MOLECULAR DISTRIBUTION WITHIN COLLAGEN GEL COLUMNS

MARION L. SHAW and ALFRED SCHY

University of Washington School of Medicine, Seattle, Wash. 98195 (U.S.A.)

SUMMARY

Purified collagen columns with collagen concentrations of 5 and 10% were constructed by modification of the procedure for the 1% gel as described by Shaw and Schy. The resulting columns were calibrated by elution of various tracers, and the observations compared with those of the 1% collagen gel column. The data were fitted to the Ogston gel model as formulated by Laurent and Killander, but the model was found to be not fully applicable. The thermodynamic treatment of Hjertén was applied to the data with more satisfactory agreement.

INTRODUCTION

The construction of a gel chromatography column comprised of purified, cross-linked collagen at a concentration of 1% was initially reported by Öbrink and Wasteson[1]. They used this column to study the interaction between the collagen and samples of chondroitin 4-sulfate and chondroitin sulfate proteoglycans applied to the column. We adapted their method of gel synthesis to make a similar 1% column[1], then modified it to produce columns of concentrations 5 and 10%.

Various substances, some used in the previous study[2], were applied as samples to the columns. The partition coefficients in the gels were calculated from the elution data and their respective steric interactions compared. We attempted to analyze the gel structures by examining the fit to the data of the Ogston gel model[3] as formulated by Laurent and Killander[4]. The model describes the partition coefficient as a function of gel and tracer parameters. Like other gel models it is not completely satisfactory, but it has become a standard by which a number of gel systems have been calibrated owing to its simplicity and few assumptions.

Our purpose in developing and describing these gels is to use them later as models for studies of interstitial transport because of analogies between the gel filtration mechanism and interstitial transport[5-7] and the technical advantages of the column system in quantitative analysis of gel properties[19]. The gels also have potential as separation media[2] and it is possible to describe adequately their separation properties with a thermodynamic treatment[8].

EXPERIMENTAL

Purified collagen gels from bovine Achilles tendon prepared and assayed as previously described[2], with covalent cross-linking of about 2% and concentrations of 5 and 10%, were formed by modification of the procedure for the 1% gel as follows.

A 1% collagen solution in the buffer 0.42 M NaCl, 5 mM phosphate was concentrated to 12 $\pm$ 2 and 33 $\pm$ 2% solutions with Aquacide II. The concentrated solutions were stirred into heptane–glutaric dialdehyde solution, and the resulting cross-linked gels were washed with heptane, 50% ethyl alcohol, and distilled water. Granulation, sizing of the gel particles, and pouring of the gel columns were accomplished as previously described[2]. The collagen gel columns were confirmed to have 5 $\pm$ 1 and 10 $\pm$ 1% concentrations by lyophilization and weighing of a known volume of column, and the dimensions were measured at 36.4 $\times$ 1.6 cm (5%) and 16.7 $\times$ 1.6 cm (10%).

To the columns were applied samples of the proteins bovine serum albumin (BSA) and ovalbumin, obtained from Pentex (Kankakee, Ill., U.S.A.) and Worthington (Freehold, N.J., U.S.A.), respectively, and narrow fractions of dextrans, FITC-dextrans, and Ficolls with measured weight-average and number-average molecular weights, given to us by Dr. Kristi Granath (Department of Polymer Chemistry, Pharmacia AB, Uppsala, Sweden).

The columns were operated in the ascending flow mode with a flow-rate of 4 ml/h. The buffer was 0.01 M phosphate, pH 7.3, and 0.15 M NaCl.

RESULTS

The results for neutral and negatively charged species on the 1, 5 and 10% columns are given in Table I. The results for the species for which values of Stokes radius were available are also presented in Fig. 1 for the discussion concerning the Ogston model and gel parameters (below) and in Fig. 2 where they are fit with eqn. 1 taken from the thermodynamic approach of Hjertén[8].

$$-\log K_{av} = A \cdot \bar{M}_w^{2/3} + B \qquad (1)$$

where K_{av} is the partition coefficient of the molecular species between the gel and the liquid phase, $\bar{M}_w$ is the weight-averaged molecular weight, and A and B are constants characteristic of the given gel.

In Table I and Figs. 1 and 2, the partition coefficient, K_{av}, is calculated from the relationship

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

where V_0 and V_t are the void volume and the total volume of the column, and V_e is the elution volume of the solute. V_0 is assumed equal to V_e for tobacco mosaic virus and V_t equal to V_e of ^3H_2O.

TABLE I

PARTITION COEFFICIENTS, K_{av}, COLLAGEN GELS

Substance		M_w [*]	Stokes radius $(\AA)$ [**]	K_{av} 1%	5%	10%
Dextran	27	26,700	40.5	0.90		
Dextran	53	53,200	53.5	0.84		
Dextran	103	103,000	66.0	0.74		
Dextran	581	581,000		0.38		
FITC-dextran	3	2900	10.7		0.98	0.64
FITC-dextran	20	19,400	33.5	0.98	0.70	0.26
FITC-dextran	40	39.000	47.5	0.89	0.52	0.16
FITC-dextran	70	67,000	59.0	0.82	0.36	0.05
FITC-dextran	150	148,000	79.4	0.70	0.16	
FITC-dextran	232	232,000	84.7	0.56	0.10	
Ficoll	9	9000	20.5		0.79	0.42
Ficoll	18	17,500	30.0		0.70	0.33
Ficoll	50	49,000	45.5	0.90	0.52	0.26
Ficoll	100	99,000	56.0	0.79	0.36	0.22
Ficoll	461	461,000		0.54	0.05	
Inulin		5200	12–15		0.90	0.53
Ovalbumin		45,000	27.6	1.00	0.72	0.26
BSA		67,000	36.1	0.98	0.63	0.17

[*] Weight average molecular weight as given by Dr. K. Granath.

[**] The values of Stokes radius for dextrans and Ficolls were obtained from Laurent and Granath[9]. The values for FITC-dextrans were assumed to be equal to those for dextrans of the same M_w because they have the same calibration curves on Sephadex gels[16]. This similarity is not unexpected because the dye-complexing is light and conformational changes are expected to be small[16]. The values of proteins are from Tanford[17]. The value for inulin was obtained from Landis and Pappenheimer[18].
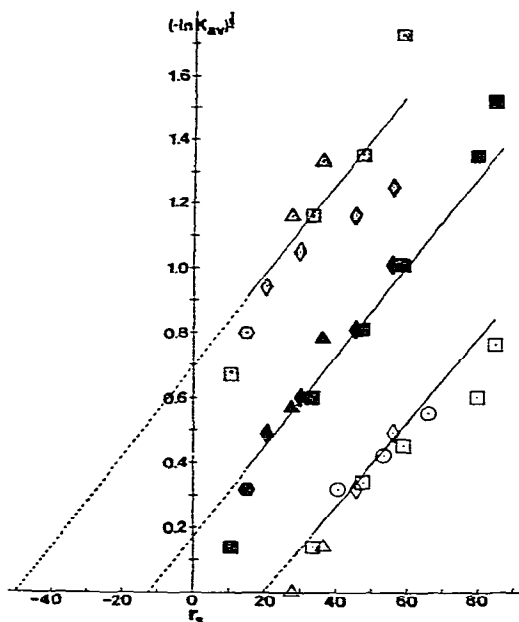

Fig. 1. $(-\ln K_{av})^{1/2}$ vs. Stokes radius, r_s is the partition coefficient (eqn. 2) for a molecule with Stokes radius r_s. The solid lines represent least-squares fits. The dotted lines represent extrapolations of the solid lines. $\Diamond$, Ficoll; $\triangle$, protein; $\bigcirc$, inulin; $\bigcirc$, dextran; $\square$, FITC-dextran. The 1% gel column corresponds to open symbols, the 5% gel column solid symbols, the 10% gel column stippled symbols. The point for inulin was not included in the data fit owing to less confidence in the value of its Stokes radius (Table I).
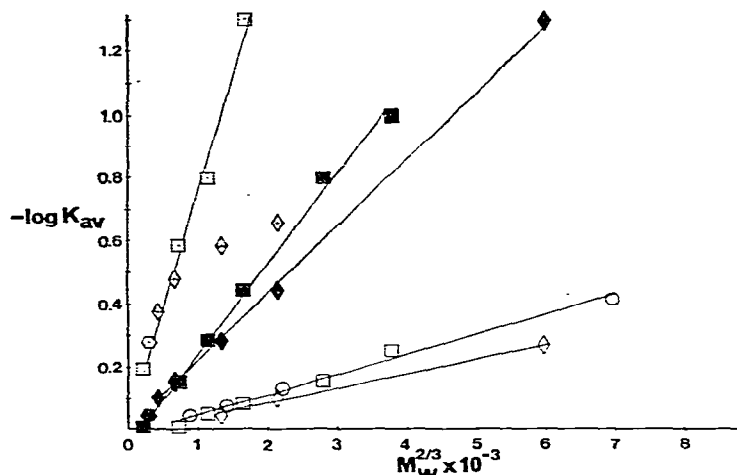
Fig. 2. $-\log K_{av}$ vs. $\bar{M}_w^{2/3}$ (see eqn. 1). M_w is the weight-average dmolecular weight of the sample species whose partition coefficient is K_{av}. $\Diamond$, Ficoll; $\Box$, FITC-dextrans; C, dextran; $\bigcirc$, inulin. The 1% gel column corresponds to open symbols, the 5% gel column solid symbols, the 10% gel column stippled symbols. The lines represent least squares fits.

DISCUSSION

A theoretical model initially developed by Ogston[3] has been adapted by Laurent and Killander[4] to explain the gel chromatography process. In this approach the gel is treated as a three-dimensional network of rigid fibers, randomly distributed and infinitely long, and the partition coefficient of a substance between the gel and a solution is assumed to be determined by the space available to the molecules in the network. For spherical molecules, the partition coefficient can be calculated from an equation derived for spherical molecules[3,4]

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

where r_r is the radius of the fiber forming the gel matrix in centimeters, L is the concentration of the fiber in centimeter fiber/cm³, and r_s is the molecular radius in centimeters of the substance. It has been shown that if the Stokes radius is used for r_s, the same relationship between K_{av} and r_s will hold on a given Sephadex gel for dextrans, which have an extended chain configuration, Ficolls, a more compact species, and globular proteins[9].

We have observed that on a 1.5% hyaluronate gel column, eqn. 3 holds only separately for FITC dextrans and for negatively charged globular proteins[2,10]. The collagen gels, however, appear to behave more like Sephadex in that a single relationship holds for substances as illustrated for the 1% gel[2]. The character of the relationship is less clear for the 10% gel owing to greater scatter in the data. This scatter may arise from the combination of hardness and irregularity of the gel particles forming the relatively more heterogeneous, 10% column.

The model summarized by eqn. 3 has been used in defining the structure of gels of dextran (5–40%) (ref. 4), of agarose (2–8%) (ref. 11), of polyacrylamide[12], and of solutions of dextran 500 (1–4%) (ref. 13) and in describing steric interactions with polysaccharide components of connective tissue[6,7].

The model (eqn. 3) was considered to be consistent with the data for all of the above substances cited where they were critically examined. For dextrans, the parameter L was proportional to gel concentration independent of degree of cross-linking, whereas the value derived for r_r was constant and reasonable (7 Å) for the dextran molecule. For agarose, the situation was slightly more complex in that the value of r_r was arther large (25 Å) and L showed some tendency to level off as a function of gel column concentration between 6 and 8%. It is, however, possible to interpret the values of L as showing an approximate proportionality with concentration (Fig. 3). No dependence on degree of cross-linking was noted. For poly-acrylamide, at a constant degree of cross-linking L was proportional to gel column concentration, and r_r, although large, was virtually independent of concentration. Increasing degree of cross-linking at constant concentration eventually caused a step-wise increase in r_r and a drop in L. This behavior was associated with fiber aggregation[12].
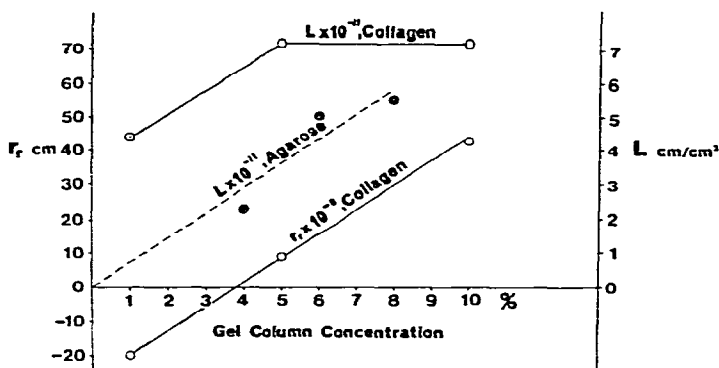

Fig. 3. Gel column concentration dependence of L and r_r. The parameters r_r and L are defined by eqn. 3. r_r and L were calculated from a least-squares fit of eqn. 3 to the collagen data (Table I). The values obtained in the data fit are represented by the points (○). Also shown are the values (●) for L for agarose gels obtained by Laurent[11].

When the collagen data are plotted as illustrated in Fig. 1, some degree of correlation is apparent. The lines drawn through the three sets of data following a least-squares fit have correlation coefficients of 0.96, 0.99 and 0.85 for the 1, 5, and 10% gel columns, respectively, and had respective slopes of 0.013, 0.015 and 0.015 Å^{-1}.

The parameters L and r_r calculated from these data fit are illustrated in Fig. 3. They show some distinct differences in gel concentration dependence from the parameters derived from the other gel types. First, the parameter L cannot be considered as proportional to gel concentration as Ogston's model[5] requires; in fact, it is virtually constant. Secondly, the parameter r_r becomes negative at 1%. The linear dependence of r_r on concentration seems to indicate a continuity between the three collagen gels, but it is not clear what this means physically.

Fawcett and Morris[12] have applied the Ogston theory to calculate a mean pore radius for the polyacrylamide gels. The expression for mean pore radius is given by eqn. 4:

$$R_p = (4L)^{-1/2} - r_r \tag{4}$$

where R_p is the mean pore radius in the gel and L and r_r are the parameters of eqn. 3. According to the Ogston theory, one-half of the gel space would be accessible to molecules of radius R_p (ref. 3). Molecules of radius R_p would thus have a partition coefficient, K_{av}, of 0.5. For the polyacrylamide gels it was found that the average value of the ratio of R_p to the radius $r_{s_{0.5}}$ of molecules with K_{av} of 0.5 was 0.91. Inspection of results of this calculation for the collagen gels (Table II) shows that R_p approximates $r_{s_0 5}$ for the 1 and 5% gels, only, although R_p decreases with gel concentration.

TABLE II

MEAN PORE RADII, COLLAGEN GELS

Gel column concentration	R_p $(Å)$ *	$r_{s_{0.5}}$ $(Å)$ **
1%	90	100
5%	50	47
10%	16	7

* R_p is calculated from eqn. 4 and values of L and r_r shown in Fig. 3.
** $r_{s_{0.5}}$ is the value of Stokes radius for which K_{av} equals 0.5 in the given column. This parameter was obtained by extrapolation of K_{av} vs. r_s data from the 1 and 10% gels and interpolation of the data from the 5% gel.

In summary, the Ogston model does not work for the collagen gels in at least two respects: (1) the derived fiber "radius" is negative for the 1% gel, and (2) the parameter L is not proportional to concentration. In addition at lower values of Stokes radius the data have a tendency to deviate systematically from a linear dependence of $(-\ln K_{av})^{1/2}$ on r_s (Fig. 1). It is recognized that the curves are more sensitive to the 1–2% experimental error in K_{av} for $(-\ln K_{av})^{1/2}$ less than 0.3.

It is possible that the inability of the model to explain all of the collagen data may relate to order present within the gel matrix or some other structural feature not occurring in the other gel types. Electron micrographs of the 1 and 5% gels did show the presence of striated fibrils.

The trend at low molecular weights may be associated with smaller molecules having a physical radius less than their Stokes radius or moving by an activated diffusion mechanism in this system as discussed by Nir and Stein[14] for other systems.

We believe that these questions need to be explored and that Ogston model must be modified for use with collagen gels.

As shown in Fig. 2, the fit of eqn. 1 to the data was found to be satsifactorily valid. This agreement illustrates the strength of the thermodynamic approach: it does not require a physical model.

REFERENCES

1 B. Öbrink and A. Wasteson, *Biochem. J.*, 121 (1971) 227–223.
2 M. Shaw and A. Schy, *J. Chromatogr.*, 170 (1979) 449–452.
3 A. G. Ogston, *Trans. Faraday Soc.*, 54 (1958) 1754–1757.
4 T. C. Laurent and J. Killander, *J. Chromatogr.*, 14 (1964) 317–330.
5 T. C. Laurent, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 25 (1966) 1127–1134.
6 W. D. Comper and T. C. Laurent, *Physiol. Rev.*, 38 (1978) 255–315.
7 P. D. Watson and F. S. Grodins, *Microvasc. Res.*, 16 (1978) 19–41.
8 S. Hjertén, *J. Chromatogr.*, 50 (1970) 189–208.
9 T. C. Laurent and K. A. Granath, *Biochim. Biophys. Acta*, 136 (1967) 191–198.
10 M. Shaw and A. Schy, *Biophys. J.*, 17 (1977) 47–55.
11 T. C. Laurent, *Biochim. Biophys. Acta*, 136 (1967) 199–205.
12 J. S. Fawcett and C. J. O. R. Morris, *Separ. Sci.*, 1 (1966) 9–25.
13 K. Hellsing, *J. Chromatogr.*, 36 (1968) 170–180.
14 S. Nir and W. D. Stein, *J. Chem. Phys.*, 55 (1971) 1598–1603.
15 K. A. Granath, Pharmacia AB, Uppsala, personal communication.
16 L. Beadling, Pharmacia AB, Uppsala, personal communication.
17 C. Tanford, *Physical Chemistry of Macromolecules*, Wiley, New York, 1967.
18 E. M. Landis and J. R. Pappenheimer, *Handbook of Physiology*, Vol. 2, Sect. 2, American Physiological Society, Washington, D.C., 1963, pp. 961–1034.
19 G. K. Ackers, in H. Neurath and R. L. Hill (Editors), *The Proteins*, Vol. I, Academic Press, London, 1975, pp. 1–94.