*Electrophoretic Studies of Tobacco Mosaic Virus**

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Since the early study of Eriksson-Quensel and Svedberg[1], the electrophoretic mobility of tobacco mosaic virus (TMV) has been measured by several investigators. However, the various recorded values of its mobility show some discrepancy (see Table I) and there have been few systematic studies under different conditions.

TABLE I

THE DATA OF ELECTROPHORETIC MOBILITY OF TOBACCO MOSAIC VIRUS

Buffer	Ionic Strength	pH	Temp. (°C)	Mobility $\left(\frac{-u\times10^5}{\text{cm}^2/\text{sec. volt}}\right)$	Investigator
Acetate	0.02	4.01	20	7.5	Ericksson-Quensel et al. (1936)[1]
〃	〃	4.35	〃	10.5	〃
〃	〃	4.65	〃	13.9	〃
〃	0.04	4.95	〃	18.9	〃
Phosphate	0.1	6.91	0-0.5	9.3	Pfankuch et al. (1940)[2]
Phosphate-KCl	〃	7.3	——	8.5	Miller et al (1941)[3]
Veronal-NaCl	〃	6.72	0	5.5	Cohen (1941)[4]
Phosphate	(0.05 M)	7.1	〃	8.8 (Des)	Knight et al. (1942)[5]
〃	(0.1 M)	7.5	——	39 (Des)	Frampton et al. (1946)[6]
〃	0.2	7.00	1	7.2	Fischer et al. (1949)[7]
〃	〃	7.7	0	7.5 (Des)	Watanabe et al. (1950)[8]
Acetate	〃	4.2	3.2	4.2	Kahler (1951)[9]
〃	〃	4.9	〃	8.0	〃

* Part of this paper was read before the 4th Annual Meeting of the Chemical Society of Japan held in Tokyo, April, 1951.

1) I.-B. Eriksson-Quensel and T. Svedberg, *J. Am. Chem. Soc.*, **58**, 1863 (1936).

2) E. Pfankuch, G.A. Kausche and H. Stuble, *Biochem. Z.*, **304**, 238 (1940).

3) G.L. Miller and W.M. Stanly, *J. Biol. Chem.*, **141**, 905 (1941).

4) S.S. Cohen, *Proc. Soc. Exptl. Biol. Med.*, **48**, 163 (1941).

5) C.A. Knight and M.A. Lauffer, *J. Biol. Chem.*, **144**, 411 (1942).

6) V.L. Frampton and W.N. Takahashi, *Phytopathology*, **36**, 129 (1946).

7) M.A. Fischer and M.A. Lauffer, *J. Am. Chem. Soc.*, **71**, 3800 (1949).

8) I. Watanabe, Y. Otsuka and S. Mizushima, *Repts. Radiation Chemistry Research Institute, Tokyo Univ.*, No. 5, 10(1950).

9) H. Kahler, private communication (1951).

In the present investigation the variations of the mobility of TMV with changes in concentration and ionic strength have been measured. The charge on the virus particle has been calculated from the mobility data, using Gorin's equation for long cylinders. It also seems to be interesting from the electrophoretic point of view to study such an elongated rod-shaped particle as TMV.

Experimental

Materials.—Of the several TMV preparations obtained, the most homogeneous preparation, TMV-9, was used in these studies. The virus was extracted from frozen and ground young White Burley tobacco leaves three weeks after the inoculation with common TMV and purified by differential centrifugation in a manner essentially similar to that described by Stanley[10], using a vacuum-type air-driven ultracentrifuge[11,12]. The details of the purification procedure have been reported in previous paper[12] with the results of several chemical and physical measurements of purified virus.

The homogeneity of TMV-9 was proved to be excellent by electrophoresis, sedimentation, and electron microscope measurements[12].

The final purified virus solution was stored in 0.01 M phosphate buffer at pH 7.0 in a refrigerator, and subjected to electrophoresis experiments.

Measurements.—Prior to electrophoresis, the virus solution in 0.01 M phosphate buffer was diluted to desired concentrations with a phosphate buffer of ionic strength 0.2 at pH 7.7, and dialyzed against a large quantity of the same buffer at least two days in a cold room. In the study of the variation of mobility with ionic strength, the ionic strength was varied by diluting the phosphate buffer of ionic strength 0.2 at pH 7.7 with water; no apparent change in pH was found even after ten-fold dilution.

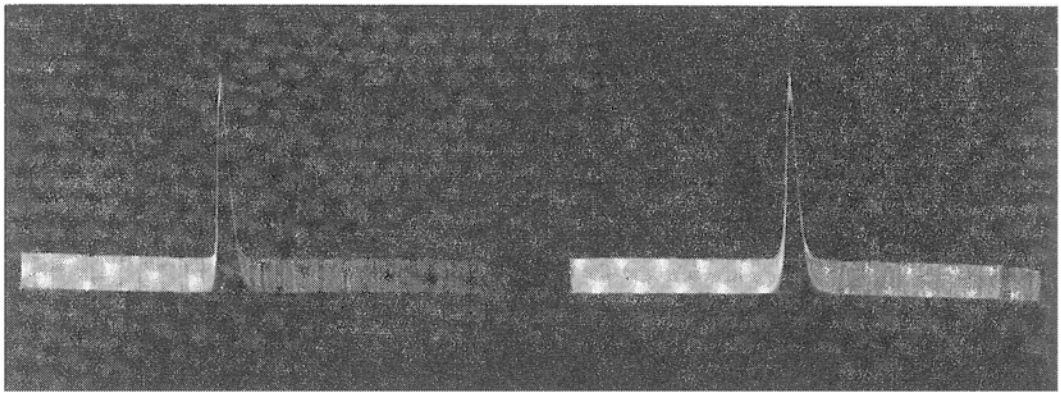
Electrophoretic measurements were carried out at 0°C in a Tiselius-type apparatus[13] equipped with a Svensson optical system[14]. The electrolytic current was automatically controlled by a constant-current regulator[15].

The movements of the boundaries were recorded photographically at appropriate intervals and the electrophoretic mobilities were calculated by the method of Longsworth[16] from the centroidal ordinates, or the first moments, of the curves of concentration gradient versus distances. Mobility values corresponding to a temperature of 0°C were obtained by using specific conductivities of solutions measured at 0°C.

Virus concentrations were determined by micro-Kjeldahl nitrogen analysis* assuming that TMV nucleoprotein contains 16.6–16.7% nitrogen[12].

Results

This preparation of TMV, TMV-9, was found to be electrophoretically homogeneous under all conditions examined and single boundaries remained sharp during the course of electrophoresis (for example, see Fig. 1).



Ascending ⟵ ⟶ Descending

Fig. 1. Electrophoretic pattern of TMV in the phospate buffer (pH 7.7, $\mu=0.2$).

A. The Variation of Mobility with Concentration of TMV.—The variation of electrophoretic mobility with concentration of TMV was examined over the concentration range 0.17–1.08% in the same phosphate buffer, pH 7.7, at a constant ionic strength of 0.2. These results are given in Table II together with the relative viscosity of solutions[17]. It can be easily seen, from Table II, that the mobility is essentially independent of the concentration although the relative viscosity of the

10) W.M. Stanley, *J. Am. Chem. Soc.*, 64, 1804 (1942).
11) S. Sasaki and O Taniguchi, *J. Japan Soc. Mech. Eng.*, 54, 255 (1951).
12) I. Watanabe and Y. Kawade, This Bulletin, 26, 294 (1953).
13) A. Tiselius, *Trans. Faraday Soc.*, 33, 524 (1937).
14) H. Svensson, *Kolloid-Z.*, 87, 181 (1939).
15) H. Noda and N. Ui, *Kagaku*, 20, 179 (1950).
16) L.G. Longsworth, *J. Am. Chem. Soc.*, 65, 1755 (1943).
* The nitrogen determinations were made by Miss T. Kitamura in our laboratory.
17) Y. Kawade, *Repts. Institute of Science and Technology, Univ. of Tokyo*, 8, 115 (1954).

TABLE II

THE VARIATION OF MOBILITY WITH CONCENTRATION OF TMV AT 0°C

(In Phosphate Buffer of Ionic Strength 0.2 and pH 7.7)

Conc. (%)	Field strength* (volt/cm.)	Mobility ($-u\times10^5$cm^2/sec. volt)		Relative viscosity of solution (η_r)
		Descending	Ascending	
0.17	3.60	7.1_5	7.2_5	1.11
0.24	3.57	7.1_5	7.3_0	1.17
0.44	3.62	6.9_5	7.1_0	1.36
0.65	3.65	7.0_0	7.1_0	1.59
1.08	3.72	7.0_0	7.1_0	2.24
Average.....		7.0_5	7.1_5	

* In our preliminary test, it was confirmed that the mobility is essentially independent of the field strength.

solutions increases by more than twofold. Similar phenomena were also observed in the electrophoretic studies of deoxypentose nucleic acid[18–20]. In this connection, it is very interesting to recall the fact that the sedimentation rates of both TMV[21–23] and deoxypentose nucleic acid[24–26] strongly depend upon their concentration and that, especially in the case of TMV, this concentration dependency of sedimentation coefficient is directly related to the viscosity of the solution[21]. This difference in dependence of sedimentation coefficient and electrophoretic mobility upon concentration is of considerable interest and, as yet, no reasonable explanations have been proposed.

The average value of descending mobilities in the phosphate buffer of ionic strength 0.2 and pH 7.7 was calculated to be $-7.0_5\times10^{-5}$cm^2/sec. volt and is in close agreement with that reported by Fischer and Lauffer[7].

B. The Dependence of Mobility on Ionic Strength.—The ionic strength was varied over the range 0.02–0.2 at constant pH. The results obtained are given in Table III and Fig. 2, in which mobilities are plotted against the square roots of ionic strengths. It is evident from Fig. 2 that the mobility increases greatly with the reduction of ionic strength.

TABLE III

THE VARIATION OF MOBILITY OF TMV WITH IONIC STRENGTH AT 0°C

(In Phosphate Buffer at pH 7.7)

Ionic Strength	Conc. (%)	Field Strength (volt/cm.)	Mobility ($-u\times10^5$cm^2/sec. volt)	
			Descending	Ascending
0.02	0.49	3.24	15.7	17.5
0.05	0.37	3.79	11.6	11.9
0.1	0.52	3.47	9.0_5	9.2_5
0.2	.....	.....	7.0_5*	7.1_5*

* Average value given in Table II.

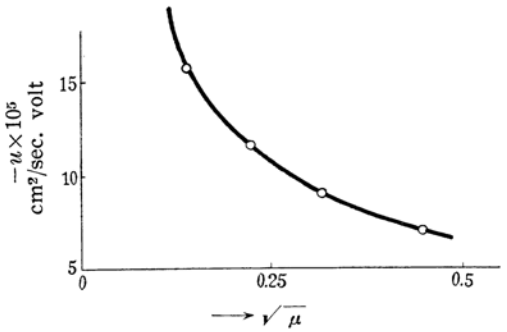


Fig. 2. The variation of descending mobility of TMV with ionic strength at 0°C (in phosphate buffer at pH 7.7).

Discussion

A. The Net Charge on TMV Molecule.—The single particles of TMV were found to be rod-shaped, of about 280 mμ to 300 mμ in length and 15 mμ in diameter, and have molecular weight of about 4×10^7 to 5×10^7 [22,27,28]. Similar molecular size and shape have also been observed for the virus preparation (TMV-9) used in these studies[12], by sedimentation, diffusion, viscosity, and electron microscopic measurements. In the following calculations TMV particles were regarded as long cylinders, 300 mμ in length and 15.2 mμ in diameter: these values correspond to a molecular weight of 4.5×10^7 [12].

The net charge, Q, on such a long cylinder as TMV can be related to the experimentally measured mobility, u, by the following equation developed by Gorin[29]:

$$Z|e|=Q=\pi\eta\cdot F'(\kappa a)\cdot(l+2a)\cdot u\Big/2\left[\frac{K_0(\kappa a+\kappa r_i)}{(\kappa a+\kappa r_i)\cdot K_1(\kappa a+\kappa r_i)}+\ln\left(\frac{a+r_i}{a}\right)\right] \quad (1)$$

18) E. Stenhagen and T. Teorell, *Trans. Faraday Soc.*, **35**, 743 (1939).
19) J.M. Creeth, D.O. Jordan and J.M. Gulland, *J. Chem. Soc.*, **1949**, 1406.
20) N. Ui and I. Watanabe, *Repts. Radiation Chem. Research Institute, Tokyo Univ.*, No. 5, 17 (1950).
21) M.A. Lauffer, *J. Am. Chem. Soc.*, **66**, 1195 (1944).
22) G. Schramm and G. Bergold, *Z. Naturforsch.*, **2b**, 108 (1947).
23) H.K. Schachman and W.J. Kauzmann, *J. Phys. & Colloid. Chem.*, **53**, 150 (1949).
24) H. Kahler, *J. Phys. & Colloid Chem.*, **52**, 676 (1948).
25) R. Cecil and A.G. Ogston, *J. Chem. Soc.*, **1948**, 1382.
26) Y. Kawade and I. Watanabe, *Biochem. et Biophys. Acta*, in press.
27) M.A. Lauffer, *J. Am. Chem. Soc.*, **66**, 1188 (1944).
28) R.C. Williams, R.C. Backus and R.L. Steere, *J. Am. Chem. Soc.*, **73**, 2062 (1951).
29) H.A. Abramson, L.S. Moyer and M.H. Gorin, "Electrophoresis of Proteins," Reinhold, New York (1942).

Here, Z is the valency of ion, $|e|$ is the electronic charge, l and a are the length and the radius of the cylinder, respectively, r_i is the average radius of the ions in the ionic atmosphere, κ is the Debye-Hückel function, i. e., the inverse of the thickness of the double layer, and η is the viscosity coefficient of the solvent. $F'(\kappa a)$ is the function developed by Gorin to account for the random orientation of cylinders, and K_0 and K_1 are Bessel functions, values of which have been given by Abramson, Moyer, and Groin[29] together with the values of $F'(\kappa a)$.

The valency at ionic strength 0.02 can be calculated directly from the equation (1) and is given in Table IV A. In this calculation the valeus of l and a were taken as 3000 Å and 76 Å respectively, and the value of r_i was assumed to be 2.5 Å. The value of κ at 0° C is $3.24\times10^8\sqrt{\mu}$, where μ is the ionic strength. In the calculations of the valencies at higher ionic strengths, however, an alternative indirect method was adopted by using Gorin's calculation of the ratio (R) of the mobility of the cylinder to that of the sphere of the same molecular volume and charge[29]. After obtaining the mobility as the sphere, the charge can be calculated from the following equation:

$$u(\text{sphere})=\frac{Z|e|(1+\kappa r_i)\cdot f(\kappa r)}{4\pi\eta a(1+\kappa r+\kappa r_i)} \qquad (2)$$

Here, r is the radius of the equivalent sphere above-mentioned, which was calculated to be 240 Å, and $f(\kappa r)$ is a function of κr given by Henry[30] (often called the Henry factor). These results were shown in Table IV B. From Table IV it is clearly shown that the valency of TMV is independent of the change in ionic strength within the error of calculation and is about $-1,800$.

In the above calculation, it was assumed that no change in the molecular state occurred with the variation of ionic strength, although the concentration of buffer was reported to have some effect on the degree of aggregation of TMV[23]. There are, moreover, many assumptions and limitations in Gorin's equation* and the meaning of the value obtained is not yet so self-evident.

This electrophoretic valency of TMV, $-1,800$, seems to be too small, compared with its very high molecular weight. Our calculations based upon the amino acid analyses of Knight[32] and the amide nitrogen analyses of Ross[33] indicate a valency of about $-800\sim-900$ at pH 7.7. In this calculation, the molecular weight of TMV was taken to be 4.5×10^7 and any contribution of nucleic acid was neglected. Although this figure appears to be in the same order of magnitude as that obtained from the mobility, the presence of the nucleic acid should be taken into consideration to obtain a correct theoretical value.

Considering the contribution made by pentose nucleic acid as free acid, the theoretical valency of TMV was calculated to be about $-9,000$. This value is very large, when compared with the surface charge calculated from the electrophoretic mobility, but is in fairly good agreement with that obtained from the titration experiments by Fischer

TABLE IV
THE NET CHARGE ON TMV PARTICLE*

A. THE VALUE CALCULATED FROM EQ. (1)

μ	$u\times10^5$ (cm²/sec. volt)	κ	$\kappa a+\kappa r_i$	$\frac{K_0(\kappa a+\kappa r_i)}{(\kappa a+\kappa r_i)K_1(\kappa a+\kappa r_i)}$	$F'(\kappa a)$	η (poise)	Z
0.02	−15.7	0.46×10^7	3.6	0.24	5.3	1.80×10^{-2}	−1.720

B. THE VALUE CALCULATED FROM EQ. (2)

μ	$u\times10^5$	R**	u(sphere) $\times10^5$	κ $\times10^{-7}$	κr	κr_i	$f(\kappa r)$	η (poise)	Z
0.02	−15.7	0.39	−40.3	0.46	11.0	0.1	0.84	1.80×10^{-2}	−1.800
0.05	−11.6	0.38	−30.5	0.72	17.3	0.2	0.88	1.81×10^{-2}	−1,800
0.1	− 9.0_5	0.38	−23.8	1.02	24.5	0.3	0.91	1.83×10^{-2}	−1,800
0.2	− 7.0_5	0.38	−18.5	1.45	34.8	0.4	0.93	1.87×10^{-2}	−1,800

* $r_i=2.5$Å; $R=2.4\times10^2$Å.

** $R=u$(cylinder)/u(sphere). To calculate these values, asymmetry$=S_r=l/2a$ was taken as 19.7.

* The limitations of Gorin's equation were discussed in detail by Abramson, Moyer, and Gorin[29] and Overbeek[31].

30) D.C. Henry, *Proc. Roy. Soc. London*, **133A**, 106 (1931).

31) J.Th.G. Overbeek, *Advances in Colloid Sci.*, **3**, 97 (1950).

32) C.A. Knight, *J. Biol. Chem.*, **171**, 297 (1947).

33) A.F. Ross, *J. Biol. Chem.*, **143**, 685 (1942).

and Lauffer[7]*.

It is very interesting to investigate the reason why the surface charge obtained from the electrophoretic experiments is much smaller than the calculated or the titration value. In this respect, it seems not inadequate to point out that the charge of horse serum albumin derived from the mobility agrees very well with the titration value[35].

B. The Dilution Factor in a Three-Ion System Containing TMV.—Strong "boundary anomalies"[36] are usually observed in electrophoretic experiments of proteins such as albumin, globulin, etc. It was found in our studies, however, that these "boundary anomalies" were greatly diminished in TMV solutions. First, the ascending mobility is not so different from descending mobility (see Table II); second, both the δ- and ε-boundaries are very small and hardly recognized (see Fig. 1); and third, the descending boundary is also as sharp as the acending one**.

All these characteristic phenomena of TMV in electrophoresis may be interpreted by the fact that the charge density of TMV is very small. Now we are trying to calculate a dilution factor[37], ξ, which is a measure of the divergence of real systems from the ideal behaviour and becomes unity at sufficiently low protein concentrations. In a three-ion system, ξ is given by the following equation[37]:

$$\xi=1+\left(\frac{r_S-r_R}{r_S}\cdot\frac{r_A}{r_A-r_R}-\frac{1}{2}\right)\frac{C_S^{\alpha}}{C_A^{\gamma}} \qquad (3)$$

Notations are the same as in the paper of Longsworth[37]. The subscript A, R, and S refer to Na^+, HPO_4^{--}***, and the negatively charged protein ion; and the superscripts γ and α mean buffer and protein solutions respectively. r is the mobility of ion relative to that of Na^+ ion (22.7×10^{-5} cm²/sec. volt) taken as unity and C is the equivalent concentration of ion.

In Table V are shown the results of the

TABLE V

THE DILUTION FACTOR AND BUFFER-CONCENTRATION BOUNDARY IN A THREE-ION SYSTEM

1% Protein Solutions in Sodium Phosphate Buffer (pH 7.7, Ionic Strength 0.2)
A: Na^+; R: HPO_4^{--}; S: Protein Ion; $r_A=1,000$ (unity); $r_R=-1.101$; $C_A^{\gamma}=0.14$; $K_{AR}=0.0148$

Protein	TMV	Horse serum albumin B
M (molecular weight)	4.5×10^7	67,100
Z (valency)	−1,800	−15.7
u (mobility)	$-7.0_0\times10^{-5}$	$-5.8_4\times10^{-5}$
r_S (relative mobility)*	−0.308	−0.257
C_S^{α} (euivalent per liter)	−0.00040	−0.00234
ξ (dilution factor)	1.005	1.035
Δn (ε-boundary)	0.000010	0.000072

* Taken the mobility of sodium ion as unity.

calculations on one per cent solutions of TMV and horse serum albumin B (according to Kekwick[38]) in sodium phosphate buffer of ionic strength 0.2 and pH 7.7. It is clear from Table V that ξ is very close to unity in the case of TMV. This low value of $\xi-1$ in TMV solution is in good accord with the fact that no marked divergence from an ideal electrophoretic behaviour can be observed. Since ξ is equal to the ratio of ascending mobility to decending one as a first approximation[37], the small differences between ascending and descending mobilities of TMV can qualitatively be explained.

The change in refractive index across the buffer-concentration boundary, so-called "ε-boundary", in the descending side can be expressed as follows[37]:

$$\Delta n=K_{AR}(\xi-1)C_A^{\gamma}$$

$$=K_{AR}\left(\frac{r_S-r_R}{r_S}\cdot\frac{r_A}{r_A-r_R}-\frac{1}{2}\right)C_S^{\alpha} \qquad (4)$$

Here, Δn is the difference in refractive index and K_{AR} is the increment of refrective index per equivalent of AR or Na_2HPO_4. Δn is also found to be very small in the case of TMV (see Table V) and small areas of both δ- and ε-boundaries may be expected from this fact.

When ξ becomes close to unity, the change in the conductivities across the boundary is also largely reduced and the boundary pattern, therefore, becomes independent of the

* Fischer and Lauffer[7] obtained a value of—6,900for the valency at pH 7 by titration studies. They reported, in their original paper, a value of—8,300 for the theoretical valency, but they re-examined their calculation and have found that the correct theoretical value should be about—1,000 instead of—8,300[34]. In their calculations, they neglected the presence of nucleic acid and assumed a molecular weight of 3.4×10^7.

34) M.A. Lauffer, private communication.

35) I. Watanabe, N. Ui and M. Nakamura, *J. Phys. & Colloid Chem.*, **54**, 1366 (1950).

36) L.G. Longsworth and D.A. MacInnes, *J. Am. Chem. Soc.*, **62**, 705 (1940); *Chem. Revs.*, **24**, 271 (1939).

** In Fig. 1 the descending boundary seems to be slightly sharper, but it is due to the difference in the sharpness of the initial boundaries.

37) L.G. Longsworth, *J. Phys. & Colloid Chem.*, **51**, 171 (1947).

*** To simplify the problem, buffer solution was assumed to consist of only Na^+ and HPO_4^{--} ions.

38) R.A. Kekwick, *Biochem. J.*, **32**, 552 (1938).

direction of the current. In this limiting case, the ascending pattern should be the mirror image of the descending one. Thus the reason why the descending boundary of TMV solution moves as sharply as the ascending one may be easily understood.

In the solution of serum albumin, on the contrary, the ascending boundary moves more rapidly than the descending one and, moreover, the later becomes more diffuse. In addition, the areas of the δ- and ε-boundaries become very significant. The values of ξ and Δn in Table V also explain these considerable "boundary anomalies" in the case of horse serum albumin.

At any rate, it may be conculded that a condition which is closer to the ideal case for electrophoresis can be easily attained in TMV solution even though its concentration is relatively high.

Summary

The variations of the mobility of tobacco mosaic virus (TMV) in phosphate buffer at pH 7.7 with changes in concentration and ionic strength have been investigated. The mobility is essentially independent of the concentration and has an average value of $-7.0_5\times10^{-5}$ cm^2/sec. volt in the phosphate buffer of ionic strength 0.2. On the other hand, the mobility increases greatly with the decrease of ionic strength. The net charge on the TMV particle, calculated from the mobility data by the aid of Gorin's equation, was found to be constant (about $-1,800$) all over the range of ionic strength examined. Although it is considerablly lower than the theoretical valency calculated from the analytical data, this low value of the valency from the mobility is quite reasonable to explain the observation that there is no serious divergence from the ideal behaviour in the electrophoresis of TMV solution.

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