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Polyacrylamide Gel Electrophoresis

A. Chrambach and D. Rodbard

Fractionation of proteins, nucleic acids, and other charged macromolecules has generally required successive use of several fractionation steps, one sensitive primarily to molecular size (such as gel filtration) and another based mainly on molecular net charge (such as free electrophoresis, ion exchange chromotography). Zone electrophoresis in polyacrylamide gel (*1, 2*), designated as polyacrylamide gel electrophoresis (PAGE), simultaneously exploits differences in molecular size and charge for purposes of fractionation.

Range of applicability. The synthetic polymer, polyacrylamide, can be made to provide an effective median pore radius of 0.5 to 3 nm (*3*) by the simple device of adjusting the total acrylamide concentration, designated (*4*) $\%T$ (3 to 30% w/v) (*5, 6*), and the concentration of cross-linking agent, designated (*4*) $\%C$ (1 to 25% of total monomer) in the polymerization reaction (Fig. 1). Larger pore sizes can be produced when the polymer is stabilized by agarose (*7–9*). The pore sizes can be selected for optimal resolution between any two species (*10*). The wide range of applicability of PAGE is illustrated by the fractionation of oligonucleotides (M.W. < 1000) (*5*) and high-molecular-weight RNA (M.W. $> 10^6$) (*6, 8, 9*). One can also optimize "charge separation" by operating at any *p*H between 3 and 11 to provide the maximal difference be-

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tween the net charges of molecules (*11*). PAGE can be carried out at 0°C as well as at higher temperatures and is therefore applicable to enzymes (*12–14*) and other thermolabile molecules (*15*). PAGE can be used for analytical fractionation in or below microgram loads or for preparative fractionation of loads in the milligram and gram range.

Experimental simplicity. The basic experimental setup (*16*) (center of Fig. 2) is simple. The gel is formed by polymerization of acrylamide in a tube or a slab mold. The gel tube is positioned between two buffer chambers. The sample is applied to the gel surface in a 10 to 50% sucrose solution, and an electrical field is applied across the gel. Each component migrates as a band with characteristic electrophoretic migration rate depending on its size and net charge (Eq. 2) (*10, 11, 17*). Fixation and staining, or slicing and assay, of the gel subsequent to electrophoresis reveals the characteristic band positions.

Versatility of polyacrylamide gels. Pore size variability, achieved by use of variable concentrations of reagents in the polymerization reaction (Fig. 1), is mainly responsible for the versatility of PAGE. Further versatility can be obtained. (i) Gel buffers containing urea (*18*), nonionic detergent (for example, Triton-X-100) (*19*), formamide (*20*), phenol (*21*), sucrose (*16*), or glycerol (*22*) can be used. (ii) Polymers such as agarose (*7–9*) or polyvinylpyrrolidone (*23*), or vinyl monomers with charged (*24*) or other hydrophilic (*25, 26*) functional groups can be introduced into the polymerization reaction. (iii) Electrophoresis can be used to introduce ionic detergents (*27*), stabilizing agents, cofactors (*28*), chelating agents (*8*), ligands (*15*), or reducing agents (*29*) into the gel.

Such versatile use of PAGE imposes restrictions with regard to apparatus design and construction. Adequate wall adherence under widely differing conditions is only obtained in Pyrex tubes or slabs. Even with glass walls, gels of lowest monomer concentrations (2 to 3 %*T*) need stabilization by hydrostatic equilibration (*30*), wall coating with linear polyacrylamide (*30*), or mechanical supports (nylon mesh or dialysis membranes) (*31*).

From qualitative to quantitative PAGE. Because of its high resolving power, applicability to the entire molecular weight range, sensitivity, versatility, simplicity, self-sufficiency, and economy, PAGE has found widespread

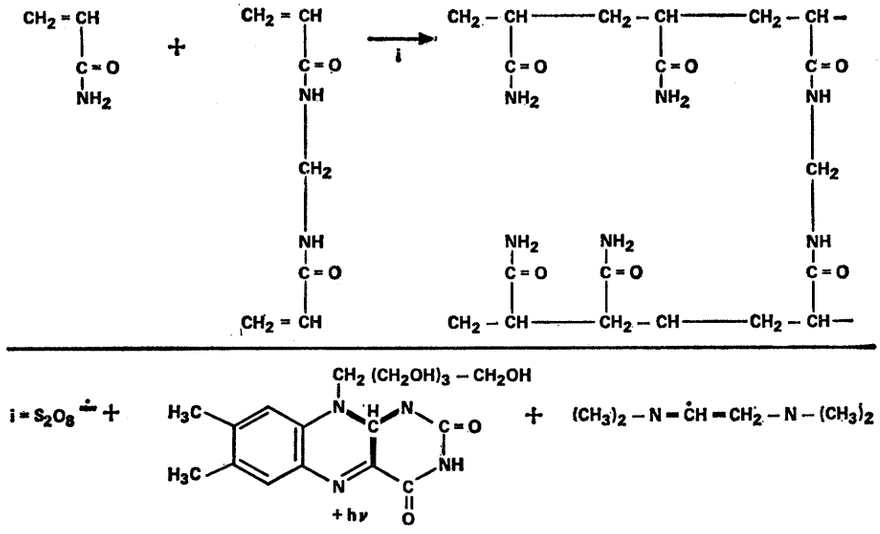


Fig. 1. The polymerization reaction of acrylamide. The structures of acrylamide, *N,N'*-methylenebisacrylamide and of a representative segment of cross-linked polyacrylamide are shown. Initiators, designated by *i*, shown are persulfate, riboflavin, and *N,N,N'N'*-tetramethylethylenediamine. Light is designated as $h\nu$.

application since its introduction 10 years ago (*1, 2*). A full account of its applications and instrumentation is given by two recent monographs (*32*) and by a periodically updated bibliography (*33*). However, most of its applications have used only qualitative pattern inspection for interpretation of results and have not taken advantage of the quantitative physical-chemical nature of PAGE, which is therefore emphasized in this review. Quantitative PAGE is based on (i) development of methods to achieve a high degree of pore size reproducibility; (ii) development of the physical-chemical theory of the movement of molecules through gels, leading to the quantitative and statistical analysis of PAGE data by computerized methods; (iii) development of an exact theory and computer program for the generation of multiphasic (discontinuous) buffer systems operative at any *p*H, at 0° or 25°C. The three advances and the development of preparative PAGE allow formulation of a general strategy applicable to the fractionation, resolution, physical-chemical characterization, and isolation of macromolecules.

The Polyacrylamide Pore

Formation of a reproducible pore. The polymerization reaction (Fig. 1) that forms the gel has to be carried out de novo prior to each fractionation, since the surface and swelling properties of the gel and buffer discontinuities do not permit storage. Since this introduces an element of irreproducibility into PAGE, it is imperative to control polymerization conditions to enable one to interpret the data quantitatively. This control is achieved in five ways: (i) Reagents are purified. Acrylamide and *N,N'*-methylenebisacrylamide (Bis) (Fig. 1) are freed of polymers and contaminating ions by recrystallization (*11, 34, 35*). Ethylenediacrylate (*36*) and *N, N, N', N'*-tetramethylethylenediamine (TEMED) (Fig. 1) are redistilled in vacuo. Buffers are purified if necessary. (ii) Optimal polymerization catalysts (initiators) and concentrations are selected for each *p*H and buffer: Catalysts [potassium persulfate (*11, 16*), riboflavin (*16, 37, 38*), TEMED (*16*), $H_2O_2 - Fe^{2+}$ (*39*), persulfate-bisulfite (*40*), and others (*26, 41*)] are used in such concentrations as needed to achieve polymerization within 5 to 15 minutes (*11, 35*) for gels 6 mm in diameter and to achieve 95% or higher conversion of monomer to polymer (*11*). (iii) Polymerization inhibitors are reduced and maintained at a constant level. The most important polymerization inhibitor is atmospheric oxygen. Oxygen concentration can be lowered by regulated and timed deaeration (*11*) or saturation with an inert gas such as argon (*42*). This becomes essential when operating at 0°C and at acid *p*H (*11*). Apparatus materials may also inhibit [polypropylene (*43*)], or enhance [rubber (*44*)] polymerization. (iv) Temperature of polymerization is kept constant. The polymerization rate is highly temperature dependent. Constancy of temperature within 1°C can

only be obtained under conditions of efficient heat dissipation since polymerization is exothermic. Limitation of gel thickness (5 to 14 mm), use of thin glass walls, immersion of the gel tubes or slab into liquid (Fig. 2), and rapid coolant flow from a reservoir of adequate capacity are required. The temperature of polymerization should be the same as the temperature of electrophoresis to eliminate thermal contraction or expansion of the gel. (v) A uniform rate of the polymerization reaction is maintained. Polymerization should be completed within 5 to 15 minutes, as judged by interface sharpening. Since completion is approached asymptotically, a practical procedure is to leave the reaction mixture undisturbed for a constant, arbitrary time, such as 30 minutes (60 minutes for preparative-size gels) (*11, 35*). Polymerization efficiency should be at least 95%; 98 ± 1% can be obtained (*11*).

Purification of the polymer to eliminate catalysts, monomer, and side products is usually unnecessary. When evidence indicates reaction between sample and catalysts (*29, 45*), or residual monomer (*46*), purification of the gel may be possible by preelectrophoresis (*8, 9*) or by sweeping of the gel with thioglycolate (*29*).

Pore size. Several models have been postulated for the geometry of the polyacrylamide gel pore (*1, 47, 48*); it is assumed in these models that all pores have the same size and shape. A more realistic and informative approach is to forego any preconception of gel structure beyond consideration of gels as random meshworks of fibers ["Ogston theory" (*49*)]. The statistical theory of such a gel structure has been extensively developed (*10, 49–51*) and provides a unified approach to gel electrophoresis and gel filtration. "Pores" are not uniform in size; rather, they are distributed according to a slightly skewed non-Gaussian distribution (*10, 49, 50*). The dimensions of the polyacrylamide pore and gel fiber have been measured against the known dimensions of macromolecules by means of gel filtration (*3, 52*) and gel electrophoresis (*10*). The median pore size, $R_{0.5}$, for any gel concentration can be estimated as the radius of a macromolecule for which the partition coefficient $K_{av} = 0.5$ (*3, 52, 53*), or for which the electrophoretic mobility is 0.5 times the free mobility (*10*). Median pore size $R_{0.5}$, is inversely related to the square root of total gel concentration, $\%T$ (Fig. 3) (*3, 10, 49, 52*). The effective length of gel fibers per unit volume, L, is proportional to gel concentration, $\%T$, for any constant cross-linking, $\%C$ (*3*). Properties of the gel can be altered significantly by changing $\%C$. Low cross-linking (1 to 5 $\%C$) yields "long fiber gels," in which the fractional volume available to a macromolecule theoretically depends on molecular surface area. A high percent cross-linking (15 to 25 $\%C$ or more) may result in "short fiber gels," which theoretically can be expected to approach a pore size which excludes macromolecules on the basis of molecular volume (*3, 10, 51*). The effective thickness of the hydrated polyacrylamide gel fiber r (Eq. 1) also depends on percent cross-linking: low (1 to 4) $\%C$ results in fiber thickness of about 1 nm, comparable to that of cross-linked dextran (*3*); high (15 to 25) $\%C$ results in a very thick fiber (about 3 nm), comparable to that of starch gels [recalculation of data from (*54*)], with an opaque gel, possibly due to "bundling" (*3*) or to fiber breakage at the points of cross-linking. Estimates of fiber thickness obtained by PAGE and gel filtration are in reasonable agreement with values obtained by independent measurement (*55*). The dimensions of a 2 $\%C$ polyacrylamide gel are comparable to those of a cross-linked dextran gel of similar polymer concentration (*3, 52*).

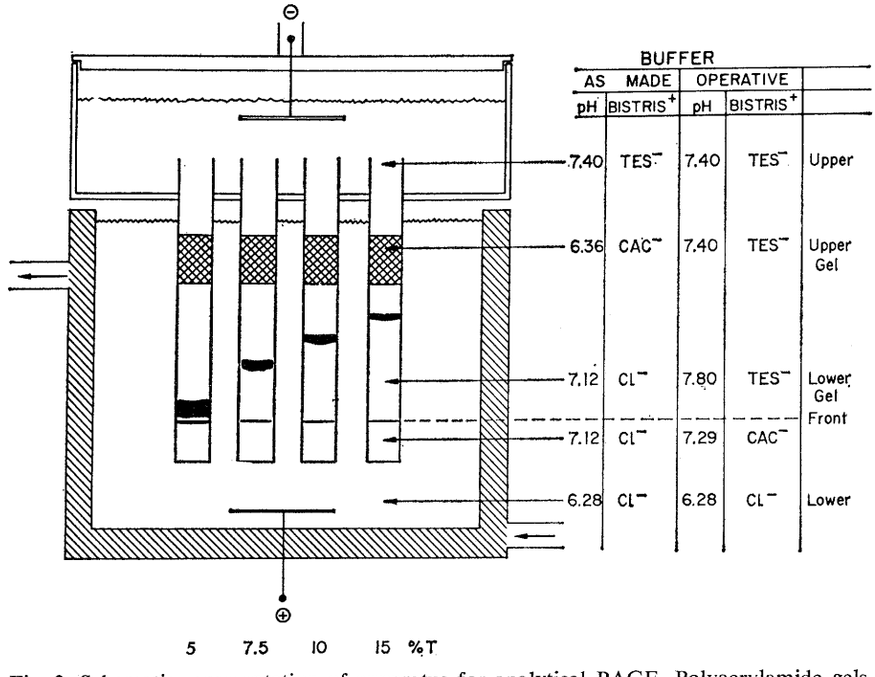


Fig. 2. Schematic representation of apparatus for analytical PAGE. Polyacrylamide gels of 5, 7.5, 10, and 15% are shown. Bovine serum albumin is fractionated. Characteristic *p*H values for the various initial and operative (*95*) buffer phases are shown for system D (*11*). Stacking gels are indicated by the cross-hatched areas above the separation gel photographs. Upper and lower buffers in their respective reservoirs are shown with waved surfaces. Coolant within the jacket of the lower reservoir is represented by stripes, and the direction of coolant flow is indicated by the short arrows. *BISTRIS* = Bis-(2-hydroxyethyl)iminotris(hydroxymethyl) methane; *TES* = *N*-tris(hydroxymethyl)-methyl-2-aminomethanesulfonic acid; *CAC* = cacodylic acid; *Cl* = hydrochloric acid; $\%T$ = gel concentration.

Pore gradients. Several workers have used pore gradients (gel concentration gradients), to provide a wide spectrum of pore sizes in a single fractionation (*56–58*). An exact theory of the behavior of macromolecules on both linear and nonlinear gradients has been developed, making it possible to predict the position and velocity for any molecular species as a function of time (*59*) (Eq. 1).

$$x = \frac{\log_e [b\, a_2\, u_0\, t + \exp(bT_0)] - b\, T_0}{b\, a_2}$$

$$v = \frac{u_0}{b\, a_2\, u_0\, t + \exp(bT_0)} \qquad (1)$$

where x is distance; t is time; v is instantaneous velocity; u_0 is free mobility; b is $K_R \cdot \log_e 10$; T_0 is gel concentration when $x = 0$ (top of the gel); a_2 is gel gradient ($\Delta T/\Delta x$); and voltage gradient is taken as unity.

The suggestion that gel gradients can

be used to determine molecular weight (*57, 58*), based on concept of a "pore limit," appears invalid (*59*). Gel gradients are not advantageous for the fractionation of one, two, or only a few molecular species (*59*) where a unique optimal pore can be defined (*10*). However, gel gradients have an important potential application in the "finger printing" of multicomponent systems (two-dimensional macromolecular mapping) (*59, 60*).

Physical-Chemical Use of PAGE

Relationships between mobility, gel concentration, molecular size and net charge. Equation 1 indicates the relation between relative electrophoretic mobility (R_f), which is equal to absolute mobility M divided by the mobility of the tracking dye or other marker (*10*), the free electrophoretic mobility M_0, and the fractional volume available to the molecule f. Several alternative, interchangeable expressions are given for f, all of which involve a negative exponential term and involve a parameter characteristic of the gel ($L = l'\ T$) and a parameter characteristic of the molecule under study [$S/4 = \pi\ (R + r)^2$] or a combination of these

$$K_R = \pi\ (R + r)^2\ l'/\log_e\ (10)$$

By expressing free electrophoretic mobility M_0 in terms of net charge Q, radius R, and counterion radius r_i through the use of equations of classical electrophoresis (the term in square brackets in Eq. 2), one is able to express mobility in gel electrophoresis entirely in terms of parameters of the macromolecule, gel, and buffer. The derivation of this result involves many assumptions and approximations (*10, 11, 17*).

$$R_f = \frac{M}{u_f} = \frac{M_0}{u_f}\ \mathrm{f}$$

$$= \frac{M_0}{u_f} \exp\{-SL/4\}$$

$$= \frac{M_0}{u_f} \exp\{K_R\ T(\log_e 10)\}$$

$$= \frac{1}{u_f}\left[\frac{Q\ X_1(\kappa R)}{6\pi\eta R} \quad \frac{(1 + \kappa r_i)}{[1 + \kappa(r_i + R)]}\right]$$

$$\times \exp\{-\pi(R + r)^2\ l'\ T\} \qquad (2)$$

When relative mobility, R_f, is measured in each of three or more gel concentrations, a linear "Ferguson plot" [plot of log R_f versus T (Fig. 4)] can be constructed (*54*). The slope of this line is a measure of molecular size (*10, 11,*

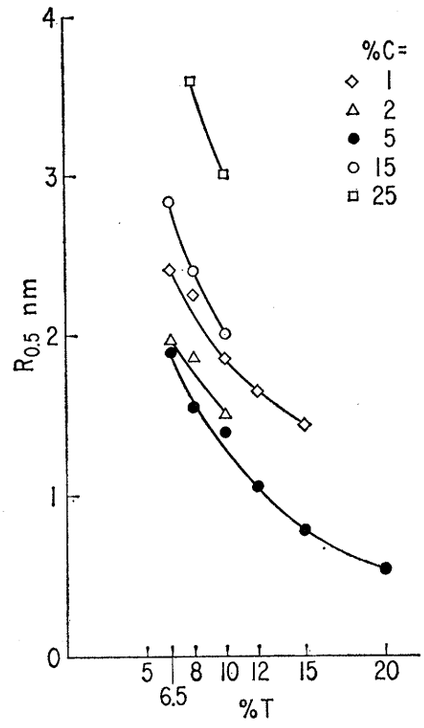


Fig. 3. Median pore radius ($R_{0.5}$) as a function of gel concentration ($\%T$), data of Fawcett and Morris (*3*); $\%C$ = degree of cross-linking.

54, 61, 62) and designated (*10*) as the retardation coefficient, K_R. The antilog of the y-intercept of this line ($Y_0 = R_f$ when $T = 0$) is a measure of the free electrophoretic mobility (*10, 11, 54, 62*) and therefore of net-charge (valence) (*11*). For globular proteins, there is a linear relationship between $(K_R)^{1/2}$ and molecular radius, R (*10, 11*), which has been predicted theoretically (Eq. 2) and confirmed experimentally (Fig. 5) over a very wide range of conditions (*11*). From a knowledge of free mobility M_0 (calculated from Y_0) and molecular radius R (calculated from K_R) one can calculate the net charge on the molecule, using classical theory of electrophoresis (Eq. 2) (*17*). These calculations have been computerized: R_f values for each gel concentration are entered, and radius R, molecular weight M.W., free mobility M_0, and valence V are provided with their 95% confidence limits (*11*). The precision of radius and molecular weight estimates depends on the number of standard proteins used to construct the calibration curve, $(K_R)^{1/2}$ versus R, and may be improved by use of experimentally determined values (instead of assumed values) for partial specific volume, hydration, and axial ratio (*11*), and by improving the precision of K_R. The latter is achieved by increasing the number of points on the Ferguson plot or by improving the precision of the R_f measurement.

The linear relation between $(K_R)^{1/2}$ and molecular radius is applicable only to spherical proteins in a long-fiber gel. For denatured "random coils," the radius of gyration R_g is proportional to (M.W.)$^{1/2}$, the effective surface area is proportional to M.W. (*63*); accordingly K_R is linearly related to M.W. Proteins treated with detergents such as sodium dodecyl sulfate (SDS) form rods or random coils with nearly constant free mobility (M_0 and Y_0). Accordingly, for a constant gel concentration, there is a linear relation between log R_f and molecular weight (*64*).

When PAGE is applied to an oligomeric series (*5, 64*) the relationship between M.W. (or the number of subunits) and K_R cannot be predicted unless one also defines a model for the quaternary structure of the aggregate. If the oligomers are a linear array of spheres (beads on a string) (*50*), then surface area (K_R) is proportional to the number of subunits (n). If the subunits coalesce into a common sphere, then the surface area (K_R) is asymptotically approaching $n^{2/3}$. Closely packed spheres will give an intermediate result. A closely packed tetrahedral tetramer will have essentially the same surface area as a linear trimer. The relation between K_R and M.W. of aggregates is influenced by the effective radius of the gel fiber r, especially when one deals with species of low molecular weight, or with high $\%C$. Gels consisting of points randomly suspended in space are, in theory (*10*), sensitive to the volume of the molecule under study. This suggests that these "O-D" gels (*10*) should be used for determination of aggregation state, since the volume of the aggregate is proportional to the number of subunits irrespective of the quaternary structure.

In addition to the above relations based on the Ogston theory (*49*), a number of empirical relations have been proposed and used for molecular weight determination by PAGE within restricted ranges (*47, 64*). When R_f is plotted versus log M.W. for nucleic acids or SDS derivatives of proteins, one obtains an excellent correlation (Fig. 6) (*8, 64, 65*). SDS virtually eliminates conformational and charge density differences among proteins and reduces the effect of variability in partial specific volume, hydration, and axial ratio. There is a sigmoidal relation between the partition coefficient K_{av} and log M.W. (or log R) (*66, 67*), representing

the integral of the probability density function for the distribution of pore sizes (*10, 49, 63*). This can be approximated by a straight line for a wide range, resulting in an apparent linear relation between K_{av} and log M.W. (*66–68*). When variability in free mobility is reduced by SDS, the R_f is proportional to K_{av} (*10, 64, 65*) resulting in a linear relationship between R_f and log M.W.

A quantitative approach to fractionation by PAGE. Since the usual application of PAGE is fractionation, one may regard calculation of K_R, Y_0, radius, molecular weight, free mobility, and valence as an optional accessory of PAGE (*11*). However, these parameters have direct bearing on fractionation. Inspection of K_R and Y_0 values allows one to discriminate between fractionation based solely on charge (parallel Ferguson plots, constant K_R), fractionation based solely on size (intersecting Ferguson plots, constant Y_0), and fractionation based in both (both K_R's and Y_0's vary) (*61*). In any given electrophoretic system [*p*H, temperature (T), ionic strength (*I*), %*C*)], knowledge of K_R, Y_0, and preferably R makes it possible to calculate the gel concentrations for maximal separation (T_{max}) and for optimal resolution (T_{opt}) of the components of interest (*10, 15, 59*). (Eq. 3)

$$T_{max} = \frac{\log_{10}(Y_1 K_1 / Y_2 K_2)}{K_1 - K_2}$$
$$T_{opt} = \frac{\log_{10}(Y_1 K_1^2 / Y_2 K_2^2)}{K_1 - K_2} \quad (3)$$

where Y_1 and Y_2, and K_1 and K_2 are the Y_0's and K_R's for species 1 and 2.

At the T_{opt} the average mobility of the two species is approximately equal to $1/e^2 = 0.135$ times the mobility at the point of intersection of their two lines on the Ferguson plot. One should attempt to optimize fractionation conditions by selecting a *p*H such that separations on the basis of size and of charge are synergistic:

$$[(K_1 - K_2)(Y_1 - Y_2) < 0]$$

If fractionation proceeds under conditions where charge and size separations are antagonistic,

$$[(K_1 - K_2)(Y_1 - Y_2) > 0]$$

or when lines on a Ferguson plot are parallel ($K_1 = K_2$), application of Eq. 3 will usually yield a T_{max} or T_{opt} at $T = 0$ in addition to the value at finite %T. Zero gel concentration may be approximated in practice by use of 20 %C, 3 %T, or on experimentally determined (*30, 31*) minimally restrictive pore size. Fractionation at 0 %T can be carried out by isoelectric focusing or isotachophoresis (see below) as well as in PAGE.

The fractionation of an oligomeric series presents a special case of charge and size antagonism (*17, 32, 64, 69*). But here the option of isoelectric focusing is barred, since in this case the *p*I's of all species are identical or very similar.

Knowledge of K_R and Y_0 makes it possible to predict the instantaneous velocity and position (R_f) for any molecule in a gel gradient (Eq. 1) (*59*), and therefore to determine whether a gel gradient is applicable to any specified fractionation problem.

The K_R and Y_0 values provide a sufficient, rigorous, and sensitive criterion for testing molecular identity and

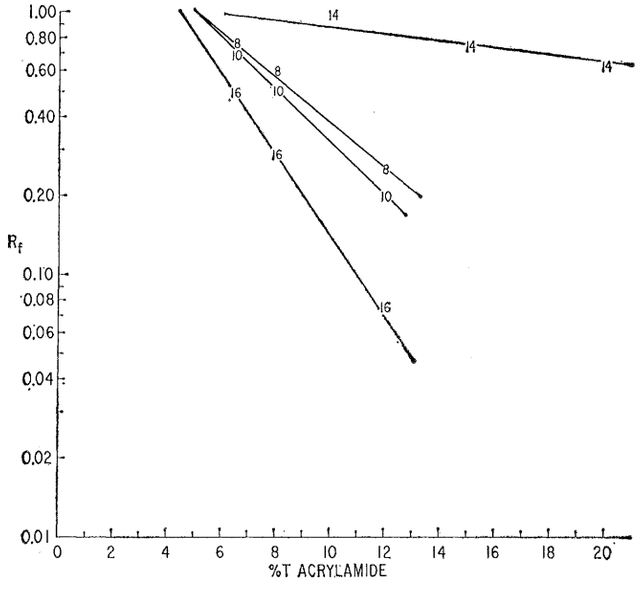
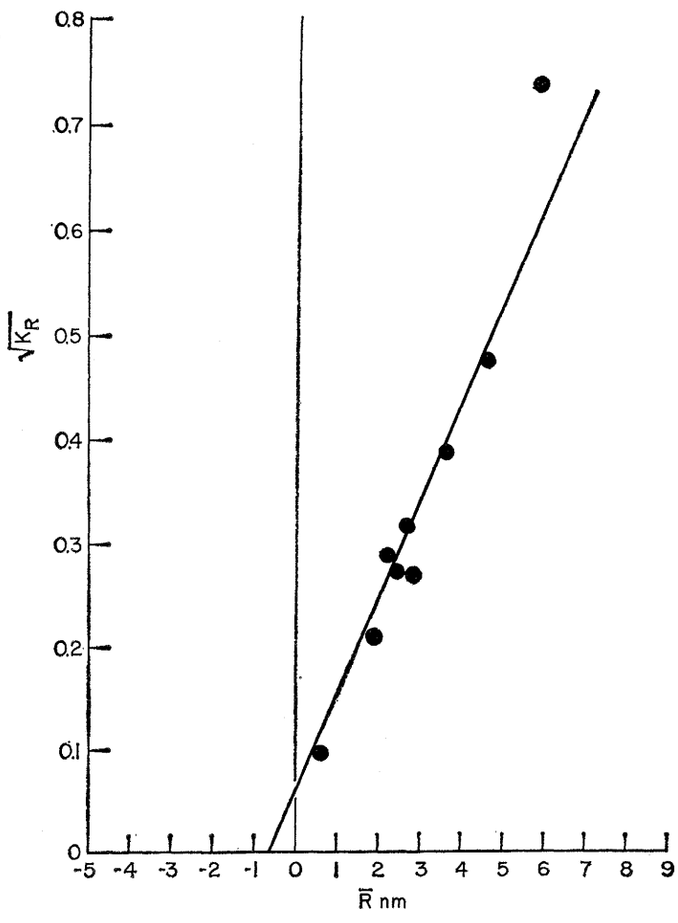


Fig. 4 (left). Plot of log R_f versus gel concentration T ("Ferguson plot"). System B (*11*), consisting of a separation gel buffer (0.3750*M* tris, 0.06*M* hydrochloric acid), a stacking gel buffer (0.0587*M* tris, 0.0320*M* phosphoric acid), a cathodic buffer (0.0547*M* tris, 0.0546*M* glycine), and an anodic buffer (0.0625*M* tris, 0.05*M* hydrochloric acid); 5 %*C*. Fractionation by PAGE of bromphenolblue (*14*), ovalbumin (*8*), bovine serum albumin (BSA) (*10*), and BSA-dimer (*16*). The slope of each line is designated as retardation coefficient, K_R. Fig. 5 (right). Standard curve for the estimation of molecular size. The abscissa is the geometric mean radius $\bar{R}$; the ordinate is the square root of the retardation coefficient K_R. Molecular sizes are, in ascending order on the standard curve, bromphenolblue, myoglobin, pepsin, ovalbumin, hemoglobin, BSA, BSA-dimer, fibrinogen, and thyroglobulin. System A (*11*), 5 %*C*.



homogeneity. The probability that two different molecules would exhibit identical K_R and Y_0 values at three divergent *p*H values is infinitesimal. PAGE thus provides the only test of identity that can be performed on microgram amounts of material, in heterogeneous systems, with sensitivity to milieu-dependent conformational changes and preservation of activity.

The resolving power of PAGE can be quantitatively assessed in any particular experiment by calculation (Eq. 4) of the number of equivalent "theoretical plates," N (*10, 70–72*), permitting comparison with other methods.

$$N = x^2/\sigma^2 = 5.55\, x^2/w^2 \qquad (4)$$

where x is the migration distance; σ is the standard deviation of peak width; and w is the width of band at 0.5 (maximal amplitude). In free electrophoresis it is possible to obtain 1000 or more theoretical plates (*72*). A similar number of "plates" can be obtained in gel electrophoresis (*71*). By contrast, gel filtration is subject to the binomial variance of any partition process, so that N values are usually below 100, and reach a maximum of about 300 with columns 1 meter long (*68, 70*).

Relationship of PAGE *to other physical methods.* Unlike sedimentation equilibrium, PAGE does not provide a direct measurement of molecular weight, but in this respect it is similar to other measurements of molecular size (gel filtration, intrinsic viscosity, light scattering, sedimentation velocity, and diffusion) which are sensitive to milieu-dependent conformational changes. In addition, PAGE can be used to measure net charge at any *p*H, and thus to construct "titration" curves (*11*). There is a direct relationship between the ratio M/M_0 in PAGE and the partition coefficient K_{av} in gel filtration (*10, 62*).

Ninety-five percent confidence limits calculated for estimates of radius and molecular weight obtained by PAGE may appear wide (*11, 15, 73*). However, classical statistical least-squares procedures have not been routinely applied to other physical-chemical methods, so that a direct comparison of precision is not available. However, the precision of estimates of R and M.W. by gel electrophoresis appears at least comparable and usually superior to that obtained from gel filtration (*11, 68*).

Measurement of band position and width. Quantitative PAGE is based on reproducible and accurate R_f measurement. This requires narrow and straight bands. Since band thickness depends on starting zone width, multiphasic buffer systems are clearly advantageous (*1*). Accuracy of measurement of migration distances of the front and the band, and of the change in gel length during staining, largely determines the accuracy of R_f (*11*). Therefore, measurement must be carried out with care, preferably on photographs (*11*) or by use of densitometers (*71*), curve analyzers, or comparators.

Proteins can be fixed in trichloroacetic acid (TCA) solutions (*74*), in amido-black in 7% acetic acid (*16*), or in methanolic solution (*65*). Nucleic acids and acid polysaccharides cannot be fixed permanently, although 15% acetic acid–1% lanthanum acetate has been used for that purpose (*75*). Proteins can be stained by Coomassie blue (*65, 74*), amido black (*16*), fast green (*76*), or the fluorescent dye amino-naphtholsulfonic acid (*77*). Quenching of fluorescence promises to be a sensitive detection tool (*78*). Nucleic acids can be stained by methylene blue (*8*), toluidine blue (*5*), acridine orange (*75*); double-stranded nucleotides may be detected fluorometrically by ethidium bromide (*79*). Polysaccharide stains include periodate–Schiff reagent (*80*) and Alcian blue or toluidine blue (*75*). Densitometric band quantitation of protein bands (*76, 81*) is feasible after amido-black or fast green staining and electrophoretic destaining. Unstained polyacrylamide gels can be subjected to ultraviolet densitometry for nucleic acids (*34*), but this is just barely sensitive enough for proteins at concentrations comparable to those required for staining (*82*). Alternative methods for measurement of R_f employ enzymatic (*13, 83*), immunological (*84–86*), or biologi-

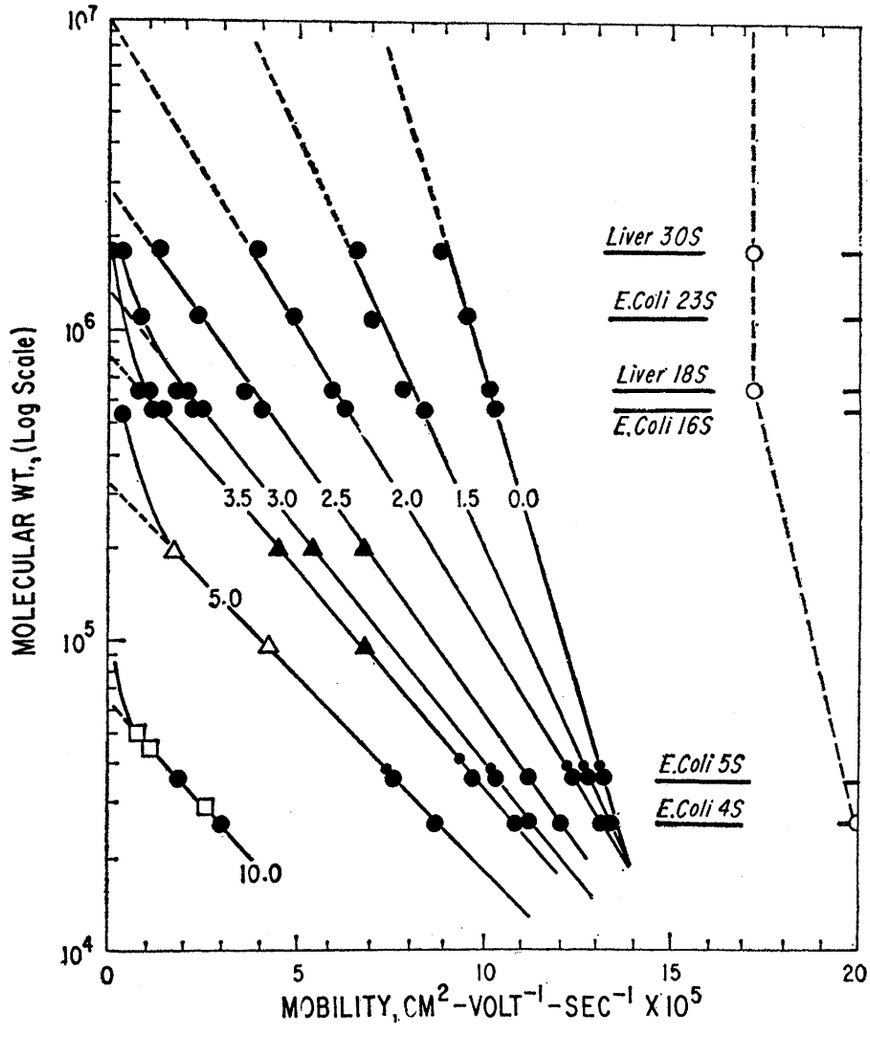


Fig. 6. Standard curve for the estimation of molecular size [A. C. Peacock and C. W. Dingman, figure 1 in (*8*)]. The abscissa represents electrophoretic mobility; the ordinate represents molecular weight. Polyacrylamide-gel concentration characteristic for each line is indicated in the figure. Agarose concentration is 0.5% throughout. RNA samples characterized by sedimentation coefficients are fractionated.

cal (*87*) assay or isotope analysis after either transverse (*83, 85*) or longitudinal (*85, 86, 88*) slicing of the gel. For activity measurement, the macromolecule (or its ligand) may be eluted from gel slices (*15, 85*), a small substrate can diffuse into the gel for enzymatic assay (*83*), gel slices can be dissolved in H_2O_2 (*89*), ethylene diacrylate cross-linked gels can be dissolved by base (*36, 90*), *N,N'*-diallyltartardiamide cross-linked gels by periodate (*91*). For isotope analysis, the material in the bands may be diffused into NCS reagent (Nuclear-Chicago) (*85, 90*), Hyamine (*86*), or Triton-X-100 (*92*), or gel homogenates may be suspended in Instagel (Packard) (*30*). Several gel slicers are useful: wire slicers (*83*) for 5 to 15 %*T* gels, blade cutters for solidly frozen, very soft (low %*T*) gels or very hard (> 15 %*T*) gels (*92*), and a nozzle device (*93*) for extrusion of the gel which lends itself to the automated processing of large numbers of gels. The precision of R_f measurement on gel slices (standard deviation of ± 0.02) is only twice that found for stained gels (*15*).

Buffer

Originally, PAGE was carried out at either very high (*1, 2*) or very low *p*H (*94*). This ensured that all proteins move in the same direction, but minimized differences in net charge and reduced efficiency of "charge fractionation" (*61*). It is desirable to perform PAGE at several *p*H values to optimize fractionation. Freedom to operate at any *p*H is required by the *p*H-activity profile and *p*H-stability characteristics of proteins, and is necessary for homogeneity testing and construction of "titration" curves. However, *p*H becomes almost irrelevant when PAGE is applied to molecules with high, fixed charge density [that is, nucleic acids (*5, 8, 34*), acid mucopolysaccharides (*75*), or the SDS-derivatives of proteins (*64, 65*)].

"Continuous" versus "discontinuous" buffer systems. When PAGE is carried out in a continuous buffer (where the buffer is the same in the gel and both electrode chambers) *p*H variation is readily obtainable. It has only recently become possible to use PAGE in discontinuous (multiphasic) buffer systems at *any p*H (*95*). This makes it possible to exploit the many advantages of multiphasic buffer systems. (i) "Stacking" (*1*) of molecules in the moving boundary between two buffer phases makes it possible to study very dilute samples. (ii) The final concentration of a component in the stack is independent of the starting concentration of the sample and may be as high as 200 mg/ml (*1, 95*). The ultrathin starting zones (about 10^{-2} cm) result in markedly improved resolution. (iii) Stacking, "unstacking," and "restacking" can be used selectively for fractionation (see below). (iv) Stacking provides a preparative separation method free of load limitations (*1*). (v) Multiphasic buffer systems provide a "front," that is, a moving boundary in front of the separation phase, which can frequently be marked by a "tracking dye" (*1*). This front is convenient in following the progress of an experiment and in the characterization of bands in terms of relative mobilities (R_f). The major disadvantages of multiphasic buffer systems have been that actual *p*H and other properties in the operative gel buffer are different from those of the buffer as prepared (Fig. 2), and that these buffer systems have not been available except at a few extreme *p*H values.

An exact theory of multiphasic buffer systems has been formulated (*95*) by extension of the treatment of moving boundaries for weak electrolytes (*96*) to multicomponent systems consisting of multiple strong and weak acids and bases. This theory makes it possible to predict, on the basis of the *pK*'s and ionic mobilities of the buffer constituents, all of the following parameters; the velocities of all moving boundaries between phases, the concentrations of all ionic constituents in the buffer phases on either side of the moving boundaries and *p*H, conductance, and buffer value in each phase. A computer program based on this theory has been developed and used to generate 4269 multiphasic systems operative in the cathodic or anodic direction, at 0.5 *p*H intervals across the *p*H scale, at 0° or 25°C (*95*). The systems utilize 45 available buffer constituents. A portion of the computer output for a single representative multiphasic buffer system is shown in Fig. 7.

Application of multiphasic buffer systems to fractionation. The complete chemical description of each multiphasic buffer system (Fig. 7) introduces a new dimension of versatility into fractiona-

DATE = 03/23/70 COMPUTER SYSTEM NUMBER = CHRAMBACH 55
POLARITY = - (MIGRATION TOWARD ANODE) TEMPERATURE = 0 DEG. C.

CONSTITUENT 1 = NO. 24 , HEPES
CONSTITUENT 2 = NO. 20 , CACODYLIC ACID
CONSTITUENT 3 = NO. 99 , CHLORIDE -
CONSTITUENT 6 = NO. 33 , BISTRIS

	PHASES					
	ALPHA(1)	ZETA(4)	BETA(2)	PI(9)	LAMBDA(8)	GAMMA(3)
C1	0.0400	0.0400		0.0256		
C2			0.0549		0.0352	
C3						0.0489
C6	0.0158	0.0158	0.0307	0.2127	0.2223	0.2361
THETA	0.396	0.396	0.560	8.304	6.320	4.823
PHI(1)	0.150	0.150		0.585		
PHI(2)			0.472		0.964	
PHI(3)						1.000
PHI(6)	0.378	0.378	0.843	0.071	0.152	0.207
RM(1)	-0.042	-0.042		-0.164		
RM(2)			-0.250		-0.511	
RM(3)						-1.626
RM(6)	0.144	0.144	0.320	0.027	0.058	0.079
PH	7.10	7.10	6.15	8.00	7.62	7.46
ION.STR.	0.0060	0.0060	0.0259	0.0150	0.0339	0.0489
SIGMA	0.381	0.381	2.275	0.955	2.977	9.475
KAPPA	96.	96.	544.	234.	703.	2201.
NU	-0.110	-0.110	-0.110	-0.172	-0.172	-0.172
BV	0.020	0.020	0.041	0.046	0.069	0.089

Fig. 7. Representative computer output descriptive of the physical properties of a multiphasic buffer system, calculated on the basis of the theory and program of T. M. Jovin (*95*). The trailing ion in the stacking phase is designated as constituent 1, the leading ion as constituent 2. The common ion is designated as 6, the counterion in the separation gel (as set) as constituent 3. Phase 1 or ALPHA designates the Upper Buffer, phase 4 or ZETA, the operative stacking gel, phase 2 or BETA, the stacking gel as set, phase 9 or PI, the operative separation gel, phase 3 or GAMMA, the separation gel as set. The symbol RM (*i,j*) denotes mobility relative to sodium ion, where *i* denotes buffer constituent, and *j* denotes phase. KAPPA designates specific conductance. Boundary displacement (cm^3/coulomb) is designated as NU. The stacking limits of this system range from 0.042 [lower stacking limit RM (1, 4)] to 0.250 [upper stacking limit RM (2, 2)]. Unstacking in the separation gel will occur for species with a relative mobility below RM (1, 9) = 0.164. Alternative stacking limits are also available from computer output not shown here (*95*).

tion. The known "upper" and "lower stacking limits" (the mobilities of the leading and the trailing ions of the stack) allow one to use stacking selectively for the purposes of fractionation. This is done by selecting the upper and lower stacking limits so that they bracket the mobility of the component of interest but are sufficiently narrow to exclude contaminants from the stack. Ions with mobilities greater than the upper stacking limit will migrate ahead of the front and are usually lost to observation. Ions with mobilities smaller than the lower stacking limit are not concentrated and give rise to diffuse bands that enter the separation gel much later than the "front" and usually do not interfere with resolution of the species of interest. Proteins have lower constituent mobilities than most buffer ions, even in the absence of molecular sieving, making it necessary to minimize the lower stacking limit. Nucleic acids, with their high fixed charge and free mobility would require an excessively high, unobtainable upper stacking limit in an open-pore gel; by an increase in gel concentration their mobility is reduced below the available upper stacking limits.

One can also use selective unstacking and restacking. "Unstacking" refers to any change of conditions which reduces the mobility of the macro-ion, concentrated at a moving boundary, below the mobility of the buffer ions delimiting the moving boundary. This can be done by changing the *p*H, by increasing the gel concentration, or by changing both *p*H and pore size.

Selective "restacking" refers to the possibility of substituting or superimposing a second trailing ion on the original upper buffer to generate a new moving boundary that will overtake, restack, and accelerate a slowly migrating species. This is potentially important for preparative PAGE because optimal resolution often occurs when net charge and mobility are small; preparative separation would therefore be associated with marked diffusion spreading and dilution unless restacking is employed.

Corollaries to the use of multiphasic buffer systems in PAGE. Fractionation in multiphasic buffer systems depends on stacking of the component of interest. This must be verified by conducting PAGE in a concentration gel and by testing for the coincidence of the stack, usually marked by a tracking dye, with the stained band or activity characteristic for the component (*11, 30, 31*). [In the absence of a tracking dye, the position of the stack can be found by analysis for the leading or trailing ion, or by determining the inflection point for the *p*H or conductivity discontinuity across the stack (*11*)].

PAGE in multiphasic buffer systems requires parallel surfaces between gel and sample phases and therefore vertical alignment of apparatus (*1, 2, 97*). The fragility of stacking gels prevents use of "sample slots" and requires mechanical sample partitions on slab apparatus.

Preparative PAGE

Load capacity of preparative apparatus. If conditions for fractionation have been properly defined and optimized, transition from the analytical to the preparative scale in PAGE only involves choice of apparatus, load, elution buffer flow rate, gel height, and current level. Load capacity in PAGE is proportional to the cross-sectional area of gel. For separation of two closely adjacent bands, load should not exceed 1 mg per square centimeter of gel [except for preparative stacking (isotachophoresis)]. In view of this limitation, the word "preparative" applied to available apparatus may imply a load capacity at the gram, milligram, or microgram level.

Gram-preparative apparatus has been described (*42, 98*) but is still developmental, cumbersome, and not generally available. Three smaller devices for milligram scale preparative PAGE have proved useful (*94, 97, 99*); they are based on a common design (*97*) and are commercially available with cross-sectional areas of 15, 10, and 5 cm^2. This apparatus contains a hollow cylindrical gel ring of up to 15 mm thickness, with an outer jacket and inner cooling core to provide constant temperature during both polymerization and electrophoresis. A symmetrical unobstructed electrical field and an elution chamber of minimal size are provided by a porous glass membrane (or dialysis membrane) as the floor of the elution chamber (Fig. 8). Elution is symmetrical, with radial flow of elution buffer from the circumference to a central capillary. By adjustment of *p*H, ionic strength, and viscosity of the elution buffer, it is possible to decelerate

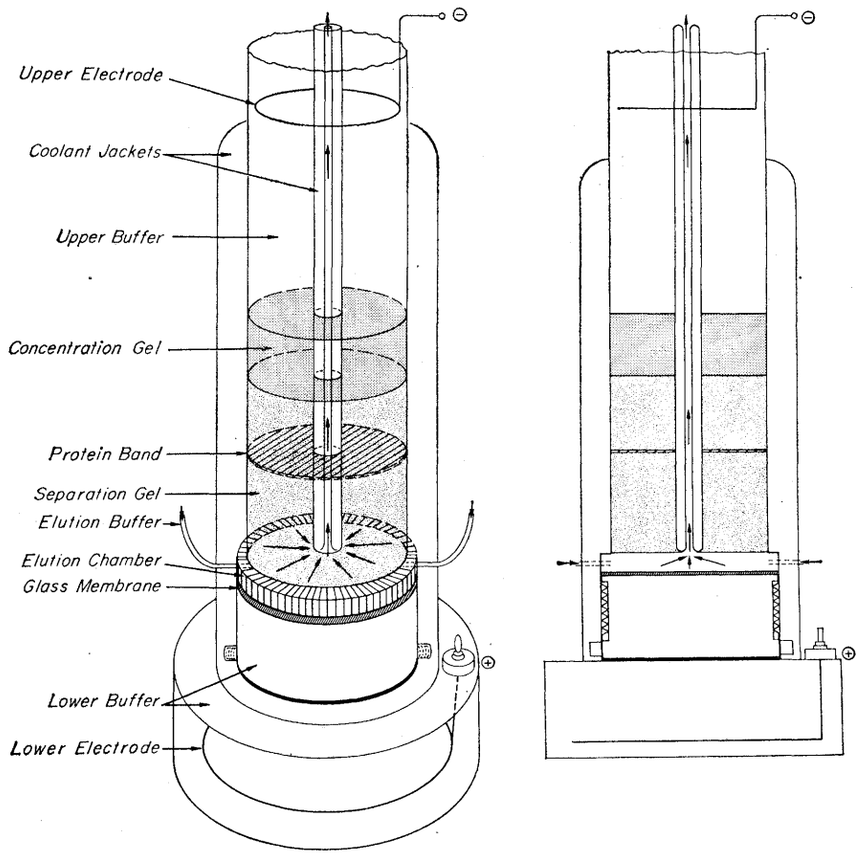


Fig. 8. Apparatus for preparative PAGE [design principle (*97*)]. Annular bands enter into the minimally sized elution chamber and are swept upward into a central capillary by radial flow of elution buffer (indicated by the arrows).

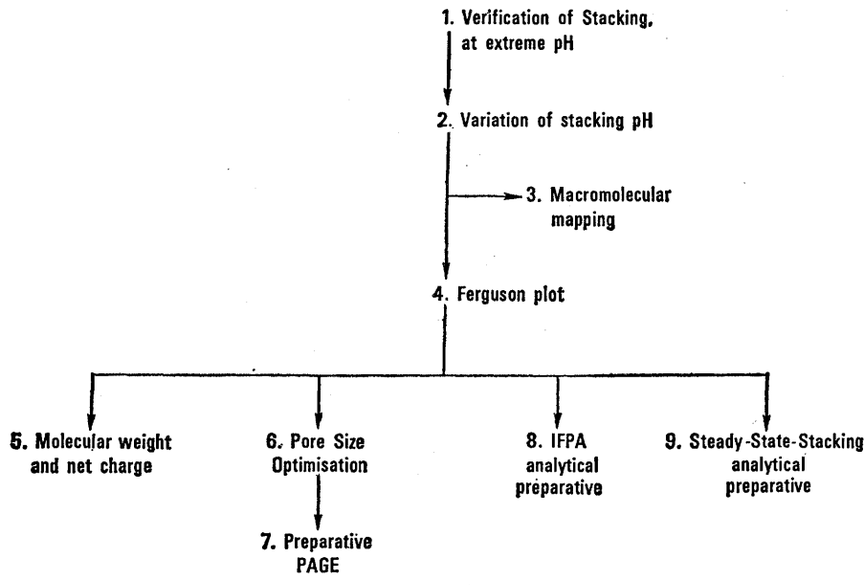


Fig. 9. General strategy for fractionation by PAGE.

the migration of the band, so that the protein is flushed from the elution chamber before it reaches the membrane and is lost.

Microgram-preparative columns based on the same design (*97*) with cross-sectional areas of 3 and 1 cm^2 are also commercially available. Devices with two other elution mechanisms are available in this load range (*44, 100*). Elution from analytical scale gels 6 mm in diameter has been utilized for microgram preparative purposes (*101*). These smaller preparative columns provide relative ease of operation at the expense of load capacity.

Problems in preparative PAGE. Recoveries from PAGE are load dependent; the recovery has been observed to increase from 60 to 90% with increasing load from 0.1 to 3 mg per square centimeter of gel and to depend on $\%T$ (*20*). Total recoveries are frequently lower in view of losses during subsequent concentration of the dilute eluates.

Another problem in preparative PAGE is the elution of a nondialyzable impurity, which is given off continuously during electrophoresis, gives a positive reaction in the colorimetric assays for protein, and may be linear polyacrylate (*20*). However, it can be separated from proteins by gel filtration on purified Sephadex G-50 (*20*).

Continuous recording of absorbance is of limited usefulness because mixtures of components generally yield overlapping distribution curves which result in a continuous broad elution pattern; and because the high dilution concomitant with continuous elution often reduces protein concentrations below detectability. Measurement of protein concentration in eluates by the Lowry procedure (*43*) is usually precluded by interfering reactions of buffer constituents, but analysis of TCA-precipitates by the Lowry method is possible. Analytical PAGE appears to be generally the best way to monitor the eluates.

The high dilution of eluates necessitates auxiliary concentrating steps, such as dialysis against volatile buffer followed by lyophilization, ultrafiltration with Diaflo (Amicon) membranes, or with dialysis tubing under pressure or vacuum, dialysis against 50% sucrose, saturated ammonium sulfate, Sephadex, Aquacide II, or Ficoll, or concentration by electrophoresis in large stacking gels (*102*).

Isotachophoresis. A promising approach to gram-preparative PAGE utilizes steady-state-stacking (*1*). The components of the sample are subjected to PAGE in a stacking gel and are eluted in order of electrophoretic mobility while still in the stack. Fractionation improves with increasing load; the distance between adjacent stacked components increases as the load becomes larger since the concentration of each component is regulated (*1*). A modification of this method, designated (*103*) "isotachophoresis," is of considerable interest. Here synthetic polyaminopolycarboxylic aliphatic ampholytes (Ampholine, LKB Produkter) are used as "spacers", with mobilities intermediate between protein components in a stack. Since the synthetic ampholytes have a broad distribution of mobilities, they can be chosen to provide spacers between any two proteins. Elution of the components of the stack, separated by spacers, should be possible within wide limits of load, with less dependence on gel surface area than in conventional PAGE.

Isoelectric Focusing in Polyacrylamide Gel

Parallel lines on a "Ferguson plot" indicate fractionation based entirely on molecular net charge and therefore optimal separation (*1, 11*), occurs at "zero" gel concentration, that is, in free electrophoresis, in sucrose density-gradient electrophoresis, or in a gel with anticonvectant but no molecular sieving properties. When this is encountered, isoelectric focusing is usually the fractionation method of choice. Isoelectric focusing in polyacrylamide gel (IFPA) (*30, 31, 104*) provides load economy, short running times, and operational simplicity. The fact that most proteins precipitate at their isoelectric points (pI) is detrimental when isoelectric focusing is conducted in sucrose solution but is turned to advantage in gels.

Under conditions of molecular sieving, migration of proteins to their isoelectric positions would require very long times. Thus IFPA should be conducted at the minimal gel concentration that provides adequate mechanical stability and wall adherence. Mechanical support of the gel and hydrostatic equilibration are frequently desirable. A chemically stable all-glass apparatus with good heat transfer and hydrostatic equilibration properties has been developed (*30*) for this purpose.

The isoelectric state of proteins is best recognized in IFPA by a time-stability of the band pattern during several hours. Usually a period of 8 to 11 hours at 40 volt/cm (regulated), 0°C, is sufficient for the attainment of "isoelectric endpoint" positions of the bands in gels of 5 cm length. The rates at which linear pH gradients and asymptotic values of specific conductance are obtained are not useful guides to the recognition of the isoelectric endpoint (*30, 31*). The band patterns at the isoelectric endpoint and pH gradients are unstable with time (*30*). This instability can be significantly reduced by the incorporation of 12 to 25% sucrose into the gel (*31*) for some Ampholine ranges. Instability of pH gradients may

Appendix

The following abbreviations are used:

a_2	gel gradient
A	acrylamide concentration (w/v)
b	retardation coefficient: (2.303) K_R
Bis	N,N'-methylenebisacrylamide concentration (w/v)
C	% cross-linking = Bis/(A + Bis)
η	viscosity
f	fractional volume available to macromolecule
IFPA	isoelectric focusing in polyacrylamide gel
I	ionic strength
K_{av}	partition coefficient (*52*)
K_R	retardation coefficient
K_1	retardation coefficient for species 1
K_2	retardation coefficient for species 2
κ	Debye-Hückel reciprocal thickness
l'	$\Delta L/\Delta T$
L	length (cm) of gel fiber per unit volume (cm^3)
M	electrophoretic mobility ($cm^2\ sec^{-1}\ volt^{-1}$)
M_0	free electrophoretic mobility
M.W.	molecular weight
n	number of subunits of an oligomeric series
N	number of theoretical plate equivalents
PAGE	polyacrylamide gel electrophoresis
pI	isoelectric point
Q	molecular net charge
R_f	mobility relative to arbitrary ion (or moving boundary)
R	radius of macro-ion
RM	electrophoretic mobility relative to Na^+
r	radius of polyacrylamide gel fiber
r_i	radius of counterion
R_g	radius of gyration
R_S	Stokes' radius
S	surface area
SDS	sodium dodecyl sulfate
σ	standard deviation of peak width
t	time
T	temperature
T	total gel concentration (T = A + Bis) (w/v)
T_{max}	gel concentration for maximal separation
T_{opt}	gel concentration for optimal resolution
TCA	trichloroacetic acid
TEMED	N,N,N',N'-tetramethylethylenediamine
T_0	gel concentration on top of gel
u	electrophoretic mobility
u_0	mobility at zero gel concentration
u_f	constituent mobility of the leading ion in the operative separation phase
v	velocity
w	width of peak at half-maximum height
w/v	weight (volume)$^{-1}$
x	distance
X_1 (κR)	Henry's function
Y_1	Y_0 for species 1
Y_2	Y_0 for species 2
Y_0	R_f when $T = 0$

relate to the nonuniform distribution of presently available Ampholines across the gel, which results in a relatively decreased conductivity of the gel center (*105*).

Activity and *p*H can be measured on the identical IFPA gel slices (*31, 106*). The staining procedures of PAGE can be applied if ampholytes are previously removed with a diffusion-destainer containing fixative (*11*); alternatively, proteins may be stained by a procedure insensitive to ampholytes (*107*). After fixation in TCA, precipitate bands are visible and allow for selective slicing of the bands, extraction of protein from the slices, and amino acids analysis (*30*). Preparative IFPA on gels with a diameter of 18 mm can accommodate as much as 2 mg of protein per band (*30*).

Strategy of PAGE

Despite the diversity of applications of PAGE, a general strategy can be developed (Fig. 9). In this discussion, the component of interest will be referred to as the protein, although it may be any other charged molecule.

1) Verification of stacking: At an extreme *p*H, the sample is applied to a nonsieving stacking gel, and the presence of the protein in the stack is determined. After stacking has been verified, the upper and lower stacking limits are narrowed to obtain as much selectivity as possible.

2) Choice of *p*H of stacking: The *p*H of the operative stacking gel is varied systematically, to determine the *p*H range available for fractionation. This can be done by changing buffer systems, or by modification of the buffer concentrations within any one system.

3) Macromolecular mapping: If the purpose is to obtain a macromolecular map of the components, fractionation is carried out in two dimensions, one of which is usually pore-gradient electrophoresis in either a slab apparatus or a preparative device.

4) Ferguson plot: At a desirable operative *p*H, a separation gel is selected in a system previously shown to provide stacking. The protein is fractionated by PAGE at five or more pore sizes, with a constant percentage of cross-linking. Nominal T values are corrected for the degree of completion of the polymerization reaction. R_f and T values are used to compute the slope and intercept of the Ferguson plots, K_R, Y_0, and their 95% confidence limits (*11*).

5) Determination of molecular size and net charge: Ferguson plots are obtained for seven or more standard proteins of known molecular weight (see 4). The molecular weight and molecular radius of the protein are computed, with 95% confidence limits, from the linear relationship between $(K_R)^{1/2}$ and R for the standard proteins. From the Y_0 and R values and the calculated or measured mobility of the buffer "front" (*95*) the net charge of the protein is computed (*11*).

6) Optimization of pore size: From the K_R, Y_0, and R values of the protein and any one contaminant, the optimal gel concentration is computed (*10, 11, 15, 59*). If the Ferguson plots of the protein and any one contaminant are parallel, then $T_{opt} = 0$ and fractionation is carried out in a nonsieving anticonvectant gel, a sucrose gradient column, by isoelectric focusing (see 8) or by isotachophoresis (see 9).

7) Isolation by PAGE: The protein is isolated by preparative PAGE in apparatus of the desired load capacity, using conditions previously optimized on the analytical scale.

8) Fractionation by IFPA: Using a very low gel concentration, or an agarose-polyacrylamide gel, one forms a maximally mechanically stable gel without significant molecular sieving. (Stacking in a multiphasic buffer system can be used as an operational criterion for absence of significant molecular sieving for proteins.) A narrow range of ampholyte *p*I's is chosen for best resolution. Moderate voltage gradients (40 volt/cm) lead to temporary pattern stability and a linear *p*H gradient in 8 to 11 hours, 0°C. Stability of gradients may in some cases be improved in gels containing sucrose. Conditions established for analytical IFPA are applicable to the milligram-preparative level.

9) Isotachophoresis: The *p*H, ionic strength, temperature, and gel concentration are selected, usually on the basis of Ferguson plots. In addition, one must select the "spacers": either synthetic ampholytes with a broad distribution of mobilities, or preferably specific ions

with mobilities intermediate between the protein and its adjacent contaminant(s). Isotachophoresis can be scaled up to accommodate gram loads relatively uninfluenced by gel size.

Summary

Polyacrylamide gel electrophoresis (PAGE) provides a versatile, gentle, high resolution method for fractionation and physical-chemical characterization of molecules on the basis of size, conformation, and net charge. The polymerization reaction can be rigorously controlled to provide uniform gels of reproducible, measurable pore size over a wide range. This makes it possible to obtain reproducible relative mobility (R_f) values as physical-chemical constants. Application and extension of Ogston's (random fiber) model for a gel allows for calculation of molecular volume, surface area, or radius, free mobility, and valence from R_f measurements at several gel concentrations, to calculate gel concentration for optimal resolution, and to predict behavior of macromolecules on gel gradients by computerized methods. Extension of classical moving boundary theory has been used to generate multiphasic buffer systems (providing selective stacking, unstacking, restacking, and preparative steady-state-stacking) with known operating characteristics for any *p*H at 0° and 25°C. A general strategy for isolation of macromolecules and for macromolecular mapping has been developed. Preparative scale PAGE is operational for milligram loads and feasible for gram quantities.

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European Astronomers Decide to Consolidate Their Journals

A new journal, *Astronomy and Astrophysics*, has resulted from the merger.

J. L. Steinberg

In the fall of 1968 European astronomers decided to merge five existing astronomy or astrophysics journals of long standing into a single new journal, with the title *Astronomy and Astrophysics, A European Journal.* The journals which merged are the following: *Annales d'Astrophysique* (France), founded in 1938; *Bulletin of the Astronomical Institutes of the Netherlands*, founded in 1921; *Bulletin Astronomique* (France), founded in 1884; *Journal des Observateurs* (France), founded in 1915; and *Zeitschrift für Astrophysik* (Germany), founded in 1930.

In a period during which new journals of varying size, scope, and quality are being founded at the rate of several per year, it might be interesting to analyze the motives behind this unusual decision which created *Astronomy and Astrophysics* and the method of operation of this journal.

In order to understand the reasons for the founding of this new journal, one has to understand the situation in astronomical publication in the mid-1960's. At that time the periodical *Astrophysical Journal* (United States) was the most prominent astronomy journal in the world. Its circulation was from three to ten times larger than that of any of the other astronomy journals. The quantity of material it published was considerably greater than that of any of the other astronomy journals, and the content was of high quality. Some medium-sized European astronomical institutes had two subscriptions to this journal, whereas one subscription to the other journals was considered sufficient. Many European astronomers subscribed to it personally, and also to their own "national" journal. It is small wonder that in such a situation some European astronomers began thinking about publishing their own work in the *Astrophysical Journal* in order that it be more widely read.

It was true that work not published in the *Astrophysical Journal* was less likely to be cited, for example, in some review articles, especially by American authors. And often articles of more limited interest published in the smaller European journals were never seen by some astronomers. Many European astronomers would therefore order 300 to 500 reprints of their articles in these smaller journals and mail them to those who might possibly be interested. That such an inefficient and time-consuming method of circulation was thought necessary was an indication that something was wrong. Another indication was that such journals as the *Annales d'Astrophysique* and the *Bulletin of the Astronomical Institutes of the Netherlands* were each cited in the list of references in the *Astrophysical Journal* only about 1 percent of the time, and the articles in English in the *Annales d'Astrophysique* were cited twice as often as the articles in French.

All of this evidence suggested that the older journals, which were "national" or "seminational," were not growing as fast as the total number of articles on astronomy or astrophysics. Astronomers solved the problem of where to publish these articles in two ways. First, some articles have been published in newly created journals, which have begun to appear over the last 10 years. These were sponsored by private publishers whose main interest was not in science. Because a certain number of libraries and astronomical institutes subscribe to all publications on astronomy, a small profit is assured to each of these publishers. But the astronomy literature can easily suffer from this kind of proliferation. Second, since the expansion of research in astronomy in the rapid tempo of the last 10 years was accomplished in part by physicists, some of them began publishing results of their research in astronomy in physics journals, mainly because they were familiar with these journals and there existed no obvious alternative astrophysics journal.

Confronted with this situation, many European astronomers took initiatives, at first independently and later jointly. In addition to the efforts of individuals

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