Agarose Gel Electrophoretic pH-Mobility (Titration) Curves of Isometric Plant Viruses

S. S. Hurtt, D. Tietz, J. S. Fawcett, and A. Chrambach

First author: Agricultural Research Service, U.S. Department of Agriculture, National Plant Germplasm Quarantine Laboratory, Bldg. 580, BARC-East, Beltsville, MD 20705; other authors: Section on Macromolecular Analysis, Laboratory of Theoretical and Physical Biology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892.

We acknowledge the technical assistance of Robert Tuscan and the horticultural assistance of John McKenzie.

Accepted for publication 4 January 1989 (submitted for electronic processing).

ABSTRACT

Hurtt, S. S., Tietz, D., Fawcett, J. S., and Chrambach, A. 1989. Agarose gel electrophoretic pH-mobility (titration) curves of isometric plant viruses. Phytopathology 79:661-667.

Electrophoretic pH-mobility curves (also known as titration curves) were used to analyze virions of six purified isometric plant viruses. Curves were generated in agarose slab gels composed of 0.8% IsoGel agarose, 10% d-sorbitol, and 2% carrier ampholyte. In the first dimension of this two-dimensional electrophoretic procedure, the carrier ampholytes were focused to a steady state, creating a pH gradient in the gel. Virus was applied in a continuous band across the pH gradient, and an electrical field was applied to the prefocused gel at right angles to the first field. This step showed the virion mobility over a range of pH values. Protein was fixed and stained to visualize the curve. Each virus gave a unique curve. The procedure established the isoelectric point (pI) of the virus and the virion mobility (that is, net surface charge) as a function of pH in a single gel experiment. Previously, these data could only be obtained by conducting numerous electrophoretic experiments. Curves also revealed virion pH instabilities and heterogeneity within preparations. Curves were standardized using a computer program to normalize nonlinear variations in the pH gradient within a gel and among gels.

Additional keywords: cucumber mosaic virus, hibiscus chlorotic ringspot virus, pelargonium flowerbreak virus, tomato bushy stunt virus, turnip crinkle virus, two-dimensional isoelectric focusing-gel electrophoresis.

Electrophoresis of intact plant viruses has been used in immunoelectrophoretic studies (8,15,30) and in comparative studies in solution, density gradients (4–6,16,18), polyacrylamide, or agar gels (10,13). These methods have been useful in distinguishing or separating viruses and strains of viruses and in showing heterogeneity within a purified virus preparation. The primary biophysical basis for these separations is net surface charge density. The variation is due largely to differences in the amino acid composition and size of the particles. Net charge historically has been derived from measurements of particle electrophoretic mobility, that is, migration rate (cm/sec) divided by electrical field strength (V/cm). Electrophoretic methods used to obtain these values have several technical limitations. Because many variables (including temperature, pH, buffer ionic strength and composition, and support medium concentration and composition) influence migration, comparative studies of virion mobility have been difficult to conduct and analyze. The optimal conditions for separating particle types often are found empirically by trying many buffers and pHs. If virus preparations have multiple electrophoretic bands, comparing the composition of the bands on parallel gels or gradients at different buffer conditions can become a complex procedure (4,16,18).

Isoelectric focusing is an established electrophoretic method (1–3,21) for analyzing amphoteric substances, that is, substances with both basic and acidic groups capable of accepting or donating hydrogen ions and thereby varying in net charge as the pH is changed. Separation in isoelectric focusing is carried out in a natural pH gradient that is established between electrodes and stabilized by carrier ampholytes. Carrier ampholytes are mixtures of synthetic, low-molecular-weight amphoteric compounds with different isoelectric points (pI). In electrophoresis, the pI is defined as the pH at which the mobility of the particle is zero. The alignment of isoelectric particles with suitably spaced pIs and adequate buffering capacity creates the pH gradient as current is passed through the mixture. The gradient reaches a steady-state (equilibrium) when each ampholyte has migrated to a position where the pH equals its pI. For analytical purposes, it is convenient to create the steady-state pH gradient in a gel medium.

The concept of the protein electrophoretic "titration curve" was developed by Rosengren et al as a simple technique to select optimum conditions for protein electrophoresis (24). The concept has been expanded by Righetti and co-workers (14,21–23,29). The technique is a two-dimensional electrophoretic technique that combines principles of isoelectric focusing and gel electrophoresis. Because the generated curves are in fact pH-mobility curves (22) rather than true titration curves (28), we use the designation pH-mobility curve.

Initially, pH-mobility curves were produced in polyacrylamide gels. The introduction of electroendosmosis-free agarose suitable for isoelectric focusing (25) has enabled us to generate pH-mobility curves of intact, large particles the size of isometric plant viruses. In applying the technique, a pH gradient is generated in a gel by focusing carrier ampholytes incorporated into the gel matrix. When an electric field is then reapplied at right angles to the focusing field, the pH gradient remains essentially unchanged for some time (24). If sample is applied to a channel that bisects the pH gradient, particles moving into the gel acquire different net charges according to the pH of the point of gel entry and therefore migrate with different velocities. The protein band (that is, the pH-mobility curve) shows the migration of sample component(s) as a function of pH. The method has found many uses in protein studies including the study of protein mutants (23) and ligands (14).

We adapted the protein pH-mobility curve method to analyze virion mobility. Virions are amphoteric particles by virtue of the acidic and basic amino acid composition of their protein coats, but isoelectric focusing has rarely been used in their study (19,20). This is the first report of the application of pH-mobility curve technique to plant viruses. Details of the procedure are described and the pH-mobility curves of six plant viruses are illustrated in this paper. Preliminary reports have been made (11,26).

MATERIALS AND METHODS

Viruses and proteins. Hibiscus chlorotic ringspot virus (HCRSV), pelargonium flowerbreak virus (PFBV), tomato bushy stunt virus (TBSV) type strain, and turnip crinkle virus (TCV) were propagated and purified as previously described (10). Cucumber mosaic virus strain D (CMV-D) and strain S (CMV-S) were kindly supplied by J. M. Kaper (Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD). Virus preparations were diluted with double-distilled water to 1 mg/ml based on an extinction coefficient of 5 absorbance units at 260 nm. Naturally colored proteins used as visual markers were the pink R-phycoerythrin (PE) (Polysciences, Warrington, PA) and the blue C-phycocyanin (PC) from *Aspergillus nidulans*, pI 4.75 and 4.85 (BDH Chemicals, Ltd., Poole, England).

Gel composition and casting. Gel preparation and protein staining procedures were modified from those recommended by the manufacturer of IsoGel (FMC Corp., Rockland, ME) (3). Double-distilled water was used throughout unless otherwise mentioned. Horizontal slab gels of 0.8% IsoGel, 10% d-sorbitol, and 2.5% carrier ampholyte were prepared by adding with continuous stirring 0.2 g of IsoGel and 2.5 g of d-sorbitol into 20 ml of water in a 50-ml Erlenmeyer flask. The flask was loosely covered with foil and the suspension was gently boiled with stirring for 10 min to dissolve the agarose. The solution was held at 65 C in a water bath. Immediately before casting the gel, 1.6 ml of 40% carrier ampholyte solution was added and the solution was brought to 25 g by adding water at 65 C. Gels, except those used for CMV-D, received equal volumes of pI range 2.5–5 carrier ampholyte (FMC Corp.) and pI range 5–8 carrier ampholyte (Pharmacia-LKB Biotechnology, Inc., Piscataway, NJ). Gels for electrophoresis of CMV-D received only pI range 3.5–9 carrier ampholyte (FMC Corp.) because initial experiments showed a pI near pH 7 for this virus.

Gels were cast on 12 × 20 cm sheets of GelBond (FMC Corp.) attached with water to a glass plate of the same size. The agarose solution was spread with a warm glass rod onto the GelBond-glass plate support warmed to 65 C on a hot plate. The support was carefully moved from the hot plate to a level surface at room temperature. A 9-cm-long Teflon strip was inserted into the gel to form a central slot 0.1 cm wide and slightly less than 0.1 cm deep across the width of the gel. After gelation, the Teflon strip was removed and the gel was refrigerated overnight in a moist chamber.

Electrophoresis. Electrophoresis was performed on an LKB Multiphor II horizontal slab gel unit (Pharmacia-LKB Biotechnology) cooled with tap water. The gel-GelBond unit was peeled from the glass support and adhered to the cooling plate of the electrophoresis unit with a few drops of 0.1% Triton X-100. Anode and cathode wicks (Pharmacia-LKB Biotechnology) were saturated with 1 M phosphoric acid and 0.1 N NaOH, respectively, and placed along the edges of the gel perpendicular to the sample slot.

Isoelectric focusing was begun at 300–350 V with 10-W limiting power. Current typically measured 25 mA and dropped rapidly as the pH gradient formed in the gel. Current was plotted against time at 5-min intervals to determine when the focusing reached the steady state (Fig. 1). This usually required 110–120 min. The electrode wicks were lifted from the gel and the underlying area was cut away.

The linearity of the gradient was checked by measuring the gel pH at 1-cm intervals along a line 1 cm from the sample slot (method described below). Care was taken to avoid damage to the gel. The pH values then were plotted against distance along the sample slot. Gels with nonlinear pH gradients were focused longer or discarded.

Approximately 80 µl of a 1 mg/ml virus suspension was pipetted into the sample slot. Electrode wicks saturated in water or dilute citric acid were placed along the edges of the gel, parallel to the sample slot. Electrophoresis was at 10 V/cm for about 90 min. Water was added to the central slot if it began to dry.

In some experiments, 80 µl of a single colored protein or a mixture of phycoerythrin and phycocyanin was added to the sample slot about 45 min after electrophoresis of the virus began. These markers could be observed as they formed a pH-mobility curve. They served as internal standards for pH determinations and helped to detect perturbations in the gel during electrophoresis.

With the gel still on the cooling plate of the electrophoresis unit, a contact pH electrode (Pharmacia-LKB Biotechnology) was used to measure pH values at points across the gel (Fig. 2). Measurements were recorded for points at 0.5-cm intervals along parallel lines 1, 2, and 4 cm from either side of the sample slot.

Viral proteins then were fixed and stained (3). Gels were immersed without agitation for 2 min in a solution of 180 ml of methanol, 30 g of trichloroacetic acid, 18 g of sulfosalicylic acid, and 300 ml of water and then agitated gently in the solution for 10–15 min. The gel was removed from the fixative and either dried and stained or stained and dried. For drying, gels were overlaid with wet filter paper (Whatman 3 MM filter paper) and several layers of paper towel. A 2-kg weight was placed on top of the stack for about 15 min. The filter paper was rewetted and carefully peeled from the gel. The gel was rinsed for 5 min in water and stained in a solution of 0.1 g of Coomassie Brilliant Blue R 250 (Bio-Rad Laboratories, Richmond, CA), 25 ml of methanol, 65 ml of water, and 10 ml of acetic acid. Destaining was in the same solvent without dye. The gel was dried at about 60 C for 30–60 min.

Computer normalization of pH-mobility curves. The position of the virion curve was apparent upon staining, but the pH gradient was lost during this procedure. The position of the virion curve had to be related to the previously recorded gel pH measurements. If the gel pH gradient had been linear and if iso-pH lines (lines drawn through points in the gel with the same pH) had been straight lines perpendicular to the sample slot, the virus migration distance could have been plotted directly against pH. However, the pH gradients were nonlinear and the iso-pH lines were irregular (Fig. 2). Therefore a computer program was created to normalize the pH-mobility curves (available upon request from the second author). The purpose of the program was to determine pH at points along the viral curve. This was achieved by nonlinear interpolation in two directions between the data points. The migration distance of the virus (assumed to be linear) then was plotted against the predicted pH.

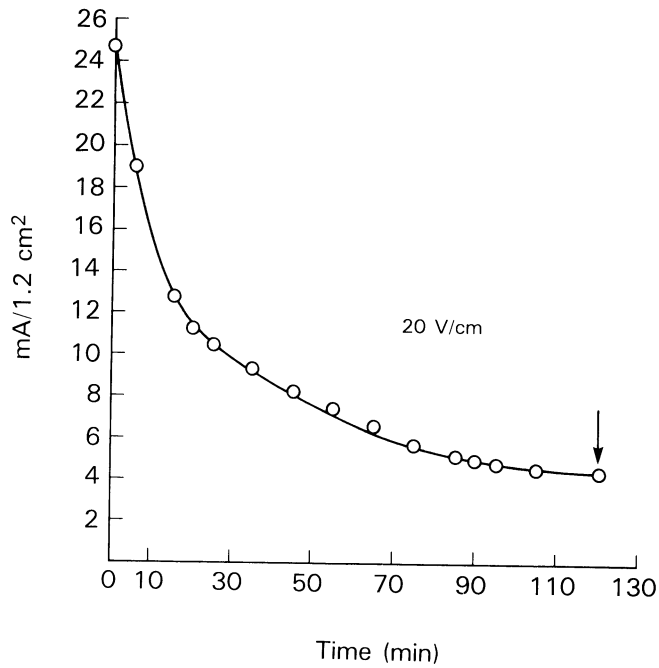

Fig. 1. Plot of the decrease in current (mA) with time (min) at constant voltage during the first dimension of the pH-mobility (titration) curve method. The pH gradient was formed when the carrier ampholytes were focused to a steady state and the rate of current decrease approached 0 (arrow).

The curves were plotted by using either absolute or relative migration distance. The plot of relative migration distance as a function of pH is shown. In each plot, the greatest distance migrated by the virus toward the anode was arbitrarily assigned a value of 100, and distance migrated toward the cathode was given a negative value.

Agarose slab gel electrophoresis. Agarose gel electrophoresis was carried out in 0.8% agarose (DNA pure agarose, Bio-Rad Laboratories) using 0.075% MES (2-[*N*-morpholino]ethanesulfonic acid) buffer brought to pH 6.3 with NaOH. Electrophoresis was conducted as previously described (10).

RESULTS AND DISCUSSION

pH gradients. Electrofocusing in the agarose slab gels (electrophoresis in the first dimension) produced near-linear, steady-state pH gradients from about pH 3 to 7 or pH 4 to 8.75 (CMV-D gels), according to the carrier ampholyte mixture incorporated in the IsoGel. Near the electrodes the pH's gradients rose or dropped sharply.

The gel pH measurements obtained after electrophoresis in the second dimension in an experiment with PC are shown in Figure 2. Comparisons of the pH gradient plots after electrophoresis in the first and second dimensions confirmed that electrophoresis in the second dimension had not perturbed the pH gradient.

pH-mobility curves of six viruses. A pH-mobility curve for each of the six plant viruses is shown in Figure 3. The stained and dried gels mounted on GelBond were durable, permanent records that were easily photographed and stored. The pH values of the gel matrix were superimposed on the gel photographs. When the gel pH gradients were compared among experiments, minor variations in the linearity and range were evident. This prevented direct comparison of the curves unless they were normalized (Fig. 4). Even so, the profiles were sufficiently different to demonstrate the unique character of each virus. Several properties of each of the viruses could be determined from these gels.

Characteristics of the pH-mobility curves. The pH-mobility curves for each virus could be divided into four regions: 1) gel pH $<$ virion pI; 2) gel pH = virion pI; 3) gel pH $>$ virion pI and a change in pH was correlated with a change in virion mobility; and 4) gel pH $>$ virion pI and a change in pH did not cause a change in virion mobility. The position in the pH gradient and the contour of the pH-mobility curve in each of these regions were different and created a unique profile or fingerprint for each virus.

Region 1 where pH $<$ pI was characterized by the absence of a band for all viruses except TBSV. Only TBSV showed a continuity of the curve on the acidic side of the virion pI. The reason(s) why the positively charged particles of many of the viruses did not migrate toward the cathode (−) is unknown. Possible explanations include particle degradation or precipitation under the low ionic strength, acidic gel conditions, or interaction of the positively charged virions with the locust bean gum component in IsoGel (7). Several viruses migrated toward the cathode at pH $<$ pI in standard agarose slab gel electrophoresis (data not shown). The

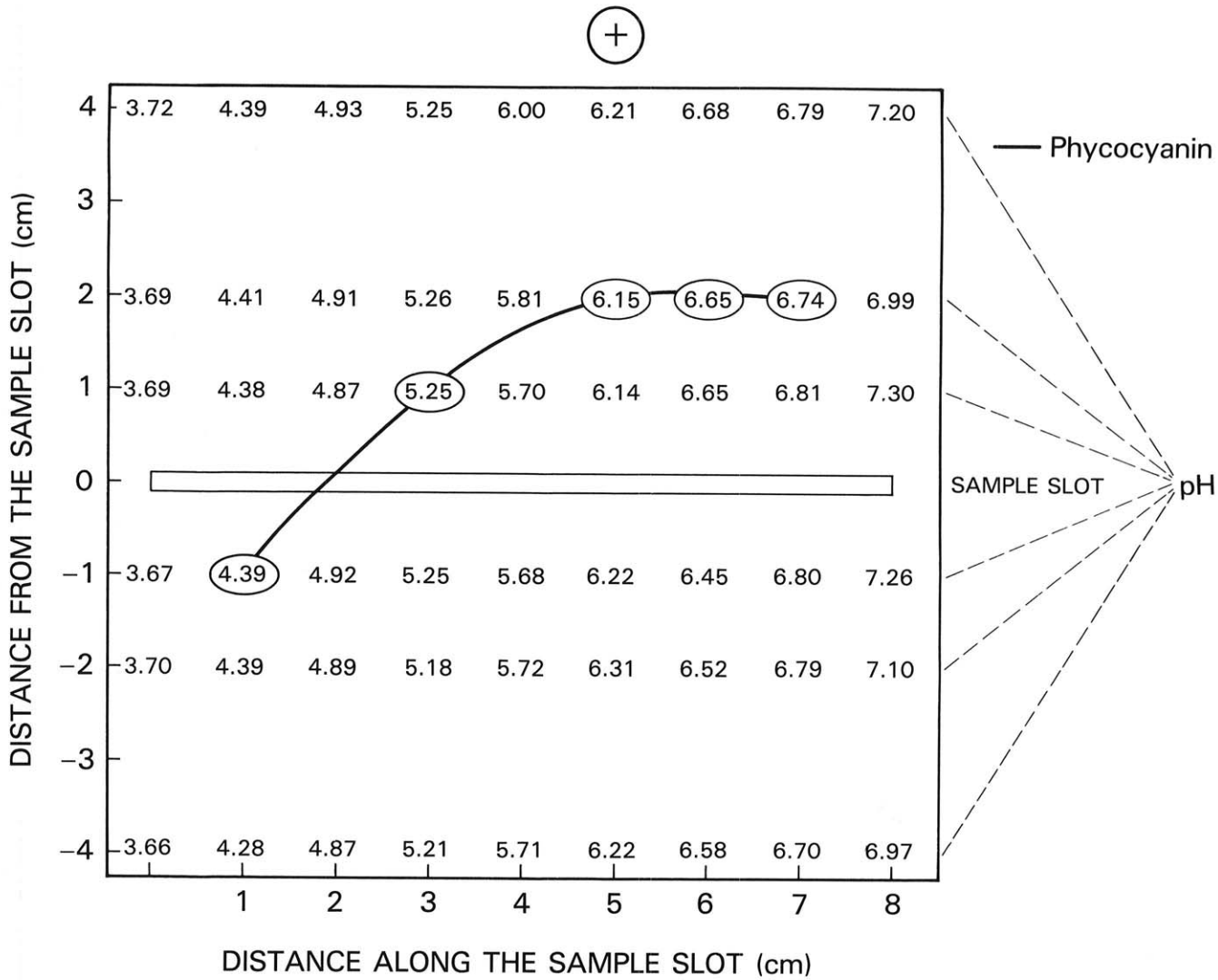

Fig. 2. Diagrammatic representation of the pH of points in an agarose gel after electrofocusing of the carrier ampholytes in the first dimension of the pH-mobility curve method. The solid line across the gel shows the position of the pH-mobility curve generated for phycocyanin after electrophoresis in the second dimension.

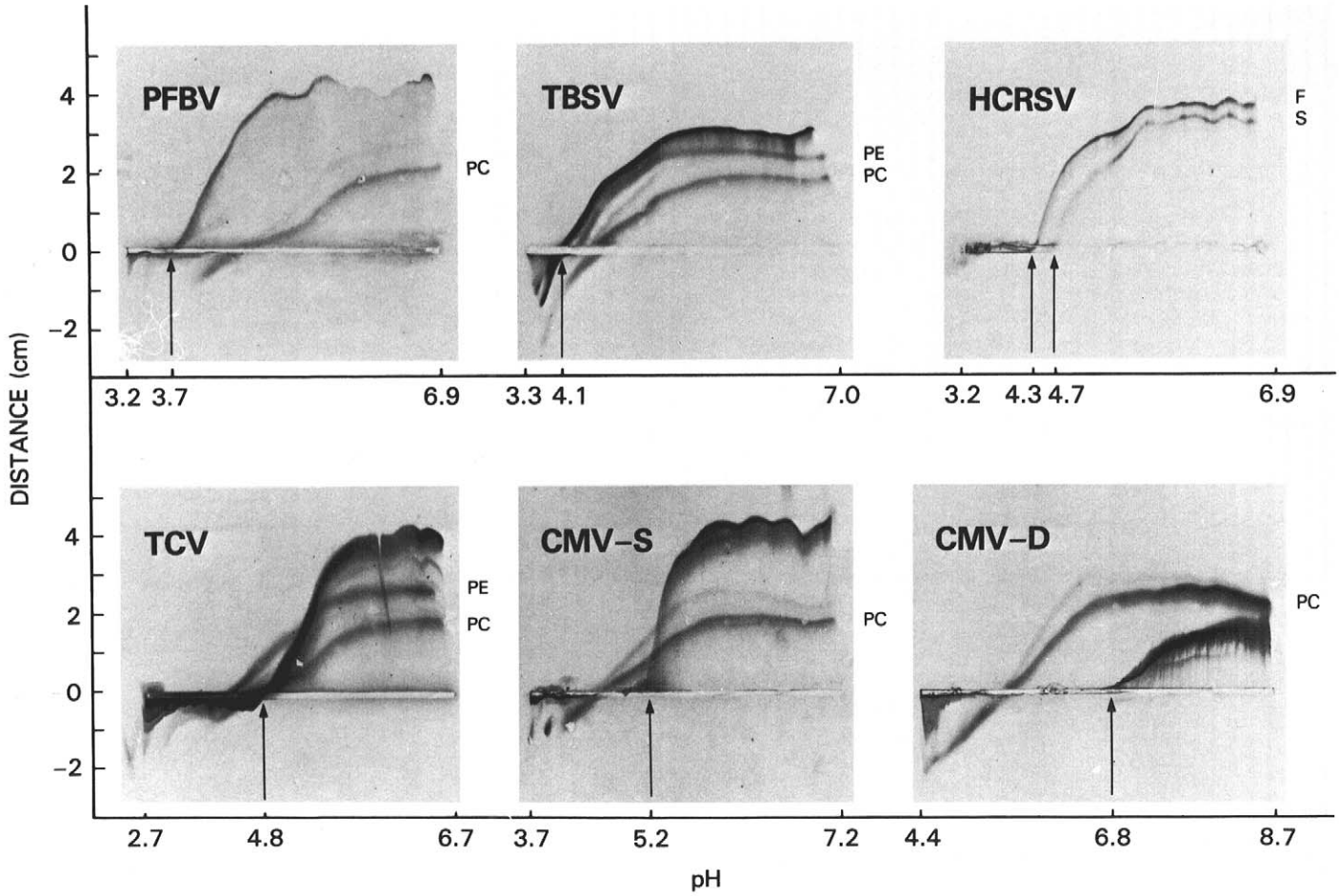

Fig. 3. Composite of the pH-mobility curves in six agarose gels for six plant viruses: pelargonium flowerbreak virus (PFBV), tomato bushy stunt virus (TBSV), hibiscus ringspot virus (HCRSV), with fast and slow migrating components marked F and S, respectively, turnip crinkle virus (TCV), cucumber mosaic virus strain S (CMV-S), and cucmber mosaic virus strain D (CMV-D), and colored protein standards (phycocyanin [PC], phycoerythrin [PE]) after electrophoresis of the samples into the pH gradient within the gel. The curves show the mobility of the virions or protein as a function of pH. Arrows indicate the isoelectric point of each virus, that is, the pH at which mobility is zero.

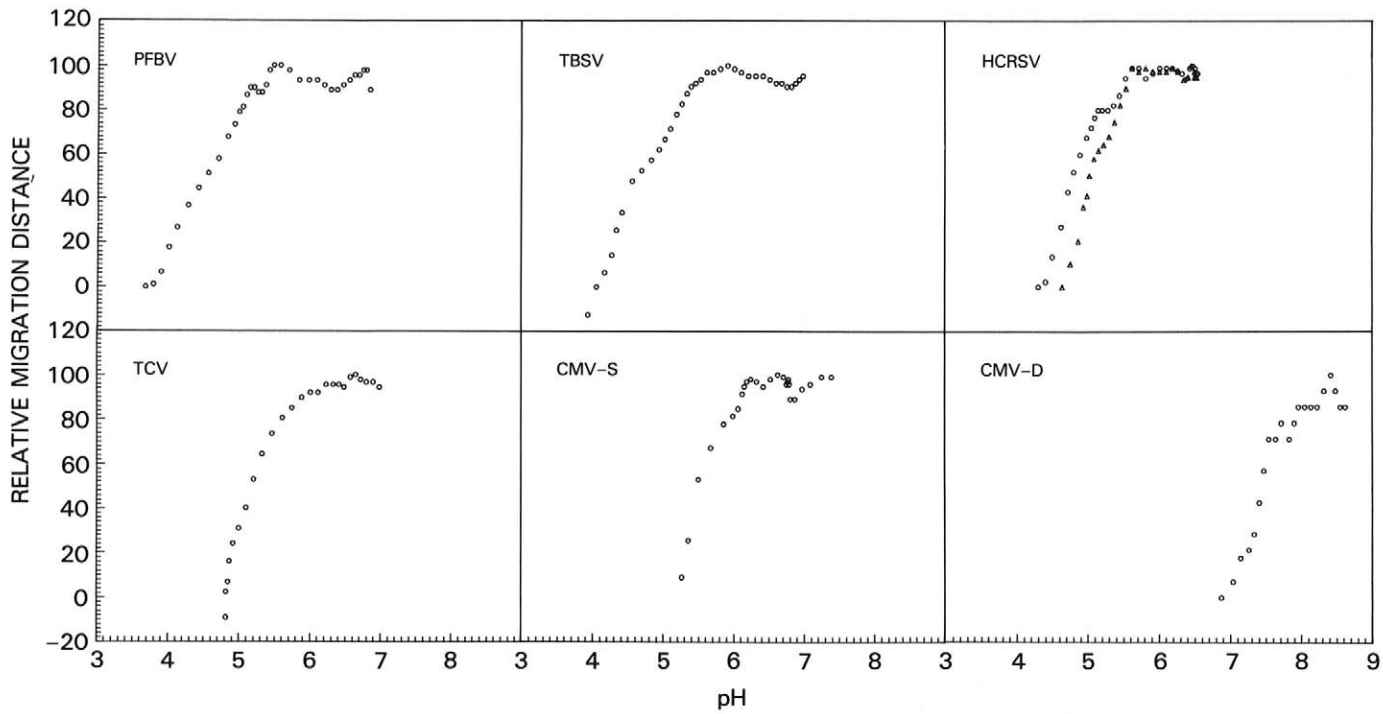

Fig. 4. Computer-generated, normalized pH-mobility curves for the curves in Figure 3 of six plant viruses: pelargonium flowerbreak virus (PFBV), tomato bushy stunt virus (TBSV), hibiscus ringspot virus (HCRSV), turnip crinkle virus (TCV), cucumber mosaic virus strain S (CMV-S), and cucumber mosaic virus strain D (CMV-D). The greatest distance migrated toward the anode was arbitrarily set as 100 for each virus. Therefore, migration rates between viruses cannot be compared. Normalization was done with a computer program (provided upon request by the second author).

critical variables that influence virion behavior in the two systems need further study.

Region 2 where pH = pI was the point of intersection of the pH-mobility curve with the sample slot. At this pH, titration of the charged groups on the virion surface produced a net surface charge and mobility of zero. The pH-mobility curves are displayed in Figure 3 in order of increasing viral pI (marked with arrows). The pIs ranged from pH 3.7 for PFBV to pH 6.7 for CMV-D.

The resolution of this methodology with gel dimensions and pH ranges used in this study was estimated to be ± 0.1 pH unit. The two charge isomers of PC with pIs of 4.75 and 4.85 were clearly resolved. Greater sensitivity should be obtainable by using a narrower pH range and/or longer gels.

Repetitive experiments with a virus produced curves that varied slightly in contour. Contour variations resulted from inter-experimental differences in the pH gradients and the second dimensional electrophoresis conditions (Fig. 5). However the standard deviations of pI were less than ± 0.1 pH units.

The pI values that we obtained for β-lactoglobulin B (5.35, data not shown) and PC were within 0.1 pH units of published values. We obtained pI values of 4.1 for TBSV and 5.2 (data not shown) for carnation mottle virus, which are in agreement with published data (17,27). Values for other viruses and PE are first reports.

Region 3 occurs where a change in pH is correlated with a change in virion mobility, that is, a change in net surface charge. The position in the gel, the slope, and the contour of each band is unique in this region. The ratio of the basic to the acidic amino acids on the surface of the coat proteins establishes the pI. Therefore, the shape of the curve is indicative of the amino acids contributing to the net charge. The change in mobility below pH 5 primarily reflects the ionization of the free −COOH residues of aspartic acid (amino acid pK = 3.8) and glutamic acid (amino acid pK = 4.2). Histidine (N) ionizes and contributes to the change in mobility in the neutral pH region (pH 6–8). The basic amino acids arginine and lysine (NH_2), cysteine (SH), and tyrosine (OH) remain fully protonated and do not contribute to the changes in mobility below pH 8.

The pH-mobility curves show that PFBV, TBSV, HCRSV, and TCV have an excess of acidic amino acids over basic amino acids. That excess of glutamic and aspartic acid is largely responsible for the net surface charge. Unique combinations of these two amino acids give rise to the unique contours of the bands in region 3. The profiles of CMV-D and CMV-S are indicative of the titration of histidine.

Region 4 occurs where a change in pH does not result in further change in particle net charge or particle mobility. In this region the virion has no buffering capacity. The region defines the pH range where experimental variation in buffer pH should have little effect on particle charge. Therefore in experiments designed to study particle mobility as a function of a parameter other than pH (for example, gel concentration), pHs from region 4 should be used. Likewise pHs from region 4 should be used for characterizing viruses by their net surface charge. Comparing viral migration rates at maximum net surface charge (at pHs in region 4) (Fig. 6) reduces interexperimental variablity. On the other hand, if maximum virus separation is desired, the separation can be enhanced by choosing a pH where maximum differences in migration rates occur.

Region 4 also provides data on particle pH stability. Band perturbations were common in this region; most of the viruses showed undulations or smearing and streaking of the bands. Fading of protein pH-mobility curves, as observed for PFBV in this region, has been interpreted as degradation. Causes for the undulations and streaking are unclear. Similar pertubations were not seen in the colored protein bands. Therefore the phenomena may reflect properties of the viruses, not the pH gradient. Swelling of virions induced by pH is documented for plant viruses (9,10,12) including several used in this study. Particle swelling may decrease mobility due to size increase. It also may affect mobility by exposing additional ionizable residues to contribute to the net surface charge. Conformational changes may also affect binding to the gel and/or ampholytes. Further studies of these phenomena are needed to determine their causes.

In principle, the curve in region 4 should show an upward slope in the adjacent alkaline region of the gel. In this alkaline area, the virions should lose positive charge as the basic amino acid, cysteine, and tyrosine residues are deprotonated, thereby increasing net negative charge and increasing migration rate

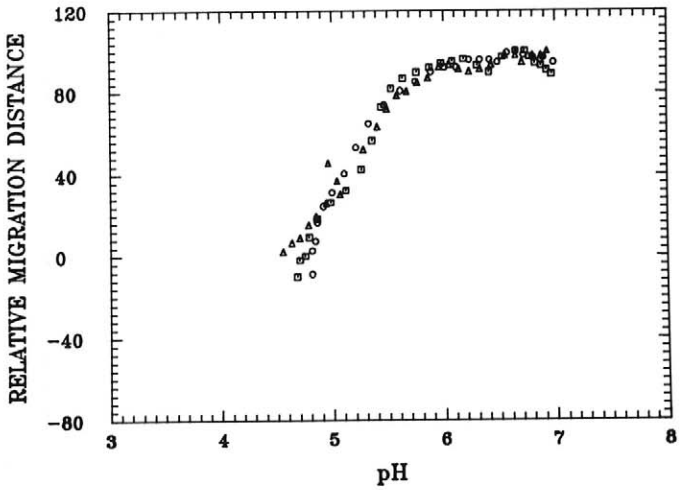

Fig. 5. Superimposed, normalized pH-mobility curves for turnip crinkle virus obtained in three experiments (each indicated by a different symbol) using two different virus preparations.

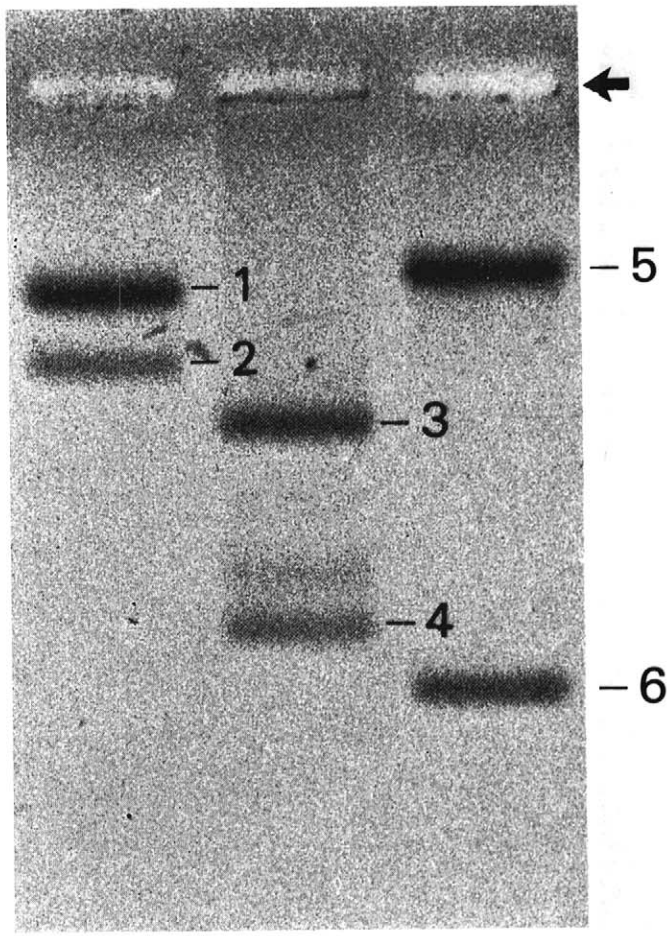

Fig. 6. Agarose slab gel showing relative electrophoretic mobilities of carnation mottle (1), turnip crinkle (2), hibiscus chlorotic ringspot virus electrophorotypes -S (3) and -F (4), tomato bushy stunt (5), and pelargonium flowerbreak (6) viruses. The origin is indicated (←). Electrophoresis was in 0.075 MES, pH 6.3, for 1 hr at 50 V constant. pH 6.3 lies in region 4 of the pH-mobility for all of the viruses.

toward the anode. Our gel gradients terminate short of these alkaline regions. Particle instablility, as previously noted, may be a problem in visualizing this portion of the curve for some viruses.

Heterogeneity among virions. Preparations of PFBV, TBSV, and TCV gave single titration curves. The preparation of CMV-S showed a minor, slower migrating component (Fig. 3). This component was subsequently detected in agarose slab gel electrophoresis. Preparations of HCRSV gave up to four pH-mobility curves, two of which are visible in Figure 3. Additional components were detectable on gels that received two to three times more virus than was applied to the gel shown in Figure 3. Multiple components, designated electrophorotypes, also were detected in the HCRSV preparations by agarose slab gel electrophoresis (Fig. 6) (10). In slab gel electrophoresis, the ratio of distance migrated by HCRSV with a slow migrating component (HCRSV-S) to HCRSV with a fast migrating component (HCRSV-F) varied with the pH of the electrophoresis buffer. The ratio was a maximum (0.65) at pHs above pH 5.8 but decreased dramatically (ratio became smaller) as the electrophoresis buffer was made more acidic. The reason for this behavior becomes evident upon examination of the pH-mobility curve for HCRSV preparations. The pH-mobility curves showed that the HCRSV purified from *Chenopodium quinoa* Willd. contained two major types of virions with different pIs. HCRSV-S had a higher pI and migrated slower (that is, it had a smaller net surface charge) than HCRSV-F at all pHs from about pH 4.4 to pH 7. The relative displacement of the curves of these two species was compatible with differences in amino acid content (9) and indicated that some of the substitutions of acidic amino acids for neutral amino acids in HCRSV-S had occurred at positions on the surface of the virion.

HCRSV-F and HCRSV-S differed least in mobility where both were fully charged; at other pHs the vertical distance between the two curves was greater than in the region of maximum mobility. The vertical distance between the curves varies from pH to pH in region 3. The change in distance between curves is related to the change in migration distance ratios observed in slab gel electrophoresis. The continuity of each of the two curves without intersection or confluency showed that each zone represented a unique, independent entity. One component was not the result of anomalies, aggregation, or degradation of the other component induced at a particular pH or range of pHs. This was evident without the biological, serological, biochemical, or physical analyses previously used in classical electrophoretic experiments to ascertain the composition of zones that vary in number or mobility as the pH varies (5,18).

Normalized pH-mobility curves. The general shape and position of the pH-mobility curve in the gradient, the pI, the pH dependence of virion stability, and the pH ranges of changing and consistent mobility can be estimated directly from the gel pattern and gel pH measurements. However, if these properties are to be compared between experiments that were not conducted under identical conditions, a procedure of normalization is needed. Comparable curves for an individual virus were achieved by computer normalization of the curves (Fig. 4). Three normalized pH-mobility curves of TCV are shown in Figure 5. They were produced in three different experiments using two different virus preparations. Discrepancies were small when normalized curves were superimposed.

The obvious shortcoming in the method we employed is the lack of a standard for comparing mobility among viruses. A comparison of the actual distances migrated by each virus was inappropriate because electrophoretic conditions, such as duration and voltage, varied among experiments. On the other hand, when maximum migration is set as 100 and all of the normalized curves are the same height, any differences in the real net charge of the viruses (Fig. 4) are obscured. For instance, it is obvious on the gels (Fig. 3), that CMV-D migrated very slowly whereas PFBV migrated very rapidly in region 4. (The position of the colored protein curve served as a rough calibration of the relative mobility of each virus.) This information is lost in the plots of the normalized curves.

Mobilities may be compared by analyzing mixtures of viruses in a manner similar to that used to compare HCRSV-F and HCRSV-S mobilities. Slab gel electrophoresis can be used to compare a number of viruses, once an appropriate pH is selected according to information obtained from the pH-mobility curve (Fig. 6). This is undesirable if large numbers of viruses are to be compared or if a purified preparation of a particular virus standard is not available. Nonetheless, the normalized curves are unique profiles that can be stored in libraries for comparative purposes. If a suitable standard is identified, computer standardized and normalized curves can be used routinely for viral identification.

This work is only an initial demonstration of the applicability of pH-mobility curves to the study of intact plant isometric viruses and a description of a protocol that worked for us. The technique is simple, rapid, and relatively inexpensive. Improved techniques for measuring and recording the pH of points in the gel will improve the sensitivity and ease of applying the technique. At present this is a tedious step in the process.

Several uses of the methodology already have been mentioned or implied. For example, data obtained from a pH-mobility curve can be used to select buffer pH for virus purification, storage, and electrophoretic separations. Additional studies might explore the use of crude sap extracts for virus detection. The nondenaturing properties of the IsoGel agarose and the reagents should permit a recovery of entities from the gels without loss of infectivity or immunological characteristics. Other applications might include studying immunological differences among strains of viruses, binding phenomena, the effect of pH on the susceptibility of virions to degrading reagents, mutation, or effects of host passage on coat protein (10). As these possibilities are explored and standards for normalization are developed, we feel that the electrophoretic pH-mobility curve technique should prove a simple and versatile tool for characterizing and identifying numerous isometric plant viruses.

LITERATURE CITED

1. Andrews, A. T. 1986. Electrophoresis: Theory, Techniques and Biochemical and Clinical Applications. Clarendon Press, Oxford. 452 pp.
2. Anonymous. 1982. Isoelectric Focusing. Principles and Methods. Pharmacia Fine Chemicals AB, Uppsala, Sweden. 163 pp.
3. Anonymous. 1982. IsoGel system: Methodology for agarose isoelectric focusing, immunofixation, and related techniques. FMC Corporation, Rockland, ME. 11 pp.
4. Ball, E. M. 1966. A technique for comparing the electrophoretic mobility rates of viruses or virus strains. Arch. Biochem. Biophys. 114:547-556.
5. Bancroft, J. B. 1962. Purification and properties of bean pod mottle virus and associated centrifugal and electrophoretic components. Virology 16:419-427.
6. Brakke, M. K. 1955. Zone electrophoresis of dyes, proteins, and viruses in density-gradient columns of sucrose solutions. Arch. Biochem. Biophys. 55:175-190.
7. Buzas, Z., and Chrambach, A. 1982. Steady-state stacking in agarose at various pH. Electrophoresis 3:121-129.
8. Hollings, M., and Stone, O. M. 1975. Serological and immunoelectrophoretic relationships among viruses in the tombusvirus group. Ann. Appl. Biol. 80:37-48.
9. Hull, R. 1977. The stabilization of the particles of turnip rosette virus and of other members of the southern bean mosaic virus group. Virology 79:58-66.
10. Hurtt, S. S. 1987. Detection and comparison of electrophorotypes of hibiscus chlorotic ringspot virus. Phytopathology 77:845-850.
11. Hurtt, S. S., and Fawcett, J. S. 1987. Titration curves of isometric plant viruses in agarose slab gel electrophoresis. Phytopathology 77:987.
12. Incardona, N. L., McKee, S., and Flanegan, J. B. 1973. Noncovalent interactions in viruses: Characterization of their role in the pH and thermally induced conformational changes in bromegrass mosaic virus. Virology 53:204-214.
13. Koenig, R., and Gibbs, A. 1986. Serological relationships among tombusviruses. J. Gen. Virol. 67:75-82.
14. Krishnamoorthy, R., Bosisio, A. B., Labie, D., and Righetti, P. G. 1978. Titration curves of liganded hemoglobins by combined isoelectric focusing-electrophoresis. FEBS Lett. 94:319-323.
15. Lovisolo, O., Ambrosino, C., Liberatori, J., and Papa, G. 1964. Studies on the tomato bushy stunt virus. I. Electrophoretic and

immunochemical differentiation of "Petunia" and "BS-3" strains. Atti Accad. Sci. Torino Cl. Sci. Fis. Mat. Nat. 98:391-406.
16. Magdoff-Fairchild, B. S. 1967. Electrophoretic and buoyant density variants of southern bean mosaic virus. Virology 31:142-153.
17. McFarlane, A. S., and Kekwick, R. A. 1938. Physical properties of bushy stunt virus protein. Biochem. J. 32:1607-1613.
18. Nelson, M. R., and Tremaine, J. H. 1975. Physiochemical and serological properties of a virus from Saguaro cactus. Virology 65:309-319.
19. Pappu, H. R., Tittle, F. L., and Hiruki, C. 1988. IEF of plant viruses with mini IEF cell. Bio-Radiation 68:6.
20. Rice, R. H., and Horst, J. 1972. Isoelectric focusing of viruses in polyacrylamide gels. Virology 49:602-604.
21. Righetti, P. G. 1983. Isoelectric Focusing: Theory, Methodology and Applications. Elsevier Biomedical Press, Amsterdam. 386 pp.
22. Righetti, P. G., and Gianazza, E. 1979. pH-mobility curves of proteins by isoelectric focusing combined with electrophoresis at right angles. Pages 23-38 in: Electrophoresis '79. B. J. Radola, ed. W. de Gruyter & Co., Berlin. 858 pp.
23. Righetti, P. G., Krishnamoorthy, R., Gianazza, E., and Labie, D. 1978. Protein titration curves by combined isoelectric focusing-electrophoresis with hemoglobin mutants as models. J. Chromatogr. 166:455-460.
24. Rosengren, A., Bjellqvist, B., and Gasparic, V. 1977. A simple method for choosing optimum pH-conditions for electrophoresis. Pages 165-172 in: Electrofocusing and Isotachophoresis. B. J. Radola and D. Graesslin, eds. W. de Gruyter & Co., Berlin. 608 pp.
25. Saravis, C. A., and Zamcheck, N. 1979. Isoelectric focusing in agarose. J. Immunol. Methods 29:91-96.
26. Tietz, D. 1987. Gel electrophoresis of intact subcellular particles. J. Chromatogr. 418:305-344.
27. Tremaine, J. H. 1970. Physical, chemical, and serological studies on carnation mottle virus. Virology 42:611-620.
28. Tremaine, J. H., and Lauffer, M. A. 1960. The charge effect in sedimentation. J. Phys. Chem. 64:568-573.
29. Valentini, L., Gianazza, E., and Righetti, P. G. 1980. pK determinations via pH-mobility curves obtained by isoelectric focusing-electrophoresis: Theory and experimental verification. J. Biochem. Biophys. Methods 3:323-338.
30. Van Regenmortel, M. H. V. 1982. Serology and immunochemistry of plant viruses. Academic Press, New York. 302 pp.