A Viscosity Model of Polyacrylamide Gel Electrophoresis

Hans-Joachim Bode

Zoologisches Institut der Universität, Im Neuenheimer Feld 230, D-6900 Heidelberg 1

Z. Naturforsch. **34 c,** 512 – 528 (1979); received March 22, 1979

Polyacrylamide, Molecular Sieve, Electrophoresis, Viscosity, Frictional Coefficient, Partition Coefficient

In current theories of polyacrylamide gel electrophoresis, the idea prevails that molecular sieving relies on different accessibility of volume fractions and of cross-sectional area fractions (denoted "pores") to different-sized ions due to the effect of "geometric exclusion". This correlates with the assumption that all elements of a polyacrylamide network occupy fixed and unchangeable positions thus forcing colliding macro-ions to diffuse laterally in order to find an "accessible pore" and to resume motion in direction of the electrical field. However, the alternative conception would be equally well justified, *i. e.* the assumption that polyacrylamide chains represent smooth obstacles cleared aside under the electrokinetic pressure of a macro-ion. This explanation would even be preferable with respect to the molecular sieving effects occuring in solutions of "liquid polyacrylamide". Yet no theory exists as to describe such effects in quantitative terms.

In the present article, a parameter is defined and discussed, which can be estimated by experiment, and which seems to be apt to characterize local resistivity of polymer structures against dislocation and deformation: the "fractional specific resistance". Definition of this parameter is based on the model of a "viscosity-emulsion" composed of two interpenetrating liquid compartments which are characterized by different levels of hydrodynamic friction and the spatial dimensions of which are inferred from Ogston's theory. This concept of "localized viscosity" may also serve as a link between theories of molecular sieving and of "macroscopic viscosity" of flexible polymers. The data of Morris, formerly taken as verifications of the "rigid-pore" concept, are now interpreted in terms of four factors responsible for sizediscrimination: collision frequency, duration of single contacts, size-dependent frictional force, and the extent of cooperation among fibres, due to crosslinking and to simultanous contacts of several fibres to a single macro-ion. Some functions relevant for problems of molecular weight determination by gel electrophoresis are discussed in relation to the suggested model.

Introduction

Polyacrylamide gel electrophoresis [1 – 8] has become one of the most wide-spread methods of macromolecular fractionation in biochemistry, developmental physiology and molecular genetics. This is the consequence of a favourable combination of polyacrylamide properties not found in other electrophoretic support materials: cheapness, stability, compatibility with various staining and evaluation procedures, adaptability to a wide range of fractionation problems, high resolving power especially when combined with discontinuous buffer systems (= disc-electrophoresis) [3, 4]. The most prominent property, however, which distinguishes polyacrylamide gel electrophoresis from "non-sieving" electrophoretic methods is the capability to discriminate sizes of macro-ions. In homologous series of macroions having identical free-solution mobilities, such as nucleic acids [9, 10] and SDS-complexed proteins [11 – 13], this leads to a monotonous relationship between size and mobility within the gel which enables molecular weight estimation of unknowns, after proper calibration, in single experiments with minimal need of material. In part, this capability is shared by starch [14, 15] and agarose gels [16, 17]. Molecular size discrimination is also found in some related laboratory techniques: dialysis [18], gel filtration [19, 20], ultracentrifugation in the presence of linear polymers [21], and molecular-sieve electroosmosis [22].

Notwithstanding the great success of polyacrylamide gel electrophoresis, it can be shown that the theoretical foundations of this method are not yet established, since important possible interpretations are neglected by current theories. Since the first papers dealing with electrokinetic molecular sieving, interpretations of the process were inspired by the idea of a congruence in the dimensions of the macroions under investigation and the dimensions of interstices left between the gel structures, *i. e.* the "pores" [1]. Selective mechanisms based on the direct

Reprint requests to Dr. H.-J. Bode.

0341-0382/79/0700-0512 $ 01.00/0

[1] Smithies (ref. 14): "... the pore size of which approaches the molecular dimensions of some of the proteins involved."

comparison of sizes of macro-ions and widths of the holes, similar to processes occuring in rigid technical sieves, seemed to be conceivable and appropriate models. The accurate description of "pore" geometry is, therefore, a major concern of many authors in the field (3 – 8, 23 –31). In a first quantitative approach, Ornstein [3] envisaged a cubic lattice of regularly arranged straight fibres. This was criticized by Raymond and Nakamichi [31] on the basis of White's physical pore size measurements [32, 33]. In contrast to Ornstein, Tombs suggested a model involving Gaussian pore size distribution [34, 35] and formulated a hypothesis still inherent in contemporary theories of electrophoretic molecular sieving in modified form: he suggested that the electrophoretic mobility of a macro-ion be proportional to the number (resp. cross-sectional area fraction) of "pores" accomodating its passage. Morris [36, 37] largely improved this concept by combining it with Ogston's [38] geometric exclusion theory known to govern partition between inner and outer gel volume fractions in gel filtration [39, 40], and by apparently verifying the above-mentioned postulate of Tombs by experiment. Subsequently, Rodbard and Chrambach [5, 7, 8, 41 – 43, 69] made efforts to generalize this concept and contributed much to its wide acceptance [6, 23, 30, 44, 45].

Nevertheless, some authors seemed to be reluctant [16, 46, 47] to accept concepts based on geometrically static "pores" [2], since vinyl polymers like polyacrylamide would rather be expected to have flexible random coil conformation [4, 48, 49]; this also underlies a hypothesis concerning swelling behaviour of gels [50]. Yet there were no strong arguments to substantiate molecular flexibility of the sieving matrix, besides some qualitative hints [27, 29, 31, 51, 52]. Consequently, flexibility of the polymers has always been treated as marginal by theoreticists [3]. Recent experiments using linear polymers stabilized between macroporous supports gave strong hints, however, that molecular sieving may not rely solely on the geometry of interstices, but rather on the kinetics of deformation and dislocation of floating polymer chains [53 – 57]. As shown in Fig. 1, very similar effects on the size-mobility relationships of nucleic acids and SDS-protein complexes can be achieved when the lengths of dissolved polymers and of single chains of crosslinked gels are varied; these effects cannot be explained by "geometric exclusion", but urge us to envisage alternative interactions between polymers and macro-ions such as "hydrodynamic" activity of the polymer matrix (for more complete discussion *cf.* refs. 55 and 56). As yet, there seemed to be no possibility to integrate the geometrical and statistical arguments of the "geometric exclusion theories" [5, 7, 41, 42] into this concept in quantitative terms. This is overcome in the hypothesis presented in the following sections. Reevaluation of the data of Morris and Morris [37] according to this hypothesis will demonstrate that a concept of dynamic interplay of pushing forces and responding polymer resistance can reasonably replace the "static pore" concept.

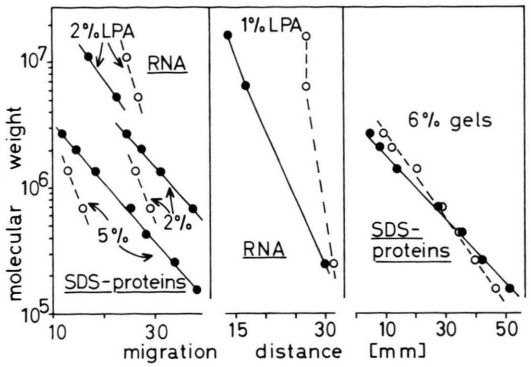

Fig. 1. Effects of polymer chain length on molecular sieving in electrophoresis. Long chain polymers are more efficient in discriminating sizes of SDS-protein complexes and of nucleic acids than short chain polymers. This is demonstrated in mixed gels composed of agar-agar and liquid polyacrylamide (= LPA, uncrosslinked polyacrylamide) (diagram at left), in solutions of liquid polyacrylamide soaked in celluloseacetate (middle diagram), and in crosslinked polyacrylamide gels (diagram at right) (compare the slopes!). Closed circles: long chain polymers; open circles: short chain polymers; % = g polymer/100 ml. Polymer chain length was varied in either case by inclusion of different amounts of initiators (tetramethylethylenediamine, ammonium persulfate, for details *cf.* refs. 54 –56) into the polymerization mixtures. The observed effects have opposite sign relative to the effects expected on the basis of purely "geometric" exclusion (see refs. 41, 42, 55, 56).

Proposed model

The following parameters are relevant for the model: electophoretic mobility u [cm^2 $volt^{-1}$ sec^{-1}], du-

[2] Hjertén (ref. 46): "The expression 'molecular sieving' ..... should not be associated with the separation mechanisms, ..."

[3] Morris and Morris (ref. 37): "... these may be locally deformed ..., but the subject undoubtedly requires further investigation ..." Rodbard (ref. 7): "It remains unclear whether such molecules "blast" or tunnel their way ..... in a ballistic manner ..."

ration of electrophoresis t [sec], migration distance s [cm], frictional coefficient f [dyn · sec · cm^{-1}]. For convenience, the electrical field is assumed to be 1 volt/cm. Consequently, the electrophoretic mobility is equivalent to migration velocity. Indices of the parameters refer to the respective conditions under which they are effective, *e. g.* $/_0$ = parameter in free solution, $/_p$ = parameter in a polymer solution or a gel. The ratio of the mobilities pertinent to gel or to free solution is given by: $u_{rel} = u_p/u_0$.

Basic assumptions

1. The distribution of gel elements in space is essentially given by Ogston's theory of randomly oriented fibres [38, 39]. Gel fibres are assumed to consist of randomly coiled polymer segments. In analogy to Ogston's theory, the total gel volume is subdivided in two fractions: compartment F which is freely accessible by diffusion for the centre of a particular macro-ion, and compartment C (= "contact zone") which surrounds the polymers and can only be entered by the centre of the ion under the condition of surface contact (Fig. 2). The minimal distance for avoidance of contact is the sum of the radii, *i. e.* $(r + R)$. Hence, it is a function of the size of the macro-ion. Parameters pertinent to the zones C and F are labelled by the indices $/_c$ and $/_f$, respectively.

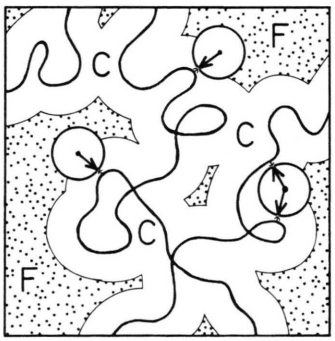

Fig. 2. Compartmentation of spaces within a random array of gel fibres. Dotted area: zone F which accomodates the centres of spherical molecules (white globules) without making surface contacts between molecules and fibres, hence, zone available for free diffusion (Ogston-theory); blank area: complementary zone C accessible only under the condition of surface contacts, hence, "contact zone" accessible only for molecules driven by a vectorial force able to dislocate the fibres. The minimal distance for avoidance of contacts is the sum of the globule radius (R) and of the fibre radius (r) (see arrows). C is largely determined by the globule radius, when $R > r$.

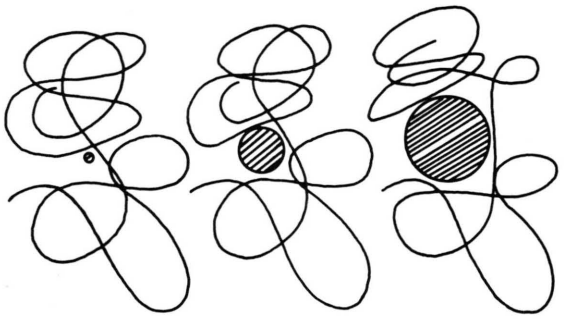

Fig. 3. Shape changes of randomly coiled polymer molecules induced by penetrating ions. a) Unperturbed conformation, very small ion; b) slightly distorted conformation during passage of an intermediate ion:; c) more strongly distorted conformation during passage of a macro-ion. Migration of ions is perpendicular to the drawing plane.

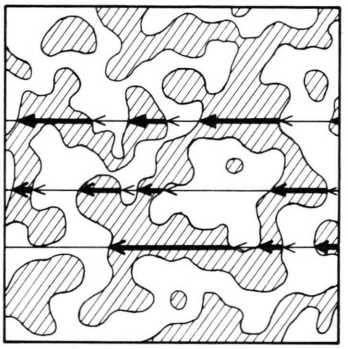

Fig. 4. Longitudinal section through a "viscosity emulsion". Blank areas: "contact-free zone" F within which the frictional coefficient corresponds to the free-solution value ($f_f = f_0$); dashed area: "contact zone" C which surrounds gel fibres according to Ogston's theory and within which the frictional coefficient is enhanced ($f_c > f_0$); arrows: migration pathways of several point-ions (centres of macro-ions) in the emulsion. According to the principle of Delesse, the "fractional contact distance" (integral of bold arrows) is equal to the volume fraction occupied by the "contact zone" ($s_c/s_p = C$).

2. The electrical field is assumed to be homogeneous. The charge and driving force of a macro-ion are constant throughout the gel.
3. Macro-ions move straight-on according to the electrical field vector.
4. The polymers yield the electrostatic pressure exerted by colliding macro-ions (Fig. 3). Their resistivity against dislocation can be quantitated by use of hydrodynamic frictional coefficients (neglecting the possible contribution of weak elastic interactions to the total resistive force) [4].

[4] under conditions of sufficiently high electrical field strength.

5. The total migration distance of an ion is composed of two fractions: the sum of distances covered in the "contact-free zone", and the sum of single stretches covered within the "contact zone". The ratio of these fractions ("fractional contact distance") is given by the ratio of the corresponding volume fractions (extended principle of Delesse; Fig. 4, this text; refs. 41 and 58).

These assumptions can be formulated as follows: complementarity of the volume fractions:

$$C = (1 - F), \tag{1}$$

constancy of the driving forces:

$$K = K_0 = K_p = K_f = K_c, \tag{2}$$

rectilinearity (= additivity) of the migration distances:

$$s_c + s_f = s_p, \tag{3}$$

application of the principle of Delesse:

$$s_c : s_f = C : F, \tag{4}$$

application of hydrodynamic frictional coefficients:

$$K = u \cdot f = u_0 f_0 = u_p f_p = u_f f_f = u_c f_c. \tag{5}$$

By using the definition of migration velocity, Eqn (3) can be transformed into:

$$u_c\, t_c + u_f\, t_f = u_p\, t_p. \tag{6}$$

The total duration of electrophoresis is the sum of the time fractions:

$$t_c + t_f = t_p. \tag{7}$$

Division of Eqn (6) by u_f and by t_p, insertion of Eqn (7) into the modified Eqn (6), and subsequent reduction of the items by t_f or by t_c, respectively, ensues:

$$\frac{u_p}{u_f} = \frac{1}{1 + t_c/t_f} + \frac{u_c/u_f}{1 + t_f/t_c}. \tag{8}$$

By using the definition of migration velocity, Eqn (4) is transformed into:

$$\frac{t_c}{t_f} = \frac{C}{F} \cdot \frac{u_f}{u_c}. \tag{9}$$

Eqn (5) ensues:

$$u_c/u_f = f_f/f_c. \tag{10}$$

When Eqns (1), (9), and (10) are inserted into Eqn (8), and the items on the right side are brought to a common denominator, we obtain:

$$\frac{u_p}{u_f} = \frac{f_f \cdot F + (f_f/f_c)\,(1 - F) \cdot f_c}{f_f \cdot F + (1 - F) \cdot f_c}. \tag{11}$$

When the term on the right is reduced by f_f, the total equation rewritten in reciprocal form, and when 1 is subtracted from either side, this ensues:

$$\left(\frac{u_f}{u_p} - 1\right) = \left(\frac{f_c}{f_f} - 1\right) \cdot (1 - F). \tag{12}$$

After dividing this equation by $(1-F)$, and after transformation of the terms in brackets, we arrive at:

$$\frac{(f_c - f_f)}{f_f} = \frac{(u_f - u_p)}{u_p} \cdot \frac{1}{(1 - F)}. \tag{13}$$

Eqn (13) is essential for our model, and can easily be transformed for practical purposes, if certain assumptions are made. The parameters u_f and f_f can plausibly be equated with u_0 and f_0. This transforms the first factor of the right side into the "microscopic specific viscosity" defined in an earlier paper [56]:

$$\frac{u_0 - u_p}{u_p} = \frac{f_p - f_0}{f_0} = \frac{\ddot{\eta}_p - \eta_0}{\eta_0}. \tag{14}$$

The volume fraction F in Eqn (13) is equivalent to the volume fraction f of Ogston's theory which can be estimated by gel filtration as K_{av}:

$$F = K_{av} = \frac{V_e - V_0}{V_t - V_0} \tag{15}$$

(V_e, V_0, and V_t = elution volume, void v., and total v., resp.). After replacing f_f by f_0, the left side of Eqn (13) may serve as definition for a novel parameter, the "fractional specific resistance" FSR, the significance of which has to be discussed in the following sections. After all these replacements, and after reduction of the right side by u_0, Eqn (13) can be rewritten:

$$FSR = \frac{f_c - f_0}{f_0} = \frac{1 - u_{rel}}{1 - K_{av}} \cdot \frac{1}{u_{rel}}. \tag{16}$$

Assuming that the frictional coefficient f_c is composed of the coefficient of the ambient fluid f_0 and of the complementary contribution of the polymer itself, and provided the propelling force of the considered macro-ion is known, we can calculate an absolute force K_{cp} directed by the macro-ion towards the polymer:

$$K_{cp} = \frac{(f_c - f_0) \cdot u_c}{f_c \cdot u_c} \cdot K_c = \frac{FSR}{FSR+1} \cdot K. \qquad (17)$$

Significance of gel compartmentation and of "fractional specific resistance" [5]

As suggested by gel filtration analyses, the internal structure of dextran gels and of granulated polyacrylamide gels is compatible with Ogston's random fibre model [38 – 41], the square radii of the interstices between the polymers being distributed according to a chi-square distribution [41, 59]. Morris found that the partition coefficients K_{av} of native proteins, as determined in granulated polyacrylamide, are numerically almost equal to the reduced electrophoretic mobilities measured in continuous gels, provided the two parameters had been estimated within gels of identical internal structure [36, 37]. This finding indicates that small proteins, which are only slightly retarded, dispose of a large volume fraction for free diffusion and, consequently, for unimpeded motion within the gel. As the electrical field is maintained by small salt ions [24] present in excess, the flow of current inevitably drives macro-ions into collisions [6] with polymers. In contrast to gel filtration, where macromolecules are presumably propelled by diffusion, *i. e.* random thermic agitation, and where weak elastic forces may prevent centres of macromolecules from diffusing into the volume fractions prohibited by "geometric exclusion" (Fig. 2), the suggested model postulates that vectorial forces enable macro-ions in electrophoresis to push flexible gel fibres aside which consist of randomly coiled segments of polymer (Fig. 3). This process divides the migration pathway into stretches where the macro-ions are opposed by accessory frictional forces and intermittent intervals where they can move according to free solution mobility. The contour line, which defines the centre positions of macro-ions in the moments where surface contact with polymers begins and where it ends can tentatively be equated with the contour line separating available and non-available zones in diffusion, *i. e.* in gel filtration. Hence, K_{av} should be complementary to the volume fraction ($C = 1 - F$), in which accessory friction takes place. Provided this is correct, the gliding motion of a macro-ion through fluid spaces interwoven with polymers can be formally replaced by the passage of a point ion (centre of a macro-ion) through a "viscosity-emulsion" composed of interpenetrating compartments of different viscosity (Fig. 4). In this model, the "fractional specific resistance" *FSR*, defined in Eqn (16), corresponds to the imaginated "specific viscosity" of the more resistive compartment (taking the less resistive compartment as reference) [7]. As random interpenetration is presumed, application of the principle of Delesse [58] is justified and the "fractional contact distance" ($= s_c/s_p$) can be equated with ($1 - K_{av}$).

According to Eqn (16), every value of u_{rel} can be interpreted as the result of two preconditions: frictional resistance of the polymers on the one hand, and geometrical interrelations between gel fibres and macro-ions on the other hand, including all details of conformation which influence K_{av} [5, 41]. Conversely, pairs of K_{av} and u_{rel} which fit Eqn (16) can be calculated for any arbitrarily chosen value of *FSR;* this provides the net of lines shown in Fig. 6, which may be used to rank empirical pairs of K_{av} and u_{rel} according to the level of frictional resistivity expressed in them.

Critical notes on the basic assumptions

The length of the "fractional contact distance" is inferred, in our model, from the contours of the two compartments of a "viscosity-emulsion" (Fig. 4). In reality, a gel is no such emulsion, and we have to look for factors in the polymer-ion interaction which are able to justify the use of this analogy. Numerically, the "fractional contact distance" should be given by [7, 39, 41, 42]:

$$s_c/s_p = C = (1 - K_{av}) = 1 - \exp(-\pi \cdot L \cdot (r + R)^2) \qquad (18)$$

(L = effective fibre length per unit volume, r = gel fibre radius, R = radius of macro-ion).

[5] The expression "fractional specific resistance" was chosen in analogy to the preexisting terms "specific viscosity" and "fractional available volume"; paradoxically, *FSR* relates to the volume fraction which is not available for free diffusion.

[6] Although in the following text "collisions" are treated as if physical surface contact between polymers and macro-ions were taking place, it would easily be possible to extend this concept by subsuming all processes in which force is only mediated by the ambient fluid.

[7] This analogy is purely imaginative, but cannot be realized physically as it would not be compatible with homogeneity of the electrical field.

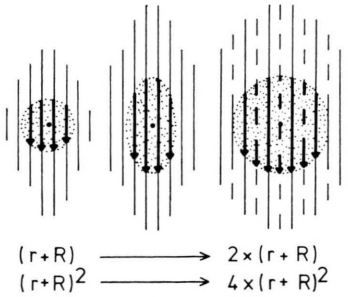

Fig. 5. Influences of the effective molecular radius $(r + R)$ on single intervals of the "fractional contact distance". Dotted areas: cross-sections of "viscous tubules" pertinent to the "contact zone"; lines: electrical field lines; bold arrows: single intervals of the migration pathways of point-ions covered within "viscous tubules". An increase in the "effective" radius $(r + R)$ not only lengthens the average intervals (see intermediate stage), but also enhances the probability of point-ions to be projected onto "viscous tubules" (additional broken arrows in the right drawing).

For low values of C, *i. e.* low polymer concentration and/or small ion, this function can be approximated by:

$$s_c/s_p = C = \pi \cdot L \cdot (r + R)^2. \tag{19}$$

It is conceivable, intuitively, that the probability of gel fibres to be hit by randomly projected spherical molecules rises linearly with concentration of the fibres (expressed in L) and with first power of the combined lateral extensions of projected molecules and gel fibres, *i. e.* $(r + R)^1$. For geometric reasons, this is equal to the probability of "viscous tubules" of radius $(r + R)$ in an emulsion to be hit by point-ions (centres of macro-ions). However, Eqn (19) suggests a square relationship. In a "viscosity-emulsion", this duplicate effect would be provided by probability of hit on the one hand, and by length of the single stretches, on the other hand, point-ions have to travel "within viscous tubules" as demonstrated in Fig. 5. In reality, polymer chains have to be dislocated by the macro-ions, and suggestions on average distances lying between the initial and final positions of macro-ionic centres contacting a polymer are pure speculation. Of course, there should be a monotonously rising relationship between radius R and that distance, but it might be non-linear. Therefore, $(1 - K_{av})$ in Eqn (16) and in Eqn (18) should be replaced by the general formulation:

$$s_c/s_p = f(C) = f(1 - K_{av}). \tag{20}$$

As function f might also reflect systematic differences between types of macro-ions (compact proteins, flexible nucleic acids), this would cause systematic variations of *FSR* independent of the properties of gel fibres. The "true" value of *FSR* relating strictly to polymer properties would be related to the "apparent" value by:

$$\text{"true" } FSR = \frac{1 - K_{av}}{f(1 - K_{av})} \cdot FSR_{app}. \tag{21}$$

This uncertainty of the model cannot be overcome, as yet.

The model attributes a constant value of *FSR* to all stages of a collision, although the resistive force of a polymer chain is likely to rise first to a maximum and then to decline again, and it is probably dependent on whether the polymer hits the macro-ion centrally or marginally. *FSR* can only give an average statistical estimate for a large population of collisions which are not yet seizable as single events.

A more important objection may be raised against one essential assumption inherent in the model, *i. e.* straight-on migration of the macro-ions through "contact-zones". In his molecular sieving theory, Morris [36] postulated quite the contrary by assuming fixed positions of the gel fibres and lateral circumventory diffusion of macro-ions according to the "barrier"-model (see Fig. 1 of ref. 60), which would result in size-dependent retardation. This mechanism is not considered in our model, although size-sensitive effects of solid polymer supports on diffusion rates have been demonstrated, recently [61]. Since "circumventions" of obstacles are obviously possible, neglection of these processes might lead, under certain conditions, to erroneous interpretation of *FSR* by underestimation of actual resistive forces. After collision, the reaction of a polymer is likely to be a function of the following variables: the level of resistive force against deformation, concavity of the surface exposed towards the macro-ion, and excentricity of the collision, *i. e.* distance of the mass centres in a plane perpendicular to the electrical field vector. Circumventory movements should be favoured by rigidity, convexity and excentric hit of the collision partners; within fibrous gels, these conditions would be met in one-to-one collisions (1 fibre/1 macro-ion). However, when the macro-ion collides with an agglomeration of flexible fibres, circumventory diffusion can be expected to be a less efficient and, therefore, less frequent mechanism as compared to straight-on "penetration" of the contact zone. Hence, "circumvention" should hardly affect the concor-

dance of *FSR* and deformation resistance under these conditions. A possible way to solve the dilemma in the case of one-to-one collisions will be considered in a subsequent chapter.

One factor effective in some electrophoretic support media is not considered since it is probably irrelevant to polyacrylamide gel electrophoresis, *i. e.* the occurence of electrically insulating and mechanically stabilizing micelles [62]. Maybe this has to be considered in agar-gels [63]. *FSR* refers only to those gel elements which do not disturb the flow of current noticeably; it cannot account for mechanical stabilization by gel structures lying outside the electrical field.

Application to empirical data

FSR can be calculated whenever the values of K_{av} and u_{rel} of a particular macro-ion are known at defined gel concentration. Application of Ogston's theory to gel filtration [39 – 41] led to:

$$K_{av} = 10^{-k \cdot T}. \tag{22}$$

(T = % gel concentration).

A formally similar, empirical relationship ascertained by numerous reports [23, 37, 64 – 69] has been formulated in electrophoresis:

$$u_{rel} = 10^{-K_R \cdot T} \tag{23}$$

(K_R = retardation coefficient).

The constants k and K_R of these equations are characteristic of the types of gel and macro-ions used. When these equations are inserted into Eqn (16), we obtain a theoretical relationship between *FSR* and gel concentration:

$$FSR = \frac{1 - 10^{-K_R T}}{1 - 10^{-kT}} \cdot 10^{+K_R T}. \tag{24}$$

At high gel concentrations, this approaches the function $10^{K_R T}$, and rises sharply. At low concentrations, a minimum of *FSR* may or may not occur, depending on the ratio of k to K_R.

For valid estimations of *FSR*, the pairs of K_{av} and u_{rel}, or of k and K_R to be inserted into Eqn (16) or Eqn (24), respectively, must have been determined in gels of identical internal structure. This prerequisite is hardly fulfilled by data combined from different publications or issued from different laboratories. As yet, the only experiments suitable for this type of evaluation are those published by Morris and Morris [37], who took great care of comparability of the gels used in gel filtration and in electrophoresis. Their electrophoretic mobilities, the corresponding K_{av}-values, and the calculated *FSR*-values of eight native proteins are listed in Table I to serve as the empirical basis for the Fig. 6 – 8. The data obtained for several arbitrarily chosen gel types of Morris are superimposed on the background of theoretical lines of constant *FSR* in Figs. 6a and 6b. As mentioned by Morris, the points are lined up near the diagonal in a plot of K_{av} versus u_{rel}. By comparison with the theoretical *FSR*-lines, one can conclude that *FSR* tends to rise concomitant with the rise of

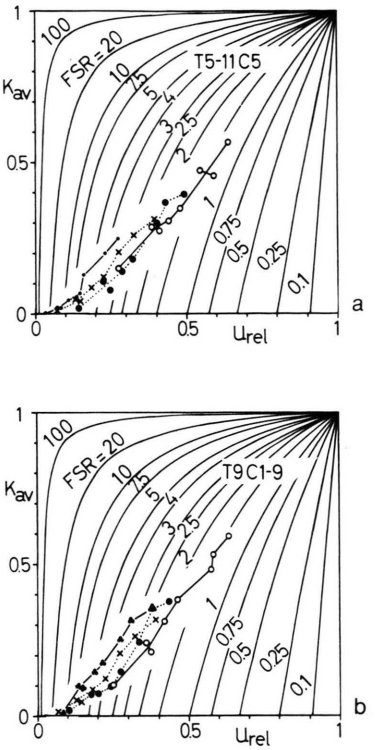

Fig. 6. Empirical data of Morris projected onto the net of theoretical lines of constant *FSR*. Empirical data have been extracted from Table I. The points pertinent to eight different-sized proteins are linked to form "gel-specific lines". Levels of *FSR* are given by the numbers beside the theoretical hyperbolas. a) "Gel-specific lines" at constant crosslinking ratio (C = 5%), but varying gel concentration T; open circles: T = 5%, closed circles: T 7%, crosses: T = 9%, small points: T = 11%. b) "Gel-specific lines" at constant gel concentration (T = 9%), but varying crosslinker content; open circles: C = 1%, closed circles: C = 3%, crosses: C = 7%, rosettes: C = 9%. The location of empirical points within the diagram is influenced by molecular size of the proteins, by gel concentration, and by the crosslinking ratio.

Table I. Relative electrophoretic mobility (u_{rel}), partition coefficient (K_{av}), and "fractional specific resistance" *(FSR)* of eight native proteins under a variety of conditions.
T = gel concentration (g monomer/100 ml solution), C = crosslinking ratio (g bisacrylamide/100 g total acrylamide). Values of u_{rel} were calculated from the single mobilities of the proteins, given by Morris and Morris (ref. 37, Table II) and from the average extrapolated free solution mobilities *) (ref. 37, Table IV, last column); K_{av} values and Stokes' radii are from the same source; *FSR* was calculated from u_{rel} and K_{av} according to Eqn (16).

Protein Stokes' radius	T % C %	7	9	11 1	13	15	7	9 3	11	5	7 5	9	11	5	7 7	9	11	5	7 9	9	11
coerulo-	u_{rel}	.384	.252	.214	.111	.072	.169	.103	.053	.274	.142	.069	.032	.244	.130	.072	.031	.244	.134	.082	.043
plasmin	K_{av}	.220	.100	.016	.0	.0	.035	.014	.0	.150	.020	.012	.0	—	.030	.0	.0	.057	.020	.0	.0
4.73 nm	*FSR*	2.05	3.29	3.73	8.00	12.88	5.09	8.83	17.86	3.11	6.16	13.65	30.25	—	6.89	12.88	31.25	3.28	6.59	11.19	22.25
transferrin		.458	.356	.255	.186	.140	.250	.178	.105	.382	.224	.131	.068	.334	.203	.136	.071	.337	.200	.134	.085
		.350	.240	.091	.043	.012	.150	.069	.0	.288	.108	.049	.020	—	.141	.053	.034	.309	.193	.096	.050
3.50 nm		1.82	2.38	3.21	4.57	6.21	3.52	4.96	8.52	2.27	3.88	6.97	13.98	—	4.57	6.70	14.58	2.84	4.95	7.14	11.33
serum		.471	.374	.287	.203	.166	.279	.201	.127	.407	.248	.148	.083	.355	.226	.148	.080	.361	.226	.150	.099
albumin		.360	.210	.072	.036	.012	.114	.071	.024	.274	.080	.043	.020	.280	.118	.040	.028	.290	.180	.091	.040
3.65 nm		1.75	2.11	2.67	4.07	5.08	2.61	4.27	7.04	2.00	3.29	6.01	11.27	2.52	3.88	5.99	11.83	2.49	4.17	6.23	9.48
hemoglobin		.552	.418	.340	.271	.202	.313	.244	.154	.439	.286	.185	.107	.424	.271	.185	.116	.391	.271	.189	.131
		.450	.310	.153	.077	.052	.192	.097	.069	.308	.144	.086	.045	—	.176	.100	.052	.385	.234	.144	.090
3.08 nm		1.47	2.01	2.25	2.91	4.16	2.71	3.43	5.90	1.84	2.91	4.81	8.73	—	3.26	4.89	8.03	2.53	3.51	5.01	7.28
ovalbumin		.548	.459	.366	.296	.232	.349	.275	.196	.478	.320	.225	.144	.440	.301	.227	.148	.433	.284	.215	.156
		.490	.380	.211	.132	.080	.269	.144	.100	.347	.180	.120	.068	.340	.204	.106	.074	.412	.250	.174	.108
2.80 nm		1.61	1.90	2.19	2.74	3.59	2.55	3.07	4.55	1.67	2.59	3.91	6.37	1.92	2.91	3.80	6.21	2.22	3.36	4.42	6.54
carbonic		.663	.572	.430	.373	.289	.400	.336	.242	.542	.400	.272	.158	.505	.363	.309	.191	.454	.326	.272	.212
anhydrase		.590	.480	.312	.235	.173	.361	.241	.167	.471	.300	.204	.132	—	.320	.207	.154	.534	.380	.251	.186
2.35 nm		1.23	1.43	1.92	2.19	2.97	2.34	2.60	3.76	1.59	2.14	3.36	6.13	—	2.58	2.81	5.00	2.58	3.33	3.57	4.87
trypsin		.661	.579	.473	.401	.345	.454	.378	.285	.590	.429	.323	.231	.552	.410	.339	.240	.538	.388	.310	.253
inhibitor		.650	.530	.367	.310	.246	.419	.353	.240	.455	.368	.260	.201	—	.406	.267	.195	.618	.431	.311	.250
2.26 nm		1.46	1.54	1.76	2.16	2.51	2.06	2.54	3.30	1.27	2.10	2.83	4.16	—	2.42	2.66	3.93	2.24	2.77	3.23	3.93
lactalbumin		.736	.630	.538	.485	.403	.500	.433	.332	.639	.490	.389	.273	.611	.471	.409	.301	.577	.434	.374	.315
		.670	.590	.442	.380	.311	.449	.378	.284	.565	.395	.314	.248	.530	.441	.320	.247	.618	.470	.359	.284
2.01 nm		1.08	1.43	1.53	2.18	2.15	1.81	2.10	2.81	1.29	1.72	2.28	3.54	1.35	2.00	2.12	3.08	1.91	2.46	2.61	3.03

*) Morris and Morris used the single extrapolated values pertinent to different cross-linking ratios to calculate u_{rel}; this causes minor differences between their diagrams and Fig. 6 (this text).

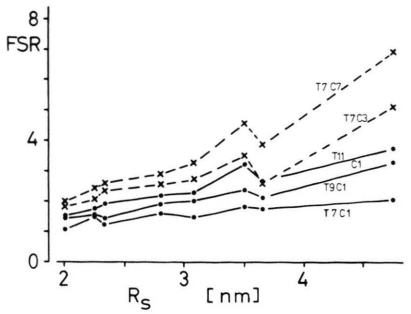

Fig. 7. Relationship between Stokes' radius and *FSR* in various gel types. R_S = Stokes' radius (*cf.* Table I for identification of the proteins); dashed lines: gels of identical crosslinking ratio, but varying gel concentration as indicated by the numbers beside the curves (*T*% *C*%); broken lines: gels with enhanced crosslinking ratios at constant gel concentration.

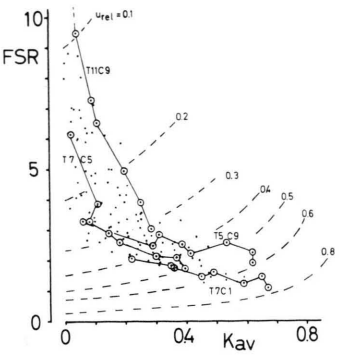

Fig. 8. Relationship between "fractional specific resistance" *FSR*, available free space K_{av}, and electrophoretic mobility u_{rel}. All data of Table I up to $FSR = 10$ are plotted. The points of some selected gel types are joined together to show that similar tendencies are found in single gels as in the total set of data.

molecular diameter, *i. e.* when K_{av} declines. Increase of gel concentration (upper diagramm) as well as enhancement of cross-linking ratios (lower diagramm) shifts the points toward the origin, thus enhancing the level of *FSR* of an ion.

These relationships are demonstrated more directly in Fig. 7. Both enhancement of the concentration of total polymer (= *T*) and enhancement of crosslinker content (= *C*) increase *FSR*; the content of bisacryamide seems to be particularly effective in this respect. Moreover, crosslinking reinforces the molecular size-dependence of *FSR* considerably. This might be due to the integration of several fibres into larger cooperative units, whereas enhancement of total gel concentration at constant content of crosslinker may leave the single polymer chains relatively independent. The lowest values of *FSR*, ranging between 1 and 2, occur under the following conditions: low values of *T* and *C*, and/or small ions. Under these conditions, *FSR* seems to approximate a lower limit which presumably characterizes single chain segments of polymer and, by analogy, chains of uncrosslinked polymer [53 – 57].

It might be helpful to consider a numerical example to make the physical meaning of *FSR* clear. A value of approximately $FSR = 2$, which stands for a considerable fraction of Morris' data, implies that the frictional coefficient of the "contact zone" is three times the free solution value: $f_c = 3 \cdot f_0$. Numerically, this is equivalent to the rise in viscosity caused by admixture of glycerol to an aqueous solution up to approximately 35% (v/v) [70]. Homologous macro-ions having identical free solution mobilities u_0 and identical $FSR = 2$ would have, by consequence, identical mobilities within their respective "contact zones": $u_{c_1} = u_{c_2} = \ldots\ldots\ u_{ci} = \ldots\ldots\ u_{cn} = 1/3 \cdot u_o$. Meantime, the absolute forces K_{cp} acting between these macro-ions and polymers (*cf.* Eqn (17)) would differ according to the different electrostatic forces propelling such ions.

All data of Table I (omitting those above $FSR = 10$) are represented in Fig. 8. Although there is considerable scatter of the data, this figure reveals a surprising congruence existing between gels of different compositions. The whole set of data can be approximated by the hyperbola:

$$y = a + b/x. \tag{25}$$

The general trends expressed in this equation can also be found in the data of single gels as shown by the lines joining the single points of selected gel types. This reveals that, irrespective of gel composition, polyacrylamide can operate two alternative mechanisms to grade electrophoretic mobility: in the upper left part of the diagram, differentiation of mobilities is brought about by an enormous rise of *FSR* induced by minute K_{av}-changes, whereas in the lower right part the overall mobilities are functions of K_{av} at nearly constant level of *FSR*. This may tentatively be interpreted as follows: under conditions where a large volume fraction is available to a macro-ion for free diffusion ($K_{av} > 0.5$) and where collisions are rare, the *FSR*-value remains virtually constant and characterizes resistivity of single fibres. This entails constant relative migration rates (u_c/u_0) of all ions within their respective "contact zones". As

different-sized macro-ions have, nevertheless, different overall mobilities u_{rel} within the gel as a whole, differences must be generated by parameters correlated with K_{av}, but not with *FSR*. Collision frequency and duration of the single contacts are candidates to account for such "geometric" interactions as shown in Fig. 5. Under conditions, however, where macro-ions are almost permanently in contact with gel fibres (at $K_{av} < 0.25$ more than ¾ of the migration pathway), the frictional resistivity grows strongly. As *FSR* of single fibres was said to be low at low crosslinking ratio (Fig. 7), the observed rise of *FSR* (*cf.* also Table I) would have to originate from double, triple, or even multiple contacts between one ion and several fibres, which are favoured under conditions of low K_{av}. Such multiple contacts should be characteristic of cases in which interstices, too small to accomodate the passage of a macro-ion, are widened under its electrokinetic pressure [7, 37, 51 – 56]. It is irrelevant for such effects whether smallness of K_{av} is due to a large size of the macro-ion or to high concentration of the polymers; this causes *FSR* to have closer correlation with K_{av} than with other geometric parameters of macro-ions (Fig. 7). The capability of polyacrylamide gels to reinforce *FSR* only in those cases when "purely geometric" discrimination according to K_{av} would fail, might be one reason for the wide operation range of single gels as well as the extraordinary adaptability of the method to different fractionation problems [5, 26, 29, 71, 72].

Molecular sieving and macroscopic viscosity

According to Henry's equation [8] [73], "molecular sieving" is no corrolary of ordinary solvent "viscosity" which affects different-sized ions to similar degrees [55]. Nevertheless, it was indicated by recent experiments using uncrosslinked, liquid polymers that the two frictional phenomena have much in common. This is suggested by the correlation between "molecular sieving efficiency" and "intrinsic viscosity" [55] and by the parallelism of "macroscopic viscosity" (= viscosimetric *v.*) and the size-sensitive "microscopic viscosity" [56], a parameter which – unlike *FSR* – does not account for compartmentation of the solution (Eqn (14), footnote 9). In these experiments, an upper limit of the molecular weights of macro-ions which can be discerned seemed to be given by the molecular weight of the polymer itself: long chain polymers were able to separate the three major components of cytoplasmic RNA (4s, 18s, 28s), whereas short chain polymers were only able to separate 4s RNA from the two heavier components without discriminating the latter (Fig. 1 b, this text, and ref. 56). Hence, short polymers seem to be "blind" towards differences existing between large macro-ions like low molecular weight substances able to generate "viscosity", and the question has to be raised what property confers size-sensitivity to the longer polymers under similar conditions.

It might be an essential requirement for size-discrimination that the reactions of single polymer molecules be gradable in relation to the sizes of the ions. This would be possible, in particular, when the polymer is large relative to the ion and when it is only partially affected by the collision process. While such a polymer may undergo deformations in close proximity of the ion's pathway (Fig. 3), the distant parts of it will not be able to contribute to friction at the molecular level, although they would take part in the generation of "macroscopic viscosity" in a viscosimeter. On the other hand, macro-ions much larger than the polymer molecules can hardly be expected to rouse graded polymer reactions, since they induce perturbations of the ambient fluid reaching farther than the domains of solvent occupied by single polymer molecules. The maximal level of overall friction (*i. e.* f_p/f_0) should, therefore, correspond to the "macroscopic viscosity".

The available empirical data do not completely conform with this hypothesis which would link the theory of molecular sieving to the general electrokinetic theory [73] [9]. "Microscopic specific viscosity" was found at its most to be one quarter of the "macroscopic" value even in those cases where the polymers were "blind" towards the ions (18s and 28s RNA in short-chain liquid polyacrylamide, Fig. 1b, this text, and ref. 56). This might be due to the fact that flexible RNA molecules are able to migrate with-

[8] $u = \frac{D \cdot \zeta}{6 \cdot \pi \cdot \eta} \cdot f(\varkappa a)$; D = dielectric constant, ζ = zeta-potential, η = viscosity of the medium, $f(\varkappa a)$ = function of the Debye-Hueckel-atmosphere and of ionic radius.

[9] According to Eqs (14) and (16), the "microscopic specific viscosity" should be equal to: $\frac{1 - u_{rel}}{u_{rel}} = FSR \cdot (1 - K_{av})$. Insertion of this term would modify Henry's equation to: $u_p = \frac{D \cdot \zeta \cdot f(\varkappa a)}{6 \cdot \pi \cdot \eta_0 \cdot (1 + FSR \cdot (1 - K_{av}))}$.

in polymer solutions at higher rates than rigid molecules of similar charge and size would do; this assumption agrees with the changes induced in the molecular weight-mobility relationship of proteins by denaturation [64], and with the differences between presumptive and "effective" radii of SDS-protein complexes and nucleic acids found in gel electrophoresis [23, 44]. Compact proteins would, therefore, provide a better test for the hypothesis.

Retrospect and prospect

It has been shown in the preceding chapters that electrophoretic molecular sieving can be deduced from "hydrodynamic" frictional effects of flexible polymers without making reference to "pores" in the classical sense of immobile structures. Instead, a combination of geometrical and dynamic interactions between gel and ions is proposed. The discrete factors which contribute to size discrimination according to this model, *i. e.* collision frequency, duration of collisions, resistive force, and cooperative action of fibres, are as concise and amenable to quantification as those inherent in the "geometric exclusion theory" [5, 7, 8, 36 – 42]. The geometrical parameters, formerly assumed to be indications for a steric hindrance mechanism according to the "barrier"-hypothesis [36, 60] play a totally different role in the novel theory: no inaccessible zones, but zones of different mobilities are postulated. The excellent treatments of the possible geometrical aspects of gel-ion interaction by Morris [36, 37], Rodbard and Chrambach [5, 7, 8, 41, 42] have, nevertheless, contributed many stimulating ideas and were prerequisites for elaboration of this model.

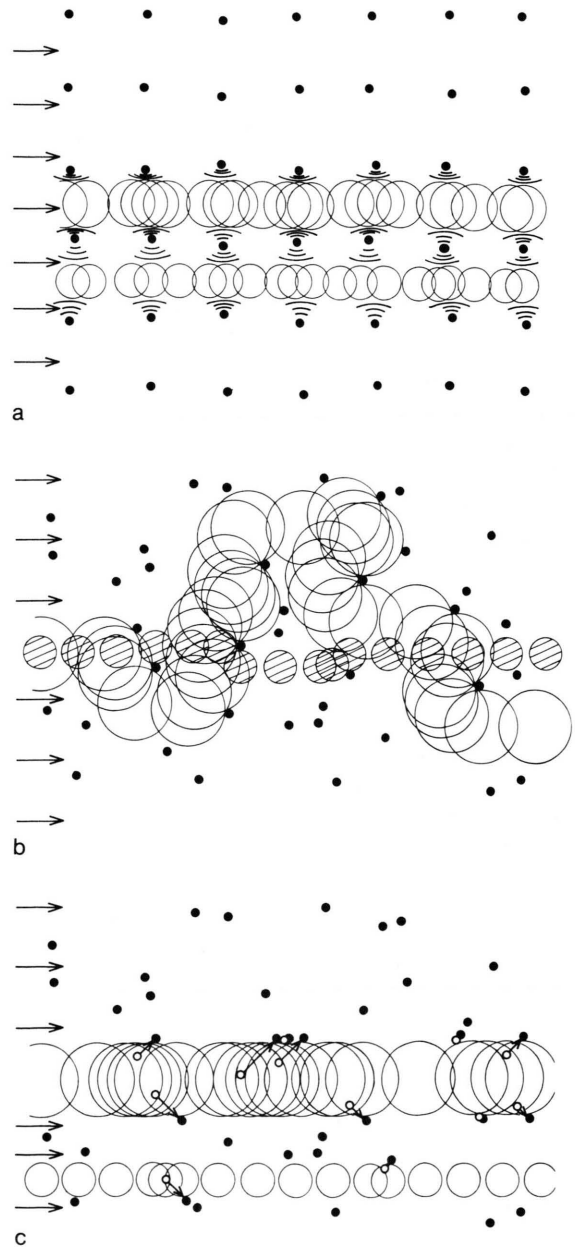

Fig. 9. Mechanisms presumed by different authors to effect "molecular sieving" in electrophoresis. a) Model of Ornstein (ref. 3): cubic lattice of equidistant, rigid fibres (small closed circles = fibre sections); differential friction originates from the shear of fluid between macro-ions (globules) and gel fibres *; macro-ions migrate in direction of the field vector (arrows on the left margin); the disadvantage of this model is to give no adequate representation of randomly polymerized gel structures. b) Model of Morris, Rodbard and Chrambach (refs. 7, 36, 41): random array of rigid gel fibres; the freedom of centres of macromolecules to move within the network is restricted by "geometric exclusion" (Ogston-theory); therefore, macro-ions of large diameter have to use time-consuming irregular side-ways; thermic agitation (Brownian movement) is assumed to enable lateral diffusion before obstacles in order to find "accessible pores" **. c) Suggested "hydrodynamic" model: random array of flexible gel fibres which can be pushed aside (see arrows between open and closed small circles) by the migrating macro-ions while exerting viscous friction; migration is along the field lines.

* This is inherent in Ornstein's arguments without being mentioned explicitly.

** Rodbard (ref. 7): ". . . the molecule will always have at least a few pores 'open' to them."

Thinking in terms of "friction" was much more popular one decade ago. Ornstein [3] used the terms "friction" and "viscosity" to denote the impeding effect of gels on electrophoretic migration. Meanwhile, he pleaded for static pores and allowed for pore enlargements only under limited and specified conditions. In his model, differential friction seemed not to be a consequence of polymer distortion, but rather to originate from the shear of fluid between travelling macro-ions and pore walls (Fig. 9a, this text; for comp. *cf.* ref. 74). While making similar assumptions on pore geometry, Raymond and Nakamichi [31] tended to postulate flexible, rather than static "pores" to account for the disparity of White's physical pore size measurements [32, 33] and known dimensions of the proteins under investigation. This line of theory aiming at "friction" was pursued by some authors for a while [75 – 77], but seemed to be obliterated by the evolving statistical rigid-pore concepts developed by Tombs [34, 35], Morris [35, 36], and Rodbard and Chrambach [5, 41, 42]. Apart from accounting for the random nature of the polymerization process and its resulting products, their models carried conviction also because of the clarity of the mathematical formalisms, thus relegating the arbitrariness of some basic assumptions to the background [10]. The main intention of this text is, therefore, to make clear that efficient size discrimination in electrophoresis and a mathematical formulation of underlying principles are equally well or even more compatible with a highly flexible polymer network as with a rigid one. [11] As shown in a previous section, the local resistivity of single polyacrylamide chains need not be higher than the viscosity of a 35% glycerol solution to represent a potent sieving agent.

Nevertheless, further evidence will be needed to make a clear-cut decision for one of the models, or to construct a compromise. Provided the postulated graded responses of gel fibres are prerequisite for molecular sieving electrophoresis, there should be a close correlation between flexibility of different types of polymers, their "fractional specific resistance", and their general suitability for highly resolving electrophoresis. The best way to test this problem would be to repeat Morris' experiments with different types of ions and with a variety of gel-forming polymers to get estimates of FSR at fixed K_{av}, and to compare these data with hydrodynamic parameters [48, 49, 78] of the same polymers in the absence of crosslinks. This should show whether or not the proportionality of K_{av} and u_{rel}, found by Morris, is universal and whether "circumvention" or "penetration" of contact zones is the predominant mechanism in polyacrylamide gel electrophoresis.

Appendix: Remarks on some functions used for molecular weight estimation

To estimate molecular weights of unknown macro-ions by gel electrophoresis, several graphic and arithmetic procedures have been suggested. Some are purely empirical, whereas others intend universal applicability. The underlying functions are rather similar in the central range of relative mobilities ($0.1 < u_{rel} < 0.9$), but diverge at the extremes where the researchers are often faced with the problem of whether "their" function allows extrapolation beyond the range covered by the standards. This has roused considerable incertainty. Therefore, it might be interesting to test the compatibility of some representative functions with our model.

Without making assumptions on the behaviour of molecules in electrophoresis, we may use the following equation, which seems to be largely accepted for homologous series of molecules, to correlate molecu-

[10] The postulated proportionality (or exponential relationship) of available volume fraction and mobility is not founded on a convincing mechanism. Geometric hindrance is only discussed with respect to "foreward" migration [36], thus neglecting its influence on lateral diffusion. Within a random network of rigid polymers, the latter effect would sometimes force macro-ions to move backwards against the potential gradient to find an accessible "pore". Moreover, Giddings and Boyack [60] were able to show that electrophoretic fractionation according to the "barrier"-concept, which underlies Morris' theory, would strongly depend on electrical field strength. In polyacrylamide gel electrophoresis, such effects occur only under specialized conditions ([44, 84] for comp. see also ref. 85) and have opposite direction.

[11] One possible reason for the wide acceptance of geometrical rigidity of the polyacrylamide network may be the intuitive idea that the number of covalent crosslinks between polymer chains were given by the molar ratio of bisacrylamide: acrylamide [47]. This would produce relatively small polymer rings extensible only to a minor degree. However, completeness of the reaction of bisacrylamide has not yet been subjected to critical examination [86]. For kinetic reasons, the reactivity of the second arm of a bisacrylamide molecule should be much reduced after incorporation of the first arm into a growing chain, as it is immobilized. Therefore, the freely floating polymer segments between points of cross-linking may be much longer than generally assumed.

lar weight with the partition coefficient [5, 7, 79 – 81]:

$$K_{av} = 10^{-c \cdot M^d \cdot T}. \quad (26)$$

(c, d = constant, M = molecular weight, T = gel concentration). The exponent d depends on the investigated molecules; moreover, differences due to the internal structure of the gel have been predicted [5, 41, 42]. Contributions of the gel fibre radius r (cp. Eqn (18)) to the "effective molecular weight" are neglected here, although they would be relevant at the lower end of the molecular weight scale.

Functions tested:

(a) $$K_{av} = e \cdot u_{rel} + f. \quad (27)$$

Functions of this type have been suggested by Morris [36, 37].

Consequently:

$$u_{rel} = \frac{10^{-cM^dT} - f}{e} \quad (28)$$

and:

$$FSR = \frac{1}{10^{-cM^dT} - f} \quad (29)$$

(b) $$K_{av} = u_{rel}. \quad (30)$$

Idealized version of (a) using $e = 1$ and $f = 0$.

Consequently:

$$u_{rel} = 10^{-cM^dT} \text{ (in analogy to ref. 66)} \quad (31)$$

and:

$$FSR = 10^{+cM^dT} \quad (32)$$

(c) $$K_{av} = (u_{rel})^g. \quad (33)$$

This was preferred over (a) by Rodbard and Chrambach [41] for theoretical reasons, namely to be compatible with the Ferguson-relationship (Eqn (23), this text, and ref. 66):

$$u_{rel} = 10^{-cM^dT/g}. \quad (34)$$

Consequently:

$$FSR = \frac{1 - 10^{-cM^dT/g}}{1 - 10^{-cM^dT}} \cdot 10^{+cM^dT/g} \quad (35)$$

(d) $$FSR = (m/K_{av}) + n. \quad (36)$$

This is an independent approximation to Morris' data (Eqn (25), this text).

Consequently:

$$u_{rel} = \frac{1}{1 + (m \cdot 10^{+cM^dT} + n) \cdot (1 - 10^{-cM^dT})} \quad (37)$$

and: $$FSR = m \cdot 10^{+cM^dT} + n \quad (38)$$

(e) $$FSR = \text{constant} = 1. \quad (39)$$

This serves as an arbitrary contrast to the other functions and might be close to the behaviour of uncrosslinked polymers in dilute solution [53 – 57].

Consequently:

$$u_{rel} = \frac{1}{2 - 10^{-cM^dT}} \quad (40)$$

and: $$K_{av} = 2 - 1/u_{rel} \quad (41)$$

(f) $$\log M = \log M_0 - p \cdot u_{rel}. \quad (42)$$

This was suggested by Shapiro and Maizel [11] and has been used, for convenience, very frequently [9, 10, 54, 55, 82], but has been subjected to criticism [7, 23, 44, 69, 70, 83].

Consequently:

$$FSR = \left(\frac{p}{\log M_0/M} - 1\right) \cdot \frac{1}{(1 - 10^{-cM^dT})} \quad (43)$$

and: $$\log K_{av} = -c \cdot (M_0 \cdot 10^{-u_{rel}})^d \cdot T \quad (44)$$

(g) $$\log M = q \cdot \log\left(\frac{1 - u_{rel}}{u_{rel}}\right) + r. \quad (45)$$

This is the modified version of a function given by Kovacic and van Holde [83], in which V_m has been replaced by u_0. The resulting formulae are equivalent to those deduced under (i).

Consequently:

$$u_{rel} = \frac{1}{1 + 10^{-r/q} \cdot M^{1/q}} \quad (46)$$

and: $$FSR = \frac{10^{-r/q} \cdot M^{1/q}}{1 - 10^{-cM^dT}} \quad (47)$$

(h) $$M^{1/2} = w \cdot \log(1/u_{rel}). \quad (48)$$

This function, which is formally related to (b) and (c), was suggested by Lehrach *et al.* [44] for agarose gels.

Consequently:

$$FSR = \frac{1 - 10^{-(1/w) \cdot M^{1/2}}}{1 - 10^{-cM^dT}} \cdot 10^{+(1/w) \cdot M^{1/2}} \quad (49)$$

and: $$\log K_{av} = -c \cdot w^{2d} \cdot (\log 1/u_{rel})^{2d} \cdot T \quad (50)$$

(i) $$u_{rel} = \frac{1}{1 + fsr\,(-\ln K_{av})} = \frac{1}{1 + 2.302 \cdot fsr \cdot c \cdot M^d \cdot T}. \quad (51)$$

This can be deduced theoretically under the following assumptions:

$$p_k \cdot s_p = s_k. \quad (52)$$

(p_k = probability of a sphere projected at random into Ogston's fibre network to be cut by k fibres simultanously; s_k = fractional migration distance of the centre of a macro-ion covered during simultanous contact with k fibres; k = zero, 1, 2, 3, ...) The number k of simultanous cuts (= contacts) is Poisson-distributed [59] and has a defined expectation μ linked to the probability of zero contacts and to K_{av} by [38, 39, 41]:

$$p_{zero} = e^{-\mu} = K_{av}. \quad (53)$$

It is further assumed that the actual value of FSR during multiple contacts ($= FSR_k$) is a multiple of its minimal value pertinent to single contacts ($=fsr$):

$$FSR_k = k \cdot fsr. \quad (54)$$

The instantanous velocity of a macro-ion during multiple contacts would then be:

$$u_k = u_0/(k \cdot fsr + 1). \quad (55)$$

The overall relative mobility would be defined by:

$$u_{rel} = \frac{u_p}{u_0} = \frac{s_p}{t_p \cdot u_0} = \frac{\sum_{k=\text{zero}}^{\infty} s_k}{u_0 \cdot \sum_{k=\text{zero}}^{\infty} t_k} \quad (56)$$

if additivity of fractional times and distances is maintained (principle of Delesse). This can be transformed as follows:

$$u_{rel} = \frac{\sum s_k}{u_0 \cdot \sum (s_k/u_k)} = \frac{s_p \cdot \sum p_k}{s_p \cdot u_0 \cdot \sum p_k\,(k \cdot fsr + 1)/u_0} = \frac{\sum p_k}{\sum p_k + fsr \cdot \sum k \cdot p_k}. \quad (57)$$

As $\sum p_k$ can be equated with "one" and $\sum k \cdot p_k = \mu$, and using Eqn (53), the foregoing equation can be transformed into:

$$u_{rel} = \frac{1}{1 + fsr \cdot \mu} = \frac{1}{1 + fsr \cdot (-\ln K_{av})} = \frac{1}{1 + 2.302 \cdot fsr \cdot c \cdot M^d \cdot T} \quad (58)$$

which is identical with Eqn (51) and resembles Eqn (46). Insertion of Eqn (58) into Eqn (16) leads to an "average" value of FSR in the "contact zone" of a particular macro-ion:

$$\overline{FSR} = \frac{fsr \cdot (-\ln K_{av})}{1 - K_{av}} = \frac{2.302 \cdot fsr \cdot c \cdot M^d \cdot T}{1 - 10^{-cM^d T}}. \quad (59)$$

According to Eqn (58), the constant d should be estimatable from the slope of a plot of $\log M$ versus $\log\left(\frac{1 - u_{rel}}{u_{rel}}\right)$ according to the suggestion of Kovacic and van Holde [83], whereas fsr should be estimatable from the slope of a plot of $(1/u_{rel})$ *versus* $(\ln K_{av})$.

In Fig. 10, each of the given preconditions (a) – (i) is represented by three curves showing the inherent relationships between different parameters. Constants were selected for the functions as to result in a high degree of concordance in Fig. 10a without intending numerical identity; these are given in the figure's legend. With the only exception of function (f), all functions are represented in Fig. 10a by sigmoidal curves in agreement with numerous experimental findings [10, 23, 44, 67, 69, 83]. Functions (a) and (e) are characterized by "limiting mobilities" [44], *i. e.* mobility approaches a positive minimum with rising molecular weight.

For a critical validation of these functions, it is necessary to examine whether they are compatible with a rational use of the term "friction". Negative values of FSR and discontinuities cannot be tolerated within normal ranges of K_{av}, u_{rel} and molecular weight. Negative values of FSR are only produced by function (f) in the lower molecular weight range (Fig. 10b); hence it has to be rejected. In contrast, function (h) causes FSR to rise in the same range of molecular weights. This can only be accepted with reservations as it implies, in terms of the "hydrody-

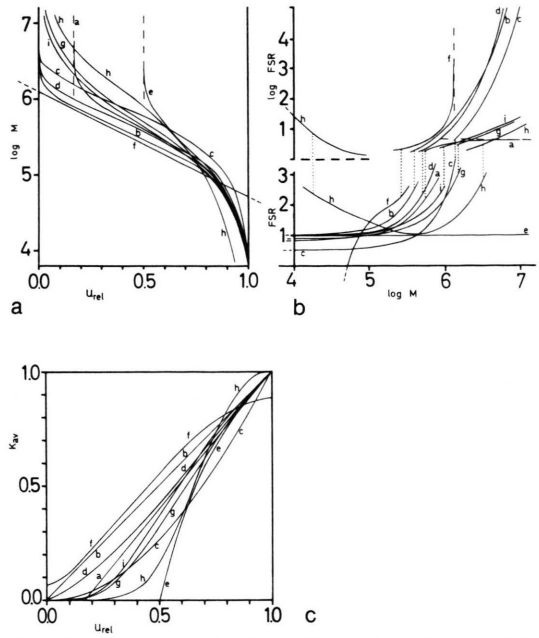

Fig. 10. Diagrammatic view of some functions used in molecular weight determination and of their counterparts in various plots. Eqn (26), which serves to link K_{av} with molecular weight M, and a set of a-priori conditions (a) – (i) are given in the text; constants of the functions were chosen as to result in approximate congruence of the curves in diagram a). Diagram a): familiar plot of the decadic logarithm of molecular weight versus mobility; diagram b): relationships between logarithm of molecular weight and *FSR* (resp. log *FSR*) inherent in the curves of diagram a) according to Eqn (16) and (26); diagram c) corresponding relationships between K_{av} and u_{rel} (Morris-plot).

Constants used for calculation: Eqn (26): $c = 10^{-7}$, $d = 1$, $T = 10\%$; condition (a): $e = 1.2$, $f = -0.2$; (b): equivalent to (a), when $e = 1$ and $f = 0$, and to (*c*), when $g = 1$, and to (h), when $d = {}^1/_2$ and $c \cdot T = 1/w$; (c): $g = 2$; (d): $m = 0.5$, $n = 0.5$; (e): FSR = constans = 1; (f): log $M_0 = 6.1$ (upper size limit of macro-ions able to intrude the gel), $p = 1.4$; (g): $q = 1$, $r = 5.6989$; equivalent to (i), when $r = 5.6389$; (h): $w = 3000$; (i): $fsr = 1$.

namic" model, a relatively higher rigidity of short polymer segments involved in collisions with small ions as compared to the longer segments; this might be a consequence of the fact that Eqn (48) applies to agarose [44] which may have a higher persistence length [78] than polyacrylamide.

In the range of high molecular weights, all functions (except the arbitrary assumption e) postulate a rise of *FSR*, as is consistent with the results shown in Fig. 7. This rise has different steepness and is even limited by a maximum in function (a). None of these functions represents a challenge of the "hydrodynamic" point of view; only function (f) remains to be critical. In the latter case, *FSR* goes to infinity already at finite molecular weight. Although this would not be intolerable, per se, it cannot easily be brought in line with the assumptions inherent in Eqn (26): according to that equation and to general theory [5, 7, 39, 41, 79], those large and immobile ions should still have some diffusional freedom within the gel. This conflicting condition would be realizable only by firm basket- or cage-like polymer structures able to trap macro-ions beyond a certain molecular size. Fig. 10c shows that despite the variability of the functions in Fig. 10b, they are rather consistent in the plot of K_{av} versus u_{rel} demonstrating that several functions might be able to fit data of the type obtained by Morris [37] (Fig. 6).

The strictly theoretical deduction (i) based on the probability of simultanous contacts between gel and macro-ion is a logical extension of the suggested model. In Figs 10a – c, it is represented by curves which are very similar to the other functions. This congruence of general form is able to reinforce some arguments of the foregoing sections concerning frequency, duration and multiplicity of fibre contacts. However, the given deduction is highly speculative and should certainly not be overstretched with respect to details.

The fact that diverse functions came out to fit experimental data of different authors optimally leads one to suppose that no singular "true" function exists to correlate molecular weight with mobility, but that the optimal function is dictated by the actual combination of gel type, method of gel casting, and type of macro-ions. Sometimes, there has been a tendency to reverse the steps in analysis of the problem by selecting gel recipes, types of ions and auxiliary assumptions to fit a favourite mathematical formula, and to make "non-ideal" practice responsible for deviations from this "theory" [12]. In the light of our hypothesis and of the known difficulties in casting gels in a reproducible fashion in different laboratories, predictions on a universally applicable relationship between molecular weight and mobility are likely to have to wait for advanced knowledge concerning the origins of "fractional specific resistance", and for the synthesis of better defined gel structures as to the number and distances of crosslinks, lengths of the side-chains, and as to non-covalent, short-range interactions and conformation in polyacrylamide.

[12] Rodbard (ref. 7): "Often a 'theory' has been developed to provide a rationale for an empirical finding."

Acknowledgments

The author is indepted to Prof. W. Hasselbach (Heidelberg) for suggestions during preparation of this manuscript and to Mrs. Suzanne Schmidt (Heidelberg/Vienna) for the final corrections.

[1] S. Raymond and L. S. Weintraub, Science **130,** 711 (1959).
[2] S. Hjertén, J. Chromatogr. **11,** 66 (1963).
[3] L. Ornstein, An. N. Y. Acad. Sci. **121,** 321 – 349 (1964).
[4] R. Maurer, Disk-Elektrophorese. Theorie und Praxis der diskontinuierlichen Polyacrylamid-Gel-Elektrophorese. Walter de Gruyter, Berlin 1968.
[5] A. Chrambach and D. Rodbard, Science **172,** 440 – 451 (1971).
[6] R. C Allen and R. Maurer, Electrophoresis and Isoelectric Focussing in Polyacrylamide Gel. Walter de Gruyter, Berlin, New York 1974.
[7] D. Rodbard, Methods of Protein Separation (Catsimpoolas, N., ed.), pp. 145 – 179 **Vol. 2,** Plenum Press, New York 1976.
[8] D. Rodbard, ibid., pp. 181 – 218.
[9] U. E. Loening, Biochem. J. **113,** 131 – 169 (1969).
[10] A. C. Peacock and C. W. Dingman, Biochemistry **7,** 668 – 674 (1968).
[11] A. L. Shapiró and J. V. Maizel, Anal. Biochem. **29,** 505 – 514 (1969).
[12] K. Shirakama, K. Tsujii, and T. Takagi, J. Biochem. **75,** 309 – 319 (1974).
[13] K. Weber, J. R. Pringle, and M. Osborn, in Methods in Enzymology (Colowick and Kaplan, eds.), **Vol. XXVI,** pp. 3 – 27 Acad. Press, New York and London 1972.
[14] O. Smithies, Biochem. J. **61,** 629 – 641 (1955).
[15] O. Smithies, Adv. Protein Chemistry **14,** 65 – 113 (1959).
[16] H. Hoch and C. G. Lewallen, Anal. Biochem. **78,** 312 – 317 (1977).
[17] K. Andersson, M. Fagerlind, and B. Daneholt, Molec. Biol. Rep. **2,** 195 – 201 (1975).
[18] K. K. Stewart, Adv. Protein Chem. **31,** 135 – 186 (1977).
[19] J. Porath and P. Flodin, Nature **183,** 1657 – 1659 (1959).
[20] G. K. Ackers, Adv. Protein Chem. **24,** 343 – 446 (1970).
[21] T. C. Laurent and A. Pietruszkiewicz, Biochem. Biophys. Acta **49,** 258 – 264 (1961).
[22] R. L. M. Synge and A. Tiselius, Biochem. J. **46,** p. XLI (Proc. Biochem. Soc.) (1950).
[23] D. M. Neville, J. Biol. Chem. **246,** 6328 – 6334 (1971).
[24] E. G. Richards and R. Lecanidou, Anal. Biochem. **40,** 43 – 71 (1971).
[25] G. Kopperschläger, W. Diezel, B. Bierwagen, and E. Hofmann, FEBS Letters **5,** 221 – 224 (1969).
[26] R. T. Swank and K. D. Munkres, Anal. Biochem. **39,** 462 – 477 (1971).
[27] J. F. Jongkind, J. H. Wisse, and H. Bloemendal, Protides Biol. Fluids 10th Colloq., pp. 77 – 79 (1963).
[28] J. Zwaan, Anal. Biochem. **21,** 155 – 168 (1967).
[29] E. Shaaya, Anal. Biochem. **75,** 325 –328 (1976).
[30] C. Manwell, Biochem. J. **165,** 487 –495 (1977).
[31] S. Raymond and M. Nakamichi, Anal. Biochem. **3,** 23 –30 (1962).
[32] M. L. White, J. Phys. Chem. **64,** 1563 –1565 (1960).
[33] M. L. White and G. H. Dorion, J. Polymer Sci. **55,** 731 –740 (1961).
[34] M. P. Tombs, Anal. Biochem. **13,** 121–132 (1965).
[35] L. Ingram, M. P. Tombs, and A. Hurst, Anal. Biochem. **20,** 24 –29 (1967).
[36] C. J. O. R. Morris, Protides Biol. Fluids **14,** 543 '551 (1966).
[37] C. J. O. R. Morris and P. Morris, Biochem. J. **124,** 517 – 528 (1971).
[38] A. G. Ogston, Trans. Faraday Soc. (Aberdeen) **54,** 1754 –1757 (1958).
[39] J. S. Fawcett and C. J. O. R. Morris, Sep. Sci. **1,** 9 –26 (1966).
[40] T. C. Laurent and J. Killander, J. Chromatogr. **14,** 317 –330 (1964).
[41] D. Rodbard and A. Chrambach, Proc. Nat. Acad. Sci. USA **65,** 970 –977 (1970).
[42] D. Rodbard and A. Chrambach, Anal. Biochem. **40,** 95 –134 (1971).
[43] D. Rodbard, G. Kapadia, and A. Chrambach, Anal. Biochem. **40,** 135 –157 (1971).
[44] H. Lehrach, D. Diamond, J. M. Wozney, and H. Boedtker, Biochemistry **16,** 4743 – 4751 (1977).
[45] C. W. Dingman, M. P. Fisher, and T. Kakefuda, Biochemistry **11,** 1242 –1250 (1972).
[46] S. Hjertén, Protides Biol. Fluids **14,** 553 –561 (1967).
[47] H. R. Maurer, Disc Electrophoresis and Related Techniques of Polyacrylamide Gel Electrophoresis. Walter de Gruyter, Berlin, New York 1971.
[48] W. Scholtan, Makromolekulare Chemie **14,** 169 –178 (1954).
[49] P. J. Flory, Principles of Polymer Chemistry. Cornell University Press, Ithaca, N. Y. 1953.
[50] E. G. Richards and C. J. Temple, Nature (Phys. Sci.) **230,** 92 –96 (1971).
[51] N. Kingsbury and C. J. Masters, Anal. Biochem. **36,** 144 – 158 (1970).
[52] J. Gressel, A. Rosner, and N. Cohen, Anal. Biochem. **69,** 84 – 91 (1975).
[53] H.-J. Bode, FEBS Letters **65,** 56 –58 (1976).
[54] H.-J. Bode, Anal. Biochem. **83,** 204 – 210 (1977).
[55] H.-J. Bode, Anal Biochem. **83,** 364 –371 (1977).
[56] H.-J. Bode, Anal. Biochem. (in press).
[57] R. Derynck and W. Fiers, J. Molec. Biol. **110,** 387 – 404 (1977).
[58] R. Weibel, Lab. Invest. **12,** 131 –155 (1963).
[59] P. W. Chun, J. K. Thornby, and J. G. Saw, Biophys. J. **9,** 163 – 171 (1969).
[60] J. C. Giddings and J. R. Boyack, J. Theoret. Biol. **2,** 1 – 6 (1962).
[61] J. Klein and B. J. Briscoe, Nature **266,** 43 – 44 (1977).
[62] J. C. Giddings and J. R. Boyack, Anal. Biochem. **36,** 1229 – 1233 (1964).
[63] E. Pines and W. Prins, Macromolecules **6,** 888 – 895 (1973).
[64] W. Thorun, Z. klin. Chemie u. klin. Biochemie **9,** 3 –12 (1971).
[65] J. L Hedrick and A. J. Smith, Arch. Biochem. Biophys. **126,** 155 –164 (1968).
[66] K. A. Ferguson, Metabolism **13,** 985 – 1002 (1964).
[67] R. N. Frank and D. Rodbard, Arch. Biochem. Biophys. **171,** 1 – 13 (1975).

[68] S. Panyim, R. Thitipongpanich, and D. Supatimusro, Anal. Biochem. **81,** 320 – 327 (1977).
[69] A. R. Ugel, A. Chrambach, and D. Rodbard, Anal. Biochem. **43,** 410 – 424 (1971).
[70] H. M. Rauen, Biochemisches Taschenbuch. Springer-Verlag, Berlin, Heidelberg, New York 1964, cf. Table 16c.
[71] E. Elson and T. M. Jovin, Anal. Biochem. **27,** 193 – 204 (1969).
[72] S. Quittner, L. A. Watts, and R. Roxby, Anal. Biochem. **89,** 187 – 195 (1978).
[73] R. A. Brown and S. N. Timasheff, Electrophoresis. Theory, Methods, and Applications, (Bier, M., ed.) pp. 317 – 367 Acad. Press, New York 1959.
[74] P. Harridt, Separ. Sci. **8,** 291 –302 (1973).
[75] W. Thorun and E. Mehl, Biochim Biophys. Acta **160,** 132 – 134 (1968).
[76] H. Demus and E. Mehl, Biochim. Biophys. Acta **203,** 291 –297 (1970).
[77] C. R. Parish and J. J. Marchalonis, Anal. Biochem. **34,** 436 – 452 (1970).
[78] H. Yamakawa and Fujii, Macromolecules **6,** 407 –415 (1973).
[79] H. Determann, Gel Chromatography. 2nd ed., Springer-Verlag, Berlin, Heidelberg, New York 1969.
[80] L. M. Siegel and K. Monty, Biochim. Biophys. Acta **112,** 346 – 362 (1966).
[81] M. Belew, J. Fohlman, and J.-C. Janson: FEBS Letters **91,** 302 – 304 (1978).
[82] A. K. Dunker and R. R. Rueckert, J. Biol. Chem. **244,** 5074 – 5080 (1969).
[83] R. T. Kovacic and K. E. Van Holde, Biochemistry **16,** 1490 – 1498 (1977).
[84] M. P. Fisher and C. W. Dingman, Biochemistry **10,** 1895 – 1899 (1971).
[85] W. L. Fangman, Nucl. Acids Res. **5,** 653 – 665 (1978).
[86] A. Chrambach and D. Rodbard, Separ. Sci. **7,** 663 – 703 (1972).