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Molecular-Sieve Chromatography of Proteins on Granulated Polyacrylamide Gels

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Summary

A method for molecular-sieve chromatography of proteins on columns of granulated polyacrylamide gels is described in detail. Distribution coefficients (K_D and K_{av}) are given for the model proteins γ globulin, bovine serum albumin, ovalbumin, ovomucoid, pepsin, myoglobin, and cytochrome c, as well as certain other proteins, between 0.5 M NaCl and polyacrylamide gels of 30 different compositions. The results are interpreted in terms of the Ogston-Laurent-Killander theory of molecular-sieve chromatography, and empirical equations are derived relating the fundamental parameters L and r of this theory to the gel composition. The results are discussed in terms of the gel structure and a method for estimating the average effective pore radius in polyacrylamide gels of different compositions is derived and compared with experimental findings.

Since its introduction by Porath and Flodin (*1*), molecular-sieve chromatography or "gel filtration" has proved to be one of the most valuable methods for the separation of macromolecules. As it operates almost exclusively on differences in the molecular dimensions of the solutes, it complements admirably such methods as ion-exchange chromatography and electrophoresis, which operate principally on differences in net charge.

The method is, however, greatly dependent on the availability of suitable materials for the chromatographic stationary phase. Although Lathe and Ruthven (2) demonstrated the principle of the method in 1956, they were unable to make much progress,

owing to the lack of suitable media at that time. The introduction of the artificially cross-linked dextrans by Porath and Flodin (*1*), and their commercial production as the "Sephadex" grades, really put the method on a practical basis. Although the seven currently available Sephadex grades can be used for chromatographic separations over a molecular weight range of 500 to 300,000, there is, however, an evident need for supplementary materials. In particular, gel media in which the effective pore size can be varied continuously, by change of concentration or of some other parameter, could be used to prepare a chromatographic stationary phase uniquely suited for a particular separation problem.

Two types of material have been suggested as alternatives to the cross-linked dextrans: agar or agarose and polyacrylamide gels. The former, although useful, appear best suited for the separation of macromolecules with molecular weights above 100,000.

The synthetic polymer, polyacrylamide, was introduced by Raymond and Wang (*3*) as a stabilizing medium for zone electrophoresis. It is prepared by polymerizing acrylamide in aqueous solution in the presence of the cross-linking agent N,N'-methylene-bis-acrylamide (BIS). The polymerization can be brought about by base-catalyzed oxidation or by a riboflavin-sensitized photoactivation. The resultant polymer is completely water-insoluble, optically transparent over a wide range of wavelengths, and is relatively resistant to chemical reaction. Hjerten and Mosbach (*4*) first described the use of granulated polyacrylamide gel for the molecular-sieve chromatography of proteins, and Hjerten (*5*) described an improved method for granulating the polymer and also discussed the effects of alterations in the polymer composition on its apparent pore size. Lea and Sehon (*6*) briefly described the use of polyacrylamide and some related polymers for the chromatography of proteins, and in a recent paper Sun and Sehon (*7*) have given chromatographic data on several proteins on gels of different compositions.

The present studies were begun in 1962 in an attempt to find a molecular-sieving medium which would be superior to the cross-linked dextrans over a limited molecular weight range. It soon became evident that an extensive survey of the effects of gel composition on effective porosity would be necessary if

this aim were to be achieved. Chromatographic data for 12 test proteins on 20 gel compositions are presented and discussed here.

A preliminary account of part of this work has been given previously [Fawcett and Morris (8)].

MATERIALS AND METHODS

Materials

The sources and effective hydrodynamic (Stokes) radii R of the proteins used in this investigation are summarized in Table 1.

TABLE 1

Sources and Properties of Model Proteins

Protein	Symbol	Source[a]	R, cm × 10^7
Thyroglobulin	Thy	K-L	8.25
Bovine γ globulin	γ	A	5.22
Bovine serum albumin	BSA	A	3.65
Haemoglobin	Hb	X	3.12
Ovalbumin	Ova	K-L	2.76
Ovomucoid	Ovm	K-L	2.68
Pepsin	Pep	A	2.40
Chymotrypsin	Chy	S	2.25
Myoglobin	Myo	S	2.00
Lysozyme	Lys	K-L	1.92
Ribonuclease	Rib	B	1.73
Cytochrome c	Cyt	S	1.65

[a] A, Armour Pharmaceutical Co., Eastbourne, England; B, B.D.H. Ltd., Poole, Dorset, England; K-L, Koch-Light Laboratories, Colnbrook, Bucks, England; S, Seravac Laboratories, Colnbrook, Bucks, England; X, prepared in this laboratory.

Acrylamide, N,N'-methylene-bis-acrylamide,* and β-dimethylamino-propionitrile were purchased from L. Light & Co., Colnbrook, Bucks, England, and were used without further purification.

Methods

Preparation of Polyacrylamide Gels. The appropriate amounts of acrylamide and BIS were dissolved in air-free water (150 ml). Then 20% (v/v) aqueous β-dimethylaminopropionitrile (0.4 ml) and 20% (w/v) aqueous $(NH_4)_2S_2O_8$ solution (0.4 ml) were added successively and the container closed so that there was no free

* Abbreviation: BIS.

air space above the solution. Polymerization proceeded with the evolution of heat, so that water cooling of the container was necessary at the higher monomer concentrations (>15%). The gels were stored overnight before granulation. This was carried out by forcing the gel successively through 100- and 120-mesh stainless-steel sieves using a Lucite cylinder and piston apparatus similar to that described by Hjerten (5). The granulated gel was collected directly into 0.5-*M* NaCl solution, and the chromatographic column packed with the suspension.

Chromatographic Technique. Chromatography was carried out by upward development in the apparatus shown in Fig. 1. Up-

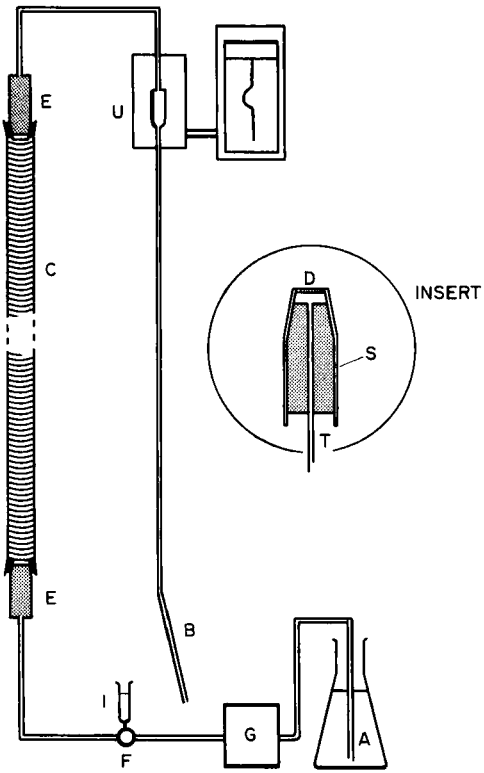

FIG. 1. Apparatus for upward-flow chromatography of proteins on granulated polyacrylamide gels. A, solvent reservoir; B, outlet tube; C, chromatographic column; D, porous polyethylene disc; E, column closures; F, three-way tap; G, peristaltic pump; I, inlet tube; S, silicone rubber tubing; U, absorptiometer flow cell.

ward development was found to obviate any reduction of flow rate with time caused by closer packing of the gel particles under gravity. It was virtually essential in order to obtain adequate flow rates with the softer gel particles (low cross-linking).

The 155 × 1 cm chromatographic column *C* was provided with B.10 sockets at both ends. The inlet and outlet closures *E*, *E* (see the insert) were fabricated from hollow B.10 polyethylene stoppers by replacement of the bottom by a tight-fitting disc *D* of porous polyethylene (Porous Plastics Ltd., Dagenham, Essex, England). The teflon connecting tube *T* was located in the stopper by silicone rubber tubing *S*, a gap of 1 mm being left between *S* and *D* to allow uniform dispersal of liquid through *D*. These closures, which do not require greasing, have been found to be very serviceable in a variety of chromatographic equipment. The present arrangement reduces free liquid space to a minimum.

The column was packed with the bottom closure in place and with an extension tube provided with a funnel attached to the upper end. Both column and extension tube were filled with 0.5-*M* NaCl. The suspension of gel particles was poured into the extension tube and allowed to settle under gravity with a slow outflow of liquid from the lower end of the column. Washing was continued with fresh solvent until the upper boundary of the gel particles had reached a stable position in the extension tube 1 to 2 cm above the upper end of *C*. The extension tube was then removed and the upper closure *E* inserted with a slight compression of the gel bed.

The lower end of the column was connected through the three-way tap *F* to the peristaltic pump *G*, which was supplied from the eluent reservoir *A* (0.5-*M* NaCl was used as eluent in all the experiments described). The upper end of the column was connected by a short length of Teflon tubing to the flow cell *U* (internal diameter 5 mm) of an LKB Uvicord absorptiometer, which recorded the ultraviolet absorption of the column effluent at 254 mμ. Electrical conductance was monitored with a Radiometer Type CDM2 conductance meter and an LKB Type 6250A recorder. All components were connected with 1.5-mm-diameter Teflon tubing.

The test solution was put in the inlet tube *I*, and admitted to the column by turning *F* to connect *I* and *C*. The exit tube *B* was then lowered below the level of *I*, so that the solution was drawn

into the column under slight negative pressure. F was then turned to connect C and G and elution begun by starting the pump G. The columns were operated at constant flow rates of 4 to 5 ml/hr.

The retention volumes V_e of the solutes were measured from the commencement of elution to the maxima of their recorded elution zones. The column void volume V_0 was measured similarly, using tobacco mosaic virus as the totally excluded marker solute. The column internal volume V_i was calculated in the earlier experiments from the retention volume of uridylic acid, and in the later experiments from the retention volume of 0.5 ml of water detected by the conductance change in the 0.5-M NaCl column effluent. Both methods gave identical results. The total packed volume of the column V_t was determined by calibration with water. In all cases the V_e values were the means of at least three independent determinations.

The partition coefficients K_D and K_{av} were calculated from the relations

$$K_D = (V_e - V_0)/V_i \quad (1)$$

$$K_{av} = (V_e - V_0)/(V_t - V_0) \quad (2)$$

RESULTS

Table 2 presents the K_D values for a number of test proteins with the 20 gel compositions studied. The gels are described by the notation of Hjerten (5), in which the first numeral (T) denotes the total weight of monomer (acrylamide plus BIS) per 100 ml of solvent, and the second numeral (C) denotes the amount of BIS expressed as a percentage (w/w) of the total amount of monomer. Thus an 8×25 gel was prepared from a solution containing 6 g of acrylamide and 2 g of BIS per 100 ml of solvent.

The range of polymers suitable for chromatography is in practice limited by their physical properties. Thus although gels can be prepared with $T = 3$ to 4%, they are so soft that deformation of the particles gives flow rates too low for satisfactory chromatography, even with upward development. On the other hand, the high-T and high-C gels are difficult to granulate. The range covered in the present investigation (T 6.5 to 20%) is adequate to allow useful generalizations to be made, while remaining quite suitable for practical chromatography.

TABLE 2

K_D Values of Proteins on Different Gels

Protein	T	6.5				8.0						10.0					12.0		15.0		20.0
	C	× 1	× 2	× 5	× 15	× 1	× 2	× 3	× 5	× 15	× 25	× 1	× 2	× 5	× 15	× 25	× 1	× 5	× 1	× 5	× 5
Thy		0.025	0.02		0.07	0.03	0.03	0.03	0.02	0.07	0.20	0.03		0.02	0.07	0.06		0	0	0	
γ		0.15	0.11	0.07	0.28	0.11	0.07	0.05	0.025	0.30	0.42	0.07	0.035	0.016	0.23	0.45	0.05	0.017	0.05	0.008	
BSA		0.29	0.22	0.18	0.42	0.22	0.14	0.11	0.115	0.38	0.51	0.14	0.09	0.06	0.32	0.50	0.10	0.034	0.08	0.013	
Ova		0.44	0.35	0.29		0.37	0.25	0.22	0.20		0.57	0.28	0.17	0.14		0.57	0.20	0.084	0.16	0.031	
Ovm		0.45	0.43	0.32	0.56	0.40	0.29	0.24	0.22		0.61	0.29	0.20	0.17	0.41	0.59	0.23	0.10	0.18	0.035	
Hb				0.39					0.24		0.53	0.33		0.18	0.44	0.58		0.12		0.058	0.05
Pep		0.54	0.44	0.37	0.58	0.49	0.35	0.31	0.25	0.54		0.35	0.27	0.20	0.45		0.30	0.13	0.25	0.062	0.06
Chy			0.52			0.55	0.45	0.41	0.41	0.69		0.47	0.37	0.32				0.24	0.28	0.14	
Myo		0.63	0.56	0.49	0.63	0.57	0.46	0.43	0.40	0.62	0.71	0.48	0.39	0.36	0.51	0.70	0.42	0.25	0.37	0.14	0.10
Cyt		0.70	0.65	0.59	0.69	0.67	0.57	0.52	0.53	0.69	0.78	0.58	0.48	0.43	0.58	0.76	0.51	0.34	0.45	0.24	0.15
Rib				0.62					0.55			0.63		0.49				0.42		0.29	0.20
Lys		0.86	0.83	0.78	0.84		0.75	0.78	0.77	0.86	0.90	0.77	0.75	0.70	0.74	0.85	0.72	0.61	0.69	0.49	0.36
DNP-Et			1.20	1.22			1.22		1.35		1.41	1.21		1.42	1.41	1.49		1.61	1.31	1.74	

During the course of this work, some of the proteins used, notably haemoglobin, chymotrypsin, and ribonuclease, were found to be unsuitable for the precise calibration of the gels, for various reasons. They were, therefore, not used in the later experiments. The incomplete results are, however, included for their intrinsic value.

Chromatographic data for dinitrophenyl-ethanolamine are included in Table 2. This solute was studied to evaluate a claim that adsorption was virtually absent on polyacrylamide gels. The K_D values were in all cases greater than 1, indicating appreciable adsorption, which in the $C = 5$ series was proportional to the total solid content (T) of the gel. The gels thus show a definite affinity for aromatic compounds, although there is no cyclic structure in the gel matrix. The same mechanism is also probably responsible for the anomalously high retention volumes (and K_D values) for lysozyme, which has a particularly high content of aromatic amino acids.

DISCUSSION

As in all other chromatographic separations, molecular-sieve chromatography depends on the relative distributions of the individual solute molecules between the mobile and stationary phases. In this case the operative mechanism is undoubtedly the differential permeability of the gel particles to solutes of different molecular dimensions, and since the introduction of the method, several attempts have been made to relate the molecular dimensions of the solutes to their chromatographic behavior. Most of these attempts have only an empirical basis and are therefore restricted to a particular set of experimental conditions. A widely used correlation is that of the logarithm of the molecular weight against some chromatographic parameter such as elution volume V_e [Andrews (*9–11*)] or V_e/V_0 [Whitaker (*12*)]. These can provide a linear relation over a restricted molecular weight range, and can thus give a simple and direct estimate of the molecular weight of an unknown solute, provided that the experimental conditions are rigidly controlled and that the necessary extrapolation is not too great.

Certain proteins may, however, exhibit anomalous behavior, giving apparent molecular weights either greater or less than those determined by reliable independent methods such as

sedimentation or light scattering. These anomalies can be due to a variety of causes, such as ion exchange of a basic solute (for example, lysozyme or ribonuclease) on residual acidic groups on the surface of the gel particle, adsorption (particularly of aromatic molecules; see above), and association or dissociation of the solutes themselves (for example, haemoglobin [Andrews (*10*)] and chymotrypsin [Winzor and Scheraga (*13*)]. All these effects can give rise to grossly erroneous estimates of molecular weight if not recognized, and constitute serious limitations to the value of the method as an independent criterion of molecular dimensions.

Two types of theoretical treatment have also been applied to the problem. In the first of these a concrete model is assumed for the gel particle, and its consequences worked out. Theories of this type have been advanced by Flodin (*14*), Porath (*15*), and Squire (*16*). On the basis of a model gel with conical pores such that the solute molecule encountered increasing resistance as it penetrated into the particle, Porath (*15*) derived a linear relation between the partition coefficient K_D and the 1.5th power of the molecular weight. Although the derivation is only strictly valid for flexible linear polymers, apparent agreement with it has been reported for proteins by Wieland et al. (*17*) and by Andrews (*10*).

The second type of theory, which makes minimal use of models, is exemplified by the investigations of Laurent and Killander (*18*). They first showed that the partition coefficient K_{av}, defined in accordance with Eq. (2), was equal to the fraction f of the total gel volume which was accessible to the solute molecule. They were further able to relate f to the properties of both the solute and the gel by making use of the earlier theoretical study of Ogston (*19*). Ogston had investigated the distribution of spaces within a uniform, *random* network of long fibers (or segments of fibers) of diameter $2r$ and concentration L (expressed as centimeters of fiber per milliliter of network). He had shown by a statistical treatment that the fraction f of the network which was available to a sphere of radius R was given by

$$f = K_{av} = \exp\left[-\pi L(R + r)^2\right] \tag{3}$$

R can be taken as the equivalent hydrodynamic radius of the solute molecule, most appropriately calculated from its diffusion coefficient by means of the Stokes-Einstein equation [see

Cohn and Edsall (*20*)]. If diffusion data are not available, R can be calculated directly from the molecular weight and the partial specific volume on the assumption of a spherical molecule.

The Ogston-Laurent-Killander theory has the advantage that it is virtually independent of any assumptions as to the nature of the gel matrix other than that it is isotropic and fibrous in nature. The latter accords well with what is known of the internal structure of many gels. The parameter f is a statistical mean, and the Ogston (*19*) derivation assumes that it is normally distributed.

Laurent and Killander (*18*) showed that Eq. (3) was in very good agreement with experiment for proteins and oligosaccharides on all the Sephadex grades G-25 to G-200, and they further showed that the values of L for the five grades were linearly related to the polymer concentration in the fully swollen gels, as required by the Ogston (*19*) theory.

It is evident from the form of Eq. (3) that the parameter L mainly affects the permeability of the gel, a low value being indicative of a permeable gel structure. The parameter r, because of its inclusion in the squared term of Eq. (3), influences the selectivity of the gel as well as its permeability, low values producing the greatest variation of K_{av} with R, and thus being most favorable for chromatography.

Laurent-Killander equations were fitted to our K_{av} data for seven well-characterized proteins, for a range of 20 gel compositions. The marker proteins used, γ globulin, serum albumin, ovalbumin, ovomucoid, pepsin, myoglobin, and cytochrome c, cover a molecular weight range from 150,000 to 13,000. Figures 2, 3, 4, and 5 show K_{av}-R diagrams for 6.5 × 1, × 5, × 15; 8 × 1, × 5, × 15, × 25; 10 × 1, × 5, × 15, × 25; 12 × 5; 15 × 5; and 20 × 5 gels. Similar data were also obtained for 6.5 × 2, 8 × 2, 8 × 3, 10 × 2, 12 × 1, and 15 × 1 gels. In these figures the points indicate experimental values; the full lines are Laurent-Killander equations with L and r values adjusted by an iterative procedure to give the best fit.

It can be seen that the agreement is in general very good and provides considerable support for the Ogston-Laurent-Killander theory, at least for the approximately spherical proteins examined. The largest discrepancies arise with certain of the values for γ globulin. However the quoted R value of 5.22 mμ for this protein is almost certainly too high, as the diffusion studies were carried out with preparations now known to contain 19 S macro-

globulins. The γ-globulin molecule is also more asymmetrical than the others studied.

The values of L and r used are collected in Tables 3 and 4. The data of these tables allow some useful generalizations to be made relating L and r to gel composition.

1. At constant C, L is a linear function of T, as demanded by

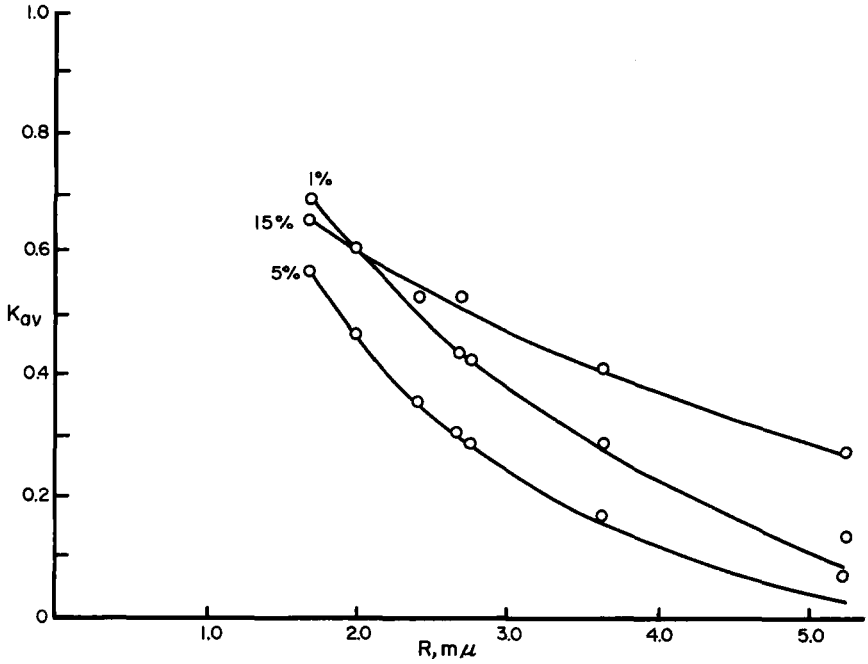

FIG. 2. Dependence of the partition coefficient K_{av} on the effective molecular radius R; 6.5% monomer series. 6.5 × 1, 6.5 × 5, and 6.5 × 15 gels.

the Ogston (*19*) theory (see Fig. 6). The empirical equations allow L values to be calculated from T to within ±5%. The values of the coefficients depend on C, and at high C values L becomes almost independent of T.

2. At constant T, L at first increases with increasing C, passes through a maximum at about $C = 5\%$, and then decreases rapidly to very low values.

3. At constant C, r is virtually independent of T.

4. At constant T, r increases linearly with C to reach a constant value of about 3.0 at $C = 15\%$. In the region of interest

below $C = 10\%$, r can be calculated from the empirical equation $r = 0.50 + 0.10C$.

The empirical equations enable L and r to be calculated with adequate accuracy for any gel composition within the range investigated. The K_{av} value of a solute of known R can then be calculated from the corresponding Laurent-Killander equation,

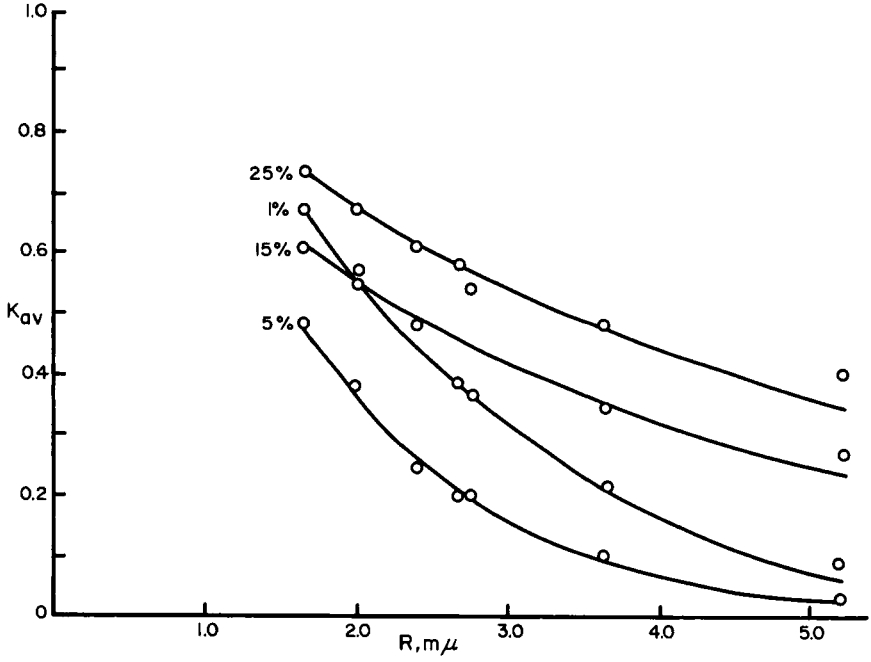

FIG. 3. Dependence of the partition coefficient K_{av} on the effective molecular radius R; 8% monomer series. 8 × 1, 8 × 5, 8 × 15, and 8 × 25 gels.

and should agree with experiment to within ±10%, except perhaps for very low values, where other factors, such as adsorption, may influence the retention mechanism.

Since the chromatographic data can be represented by Ogston-Laurent-Killander equations, the theory can be applied to give an estimate of the mean effective pore size of the gels. Ogston (*19*) showed that the mean pore radius p in his random fiber network was given by

$$p = (4L)^{-1/2} - r \qquad (4)$$

Since the pore radii in the gel are assumed to have a Gaussian distribution, half the total gel space should be accessible to a molecule of radius p or slightly less. Values of p calculated from Eq. (4) are compared in Table 5 with the values of R, $R_{0.5}$, at which $K_{av} = f = 0.5$, i.e., where 50% of the total gel volume is available to the solute molecule. The values of $R_{0.5}$ were interpolated from the experimental data and are thus independent of any theory. It can be seen from Table 5 that molecules with a $R_{0.5}/p$ ratio of 0.91, and thus with an equivalent radius of $0.91p$, do in fact have a 50% probability of penetration into the gel. The values of p given in Table 5 are comparable to those obtained by White (*21*) from water-permeability measurements on a series of polyacrylamide gels with $C = 5$ and T from 5 to 35%. Exact

TABLE 3

Values of the Parameter L for Different Gel Compositions
(cm/ml $\times 10^{12}$)

	C = cross-linking agent as % total monomer					
T = % total monomer	1	2	3	5	15	25
6.5	2.24	2.70		3.00	0.65	
8.0	2.80	3.51	3.70	3.66	0.73	0.52
10.0	3.45	4.10		4.47	0.90	0.61
12.0	4.15			5.30		
15.0	5.15			6.96		
20.0				9.14		

TABLE 4

Values for the Parameter r for Different Gel Compositions
(cm $\times 10^{-7}$)

	C = cross-linking agent as % total monomer					
T = % total monomer	1	2	3	5	15	25
6.5	0.60	0.79		0.90	2.92	
8.0	0.53	0.65	0.80	1.0	3.0	2.75
10.0	0.71	0.83		0.93	3.0	3.0
12.0	0.65			1.0		
15.0	0.62			1.01		
20.0				1.02		

correspondence is not to be expected, since permeability measurements take no account of "blind" channels, which could, however, make a contribution to f in molecular-sieve chromatography.

A striking and unexpected feature of the present results is the marked increase in gel permeability at high cross-linkings. This is accompanied by a sharp decrease in L and by an increase in

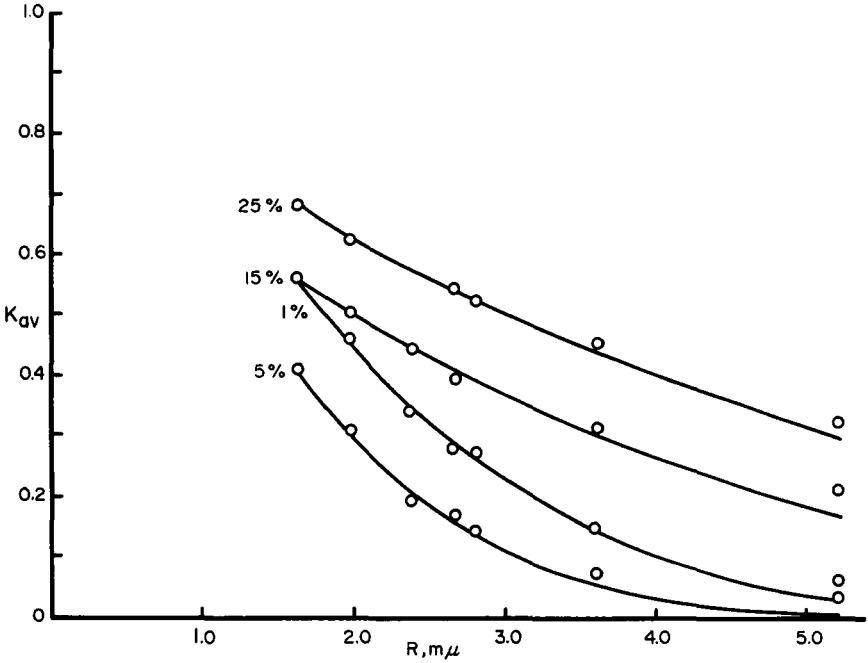

FIG. 4. Dependence of the partition coefficient K_{av} on the effective molecular radius R; 10% monomer series. 10 × 1, 10 × 5, 10 × 15, and 10 × 25 gels.

r to a constant value. It can be calculated that a 10% solution of a non-cross-linked polyacrylamide, with a monomer segment length of 2.5 A, should have an L value of 21×10^{12}. The experimental values of 3.45×10^{12} for 10 × 1, and 4.5×10^{12} for 10 × 5 gels are only 15 to 20% of the nonlinked value, and reflect the effect of the cross-linkages in binding the polymer chains into multiple stranded fibers. At $C = 25$, the experimental L value of 0.6×10^{12} has decreased to only about 3% of the unlinked value. Similarly, the radius of an uncoiled unlinked polyacrylamide chain can be estimated to be 2 to 2.5 A. The experimental r value

of 30 A for a 10 × 25 gel also suggests an aggregate of polymer chains bound together by the numerous cross-linkages to form a thick rigid fiber which apparently has a maximum stable diameter of about 60 A. The accumulation of material in the fibers results in larger spaces between them, with lower L values and higher permeabilities.

This change in structure is accompanied by striking changes in the optical and other physical properties of the gels. Highly cross-linked gels have a characteristic opaque white appearance, probably due to enhanced scattering of light by the thick fibers. They are also markedly hydrophobic.

At the present time the gels with T values of 5 to 10%, and C values of 1 to 5%, offer most promise for the chromatographic fractionation of proteins, as they combine low r values (good selectivity) with a useful range of permeabilities (L values). The usefulness of the gels at the bottom end of the range is, however, at present limited by their physical properties. The prepa-

TABLE 5

Mean Pore Radii (p) and $R_{0.5}$ Values
($cm \times 10^{-7}$)

	$T = 6.5$			$T = 8.0$		
C	$R_{0.5}$	p	$R_{0.5}/p$	$R_{0.5}$	p	$R_{0.5}/p$
1	2.41	2.73	0.88	2.25	2.47	0.91
2	1.97	2.25	0.88	1.85	2.03	0.91
5	1.90	2.00	0.95	1.56	1.62	0.96
15	2.83	3.28	0.86	2.40	2.85	0.84
25				3.60	3.90	0.93
	$T = 10$			$T = 12$		
1	1.85	2.00	0.93	1.65	1.79	0.92
2	1.50	1.62	0.93			
5	1.40	1.41	0.99	1.05	1.16	0.91
15	2.0	2.25	0.89			
25	3.0	3.45	0.87			
	$T = 15$			$T = 20$		
1	1.44	1.58	0.91			
5	0.78	0.85	0.92	0.54	0.64	0.85

Mean ratio $R_{0.5}/p = 0.91$

ration of the gel particles in bead form (Biogel P beads, Bio-Rad Laboratories, Richmond, California), or the use of somewhat larger particles with correspondingly lower flow rates, may overcome some of these difficulties, however.

The gels with T values above 12% may prove of value for the chromatography of smaller molecules such as peptides. Gels

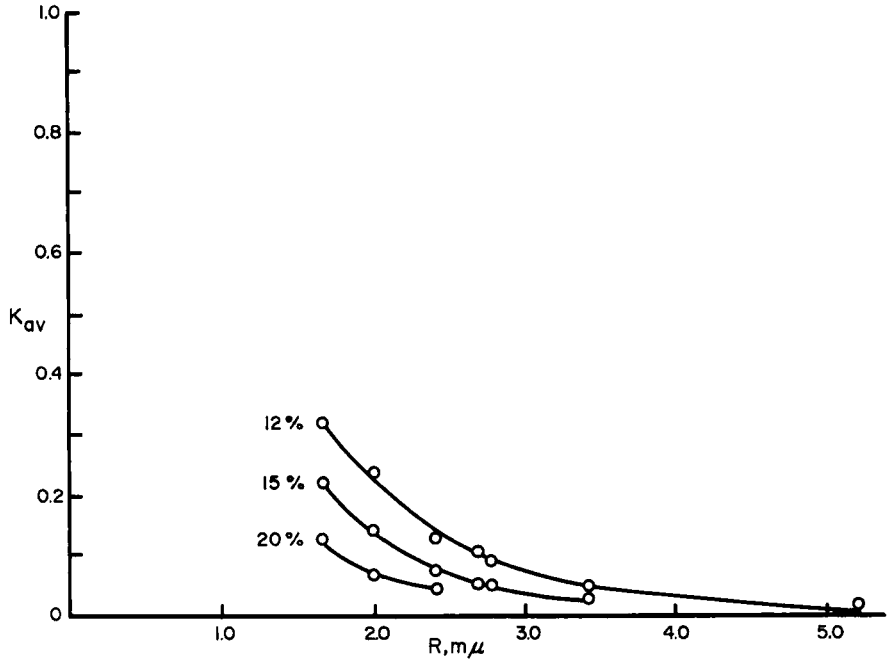

FIG. 5. Dependence of the partition coefficient K_{av} on the effective molecular radius R; 12, 15, and 20% monomer series, 5% cross-linking. 12 × 5, 15 × 5, and 20 × 5 gels.

with T values of 30% are virtually impermeable to proteins as small as cytochrome c, and may provide an alternative to cellulose membranes in some electrophoretic methods, because of their favorable electrokinetic properties.

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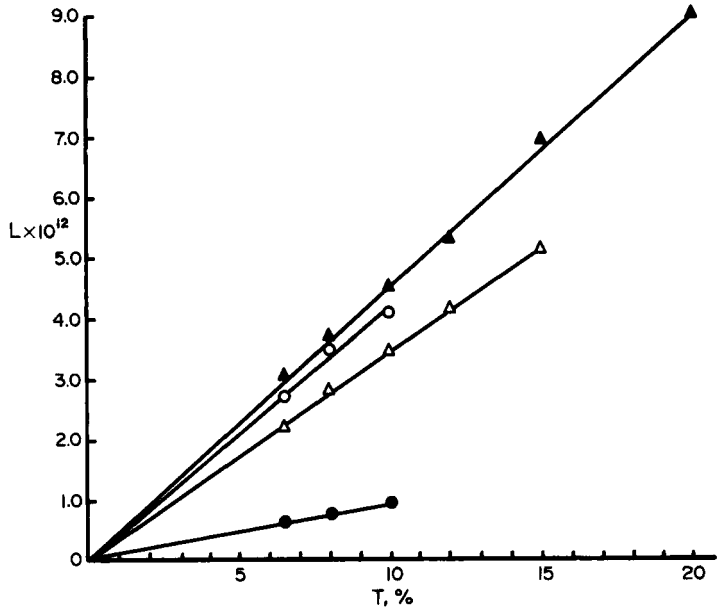

FIG. 6. Dependence of L on the total monomer concentration T. Open triangles: $C = 1\%$, $L_1 = 0.345T$. Open circles: $C = 2\%$, $L_2 = 0.412T$. Solid triangles: $C = 5\%$, $L_5 = 0.454T$. Solid circles: $C = 15\%$, $L_{15} = 0.092T$.

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