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Adsorptive interaction of Ficoll standards with porous glass size-exclusion chromatography columns

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Abstract

Ficoll, a densely branched non-ionic polysaccharide, whose hydrodynamic properties resemble those expected for compact spheres, is an interesting material for fundamental studies of permeation in chromatographic and porous media. Calibrating porous glass size-exclusion chromatography columns with Ficoll, we found unexpected retention of this solute in salt solution, but normal behavior in nearly pure water (0.003 M, pH 7.0 sodium phosphate buffer). These chromatographic results, along with the findings of adsorption studies and viscometric measurements, can be best explained on the basis of sodium ion binding to Ficoll and consequent adsorption of the transient pseudo-polycation thus formed.

1. Introduction

A wide variety of packing materials are currently available for aqueous size-exclusion chromatography (SEC), and many of them provide satisfactory attributes in the four categories of mechanical properties, pore size range, chromatographic efficiency, and low affinity for adsorbing solutes such as proteins [1–4]. Porous glass, initially developed by Haller [5] and commercialized first by Corning (hence the term “CPG”), and then by Electronucleonics and recently also Schott, performs well in the first two categories but not in the third and fourth. Consequently, its practical use is restricted. Nevertheless, the facts that its pore structure can be well defined by porosimetry and microscopy

and that the composition is very well known (in contrast to other commercial packings) make it quite well suited for fundamental studies [6–11]. In such investigations, it is commonplace to employ neutral polysaccharide as model polymers; their elution on this and on all other SEC packings are always observed or assumed to be “ideal”, i.e. controlled by steric repulsion alone, and no other form of interaction [12]. While the principal polysaccharides so employed have been dextrans, and subsequently pullulan [13], an additional solute of interest is Ficoll [14–16], a densely branched polysaccharide that more nearly approximates a compact sphere [17]. Because of its well-defined geometry, Ficoll has been extensively used in studies of permeation of both synthetic membranes and biological media [18–22]. Our investigation of Ficoll on CPG disclosed unexpected adsorption behavior in the presence

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Table 1
Characteristics of pullulan standards

Sample	M^a	$\overline{M}_w/\overline{M}_n^a$	$[\eta]$ (cm ³ /g) ^b	R_η (Å) ^c	$[\eta]$ (cm ³ /g) ^d	R_η (Å) ^e
P-800	853 000	1.14	187.0	294	171	285
P-200	186 000	1.13	70.4	128	55	118
P-100	100 000	1.10	45.9	90	39.8	86
P-50	48 000	1.09	28.6	60	23.4	56
P-20	23 700	1.07	18.1	41	15.5	39
P-10	12 200	1.06	11.9	28	9.7	27
P-5	5 800	1.07	7.9	19	6.3	18

\overline{M}_w = Weight-average molecular mass; \overline{M}_n = number-average molecular mass.

^a Supplied by the manufacturer.

^b In water, at 25°C (supplied by the manufacturer).

^c From columns 2 and 4, via Eq. 1.

^d From Ref. [24], in 0.2 M sodium phosphate buffer (pH 7.0) at 25°C.

^e From columns 2 and 6, via Eq. 1.

of added electrolyte, which is the subject of this communication.

2. Experimental

2.1. Materials

Pullulan standards (Shodex Standard P-82) with polydispersity of about 1.1 were obtained from Shodex (New York, NY, USA). Ficoll fractions were obtained from Dr. K. Granath of Kabi Pharmacia (Uppsala, Sweden), and were characterized at Uppsala by a combination of

light scattering and SEC on Sepharose 4B (fractions 9, 12, 15 and 20) and Sephadex G-200 Superfine (fractions 2 and 11). The characteristics for pullulan and Ficoll are described in Tables 1 and 2, respectively. Reagents were ACS grade. The water used throughout was deionized water from a Millipore system.

2.2. Methods

Viscometry

Intrinsic viscosities were measured with a Schott Geräte AVS-400 equipped with a 2-ml capacity capillary viscometer (capillary

Table 2
Characteristics of Ficoll fractions

Fraction	M_r^a	$[\eta]$ (cm ³ /g) ^b	R_η (Å) ^c	$[\eta]$ (cm ³ /g) ^d	R_η (Å) ^e
T1800, fraction 9	714 000	20.0	131	23.9	139
T1800, fraction 12	461 000	17.5	109	20.8	115
T1800, fraction 15	321 000	16.2	94	17.5	96
T1800, fraction 20	132 000	12.6	64	13.4	65
T2580-IVB, fraction 2	71 800	9.9	48	10.9	50
T2580-IVB, fraction 11	21 800	7.0	29	7.8	30

^a From supplier.

^b In water at 25°C.

^c From columns 2 and 3, via Eq. 1.

^d In 0.2 M sodium phosphate buffer (pH 4.5) at 25°C.

^e From columns 2 and 5, via Eq. 1.

constant = 0.008352) at $25.0 \pm 0.02^\circ\text{C}$. Ficoll was dissolved in water or ionic strength 0.2 M, pH 4.5 sodium phosphate buffer, at concentrations ranging from 10 to 24 mg/ml, and filtered through 0.45- μm Gelman filters. The average efflux times for solvents were in the range of 100–120 s with a precision of ± 0.02 s. The intrinsic viscosity $[\eta]$ was obtained from the intercept of the plot of the reduced specific viscosity, $\eta_{sp}/\text{concentration}$, vs. concentration (cm^3/g), and the viscosity radius R_η (cm) from the following equation [23]

$$R_\eta = \left[\frac{3[\eta]M_r}{10\pi N_A} \right]^{1/3} \quad (1)$$

where M_r is the molecular mass of the polymer and N_A is Avogadro's number.

Separate measurements for intrinsic viscosity were carried out with a Schott Geräte AVS-300 equipped with a dilution viscometer (capillary constant ca. 0.01) at 25.0°C for pullulan-1600 K (Shodex P-82) and Ficoll fraction 9 in different solvents. The average solvent efflux times were in the range of 90–98 s and the Hagenbach kinetic correction was applied to all measured efflux times as recommended by the manufacturer of the capillary viscometer.

Chromatography

A stainless-steel column (50 cm \times 5.5 mm I.D.) was dry packed with Bioran-CPG, pore diameter 330 \AA , 30–60 μm grain size, and 86 m^2/g surface area, from Schott Geräte (Mainz, Germany). The number of theoretical plates, 800, was obtained by injecting 10% $^2\text{H}_2\text{O}$ in water as a mobile phase. The chromatography system consisted of a Mini Pump from Milton Roy (St. Petersburg, FL, USA). A 75- μl injecting loop from Valco (Houston, TX, USA), differential refractometer R401 from Waters (Milford, MA, USA), and Omniscribe Recorder from Houston Instruments (Austin, TX, USA).

Mobile phases were prepared to the required ionic strength and pH from (1) NaH_2PO_4 and Na_2HPO_4 , (2) CH_3COOH and CH_3COONa and (3) H_3PO_4 and Li_3PO_4 , and filtered through a 0.45- μm filter. All chromatograms were ob-

tained by injecting 3 to 5 mg/ml of polymer solution at a flow-rate of 0.8 to 0.9 ml/min. Flow-rate and chart speed were measured periodically by weighing the column eluent, and by measuring the chart paper for about 20 to 25 min. respectively. Total volume (V_t) and void volume (V_0) were obtained by injecting $^2\text{H}_2\text{O}$ and pullulan-800K, respectively.

Adsorption experiments

TLC standard-grade silica gel with an average particle size 2–25 μm , pore diameter 60 \AA , and surface area ca. 500 m^2/g was from Aldrich. Small-particle-size silica was removed by settling in water and discarding the supernatant. The remaining silica was dried at 80°C overnight. A 5 mg/ml polymer solution was prepared in the required buffer and divided into two equal parts. To one part, 20% (w/w) of dried silica gel was added. A separate polymer-free solution of 20% (w/w) of silica gel in buffer served as a blank. All three solutions were tumbled for two days, centrifuged, filtered (0.45 μm), and injected onto the column in the same buffer as the mobile phase. Comparisons of the peak areas of the chromatograms indicated the magnitude of the adsorption effect.

3. Results

The partition coefficient, K_{SEC} , is given by

$$K_{SEC} = \frac{V_e - V_0}{V_t - V_0} \quad (2)$$

where V_e is the solute elution volume, V_0 is the interstitial volume or void volume and V_t is the total mobile phase volume.

Fig. 1 shows the "universal calibration" [25] plot of $\log [\eta]M_r$ vs. V_e for pullulan and Ficoll fractions in sodium phosphate buffer of different ionic strength, I , and pH. Both polymers conform to a single plot at $I = 0.003$ M, pH 7.0, but late elution is observed in $I = 0.2$ M, pH 7.0 buffer. This anomalous retention is significantly higher for Ficoll fractions than for pullulan, suggesting some interaction between CPG and Ficoll. This interaction is also evident from the

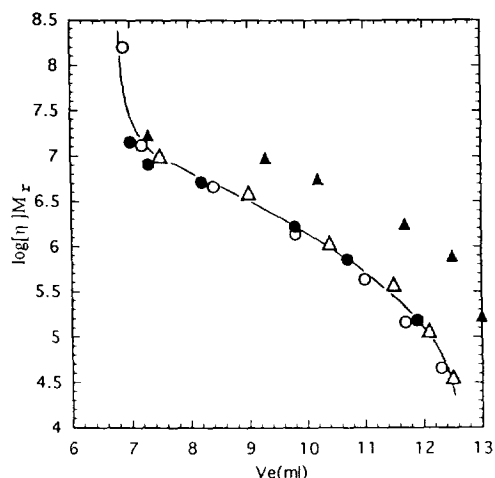


Fig. 1. Universal calibration plot for pullulan (open symbols) and Ficoll (filled symbols) on CPG in $I=0.003$, pH 7.0 phosphate buffer (O, ●), and in $I=0.2$, pH 4.5 phosphate buffer (Δ , ▲).

tailing of the peaks of Ficoll fractions 9 and 12 in $I=0.2$ M, pH 4.5 buffer.

The partition coefficient, K_{SEC} , is given by

$$K_{SEC} = \frac{V_e - V_0}{V_t - V_0} \quad (3)$$

K_{SEC} for spherical solutes in pores of well defined geometry [26] is given by

$$K_{SEC} = \left(\frac{r_p - R}{r_p} \right)^\lambda \quad (4)$$

where $\lambda=1, 2$ or 3 for slab, cylindrical or spherical geometry of the pore, r_p is pore radius, and R is solute radius. If the plot of $K^{1/2}$ vs. R ($R=R_\eta$) gives a straight line with an intercept of 1, then the value of λ is 2 and the pores behave as if they were cylindrical. The slope of this plot is $1/r_p$. The value of r_p so obtained from the upper part of Fig. 2 was around 190 Å, in close agreement with the mercury porosimetry data, supplied by the manufacturer, which yield 199 Å. For the present work, the principal value of Eq. 4 is the linearization of the data which simplifies comparisons between pullulan and Ficoll, and also among the different mobile phases studied.

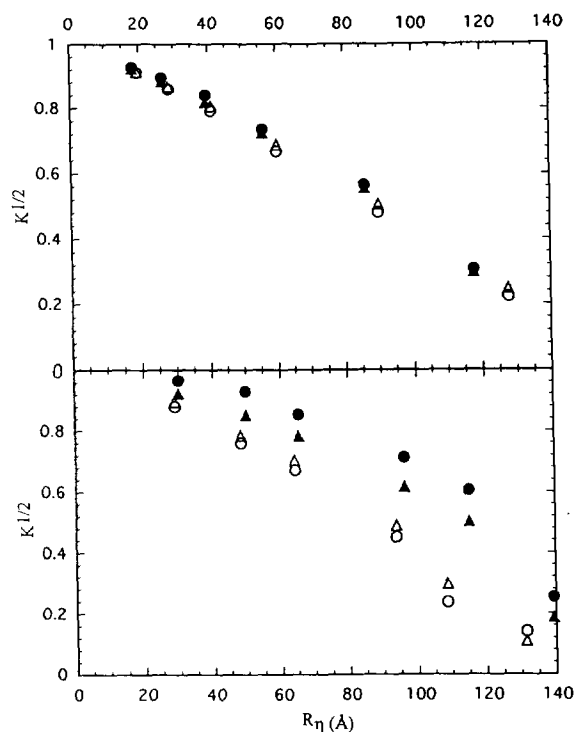


Fig. 2. Dependence of K_{SEC} on solute radius for pullulan (upper) and Ficoll (lower) in sodium phosphate buffers: (O) $I=0.003$, pH 7.0; (●) $I=0.2$, pH 4.5; (Δ) $I=0.003$, pH 4.5; and (▲) $I=0.2$, pH 7.0.

Fig. 2 shows $K^{1/2}$ vs. R_η for pullulan and Ficoll fractions in sodium phosphate buffer of different ionic strengths and pH. From Tables 1 and 2 we observe that the effect of salt on the dimensions of pullulan and Ficoll is negligible on the scale of Fig. 2; therefore, R_η was calculated using the values of $[\eta]$ measured in pure water for all 0.003 ionic strength buffers. Similarly, $[\eta]$ measured for pullulan and Ficoll in $I=0.2$, pH 4.5 buffer was used to calculate R_η in all high-ionic-strength buffers, regardless of pH. Plots of $K^{1/2}$ vs. R for Ficoll fractions are seen to show strong deviations from Eq. 3 especially at high ionic strength. The effect of ionic strength is larger at pH 4.5 than at pH 7.0, and the effect of pH is larger at high ionic strength (0.2 M) than at low ionic strength (0.003 M). That is, the anomalous retention is strongest at high ionic strength and lower pH. These correspond to the conditions

under which peak tailing was most clearly observed.

Fig. 3 shows the effect of the buffer anion on the dependence of $K^{1/2}$ on R for both polymers in phosphate and acetate buffers of different ionic strength and pH. Relatively little effect is seen for pullulan. For Ficoll, 0.003 *M* ionic strength phosphate buffers give nearly normal behavior at both pH 4.5 and 7.0. Anomalous retention appears for low-ionic-strength acetate buffer, high-ionic-strength phosphate at pH 7.0, or high-ionic-strength acetate at pH 4.5. The most striking effects are observed for high-ionic-strength pH 4.5 sodium phosphate.

The effect of cation is shown in Fig. 4. There is no measurable difference between lithium and sodium phosphate for pullulan at different ionic strengths and pH. For Ficoll, however, K_{SEC} is

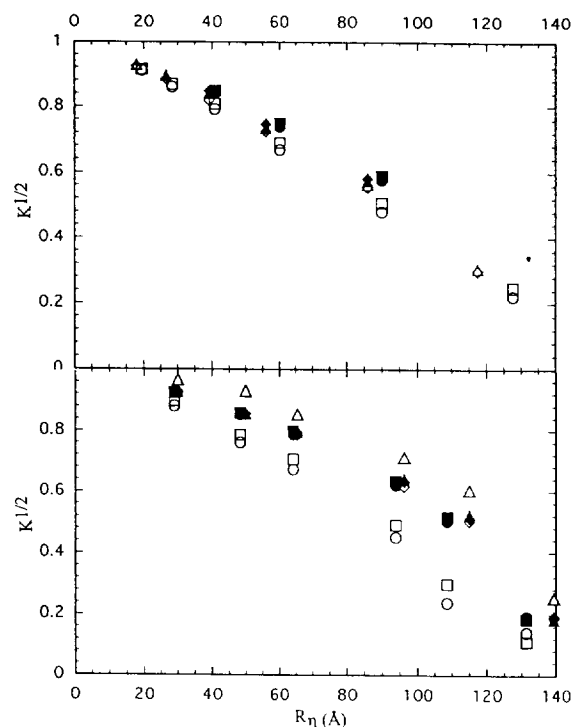


Fig. 3. Dependence of K_{SEC} on solute radius for pullulan (upper) and Ficoll (lower) in mobile phase buffers of (○, ●) $I = 0.003$, pH 7.0; (△, ▲) $I = 0.2$, pH 4.5; (□, ■) $I = 0.003$, pH 4.5; (◇, ◆) $I = 0.2$, pH 7.0. Open symbols for phosphate buffer, filled symbols for acetate buffer.

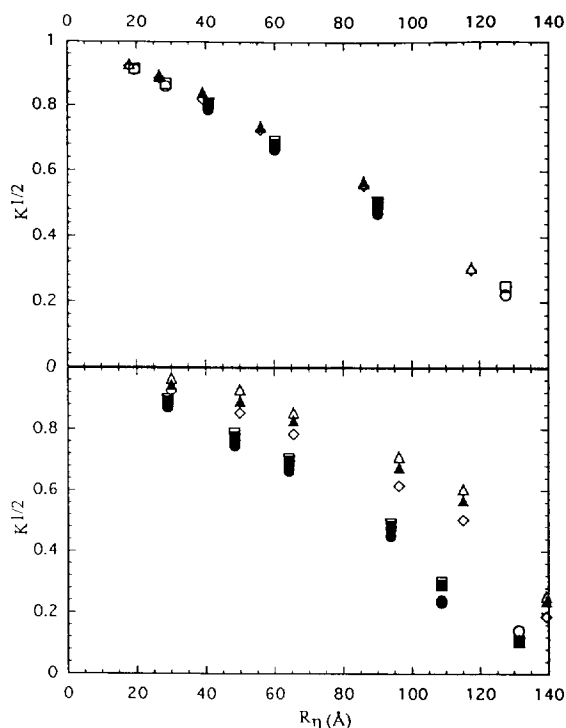


Fig. 4. Dependence of K_{SEC} on solute radius for pullulan (upper) and Ficoll (lower) in sodium phosphate buffer and lithium phosphate buffer. Open symbols as in Fig. 3; filled symbols are for lithium phosphate buffer of same ionic strength and pH as corresponding open symbols.

observed to increase when 0.2 *M*, pH 4.5 lithium phosphate is replaced by 0.2 *M*, pH 4.5 sodium phosphate.

The adsorption of Ficoll was studied using Ficoll-400 instead of the more precious Ficoll fractions. No adsorption could be observed on CPG glass, but a strong effect could be seen using silica gel as a model siliceous packing. Ficoll-400 was tumbled in sodium phosphate buffer of $I = 0.2$ *M*, pH 4.5 with and without silica gel. Both samples were centrifuged, filtered and injected onto the analytical column. Fig. 5 shows the resulting chromatograms. The peak area for Ficoll-400 after tumbling with silica is less than for the control sample, showing adsorption of the sample on silica. Equilibration with silica had no effect on peak shape or area for pullulan-100 in this buffer, or for either

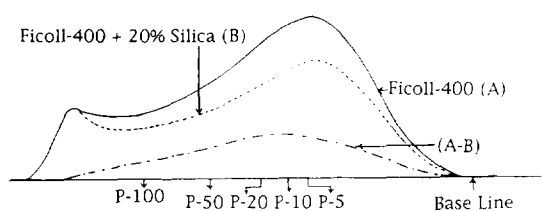


Fig. 5. Chromatograms of Ficoll-400: (A) untreated polymer; (B) polymer after equilibration with silica (supernatant). The difference (A – B) corresponds to the amount of Ficoll adsorbed. The horizontal axis corresponds to elution volumes, here parametrized using the elution volumes of pullulan standards (shown by vertical arrows).

pullulan or Ficoll at $I = 0.003 M$, pH 7.0. Thus adsorption was seen only for Ficoll in high-ionic-strength, pH 4.5 phosphate buffer, which corresponds to the conditions for anomalous retention as seen in Figs. 2 and 3.

Table 3 shows the intrinsic viscosity of pullulan-1600K and Ficoll fraction 9 in deionized water sodium phosphate buffer and tetramethylammonium chloride (TMACl). Despite its compact configuration, Ficoll exhibits the largest viscosity effect: a 15% increase upon addition of 0.1 M sodium phosphate at pH 4.0. On the other hand, no change in $[\eta]$ is observed upon addition of TMACl. The increase in viscosity of pullulan upon addition of salt is smaller, about 3% and not counterion-specific.

4. Discussion

Marked deviations from universal calibration are seen for Ficoll on CPG, particularly in the presence of added salt. These effects are not

exhibited by pullulan. The results of the adsorption measurements with silica gel as shown in Fig. 5 suggest that, while adsorption on CPG is clearly not as strong as on silica gel, it could account for the observed chromatographic effects. Some aspects of these observations are readily explained. In general, silica is more adsorptive towards hydrophilic solutes than glass, because of its higher surface density of silanol groups. We can also account for the enhanced adsorption of the lower- M_r Ficoll fractions observed in both the chromatography and the equilibrium experiments, by the greater access of smaller molecules to any porous surface. On the other hand, neutral polysaccharides in general do not display adsorption on SEC packings, and it is furthermore difficult to understand why Ficoll should be unique in this regard. A possible explanation could be that Ficoll might interact with cations like Na^+ leading to a transient polycation which then adsorbs onto the negatively charged surface of CPG. Although the nature of this interaction is not fully understood, the metal ion could be weakly coordinated to the ether and hydroxyl groups in Ficoll. Johansson [27] has in fact observed the formation of weak complexes between dextran and Na^+ and K^+ .

The intrinsic viscosity, a very sensitive measure of intramolecular expansion, increases by 15% for Ficoll fraction 9 upon addition of sodium phosphate as seen in Table 3. The absence of any viscosity change in tetramethylammonium chloride is consistent with a specific Na^+ ion interaction. Despite the fact that densely branched Ficoll should be more resistant

Table 3
Intrinsic viscosities of pullulan-1600K and Ficoll fraction 9

Solvent	$[\eta]$ (cm^3/g) (at 25°C)	
	Pullulan-1600K	Ficoll fraction 9
Deionized water	262	2.4
Sodium phosphate buffer, $I = 0.1$, pH 4.0	270	2.8
Tetramethylammonium chloride, 0.1 M	279	2.4

to conformational change than pullulan, the relative influence of salt on Ficoll viscosity is much larger. We suggest that transient binding of Na^+ to Ficoll to produce a pseudo-polycation results in intramolecular repulsion which leads to the observed intrinsic viscosity change.

We believe that the unusual effects of salt on both Ficoll adsorption and Ficoll viscosity are related, and that both arise from specific interactions between macromolecules and cation. While Johansson [27] has referred to weak complexation of this type for dextran, the effects seems to be stronger for Ficoll. Perhaps the closer proximity of saccharide units in densely branched Ficoll might facilitate intramolecular coordination of Na^+ with a saccharide residue.

Acknowledgement

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