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ESTIMATION OF FIBER RADII AND LENGTHS OF SEPHADEX GEL BY THE MODIFIED OGSTON MODEL

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Abstract—The classical Ogston-Laurent gel model of size exclusion chromatography was modified to include both dextran fibers and cross-links. The dimensions of Sephadex gel fibers were determined from the experimental data in the literature and the microstructural study of Sephadex gel. The corresponding radii of the dextran chain and the cross-linking were calculated to be 7.8×10^{-8} and 4.8×10^{-8} cm, respectively. The lengths of the dextran chain varies from 1.45×10^{12} (G-200) to 9.46×10^{12} cm/ml (G-25) while the length of the cross-link varies from 0.14×10^{12} (G-200) to 3.18×10^{12} cm/ml (G-25). The partial specific volume of swollen Sephadex gel material was calculated to be 0.586 ml/g.

INTRODUCTION

Chromatography quickly becomes a method of choice in the field of bioseparations. Size exclusion chromatography is a chromatographic technique utilizing the molecular sieving properties of porous gel particles. Detailed knowledge of three dimensional gel structure is imperative for a better prediction of separation performances of size exclusion chromatography.

The Ogston-Laurent gel model (OLM) is one of the most popular geometric models which predict the gel pore volume available to solute in size exclusion chromatography. The model originally derived by Ogston [1] considers a three-dmensional network of gel polymer as randomly distributed rigid fibers. According to the model the fraction of volume available to a spherical particle (K_{av}) is expressed as follows.

$$K_{av} = \exp[-\pi L (r+R)^2] \quad (1)$$

where L, R are the length [cm/ml] and the radius [cm], respectively, of the polymer rods and r is the radius [cm] of a solute.

Sephadex and Bio-Gel gels are two of the most popular commercial gels used in size exclusion chromatography. These gels are produced by bridging the backbone polymer chains, dextran and polyacrylamide, respectively, with proper crosslinking materials. Therefore it is rigorous to investigate the three-dimensional network of these gel polymers as randomly distributed fibers of two origins, backbone polymer chain and crosslinking bridge, instead of one as in OLM. A schematic drawing of two fiber system for Sephadex gel is presented in Fig. 1. The mathematical derivation for the modified Ogston model (MOM) is straightfowards from the original work of Ogston [1]. Koo and Wankat [2,3] already studied the microstructure of Sephadex gel by using the consistent force field theory. We obtained the dimensions of two fiber units in vacuum to be 2.54×10^{-8}, 1.54×10^{-8} cm in radii and 5.61×10^{-8}, 4.84×10^{-8} cm in length, respectively, as a result of computer calculations. The dimensions of fibers in aqueous solution were determined by curve-fitting the experimental data in Laurent and Killander [4] into MOM. The results were compared with those of Laurent and Killander and the previous work of the present author [5].

THEORETICAL DEVELOPMENT

The derivation of the original Ogston model was clearly described in the literature [1]. Only the last part of the derivation for MOM is presented here as the whole derivation is very similar to the original work.

When a network is composed of very long straight fibers, the probability distribution (dP/dD) of spaces of size D is the probability that there shall be no contact within D and at least one tangential or end contact between D and D + dD. D is the tangential distance of fiber or the distance of one of its ends from the origin of a space [see Eqn. (12a) of [1]].

$$\frac{dP}{dD} = 2\pi(\nu_1 l_1 + \nu_2 l_2)\exp[-\pi(\nu_1 l_1 + \nu_2 l_2)D^2] \quad (2)$$

where ν_1, ν_2 are the average numbers of center of fibers with R_1, R_2 as radii and l_1, l_2 as lengths, respectively. The fraction of the total volume of the suspension which can accomodate a spherical particle with

radius r is the probability that D of a space is greater than r.

$$P_{D>r}=\int_r^\infty \frac{dP}{dD}dD$$

$$=\exp\{-\pi(\nu_1 l_1 r^2-\nu_2 l_2 r^2)\} \quad (4)$$

Finite thickness of fibers are allowed for by adding the half thickness to the value of r.

$$K_{av}=\exp\{-\pi(\nu_1 l_1 (r-R_1)^2+\nu_2 l_2 (r+R_2)^2)\} \quad (5)$$

This is the expression for MOM with fiber units with R_1, R_2 as radii and l_1, l_2 as lengths. MOM can easily be extented to the multi(n)-fiber system.

$$K_{av}=\exp\{-\pi\sum_{i=1}^{n}\nu_i l_i (r+R_i)^2\} \quad (6)$$

where subscript i is for i-th fiber.

METHODS

1. Internal pore volume of swollen gel

A simple way to determine the pore volume of swollen gel is based on the mass balance of gel material and liquid.

$$(V_m+V_w)\,d=V_m d_m+V_w d_w \quad (7)$$

where V_m, V_w are the volumes occupied by the swollen gel material and the free liquid in a gel particle, respectively, d is the density of swollen gel particle, d_m, d_w are the densities of the swollen gel material and liquid, respectively. The fraction of volume available to very small molecules [$K_{av}(r=0)$] can be considered to be the fraction of the free liquid volume in the swollen gel particle [$V_w/(V_m+V_w)$]. Water is usually the liquid of choice ($d_w=1$). Therefore $K_{av}(r=0)$ can be calculated from d_m and d.

$$K_{av}(r=0)=\frac{d_m-d}{d_m-1} \quad (8)$$

2. Concentration of fibers in gel particle

Sephadex is a cross-linked dextran gel and schematically considered to consist of two types of fibers. One is glucose with α-1,6-glycosidic linkage and the other is cross-linking of glycerin ether bond. It is important to know the average number of fibers in 1 ml (ν) to quantify MOM. Granath [6] reported the degree of substitution during the cross-linking reaction of Sephadex gels. The number of glucose units for one cross-linking(n_g) can be calculated from the fractions of glucose with different substitutions.

$$n_g=\frac{100}{(P_1+1.5\times P_2)/2} \quad (9)$$

where P_1, P_2 are the fractions of glucose substituted in position 4 or 2, in position 4 and 2 or in position 3, respectively. The fraction of unsubstituted glucose unit is $1-P_1-P_2$. For P_2, average 1.5 linkages are assumed to connect to a glucose unit. As each linkage connects to two glucose units, the denominator must be divided by 2. From these data the fiber concentrations were calculated as follows.

$$\nu_1=\frac{n_g N}{(n_g M_1+M_2)V_s} \quad (10)$$

$$\nu_2=\frac{N}{(n_g M_1+M_2)V_s} \quad (11)$$

where N is the Avogadro number, M_1, M_2 are the molecular weights of the glucose unit and the cross-linking unit, V_s is the volume of swollen gel particle [ml/g dry gel]. The V_S values were calculated from the water regains [4] and the densities of swollen gels [7]. The molecular weight of the glucose unit with no, one and two substitutions (loss of hydroxyl groups) were set to be 162 ($C_6H_{10}O_5$), 145($C_6H_9O_4$) and 128($C_6H_8O_3$), respectively. The molecular weight of the cross-linking unit was fixed to 90($C_3H_6O_3$).

3. Forcing function

A least square method was used to fit the experimental data into MOM (K_{av} versus r). A forcing function was formulated from MOM to have the curves pass through the precalculated K_{av} when r is zero.

$$L_2=\frac{-\log[K_{av}(r=0)]}{\pi(\alpha_1\alpha_2^2+1)R_2^2} \quad (12)$$

where L_2 is the fiber length of cross-linking [cm/ml] and equals $\nu_2 l_2$. α_1 and α_2 are the ratios of the lengths (L_1/L_2) and the radii (R_1/R_2), respectively, of the dextran chain and the cross-linking. L_1 is the fiber length of dextran backbone chain and equals $\nu_1 l_1$. The ratios are assumed to be the same both in vacuum and in aqueous solution. With this arrangement, the number of parameters to be optimized reduces to one. Series of experimental data [4] were curve-fitted into MOM by using RNLIN (IMSL subroutine) with R_2 as the only parameter.

4. Radii and lengths of fibers

The radii of the dextran chain and the cross-linking are assumed to be constant while the lengths of which vary for different Sephadex gel types, following the description of MOM. The R_2 value which yields the least squares of discrepancies for all types of Sephadex G gel was chosen as the radius of cross-linking. Then the length of cross-linking (L_2) for each gel type was calculated from the forcing function with this R_2. The radius (R_1) and the length (L_1) of dextran chain were calculated from R_2 and L_2, respectively, by multiplying

the ratios, α_2 and α_1, respectively.

5. Partial specific volume of dextran gel

The partial specific volume of dextran gel can be obtained from the correlation between the lengths of gel fibers and the dextran concentration.

$$v_{s_1} W = \pi R_1^2 L_1 \quad (13)$$

$$v_{s_2} W = \pi R_2^2 L_2 \quad (14)$$

where v_{s_1}, v_{s_2} are the partial specific volumes of the dextran chain and the cross-linking [ml/g], W is the dextran concentration in gel particle [g dry gel/ml]. Therefore v_{s_i} can be calculated from the slope ($v_{s_i}/\pi R_i^2$) of a line correlating L_1 and W, where i is for the i-th fiber. The W values for each Sephadex gel type can be calculated from the water regain (W_r) and the density of swollen gel (d).

$$W = \frac{d}{1+W_r} \quad (15)$$

The water regain data in Laurent and Killander [4] were used in this study. W is the inverse of the volume of swollen gel particle (V_s). The inverse of the sum of v_{s_1} and v_{s_2} is the density of the swollen gel material and to equal the d_m value assumed to calculate K_{av} (r = 0) at the beginning for the consistancy.

RESULTS AND DISCUSSION

$K_{av}(r = 0)$ is a part of a forcing function and depends on the density (d_m) or the partial specific volume ($1/d_m$) of swollen gel materials. The partial specific volume was determined to be 0.586 ml/g by trial and error. Fiber concentrations for dextran chain and cross-linking were calculated from the number of glucose units for a cross-linking and presented in Tables 1 and 2. The fiber concentrations change from 1.7×10^{20} to 11.22×10^{20}/ml for dextran chain and from 0.20×10^{20} to 4.37×10^{20}/ml for cross-linking as the gel type number varies from 200 to 25 as expected because the gel concentration decreases as gel type number increases. The ratio of fiber concentrations of cross-linking to dextran chain (degree of cross-linking) decreases from 0.39 to 0.11 as gel type number increases.

Table 1. Degree of substitution and number of glucose units for a cross-linking

Sephadex type	P_1[1]	P_2[2]	n_g[3]
G-25	0.264	0.343	2.57
G-50	0.200	0.220	3.77
G-75	0.182	0.246	3.63
G-100	0.116	0.167	5.46
G-200	0.098	0.091	8.53

1; Fraction of glucose units linked in position 4 or 2

2; Fraction of glucose units linked in positiosn 4 and 2 or in position 3

3; Number of glucose units for a cross-linking

The experimental data and the curvefitting by MOM are presented in Figs. 1,2 and 3. Sizable discrepancies between the experimental data and the curves are noticed for Sephadex G-25 and G-50. This is partially due to the forcing functions which have the curves pass through $K_{av}(r = 0)$'s at r = 0 which are not the experimental data but the calculated values from the densities of the swollen gel particle and gel material. The radius of the cross-linking was calculated to be 4.81×10^{-8} cm from the experimental data by linear regression. It is the value when v_s is 0.586 ml/g. The corresponding radius of the dextran fiber is 7.80×10^{-8} cm. The lengths of dextran and cross-linking fibers were

Table 2. Calculated fiber concentrations of Sephadex G gels

Sephadex type	Wr[1] (g/g dry gel)	d[2] (g/ml)	V_s[3] (ml/g dry gel)	M_1[4] (g/mole)	Fiber concentration($\times 10^{20}$/ml)	
					Dextran chain	Cross-linking
G-25	2.3	1.13	2.92	148.8	11.22	4.37
G-50	4.3	1.07	4.95	153.0	6.88	1.82
G-75	8.7	1.05	9.23	153.0	3.67	1.01
G-75[5]	7.9	1.05	8.48	153.0	3.99	1.10
G-100	9.7	1.04	10.29	155.8	3.40	0.62
G-200	19.9	1.02	20.49	158.0	1.74	0.20

1; Water regain [5]

2; Density of swollen gel [8]

3; Volume of swollen gel [= (1 + Wr)/d]

4; Molecular weight of a glucose unit [= $162(1-P_1-P_2) + 145\ P_1 + 136.5\ P_2$]

5; Two sets of experimental data for G-75 [5]

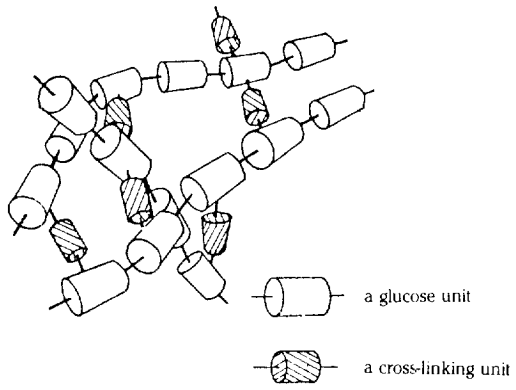

Fig. 1. A schematic drawing of Sephadex G gel network of the two fiber system.

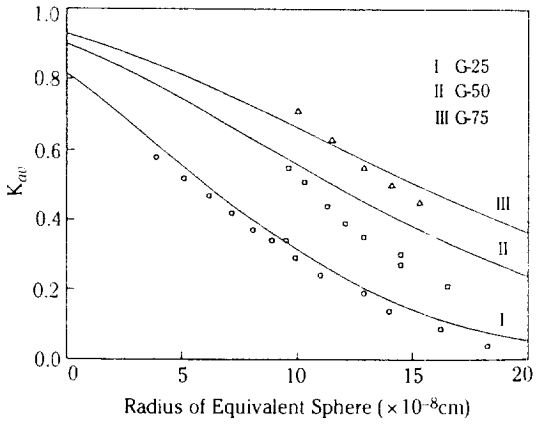

Fig. 2. K_{av} values calculated from the experimental data of Laurent and Killander obtained on Sephadex G-25, 50 and 75.
Curves are drawn according to MOM with values appearing in Table 3.

calculated from the radius of cross-linking and the forcing function. The results are presented in Table 3. The lengths of dextran and cross-linking fibers are ranging from 1.5×10^{12} to 9.5×10^{12} cm/m*l* and from 0.14×10^{12} to 3.2×10^{12} cm/m*l*, respectively.

Although no direct measure to compare the results for MOM and OLM is available the fact that the sum of L_1 and L_2 is approximate to L in OLM is encouraging as the lengths of two different fibers are additive in nature. Note that the R value (7×10^{-8} [4] or 7.21×10^{-8} cm [5]) is between R_1(7.8×10^{-8}cm) and R_2(4.8×10^{-8}cm). A probably more apparent way to validate the calculated dimensions of the two fibers is to compare the $K_{av}(r=0)$ value and the volume fraction which is not occupied by the gel fibers of concern. The comparison is made

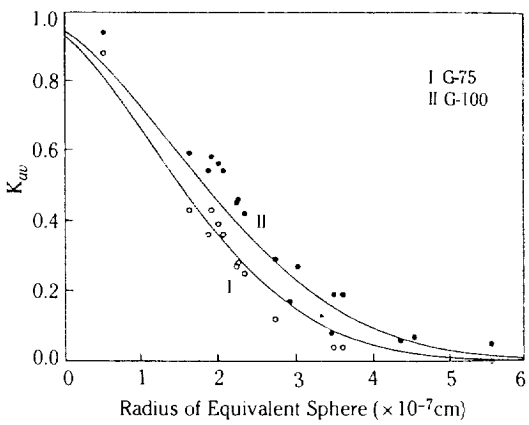

Fig. 3. K_{av} values calculated from the experimental data of Laurent and Killander obtained on Sephadex G-75 and 100.
Curves are drawn according to MOM with values appearing in Table 3.

Table 3. Comparison of fiber lengths between MOM and OLM[1]

Sephadex type	MOM[2]		OLM	
	L_1	L_2	Koo[3]	Laurent & Killander[4]
G-25	9.5	3.2	12.5	14.0
G-50	5.0	1.2	6.4	8.2
G-75	3.5	0.84	4.5	4.8
G-75	3.5	0.84	4.5	4.6
G-100	2.9	0.45	3.6	2.9
G-200	1.5	0.14	1.8	1.6

1; Unit, $\times10^{12}$cm/m*l*. Experimental data in Laurent & Killander [4] [Killander et al. (G-200, Wr = 1.99), Andrews (G-75, G-100)]
2; $R_1 = 7.80\times10^{-8}$cm, $R_2 = 4.81\times10^{-8}$cm
3; $R = 7.21\times10^{-8}$cm [5]
4; $R = 7\times10^{-8}$cm [4]

Table 4. Comparison between K_{av} (r = 0) and volume fraction not occupied by gel fibers

Sephadex type	$K_{av}(r=0)$	$(1-\pi R_1^2L_1-\pi R_2^2L_2)/1$[1]
G-25	0.816	0.796
G-50	0.901	0.895
G-75	0.929	0.926
G-100	0.943	0.942
G-200	0.972	0.971

1; $R_1 = 7.80\times10^{-8}$cm, $R_2 = 4.81\times10^{-8}$cm

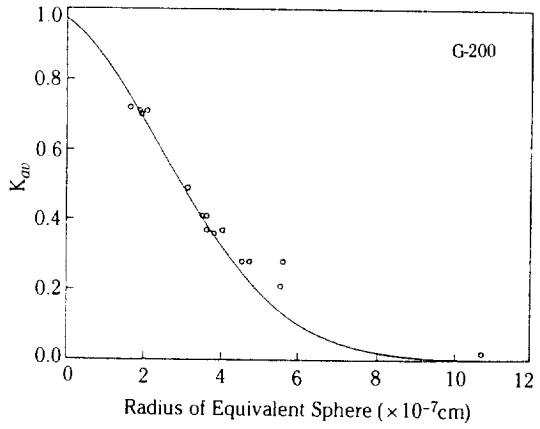

Fig. 4. K_{av} values calculated from the experimental data of Laurent and Killander obtained on Sephadex G-200.
Curves are drawn according to MOM with values appearing in Table 3.

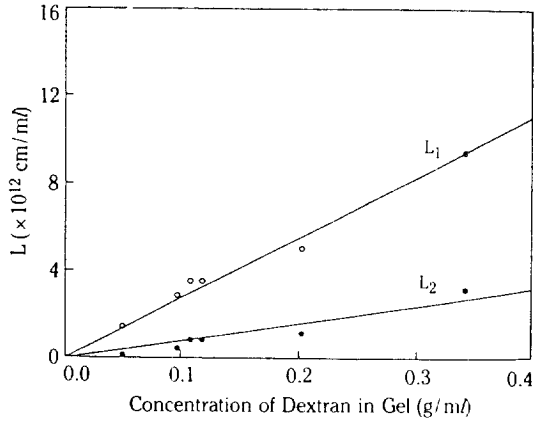

Fig. 5 The length of dextran chain(L_1) and cross-linking(L_2) in Sephadex G gels calculated from the experimental data.

in Table 4. Two values are very close when gel type numbers are large while the difference increases as gel type number decreases. This increasing discrepancy can be explained by the series expansion of MOM as described in the previous work [5].

The partial specific volumes of dextran chain (v_{s_1}) and cross-linking (v_{s_2}) were calculated from the correlation between L_1, L_2 and W as shown in Fig. 4. v_{s_1} and v_{s_2} calculated from the slopes are 0.528 and 0.058 ml/g, respectively. The fact that the sum of the partial specific volumes of two fibers equals 0.586 ml/g which was used to calculate $K_{av}(r = 0)$ at the beginning shows the consistancy of the present analysis.

This study is mainly based upon a specific series of experimental data [4] and such assumption as the constant ratios of radii and lengths of two fibers in vacuum and in aqueous solution. The calculated dimensions of fibers may vary for different data employed and according to the description for the structural changes of gel as it hydrates in real environment.

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REFERENCES

1. Ogston, A.G.: *Trans. Faraday Soc.*, **54,** 1754 (1958).
2. Koo, Y.M.: Ph. D. Dissertation, Purdue University, West Lafayette, Indiana, U.S.A. (1985).
3. Koo, Y.M. and Wankat, P.C.: *Korean Biochem. J.*, **21,** 22 (1988).
4. Laurent, T.C. and Killander, J.: *J. Chromatogr.*, **14,** 317 (1964).
5. Koo, Y.M.: *Korean J. of Biotech. and Bioeng.*, **4**(1), 15 (1989).
6. Granath, K., in James, A.T., and Morris, L.J. (Editors): "New Biochemical Separations", D. Van Nostrand, London, (1964).
7. Determann, H.: Gel Chromatography, Springer-Verlag, New York, NY (1968).