ELSEVIER

Biochimica et Biophysica Acta 1571 (2002) 1–8

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.bba-direct.com

Measuring the dielectric properties of herpes simplex virus type 1 virions with dielectrophoresis

Michael P. Hughes [a,1], Hywel Morgan [a,*], Frazer J. Rixon [b]

[a] *Department of Electronics and Electrical Engineering, Bioelectronic Research Centre, University of Glasgow, Rankine Building, Glasgow G12 8QQ, Scotland, UK*

[b] *MRC Virology Unit, Institute of Virology, Glasgow G11 5JR, Scotland, UK*

Received 2 July 2001; received in revised form 15 January 2002; accepted 17 January 2002

Abstract

An investigation has been performed into the biophysical properties of the enveloped mammalian virus, herpes simplex virus type 1 (HSV-1). The dielectrophoretic behaviour of the virus particles was measured as a function of applied frequency (over the range 100 kHz–20 MHz) and conductivity of the suspending medium (over the range 1–100 mS m^{-1}). The dielectric properties of the virus were determined from the dielectrophoretic data using the smeared-out shell model. The data suggest that the intact particle has a surface conductance of 0.3 nS, an internal and membrane permittivity of $75\varepsilon_o$ and $7.5\varepsilon_o$, respectively, an internal conductivity of approximately 0.1 S m^{-1} and a zeta potential of 70 mV. The dielectric properties were measured for intact, fresh virus particles and also for particles following exposure to various modifying agents, such as treatment with enzymes, ionophores and ageing. It is shown that the observed changes in the dielectrophoretic spectrum, and the variations in the dielectric properties of the virus concur with the expected physiological effects of these agents.

Keywords: Herpes simplex virus type 1; Virion; Dielectrophoresis; AC electrokinetics

1. Introduction

Pathogens such as viruses are a major cause of human disease, and attempts to find either treatments or rapid means of identification form a significant part of global scientific research. The majority of research is directed towards studying the virus by biochemical means. However, in many cases, physics can offer alternative methods of probing viral structure and obtain new insights into viral function.

In this paper, dielectrophoretic measurements of herpes simplex virus type 1 (HSV-1) have been used to estimate the dielectric properties of the virus. Dielectrophoresis (DEP) describes the motion of polarisable particles in nonuniform electric fields [1,2]. The DEP force is generated through the interaction of an induced dipole and the nonuniform field. The magnitude and direction of the force is related to the dielectric properties of the particle and the suspending medium. For a spherical particle of radius r the DEP force is given by:

$$F_{\mathrm{DEP}} = 2\pi r^3 \varepsilon_{\mathrm{m}} \mathrm{Re}[f_{\mathrm{CM}}] \nabla |E|^2 \quad (1)$$

where ε_{m} is the absolute permittivity of the suspending medium, E is the rms electric field, ∇ is the del vector operator and $\mathrm{Re}[f_{\mathrm{CM}}]$ is the real part of the Clausius–Mossotti factor, defined as:

$$f_{\mathrm{CM}} = \frac{\varepsilon_{\mathrm{p}}^* - \varepsilon_{\mathrm{m}}^*}{\varepsilon_{\mathrm{p}}^* + 2\varepsilon_{\mathrm{m}}^*} \quad (2)$$

In Eq. (2) $\varepsilon_{\mathrm{p}}^*$ and $\varepsilon_{\mathrm{m}}^*$ are the complex permittivities of the particle and medium, respectively, where $\varepsilon^* = \varepsilon - \mathrm{j}(\sigma/\omega)$, ε is the permittivity, σ the conductivity, ω the angular frequency of the applied field and $j = \sqrt{-1}$. The factor $\mathrm{Re}[f_{\mathrm{CM}}]$ is frequency-dependent and for a sphere can vary between -0.5 and $+1$, depending on the relative magnitudes of $\varepsilon_{\mathrm{p}}^*$ and $\varepsilon_{\mathrm{m}}^*$. Thus, the DEP force moves the particle either towards or away from high field strength regions, effects termed positive and negative DEP, respectively. At certain

* Corresponding author. Tel.: +44-141-330-5237; fax: +44-141-330-4907.

E-mail address: h.morgan@elec.gla.ac.uk (H. Morgan).

[1] Present address: Centre for Biomedical Engineering, University of Surrey, Guildford GU2 7KH, Surrey, UK.

0304-4165/02/$ - see front matter
PII: S0304-4165(02)00161-7

frequencies the polarisability of the particle and medium are the same (i.e. $\mathrm{Re}[f_{CM}]=0$) and the force on the particle becomes zero. This frequency is termed the zero-force or crossover frequency.

Measurement of the DEP spectrum can be used to measure the dielectric properties of biological particles [3–5]. Over the last 4 years, dielectrophoretic manipulation and characterisation of viruses has been reported. For example, Fuhr and co-workers demonstrated that Sendai and Influenza viruses could be trapped in field funnels by negative DEP [6,7]. Green and Morgan used positive DEP collection at electrode edges to measure the polarisability of Tobacco Mosaic virus [8,9]. Subsequently, Hughes et al. [10] demonstrated both positive and negative DEP of HSV-1 particles, depending on the applied frequency. Measurements of the frequency at which the DEP force is zero [10] were used to obtain approximate values for the polarisability of the virus. Gimsa [11] used dynamic light scattering techniques to observe electrorotation of Influenza viruses, and derived a dielectric model of the properties of that virus. Further work showed that DEP can even be used to separate different viruses [12]. In this work, we show for the first time that DEP can be used to characterise the dielectric properties of a complex virus.

HSV-1 is a human pathogen that is associated with a range of conditions but is best known for causing facial cold sores. The complete virus particle, or virion, is approximately 200–250 nm in diameter. It has a layered structure consisting of a number of "shells" [13]. The virion is enclosed by a membrane (or envelope) which is a lipid bilayer containing a large number of glycoproteins. These extend outwards for a distance of approximately 10–20 nm [14]. The envelope encloses a thick amorphous protein gel called the tegument, which contains the capsid [15]. The capsid is a robust icosahedral protein structure that contains the viral DNA. The dielectrophoretic and dielectric properties of the purified capsid have been characterised previously [16]. A transmission electron micrograph of an HSV-1 virion is shown in Fig. 1a, together with a diagrammatic representation, Fig. 1b.

In this paper we report measurements of the dielectrophoretic behaviour of HSV-1 as a function of the suspending medium conductivity, over the range 1 to 100 mS m^{-1}. In addition, the effect of chemical agents (exposure to saponin, valinomycin and trypsin), and storage at +4 °C has been investigated. The effect of these chemical agents on the biochemistry of the virus has also been examined. Results provide insights into the biophysical processes initiated by such treatment, and a simple model of the viral interior has been developed which accounts for the observed effects.

2. Materials and methods

2.1. Virus preparation

HSV-1 virions were purified using protocols established by Szilagyi and Cunningham [17]. The virus particles were pelleted from the tissue culture medium (Glasgow modified Eagles medium supplemented with 10% newborn calf serum) by centrifugation at 23,000 × *g* for 2 h at 4 °C. They were resuspended in Eagles medium without calf serum and centrifuged through a 35-ml gradient of 5–15% w/w Ficoll 400 in Eagles medium without phenol red (E – PR) at 12,000 rpm in a Sorvall AH629 rotor. The virion band was collected, diluted and pelleted at 20,000 rpm in AH629 tubes, before being resuspended in an appropriate volume of E – PR. Virions made in this way are essentially pure and appear largely undamaged [17].

The virions were fluorescently labelled with NBD-dihexadecylamine (Molecular Probes, Inc.). The dye was dissolved in DMF at a concentration of 2 mg/ml and then added to the virus suspension at a 1:100 dilution. Particles were incubated for 20 min at room temperature, pelleted at

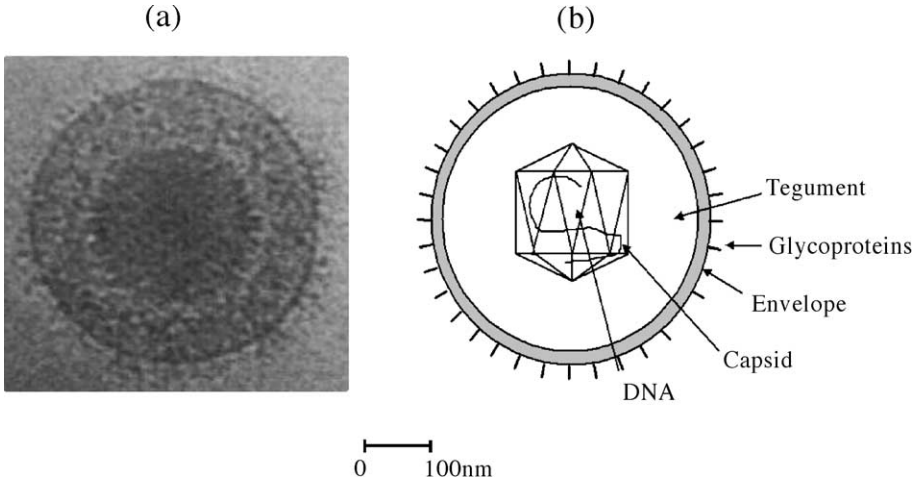

Fig. 1. (a) A transmission electron micrograph of an HSV-1 particle. (b) Schematic diagram of the HSV-1 virion showing the DNA, capsid, tegument and membrane.

20,000 rpm for 10 min in a Sorvall TLA 100.2 rotor and resuspended in 1 ml iso-osmotic (280 mM) mannitol solution; 5 μl aliquots of virions were diluted into mannitol/KCl solution (pH = 6.4 ± 0.2) for experiments.

When samples were to be treated with saponin, valinomycin or trypsin, the pelleted, fluorescently labelled virions were resuspended in 2 ml of E − PR and divided into 4 × 500 μl aliquots. One aliquot was kept at 0 °C as an untreated control, and the remaining three were treated with trypsin, saponin and valinomycin, respectively. Trypsin was added to the virion suspension at 40 μg/ml final concentration followed by incubation at 37 °C for 1 h. Saponin treatment was performed by adding the agent to the sample to a final concentration of 40 μg/ml as described by Gascoyne et al. [5], followed by incubation at 0 °C for 10 min. Valinomycin (dissolved at 10 mg/ml in ethanol) was added to a final concentration of 5 μM as described by Harold and Baarda [18] with incubation at room temperature for 30 min.

After treatment, 50 μl of each sample was removed for titration, protein analysis and electron microscopic examination. The remaining virions were pelleted at 20,000 rpm in a Sorvall TLA 100.2 rotor, resuspended in iso-osmotic mannitol solution, divided into 11 aliquots and mixed with mannitol solutions containing a range of concentrations of KCl, such that the final solutions had conductivities across the range 1–100 mS m^{-1} at five points per decade.

2.2. Experimental

Dielectrophoretic manipulation and characterisation of the virus particles were performed using electrodes of the polynomial design [19]. Electrodes were fabricated on glass slides using photolithography. They consisted of a 100-nm Au layer evaporated over a 10-nm Ti seed layer. The electrode size was such that the gap between nearest neighbour electrodes was 2 μm and the gap across the centre was 6 μm. Prior to experimentation, 25 μl of virus solution was pipetted onto the electrodes and the sample sealed with a cover slip. Particle concentration during experimentation was sufficiently low for particle–particle interactions to be ignored. Viruses were observed to be generally monodisperse, and clusters of viruses were ignored when observations were made.

Sinusoidal signals of magnitude $5V_{pk-pk}$ were applied to the electrodes over the frequency range 1 kHz–20 MHz using a Hewlett Packard function generator. Potentials were applied to give a 180° phase difference between adjacent electrodes. Experiments were observed using a Nikon Microphot microscope with a × 40 fluorescence lens. Conductivity measurements were performed using a Hewlett-Packard 4192A-impedance analyser and a Sentek conductivity cell in the range 100 kHz to 1 MHz.

Table 1
Titres of purified HSV-1 following fluorescent labelling and treatment with various envelope-modifying agents

Treatment	Titre
None	8.2×10^9
Fluorescent labelling	2.7×10^9
Trypsin	1.0×10^6
Saponin	3.5×10^9
Valinomycin	2.8×10^9

Titres are presented as the number of infectious virions per milliliter. Virus samples were diluted through 10-fold serial dilutions which were used to infect duplicate monolayers of BHK cells. After incubation for 2 days at 37 °C, the cell monolayers were fixed and stained, and the numbers of viral plaques counted.

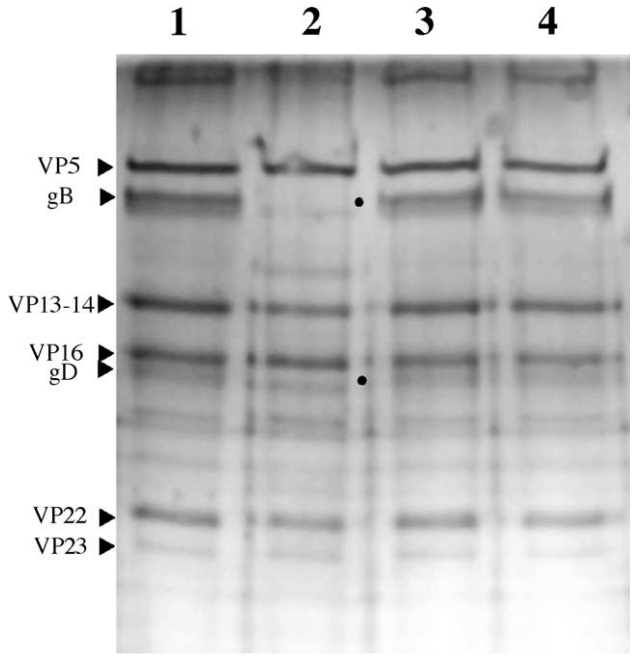

Fig. 2. Protein composition of treated virions. A 10% polyacrylamide gel showing Coomassie brilliant blue-stained profiles of purified HSV-1 virions (lane 1) and of virions treated with trypsin (lane 2), saponin (lane 3) or valinomycin (lane 4) as described in Section 2. Some examples of capsid (VP5, VP23), tegument (VP13-14, VP16, VP22) and envelope (gB, gD) proteins are indicated. The expected positions of gB and gD are marked to the right of lane 2 (•).

3. Results

3.1. Biochemical and morphological examination

The behaviour of virions undergoing DEP would be expected to be influenced by their structural integrity. The virions were therefore examined by electron microscopy after negative staining. Images showed that treatment with saponin, valinomycin or trypsin did not alter their overall structure (data not shown).

To determine whether these treatments had affected the viability of the virions, titrations were carried out to determine infectivity. Table 1 shows the result of this experiment. Fluorescent labelling resulted in a slight (two- to threefold) reduction in virus titre. Subsequent treatment with saponin or

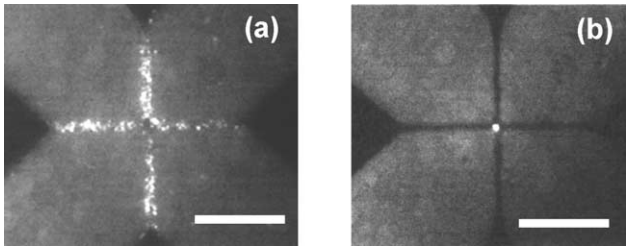

Fig. 3. Photographs showing HSV-1 undergoing DEP in a suspending solution of conductivity 1 mS m^{-1}. (a) Positive DEP, $5V_{pk-pk}$, 1-MHz signal applied (b) Negative DEP, $5V_{pk-pk}$, 20-MHz signal applied. Scale bar: 20 μm.

valinomycin had no further effect, but treatment with trypsin resulted in a 3 log reduction in titre. This is expected since trypsin removes the surface glycoproteins that are necessary for virus attachment to cells and subsequent penetration of the plasma membrane. Analysis of the protein compositions by SDS-PAGE is shown in Fig. 2 and indicates that valinomycin or saponin treatment did not affect the protein composition of the virions, whilst trypsin digestion removed the surface glycoproteins (gB and gD), but did not affect the tegument (VP13-14, VP16, VP22) or capsid proteins (VP5, VP23), which are protected by the lipid envelope.

3.2. DEP

Virus particles were observed to undergo both positive and negative DEP depending on the applied frequency, as reported in detail elsewhere [10]. At low frequencies, particles collected at electrode edges under positive DEP, and at high frequencies particles experienced negative DEP. Photographs showing the dielectrophoretic behaviour of HSV are shown in Fig. 3. At a particular suspending medium conductivity, a single crossover frequency was measured. At frequencies below approximately 100 kHz, fluid motion due to electrohydrodynamic effects interfered significantly with observation of dielectrophoretic forces [10,20,21].

DEP crossover spectra were measured by adding 5 μl of low-concentration virus/mannitol solution to 500 μl of mannitol/KCl solutions of known conductivity; mannitol was used to maintain osmolality. Particle behaviour was measured in suspending medium with conductivities over the range 1 to 100 mS m^{-1} at five conductivities per decade.

The frequency at which the DEP force is zero (the crossover point) was determined by sweeping the frequency of a $5V_{pk-pk}$ signal from 100 kHz up to 20 MHz, thus determining the approximate crossover frequency. The frequency of the applied signal was then varied between frequencies above and below this approximate value, until a single frequency was reached where no motion of the virus could be observed. At the higher medium conductivities, fluid motion was found to interfere with positive dielectrophoretic collection for frequencies up to 500 kHz, as described by Hughes et al. [10].

The DEP crossover spectrum for virions, measured within 5 h of harvesting is shown in Fig. 4a. The data are plotted as a

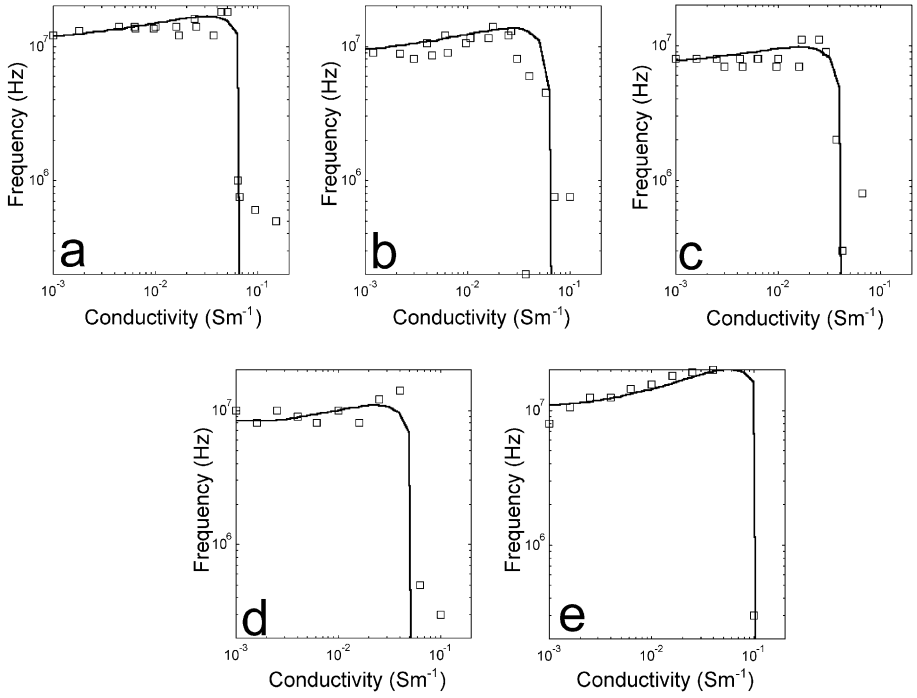

Fig. 4. Dielectrophoretic crossover spectra of HSV-1, with squares indicating data points and best-fit lines derived using the parameters in Table 2. HSV-1 was analysed under the following conditions: (a) 5 h after virus preparation; (b) 24 h after virus preparation, following storage at +4 °C in the suspending media (i.e. iso-osmotic mannitol solution containing varying amounts of KCl); (c) after treating the virus with trypsin; (d) after treating fresh virus with saponin; (e) after treatment of the viruses with valinomycin.

function of the suspending-medium conductivity. Also shown (solid line) is a best-fit curve from theory (see later). It can be seen that the crossover frequency increases with suspending medium conductivity, from 12 MHz at low conductivities, to a peak of 18 MHz at a conductivity of 50 mS m^{-1}. Thereafter, the response falls sharply to approximately 1 MHz. This later effect has been attributed to double-layer polarisation mechanisms [22]. Fig. 4b shows the behaviour of virions after 24-h storage at +4 °C. Again the solid line indicates a best fit to the data. The crossover spectra for fresh particles treated with saponin, trypsin and valinomycin are shown in Fig. 4c–e, respectively.

The sign of the surface charge on the virions in the experimental medium was determined by electrophoresis. Particles were suspended in 280 mM mannitol without KCl and pipetted onto an electrode array consisting of parallel strip electrodes spaced 50 μm apart. A DC potential of 1 V was applied across this array, and particles were observed to move towards the anode by electrophoresis, indicating that they carried a net negative charge.

4. Discussion

DEP crossover methods have been shown to be effective in measuring the dielectric properties of sub-micron particles. DEP crossover methods are valuable because of the ease with which an accurate value of the zero-force frequency can be determined for a large population of particles (of the order of hundreds at a time). In this work, the crossover frequencies of individual particles were observed to vary by up to approximately ±10% of the mean value, indicative of small variations between particles within the population. A few particles were occasionally observed behaving in a radically different manner to the norm, possibly due to damage. These were not considered in the analysis.

The dielectric properties of the virions were estimated by fitting the data to a model which describes the virus as a series of concentric shells, the ''multi-shell'' model [4,23,24]. This model can be used to predict both the frequency dependence and conductivity dependence of the DEP response, so that the properties of the separate layers can be estimated by comparison with experimental data. For example, this model has been used to determine the dielectric properties of viable and nonviable yeast cells [24], to measure membrane changes in leukemic cells [5] and the time course of HSV-1 infection in mammalian cells [25].

The virus particle consists of several compartments viz. membrane, tegument, capsid and capsid interior. From the zero-force vs. conductivity data, it is not possible to obtain unique values for the permittivity and conductivity of these separate compartments; only the overall particle permittivity and conductivity can be unambiguously determined. However, by analysing the data under a wide range of experimental conditions, the dielectric properties of the virus membrane and virus interior can be estimated. The dielectric parameters of the virus were obtained by fitting the data (zero-force vs. conductivity) using a programme written in MATLAB (the Math Works). Values of the permittivity and conductivity of the different regions of the virus were changed to give a best fit for the range of different experimental conditions.

Dielectrophoretic spectra for the virus were calculated using the shell model, modelling the particle as an insulating envelope surrounding a conducting tegument. In previous work, the dielectric properties of the HSV-1 capsid were determined from analysis of capsid crossover data [16]. Analysis showed that including the capsid (using previously determined dielectric properties) into the model had little or no effect on the overall response of the virus particles. Therefore, the shell model was simplified by setting the dielectric properties of the capsid equal to that of the interior. For analysis, the following assumptions were made:

(i) The virus particle was modelled as a sphere with a radius of 125 nm with a lipid envelope 7 nm thick.
(ii) Treatment with trypsin alters only the virus surface (gel electrophoresis indicated that no internal viral proteins were lost as a consequence of trypsin treatment).
(iii) Saponin and valinomycin increases the virus membrane permeability, leading to changes in the internal conductivity [5].

Although the best fit to the data for any one data set is not unique, it was found that the best-fit data presented here were uniquely consistent when considering the known changes to the biophysical state induced by the treatments described, and the known dielectric properties of cells. The variation in the best-fit line determined by varying the derived parameters, one at a time, for maximum upper and lower limits is shown for the fresh virus data in Fig. 5.

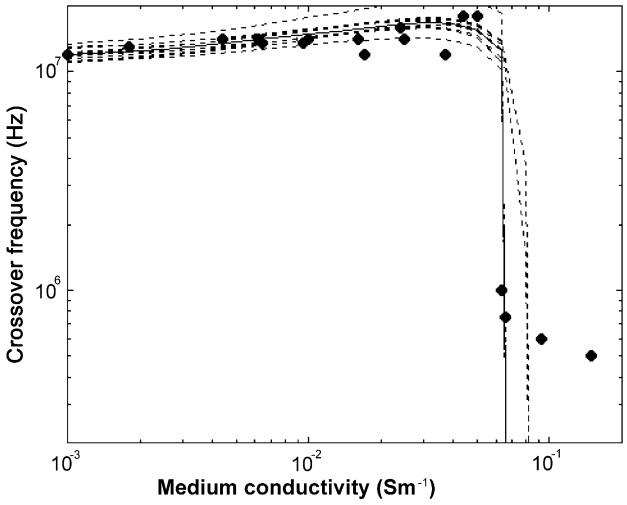

Fig. 5. A graph showing the effect on the best-fit line of varying the parameters for fresh virus across the ranges indicated in Table 2.

The dielectrophoretic properties of sub-micrometre particles are dominated by surface conductance effects [22,26–28]. If a given population of particles has a constant surface conductance, then the crossover data shown in Fig. 4 should be independent of the suspending medium conductivity. However, it can be seen that the crossover frequency increases as the suspending medium conductivity increases; in the conductivity window 1 to 10 mS m^{-1}, the crossover frequency can rise by up to 50%. To account for this, the surface conductance can be separated into two independent components, a component through the Stern layer and another through the diffuse part of the double layer, where electroosmotic effects have to be considered [29]. The total conductivity of the particle, σ_p, can then be written as:

$$\sigma_p = \sigma_{pbulk} + \frac{2K_s^i}{r} + \frac{2K_s^d}{r} \quad (3)$$

where σ_{pbulk} is the conductivity through the particle, and K_s^i and K_s^d are the conductances due to the Stern and diffuse part of the double layers, respectively. The Stern layer conductance depends on the surface charge density of the particle, whilst the diffuse double layer conductance is related to the zeta potential (ζ) and the suspending medium conductivity [29].

4.1. Freshly harvested virus

By combining Eqs. (1), (2) and (3), and equating the effective complex permittivity with that given by the shell model, a theoretical crossover vs. conductivity plot can be generated. For virus particles measured within 5 h of harvesting, the best fit to the data is shown by the solid line in Fig. 4. In this case, the virus was modelled as a particle with an internal conductivity of 100 ($\pm$ 5) mS m^{-1}, internal permittivity = 75 ($\pm$ 25)ε_o, membrane permittivity = 7.5 ($\pm$ 1.5)ε_o, Stern layer surface conductance, K_s^i = 0.3 ($\pm$ 0.1) nS and ζ = 70 ($\pm$ 5) mV. A summary of all dielectric parameters for virus subject to different chemical treatments is shown in Table 2.

Owing to the inverse dependence of conductivity with particle radius (Eq. (3)), surface conductance effects dominate over the trans-membrane conductivity, so that in general, the membrane conductivity of sub-micrometre particles cannot be determined and for the analysis was set equal to zero [10]. However, all other dielectric parameters are consistent with values previously reported for cells, e.g. K_s^i is comparable with a value of 0.54 nS determined for erythrocytes [30]; also the membrane permittivity is similar to a value 6.8ε_o determined for erythrocytes [5]. The internal conductivity is lower than that measured for cells (typically in the range 0.2 to 1 S m^{-1} for cells such as the BHK cells in which the virus was grown [25]). The reasons for this are not clear but it may be due to the exclusion of ions from the virion during maturation within the host cell. The relative permittivity value of 75 is higher than would be expected for a cell, which is typically 60–65. However, the sensitivity of the model to this parameter is sufficiently low so that in all cases (except for virus particles treated with valinomycin), the measured value fall can be considered similar to those obtained for cells.

Table 2
Dielectric parameters for virus estimated by fitting the data shown in Figs. 4 and 5 using the single shell model

	K_s (nS)	σ_{int} (mS m^{-1})	ε_{int}	ε_{mem}	ζ (mV)
Fresh	0.3 ± 0.1	100 ± 5	75 ± 25	7.5 ± 1.5	70 ± 5
+1 day	0.2 ± 0.02	85 ± 1	75 ± 15	7 ± 0.5	67 ± 2
Trypsin	< 0.05	83 ± 3	75 ± 20	7.5 ± 0.5	63 ± 7
Saponin	0.3 ± 0.05	40–60	65 ± 10	10 ± 5	75 ± 20
Valinomycin	< 1 (ins.)	σ_{med} + 30	78 ± 2	26 ± 2	74 ± 4

The best-fit values are shown in bold; limits are shown in normal type. "ins." means that the model was insensitive to the parameter below a certain threshold.

4.2. Aged virus

Virus particles were also stored in a range of KCl/iso-osmotic mannitol solutions of varying conductivities, (as prepared for the experiments) at 4 °C for 24 h. The spectrum determined after storage (Fig. 4b) indicates that the average crossover frequency is lower, although the suspending medium conductivity dependence is still apparent. The best fit to the data is obtained with K_s^i reduced to 0.2 nS, and a much lower internal conductivity of 85 mS m^{-1}. The observed reduction in internal conductivity is consistent with the work of Gascoyne et al. [5] in which cells suspended in nonionic, iso-osmotic media experienced a gradual loss of internal ions into the surrounding media over time. Comparing Figs. 4a and 4b, the main difference is in the suspending medium conductivity window between 30 and 60 mS m^{-1}. This may indicate that storing aliquots of virus particles in solutions of higher conductivities leads to a greater variation in their dielectric properties. Storage also produced a small change in surface conductance, possibly indicative of a gradual loss of surface glycoproteins over time.

4.3. Trypsin treatment

Trypsin removes the surface glycoproteins, whilst leaving the remainder of the virus intact. Fig. 4c shows that for a suspending medium with a conductivity less than 17 mS m^{-1}, the zero-force frequency is constant at a value of approximately 8 MHz, thereafter increasing to a peak of 11 MHz at approximately 20 mS m^{-1}; falling below 200 kHz for conductivities above 30 mS m^{-1}. The absence of any significant rise in the zero-force frequency with increasing suspending medium conductivity indicates a small diffuse layer conductance, and the best-fit model indicates that ζ has fallen to 62 mV, and that the Stern layer conductance, K_s^i, is negligibly small. This reduction in the overall surface charge density is

consistent with gel electrophoresis data for trypsinised viruses, which showed that the surface glycoproteins had been removed, but that the viral membrane and interior were unchanged; see Fig. 2. The other dielectric parameters were similar to that of the 1-day old virions, indicating that some slight ion leakage had occurred.

4.4. Saponin treatment

The zero-force spectrum of virions treated with saponin (shown in Fig. 4d) also exhibited a reduction in crossover frequency with respect to the untreated fresh virions, although the treated particles exhibited crossovers over a wider frequency band than other particle types. Saponin permeabilises the membrane, allowing the internal ions to escape [5]. Experiments with mouse erythrocytes [5] have shown that treatment with saponin causes cells to lose some, but not all of their interior ions, leading to a reduction in the internal conductivity (and an increase in the conductivity of the suspending medium). Since the DEP spectra were collected immediately following exposure of the viruses to saponin, the conductivity of the virus interior may not have had time to equilibrate with the exterior. The observed variability in the data probably indicates that the effect of saponin was not the same on every virus particle. The best fit to the data is with an internal conductivity of between 40 and 60 mS m^{-1}. Within the constraints of the model, there was no difference between the other dielectric parameters and those determined for the fresh virus, indicating that the saponin had no measurable effect on the membrane proteins. Saponin treatment did not significantly increase membrane conductance, which would have appeared as an increase in the surface conductance over that measured for the fresh virus. This indicates that any increase in trans-membrane conductance is small compared to surface conductance effects.

4.5. Valinomycin treatment

As shown by Fig. 4e, the data for particles treated with valinomycin is different from all the other results in that the frequency rises rapidly with suspending medium conductivity, from 8 MHz in low conductivity media, to over 20 MHz at higher conductivities. Valinomycin is a K^+ ionophore [18], transporting potassium ions across the membrane. It is expected that exposure of the virus particles to valinomycin would lead to rapid equilibrium of the K^+ concentration on either side of the membrane. The rise in the crossover frequency with increasing suspending medium conductivity can be accounted for by assuming that the conductivity of the virus interior mirrors the suspending medium conductivity. The best fit to the data was obtained by setting the internal conductivity of the virus equal to the suspending medium, with an additional constant offset of 30 mS m^{-1}. The data could only be fitted by invoking a value of surface conductance greater than 1.2 nS (the model is insensitive to values above this). In the preceding analysis, the trans-membrane conductance was assumed to be negligible but owing to the action of the valinomycin, it is probably much higher in this case. This could account for the marked change in the apparent surface conductance; see Eq. (3). Similarly, the relative permittivity of the membrane, at 26, is also higher than in previous analyses. This is a high value for a lipid membrane but may be related to the presence of valinomycin molecules allowing transport across the membrane. The data following valinomycin treatment is sensitive to the internal permittivity, which 78 (± 2)ε_o. All other parameters are within the ranges indicated for fresh viruses. The parameters are summarised in Table 2.

It is noteworthy that the increased permeability of the virion membrane following exposure to saponin or valinomycin does not have a significant effect on the infectivity of the virus. This suggests that possession of an intact envelope, impermeable to ions, is not a prerequisite for infection and indeed, purified herpesvirus particles often appear to have damaged or incomplete membranes. In some viruses the ability of the membrane to control the flow of ions is important for infection. For example, the influenza virus envelope contains an ion channel protein that plays an essential role in the virus life cycle [31]. The ion channel transports hydrogen ions into the virus particle, thereby ensuring that the interior of the virion adopts the low pH condition of the endocytotic vesicle in which membrane fusion takes place. The lowered pH weakens the interactions between the virion proteins, triggering disassembly of the virus particle and releasing the viral genome into the cell. However, the envelopes of HSV-1 virions do not appear to contain ion channel proteins and there is no evidence that pH or ionic changes are important for the disassembly of the herpesvirus particle, which takes place under approximately neutral conditions within the cytoplasm of the cell following fusion of the viral envelope with the external cell membrane.

5. Conclusions

The dielectric characteristics of HSV-1 virus have been characterised, both before and after exposure to a range of chemical agents. By modelling the virus as a single-shelled sphere, the data have indicated that the intact particle has a surface conductance of 0.3 nS and internal permittivity = 75ε_o, a membrane permittivity of 7.5ε_o, and an internal conductivity of approximately 100 mS m^{-1}. These dielectric parameters vary as a function of storage time, which may have implications in determining the infectivity of viruses following storage. Treatment with trypsin reduces the surface charge density, consistent with its action in stripping glycoproteins from the viral membrane. Saponin causes a leakage of ions from the virus interior, whilst valinomycin facilitates the free flow of potassium ions across the membrane, allowing rapid equilibrium with the suspending medium. This means that the internal conduc-

tivity of the particle is a function of the suspending medium conductivity. Significantly this has no effect on virus infectivity.

Acknowledgements

The authors would like to thank Mrs. Joyce Mitchell and Ms. Mary Robertson for virus preparation and Mr. Bill Monaghan for electrode fabrication. We also wish to thank Dr. Nicolas Green for valuable discussions. The electron cryomicroscopic image of the HSV-1 virion shown in Fig. 1A was supplied by Wah Chiu of Baylor College of Medicine, Houston, TX. This work was supported by the Biotechnology and Biological Sciences Research Council (UK) grant no. 17/T05315.

References

[1] H.A. Pohl, Dielectrophoresis, Cambridge Univ. Press, Cambridge, 1978.
[2] T.B. Jones, Electromechanics of Particles, Cambridge Univ. Press, Cambridge, 1995.
[3] P. Marszalek, J.J. Zielinsky, M. Fikus, T.Y. Tsong, Biophys. J. 59 (1991) 982–987.
[4] J. Gimsa, P. Marszalek, U. Löwe, T.Y. Tsong, Biophys. J. 60 (1991) 749–760.
[5] P.R.C. Gascoyne, R. Pethig, J.P.H. Burt, F.F. Becker, Biochim. Biophys. Acta 1149 (1993) 119–126.
[6] T. Schnelle, T. Müller, S. Fiedler, S.G. Shirley, K. Ludwig, A. Hermann, G. Fuhr, B. Wagner, U. Zimmerman, Naturwissenschaften 83 (1996) 172–176.
[7] T. Müller, S. Fiedler, T. Schnelle, K. Ludwig, H. Jung, G. Fuhr, Biotechnol. Tech. 4 (1996) 221–226.
[8] H. Morgan, N.G. Green, J. Electrost. 42 (1997) 279–293.
[9] N.G. Green, J.J. Milner, H. Morgan, J. Biochem. Biophys. Methods 35 (1997) 89–102.
[10] M.P. Hughes, H. Morgan, F.J. Rixon, J.P.H. Burt, R. Pethig, Biochim. Biophys. Acta 1425 (1998) 119–126.
[11] J. Gimsa, Ann. N. Y. Acad. Sci. U.S.A. 873 (1999) 287–298.
[12] H. Morgan, M.P. Hughes, N.G. Green, Biophys. J. 77 (1999) 516–525.
[13] A.C. Steven, P.G. Spear, Herpesvirus capsid assembly and envelopment, in: W. Chiu, R.M. Burnett, R. Garcea (Eds.), Structural Biology of Viruses, Oxford Univ. Press, Oxford, 1997, pp. 312–351.
[14] L.M. Stannard, A.O. Fuller, P.G. Spear, J. Gen. Virol. 68 (1987) 715–725.
[15] F.J. Rixon, Semin. Virol. 4 (1993) 135–144.
[16] M.P. Hughes, H. Morgan, F.J. Rixon, Eur. Biophys. J. (2001) 268–272.
[17] J.F. Szilagyi, C. Cunningham, J. Gen. Virol. 72 (1991) 661–668.
[18] F.M. Harold, J.R. Baarda, J. Bacteriol. 94 (1967) 53–60.
[19] Y. Huang, R. Pethig, Meas. Sci. Technol. 2 (1991) 1142–1146.
[20] A. Ramos, H. Morgan, N.G. Green, A. Castellanos, J. Colloid Interface Sci. 217 (1999) 420–422.
[21] N.G. Green, A. Ramos, H. Morgan, J. Phys. D: Appl. Phys. 33 (2000) 632–641.
[22] N.G. Green, H. Morgan, J. Phys. Chem. 103 (1999) 41–50.
[23] A. Irimajiri, T. Hanai, A. Inouye, J. Theor. Biol. 78 (1979) 251–269.
[24] Y. Huang, R. Hölzel, R. Pethig, X.-B. Wang, Phys. Med. Biol. 37 (1992) 1499–1517.
[25] S. Archer, H. Morgan, F.J. Rixon, Biophys. J. 76 (1999) 2833–2842.
[26] M.P. Hughes, H. Morgan, Anal. Chem. 71 (1999) 3441–3445.
[27] M.P. Hughes, H. Morgan, J. Phys. D: Appl. Phys. 31 (1998) 2205–2210.
[28] M.P. Hughes, H. Morgan, M.F. Flynn, J. Colloid Interface Sci. 220 (1999) 454–457.
[29] J. Lyklema, Fundamentals of Interface and Colloid Science, vol. 2, section 4.3f, Academic Press, San Diego, 1995.
[30] P.R.C. Gascoyne, R. Pethig, J. Satayavivad, F.F. Becker, M. Ruchirawat, Biochim. Biophys. Acta 1323 (1997) 240–252.
[31] A. Helenius, Cell 69 (1992) 577–578.