 mg ml^{-1} with some deviation at higher concentrations. The relaxation time is almost independent of concentration.

Electrode polarisation effects can often lead to anomalies in the dielectric data of suspensions of particles in electro-

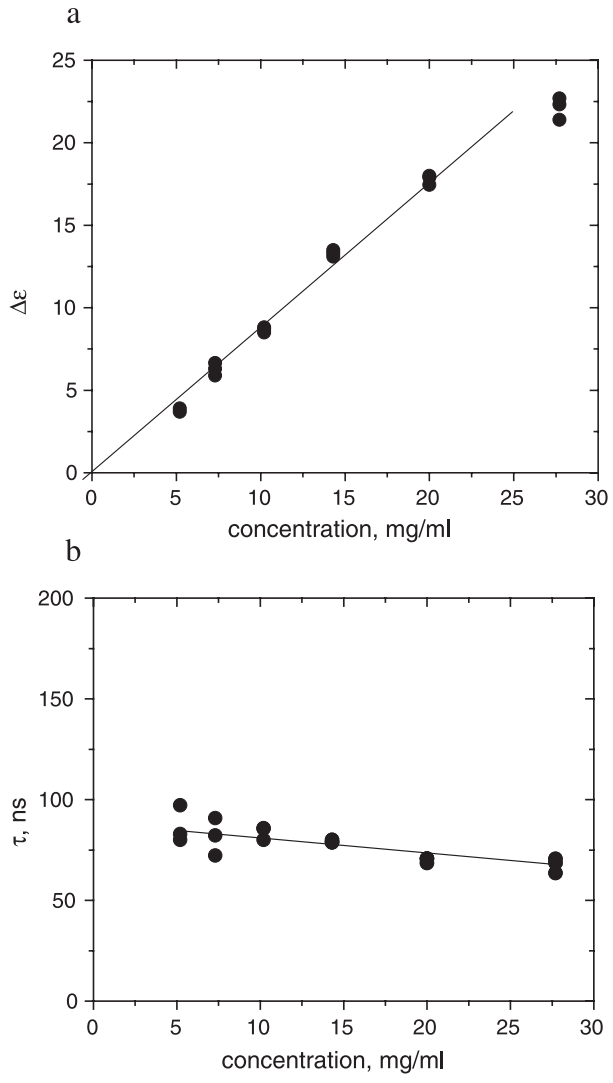

Fig. 2. The magnitude of dielectric dispersion (a) and relaxation time (b) of TMV suspended in KCl (conductivity 3 mS m^{-1}) as a function of concentration.

lytes, particularly at low frequency and/or high suspending medium conductivities. The influence of electrode polarisation on the data was determined by measuring TMV suspensions at different electrolyte conductivities. An experiment was performed with the highest TMV concentration of 27.7 mg ml^{-1} using different conductivities of KCl, in the range 3–150 mS m^{-1}. This showed that for conductivities of less than 10 mS m^{-1}, the effect of electrode polarisation is very small and can be neglected. However, at higher conductivities, electrode polarisation can affect the data and a correction method was applied, which is effective for values of conductivity up to 100 mS m^{-1} [25]. Much higher electrolyte conductivities would require a different approach.

Fig. 3 shows the dielectric parameters of TMV (at 32.9 mg ml^{-1} in 1 mM HEPES buffer, pH 7.0), as a function of temperature, with and without electrode polarisation correction. Fig. 3(a) is an Arrhenius plot of the relaxation time. It shows that after polarisation correction, the value of the relaxation time is reduced by ~ 20%, and the activation energy has a value of 1.5 kcal mol^{-1}. This value is very low and typical of charge polarisation processes, indicating that rotational orientation of the virus is unlikely to be responsible for the observed dielectric loss. For example, the typical activation energy for the rotational diffusion of proteins in aqueous solution is approximately 18 kcal mol^{-1} [19] and connected with the breaking of hydrogen bonds in solution. For charge polarisation mechanisms, low values of activation energy are more typical. Fig. 3(b) shows the magnitude of the dielectric dispersion vs. temperature. After correcting for electrode polarisation effects, it can be seen that $\Delta\varepsilon$ decreases slightly with increasing temperature, a characteristic of aqueous electrolytes due to the increasing rotational Brownian motion of the water dipoles.

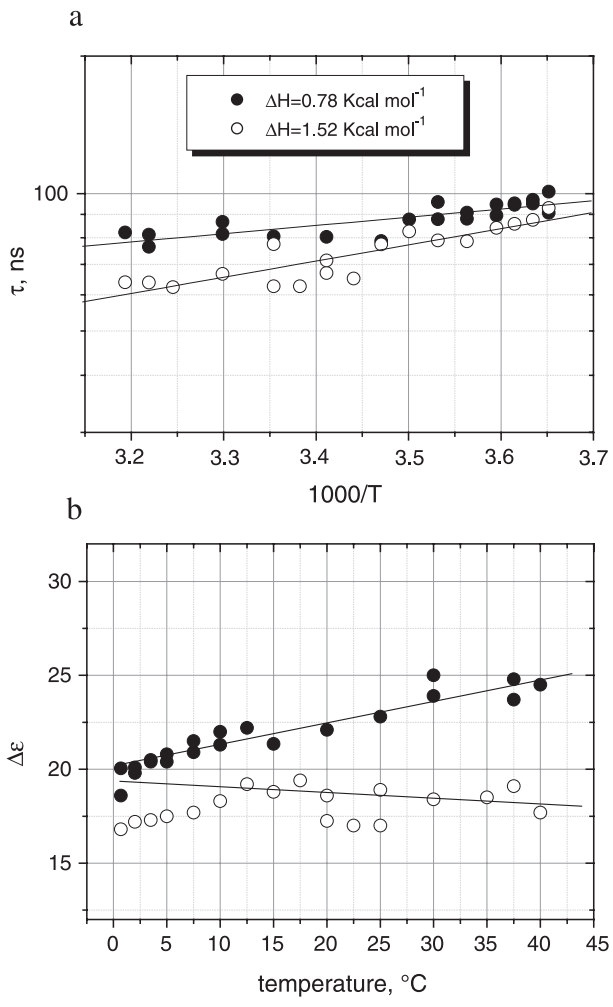

Fig. 3. The relaxation time (a) and magnitude of dielectric dispersion (b) of 32.9 mg/ml TMV in 1 mM HEPES buffer at different temperatures with (○) and without (●) electrode polarization correction. The lines are least square fits to the data giving $\Delta H = 1.52 \pm 0.21$ kcal mol^{-1} for the data following electrode polarisation correction.

4. Discussion

The mechanisms responsible for the dielectric loss of TMV are unknown. The TMV particle contains a double-stranded RNA molecule, which is enclosed by a cylinder of protein molecules. This system is symmetric, the permanent dipole moments of the proteins are expected to cancel and the RNA, being double stranded, is unlikely to possess a permanent dipole moment. As indicated above, the measured activation energy of the loss process is very small, so that we can exclude permanent dipole orientation as the main mechanism of the relaxation.

In their studies of Alfalfa Mosaic Virus, van der Touw et al. [21] concluded that the polarisation of the counterions surrounding the particle was the most likely explanation for the dielectric loss. The relaxation process of TMV may be interfacial polarisation (Maxwell–Wagner) or the formation of an induced dipole through some form of counterion fluctuation mechanism.

4.1. Interfacial polarisation

In previous work [26], the dielectrophoretic properties of TMV were measured and the data were analysed according to the Maxwell–Wagner interfacial polarisation mechanism. Dielectrophoresis is the movement of a polarisable particle in a nonuniform alternating electric field [23,26]. In order to observe the dielectrophoretic behaviour of the particle, the virus was fluorescently labelled. The effective dipole moment of the TMV particle was determined from the measured dielectrophoretic properties [26]. The data could be explained by modelling the virus as a long, thin rod with a homogeneous bulk permittivity of 55.

The model presented in Ref. [26] was used to analyse the data presented in this paper by fitting the dielectric loss data to the general expression for the dielectric properties of a suspension of ellipsoidal particles [12,26]. The complex dielectric constant ε^* for a mixture of volume fraction Φ, is given by:

$$\frac{\varepsilon^* - \varepsilon_m^*}{\varepsilon^* + 2\varepsilon_m^*} = \frac{1}{9}\Phi \sum_{\alpha=x,y,z} \frac{\varepsilon_{p,\alpha}^* - \varepsilon_m^*}{\varepsilon_m^* + (\varepsilon_{p,\alpha}^* - \varepsilon_m^*)A_\alpha} \tag{2}$$

where ε_m^* is the complex permittivity of the medium, ε_p^* is the complex permittivity of the particle, and a general complex permittivity is given by $\varepsilon^* = \varepsilon - j\sigma/\omega$ with $\omega = 2\pi f$. The depolarisation factors A_α along each axis are given by:

$$A_\alpha = \frac{1}{2}abc \int_0^\infty \frac{ds}{(s + i_\alpha^2)R} \tag{3}$$

$$i_x = a, \quad i_y = b, \quad i_z = c,$$

$$R = \sqrt{(s + a^2) + (s + b^2) + (s + c^2)} \text{ and } \sum_\alpha A_\alpha = 1.$$

Eq. (2) is valid for an isotropic dielectric where the complex permittivities are the same along each axis. If the TMV particle is approximated to a prolate ellipsoid, with axes $a > b = c$ and shape factor $q = a/b$, then the depolarisation factors can be written as:

$$A_\alpha = -\frac{1}{q^2 - 1} + \frac{1}{(q^2 - 1)^{3/2}} \ln\left\{q + (q^2 - 1)^{1/2}\right\} \tag{4}$$

and $A_y = A_z = (1 - A_x)/2 \approx 1/2$ for $a/b \gg 1$.

The dielectric data for TMV were fitted for a range of concentrations from 5.2 to 27.7 mg ml^{-1}. The suspending medium conductivity and permittivity, together with the particle permittivity, were kept constant; the particle conductivity and shape factors were varied to produce a best fit. Simulation of model spectra shows that two relaxation processes occur, corresponding to the orientation of the major or minor axis of the rod with the electric field. The low-frequency process is in a good agreement with experimental data, but the magnitude of the high-frequency dispersion is small; it occurs at frequencies above 10 MHz and could not be quantified from the experimental data.

The best fit to the data for all volume fractions was obtained with a particle conductivity of 0.4 S m^{-1}, which predicted a relaxation time for the low-frequency process of 75 ns, as observed experimentally. To establish the validity of this model for higher suspending medium conductivities, the value of $\Delta\varepsilon$ was calculated as a function of conductivity, as shown in Fig. 4. Also plotted on this figure are the experimental values of $\Delta\varepsilon$ for 20 mg ml^{-1} of TMV concentration for different conductivities of KCl solution and HEPES buffer. Two theoretical plots are shown, one with the shape factor $q = 15.56$ (corresponding to the ratio of the mean dimensions of TMV = 280/18) and a second curve with the optimum value of $q = 10.8$ determined from the best

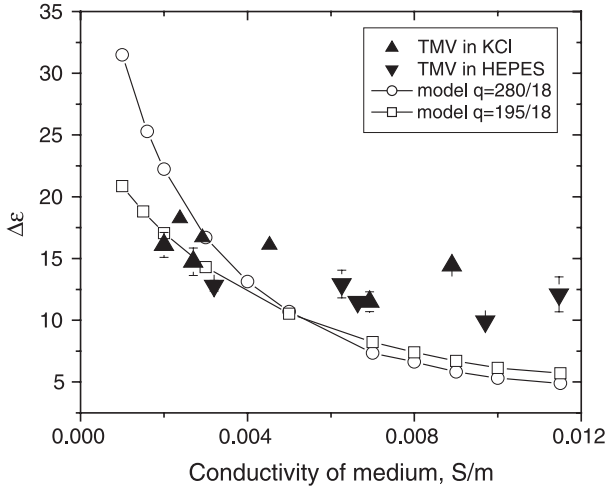

Fig. 4. Variation of $\Delta\varepsilon$ with conductivity of suspending medium for TMV in KCl solution (▲) and HEPES solution (▼) at a concentration 20 mg ml^{-1} showing agreement between the predicted value and the experimental data for two different values of shape factor q. The dielectric parameters of the TMV was $\varepsilon_p = 55$, $\sigma_p = 0.4$ S m^{-1}.

fit to the data at the low-conductivity end. This value of q would imply either a shorter particle and/or larger diameter. If the dimensions of the double layer are included in the estimate of particle diameter, then the effective diameter could indeed be larger than the physical size of the particle. The correlation between the measured and predicted values of $\Delta\varepsilon$ at the higher suspending medium conductivities is less sensitive to the shape factor (Fig. 4).

The large value of particle conductivity is consistent with previous measurements of the properties of the TMV determined by dielectrophoresis [26]. Dielectrophoretic measurements can only be performed if the particle is visible, i.e. if it is fluorescent. In a previous work, TMV was fluorescently labelled using two different biochemical techniques; results showed that the conductivity of the particle was either 0.085 S m^{-1} [26] or 0.17 S m^{-1} [27] depending on the number of charges on the virus coat protein neutralised during the labelling procedure. The virus used for dielectric spectroscopy had not been biochemically modified and would be expected to have a high surface charge density. TMV has a pI of 3.5 and contains a large number of acidic groups. At pH 7.0, it is expected that all the amino acids on the coat protein in contact with the surrounding suspending medium are fully ionised and that the TMV will have a net negative charge.

It has been shown that the dielectric properties of small particles undergoing interfacial relaxation are dominated by surface conductance [28,29]. O'Konski [30] gave an expression for the surface conductance of ellipsoids of different eccentricity, and stated that for a prolate ellipsoid or rod, the axial conductivity is given by $\sigma_p = 2K_s/b$, where b is the transverse axis (9 nm for TMV). If the conductivity of TMV is considered to arise solely from surface conductance, then this can be estimated to be 1.8 nS, which is similar to that measured for carboxylated latex particles [29].

TMV has approximately six negative charges and four positive charges on the surface of each protein [31]. With a total of 2130 protein molecules, this equates to a net surface charge density of 0.043 Cm^{-2}, assuming all groups are ionised. The surface conductance K_s is related to surface charge density σ_s through the ion mobility μ, by $K_s = \sigma_s\mu$. Assuming that the dominant charge carrier is the K^+ ion, ($\mu = 8 \times 10^{-8}$ m^2 V^{-1} s^{-1}), then K_s can be calculated to be 3.4 nS, twice the measured value. This discrepancy could be due to the assumption that the mobility of the potassium ion is the same as the bulk value, which may not be true. Also, not all the charged groups will be fully ionised on the TMV surface.

4.2. Counterion polarisation

In this model, the particle is considered to be surrounded by an ion cloud, with specific association or interaction between the ions and corresponding binding sites on the particle surface. At the pH of the electrolytes used, the amino acid groups on the virus coat protein will all be ionised with a percentage exposed to the surrounding electrolyte. The charge on these groups will be electrically compensated by charges of opposite sign in the surrounding medium, forming the electrical double layer. These counterions can move along the length of the TMV rod under the influence of an applied electric field giving rise to an induced dipole moment. In the case of polyelectrolytes, such as DNA, the high intrinsic charge density gives rise to a very large induced dipole, which is the origin of the extremely large, low-frequency dielectric constant of polyelectrolyte solutions.

The polarisation of the counterion atmosphere, particularly in relation to DNA, has been the subject of many papers [32–42]. In the counterion condensation model attributed to Manning [34–36], there is a well-defined cloud of positive ions a few angstroms thick immediately next to the DNA, and this cloud compensates for most of the charge on the DNA. The remaining ions exist in the diffuse part of the Debye double layer, which surrounds the molecule. Thus, there are two separate ionic phases surrounding the molecule, and both polarise to give rise to the observed dielectric loss. However, such a model is unlikely to be applicable to a virus particle, where a protein coat surrounds the RNA and the charge on the RNA is compensated by these proteins and their associated charges.

However, in their study on Alfalfa Mosaic Virus, van der Touw et al. [21] concluded that the counterion polarisation mechanism best accounted for the observed dielectric properties of this virus. In the models of Mandel [38,39] and Oosawa [40], the polarisability of the molecule, α, is given by:

$$\alpha = \frac{ne_o^2 l^2 A}{12kT} \tag{5}$$

where e_o is the electronic charge, n is the number of counterions on the virus, l is the length of the rod, and A is a parameter that describes the degree of interaction of counterions with the charges on the rod. In Mandel's model, this factor is neglected and set to 1, an assumption that van der Touw showed to be valid in their analysis of the Alfalfa Mosaic Virus.

The polarisability α is related to the magnitude of dielectric dispersion by, e.g. [21,41]:

$$\varepsilon_0\Delta\varepsilon = 1/3\rho\alpha \tag{6}$$

where ρ is the number density of particles in solution. For a solution of 10 mg ml^{-1}, the number density of TMV is $\rho = 1.0 \times 10^{20}$ m^{-3} so that the average polarisability of the TMV can be estimated to be 2.4×10^{-30} F m^2, which from Eq. (5) gives a figure of $n \approx 60$ ions associated with the virus particle. In their observations of Alfalfa Mosaic Virus, van der Touw assumed that all ions in the virus contributed to the potential outside the particle. This is unlikely since individual charged amino acid groups on the inside of the

coat protein bind to nucleotides, both stabilising the structure and neutralising the charge. Furthermore, the protein is also likely to screen a large component of any remaining free charges on the RNA. Thus, it is likely that only the charges on the surface of the virus will contribute to the surface potential and therefore to the counterion cloud. TMV has six negative charges and four positive charges on the surface of each protein, leaving a net surface charge of 4260 negative. This figure is much higher than that estimated from the analysis of Eq. (5).

Mandel [38,39] and Oosawa [40] also derived an expression for the relaxation time of the counterion dispersion, given as:

$$\tau = \frac{l^2 e_o A}{(2\pi)^2 \mu k T} \tag{7}$$

where μ is the mobility of the ion that polarises. At 25 °C, the relaxation time of TMV in KCl solution can be calculated from this expression as 1 μs, where the dominant counterion is again assumed to be K^+. This relaxation time is approximately 10 times larger that the measured relaxation time, which is of the order of ~ 0.1 μs. To account for this discrepancy, the mobility of the K^+ ions would need to be 10 times higher than the mobility of potassium ions in water (assuming $A=1$). If the ions that polarise are protons rather than potassium ions, then using the mobility of the free proton (36.3×10^{-8} $m^2V^{-1}s^{-1}$), the relaxation time can be calculated as 0.2 μs, which is closer to the experimental value.

5. Conclusions

The observed dispersion can be partly described using the simple counterion polarisation models of Mandel and Oosawa. However, the number of charges predicted by the counterion polarisation model is much lower than the charge density of TMV estimated from the known protein structure.

The activation energy data points to some mechanism involving charge polarisation. The measured value of ~ 1.5 kcal/mol is similar to that measured for systems where polarisation of an ion cloud is considered to be responsible for the dispersion of systems such as DNA [42]. However, this is not a verification of the counterion fluctuation model as the same physical process of charge polarisation occurs in the Maxwell–Wagner model, where the activation energies are similar.

The interfacial polarisation model (Maxwell–Wagner) predicts an accurate relaxation time for the dispersion and also a surface conductance for the virus which is in agreement with the known structure and charge density. However, there is a small discrepancy in the estimated magnitude of the dielectric dispersion. The counterion fluctuation model provides good agreement with experiment for the magnitude of the dielectric dispersion, but gives a relaxation time significantly higher than experimentally determined.

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