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NEW CELLULOSE GEL FOR CHROMATOGRAPHY

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SUMMARY

Cellulose gel particles of spherical or irregular shape were prepared by dispersing cellulose–aqueous calcium thiocyanate solution or gel in organic solvents followed by regeneration of cellulose with methanol. These gel particles were tested as column packings for gel chromatography. The gel showed high porosity and sufficient rigidity to be used for gel filtration of large solutes. The pore size of the gel ranged from about a hundred to several thousand Ångströms, depending on the concentration and the molecular weight of the starting cellulose materials. In comparison with conventional macroporous gels such as agarose or cross-linked dextran gels, the cellulose gel has similar low adsorptivity and chemical stability, higher thermal resistance and remarkably improved mechanical strength.

INTRODUCTION

The use of gel substances as column packing in aqueous chromatography, such as gel filtration, ion-exchange or affinity chromatography, is becoming more important as a separation technique in industrial processes as well as in laboratories. Column packing materials for these chromatographies have conventionally been made of cross-linked dextran, agarose or polyacrylamide. However, these products are generally too expensive to be used in industrial applications since they are made from rather uncommon natural substances or they require carefully controlled processes for preparation.

On the other hand, cellulose, the most abundant natural material, has mainly been used in the form of a so-called "microcrystalline" powder for partition or ion-exchange chromatography. Since this material is a dense and very fine fibrous powder of irregular shape, it cannot accommodate large solutes and the packed gel bed suffers from clogging or contraction. Several attempts to prepare modified cellulose packing have been reported. In one method, cellulose powder is reinforced by graft cross-linking[1]; in another, cellulose is dissolved in an aqueous solution such as viscose[2] or Schweizer's solution[3], then dispersed in a non-polar liquid to generate spherical gel particles. Although several products prepared by these procedures are now available, the potential of cellulose as a chromatographic material has not been fully developed.

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In this study a new procedure of cellulose gel preparation is developed which is similar to that of the second method above except for the use of a concentrated aqueous solution of calcium thiocyanate as solvent. The dissolution behaviour of cellulose in this salt solution and the porous structure of the regenerated cellulose gel have been reported[4]. Here, the cellulose gel was prepared in granular or bead form and its performance for gel filtration was examined and compared with conventional gel packings.

EXPERIMENTAL

Preparation of cellulose gel particles

Material. Two kinds of cellulose material of different degrees of polymerization (DP) were used as starting materials: (a) Whatman CF-1 cellulose powder, DP 180; (b) Cotton linter cellulose, DP 1620. Characterization of the starting and the regenerated cellulose has been reported previously[4].

Dissolution of cellulose. Dry cellulose material was weighed and dissolved in an aqueous solution of 59% (w/w) calcium thiocyanate (Wako, Osaka, Japan) at 120–140°C in the absence of air. The solution obtained formed a gel when cooled below about 80°C.

Dispersion of cellulose solution. Three methods were examined for preparation of cellulose gel particles.

(a) The salt–cellulose solution was cooled to room temperature to form a salt–cellulose gel. This gel was dispersed in methanol with a laboratory blender. Since methanol dissolves the salt, dispersion and regeneration take place simultaneously in this procedure.

(b) The salt–cellulose gel was dispersed in 3–4 times its volume of a non-polar liquid containing a small amount of dispersion aid (sorbitan monooleate) at room temperature. The suspension was poured into a large volume of methanol.

(c) The same procedure as (b) was carried out at a high temperature at which the salt–cellulose solution remained liquid. The suspension was poured immediately into cold methanol.

In the last two methods, *o*-dichlorobenzene was chosen as the dispersion medium because of its high boiling point and low flammability. A conventional laboratory blender was used for dispersion. The cellulose gel particles obtained by these methods were collected on filter-paper and thoroughly washed with methanol and then with tap-water. Finally, the particles were fractionated by wet-sieving.

Gel filtration chromatography

The gel particles suspended in deionized water were packed in a glass column (500 × 10 mm with plungers; Pharmacia) to form a *ca.* 30-cm long gel bed. Approximately 0.2% solutions of standard solutes were separately injected into the column through a switching valve connected to a sampling port. The flow-rate was adjusted to 0.15–0.30 ml/min and kept constant within ±5% by a peristaltic pump fitted at the drain. Elution of solutes was monitored by a refractometer (Waters R-403). For comparison, the same test was carried out on two kinds of commercial cellulose packings (Whatman DE-52 and Pharmacia DEAE-Sephacel) and an agarose gel reinforced by cross-linking (Sepharose CL-2B, Pharmacia).

Table I lists the probe solutes used and their molecular diameters in water. The latter values were calculated from the diffusion coefficients or limiting viscosity numbers according to the well-known relations

$$R_d = \frac{kT}{6\pi\eta_w D} \quad (1)$$

$$R_v = 0.54\,(M[\eta])^{1/3} \quad (2)$$

where R_d and R_v are the radii of hydrodynamically equivalent spheres based on diffusion and viscosity respectively, k is the Boltzmann constant, T is the absolute temperature, η_w is the viscosity of water and D and $[\eta]$ are respectively the diffusion coefficient and the limiting viscosity number of the solute in water. The values of D, and $[\eta]$ were obtained from reported data and from the molecular weight of dextran, poly(ethylene oxide) and oligosaccharides[5].

TABLE I

MOLECULAR WEIGHTS AND SIZES OF STANDARD SOLUTES

Polymer	*Molecular weight, M_w**	*M_w* / M_n*	*Molecular diameter (Å)*	*Manufacturer*
Dextran				
T-2000	$2\cdot10^6$	—	580	Pharmacia (Uppsala, Sweden)
T-500	$5.11\cdot10^5$	2.67	330	
T-70	$6.85\cdot10^4$	1.70	130	
T-40	$3.95\cdot10^4$	1.34	100	
T-10	$9.4\cdot10^3$	1.71	50	
Poly(ethylene oxide)				
RE-7	$1.52\cdot10^6$	1.12	1140	Toyo Soda (Tokyo, Japan)
RE-6	$8.76\cdot10^5$	1.10	810	
RE-5	$3.48\cdot10^5$	1.05	460	
RE-4	$1.76\cdot10^5$	1.04	310	
Uniform Latex**				
0.5 μm	—	—	5000	Dow Chemical (Midland, MI, U.S.A.)
0.087 μm	—	—	870	
Raffinose	504	1.00	12	
Glucose	180	1.00	8	

* The values for Dextran T fractions and standard poly(ethylene oxide) were as given by the manufacturers.

** Used for void volume determination of large-pore gels.

RESULTS AND DISCUSSION

Table II gives the preparation conditions of the cellulose gel particles. It has been shown that cellulose does not significantly decompose under these conditions except for chain scission in long molecules[4]. Since spherical shape is desirable for chromatography packings, dispersion method (c) was mainly adopted. However, CF-1:9% gel*, the highest concentration grade prepared here, failed to form spherical particles because of its tendency to re-aggregate after dispersion at high temperatures.

* The cellulose gels are designated as: (cellulose material): (initial concentration (%, w/w)).

TABLE II

PREPARATION CONDITIONS OF CELLULOSE GEL PARTICLES

o-DCB = *o*-Dichlorobenzene.

Cellulose material	*Initial concentration of cellulose (%, w/w)*	*Dissolving condition*		*Dispersing condition*		*Particle shape*
		Temperature (°C)	*Heating time (min)*	*Liquid*	*Temperature (°C)*	
Whatman CF-1 cellulose powder	3.0	120–140	60	*o*-DCB	100–120	Spherical
	6.0	130–150	65	*o*-DCB	100–120	Spherical
				Methanol	25	Irregular
	9.0	130–150	80	*o*-DCB	25	Irregular
Cotton linter	1.0	120–140	30			
	1.5	120–140	30	*o*-DCB	100–120	Spherical
	3.0	140–160	60			

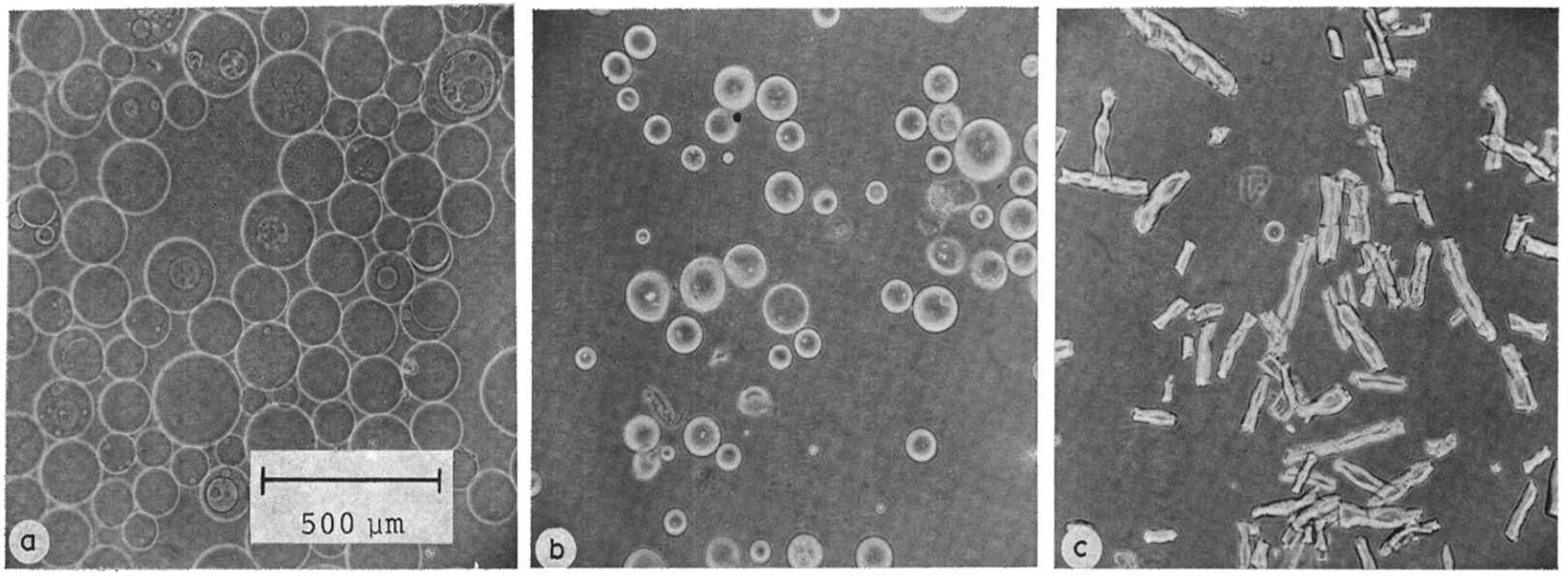

Fig. 1. Phase-contrast photomicrographs of commercial gel packings. (a) Sepharose CL-2B, (b) DEAE-Sephacel and (c) DE-52. Magnification as shown in (a) for all parts of Figs. 1 and 2.

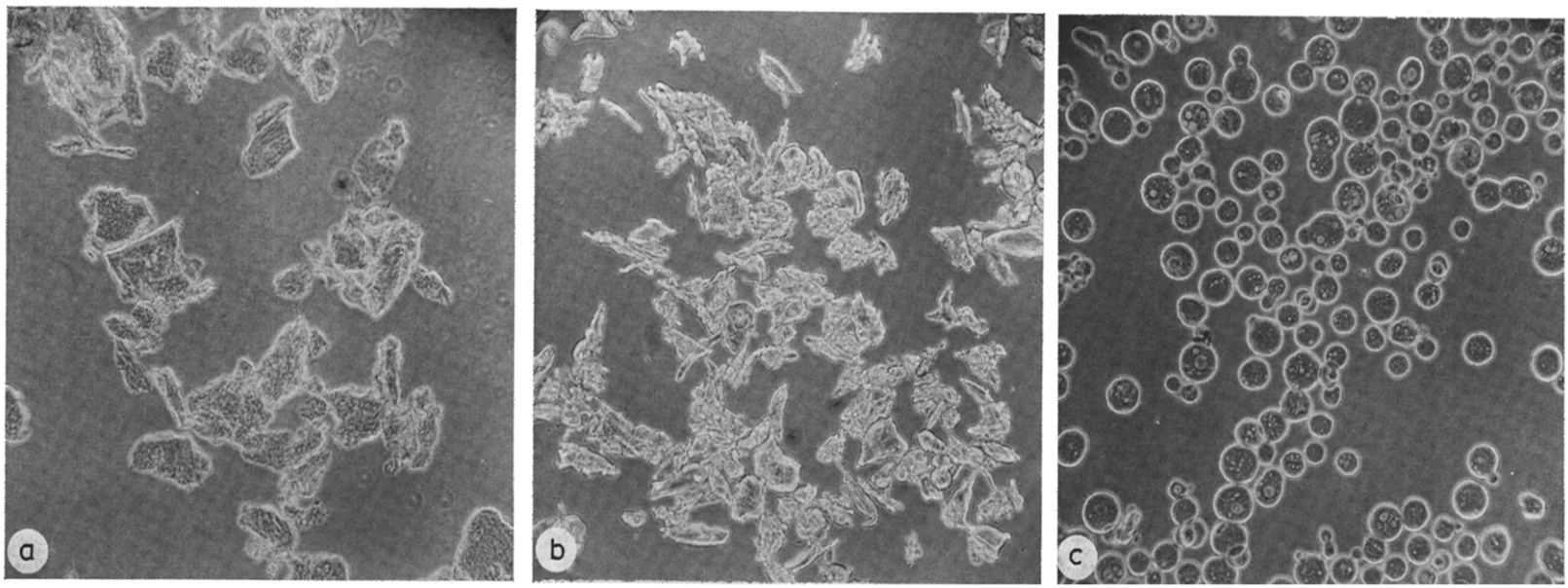

Fig. 2. Phase-contrast photomicrographs of CF-1: 6% cellulose gel particles. Prepared by dispersing in (a) cold methanol, (b) cold o-dichlorobenzene and (c) hot *o*-dichlorobenzene.

Figs. 1 and 2 show phase-contrast photomicrographs of the cellulose gel particles obtained, together with those of commercial gel particles examined. The gel particles obtained by dispersing the salt–cellulose gel in methanol or in cold *o*-dichlorobenzene are of irregular shape as expected (Fig. 2a and b). On the other hand, the particles obtained by dispersing the cellulose solution in hot *o*-dichlorobenzene (Fig. 2c) were nearly spherical, similar to DEAE-Sephacel or Sepharose CL-2B (Fig. 1).

Rigidity of the cellulose gel particles depends on both cellulose concentration and DP of the cellulose material. In general, increase in concentration and DP of the cellulose material results in higher rigidity of the gel obtained. The packed gel bed of the spherical cellulose particles showed sufficient mechanical stability under flow at cellulose concentrations greater than 3% for CF-1 and 1% for cotton linter. The packed gel bed of CF-1:6% or cotton linter:3% gel was markedly more stable to eluent flow than Sepharose CL-2B, which has similar pore and particle sizes to these gels. The bed of an irregular-shaped gel tends to contract and clog more readily than that of a spherical gel of the same grade.

Figs. 3–6 show the chromatograms obtained. Table III summarizes the values of capacity ratio and height equivalent to a theoretical plate. The irregular-shaped gels show lower capacity ratios and poorer resolution than the spherical gels. The latter show as good performance as the commercial agarose or dextran gels. The resolution could be improved by preparing smaller and more uniform particles. Since a gel of high cellulose concentration is quite rigid, it is potentially useful for high-pressure chromatography.

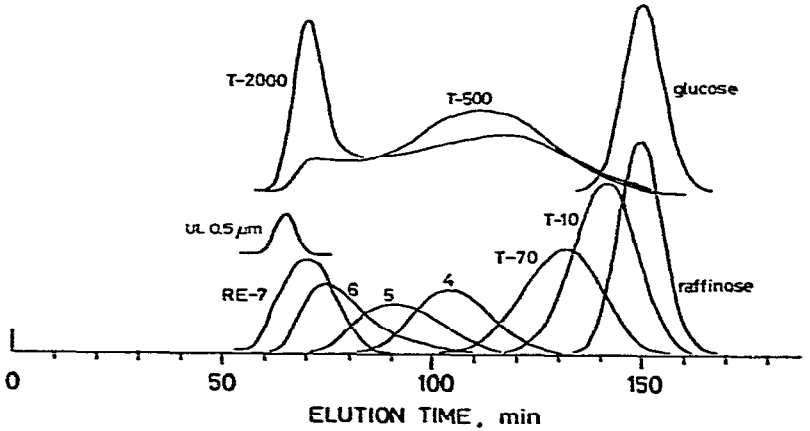

Fig. 3. Gel filtration chromatogram of standard polymers on CF-1: 6% cellulose gel (74–210 μm fraction of spherical beads). Column dimensions: 29.5 × 1.0 cm. Sample volume: 0.5 ml. Flow-rate: 0.162 ml/min.

Figs. 7 and 8 show the calibration curves determined from the chromatograms. Elution times for symmetrical curves were determined from peak positions, for unsymmetrical curves from the position of the vertical line dividing the area under the curve into two parts of equal area. The results were plotted against molecular diameter of the solutes instead of molecular weight so that a universal single curve can be drawn for the different series of polymers. This representation seems pertinent

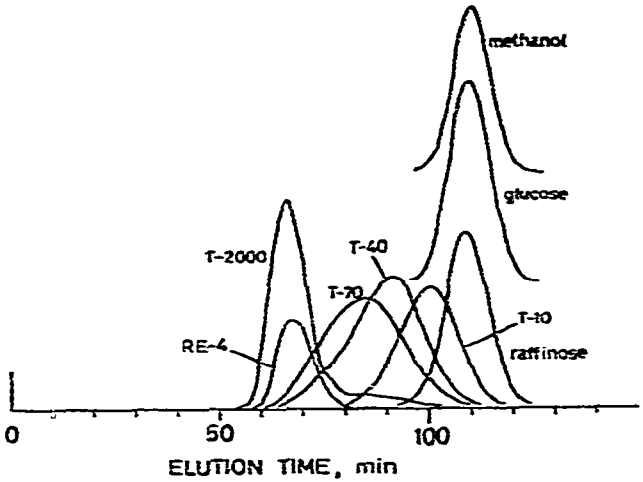

Fig. 4. Gel filtration chromatogram on CF-1: 9% cellulose gel (105–210 μm fraction of irregular-shaped particles). Column: 31.2 × 1.0 cm. Flow-rate: 0.242 ml/min.

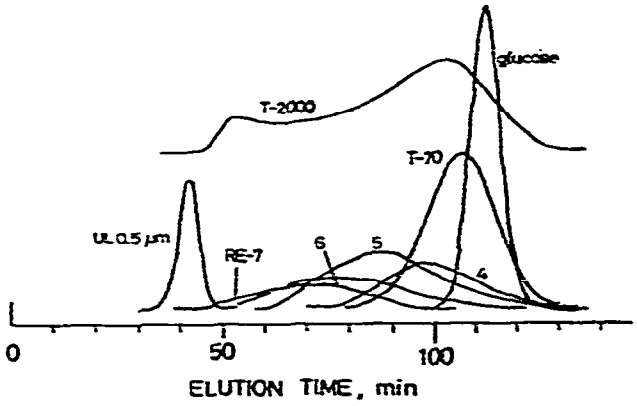

Fig. 5. Gel filtration chromatogram on cotton linter: 1.0% gel (44–210 μm fraction of spherical beads). Column: 29.6 × 1.0 cm. Flow-rate: 0.224 ml/min.

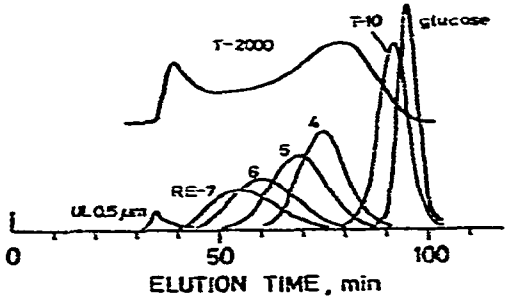

Fig. 6. Gel filtration chromatogram on cotton linter: 1.5% gel (44–149 μm fraction of spherical beads). Column: 25.5 × 1.0 cm. Flow-rate: 0.229 ml/min.

TABLE III

GEL FILTRATION PERFORMANCES OF CELLULOSE AND OTHER GEL PACKINGS

HETP = Height equivalent to a theoretical plate; V_i = internal volume of gel; V_0 = void volume.

Gel	*Shape and size (μm)**	*HETP (mm)*	*Flow-rate (ml/min)*	*Capacity ratio, V_i/V_0*
CF-1: 3%	Spherical, 74–210	0.69	0.164	1.86
6%	Irregular, 149–297	1.13	0.169	0.69
6%	Spherical, 74–210	0.30	0.162	1.27
9%	Irregular, 105–210	0.46	0.242	0.82
Cotton linter: 1.0%	Spherical, 44–210	0.31	0.224	1.22
1.5%	Spherical, 44–149	0.12	0.229	1.94
3.0%	Spherical, 105–210	0.23	0.227	1.00
DEAE-Sephacel	Spherical, 40–130**	0.44	0.157	1.94
DE-52	Fibrous, 30–60 × 100–300**	1.04	0.157	1.00
Sepharose CL-2B	Spherical, 60–250**	0.23	0.155	1.78

* Nominal value from sieve opening.

** Obtained by microscopic observation. For DE-52, width × length.

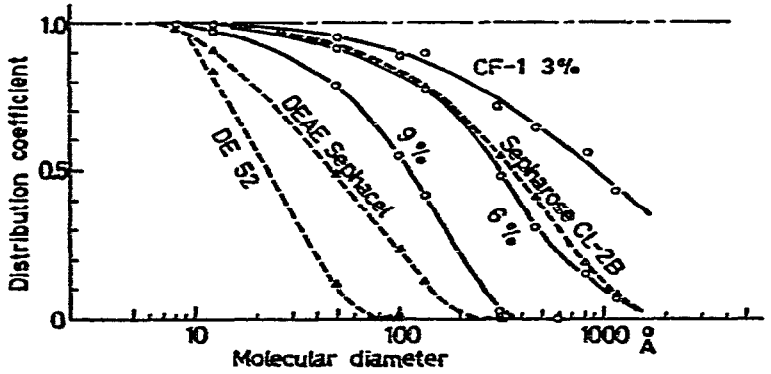

Fig. 7. Calibration curves for CF-1 cellulose gels and commercial cellulose and agarose gels.

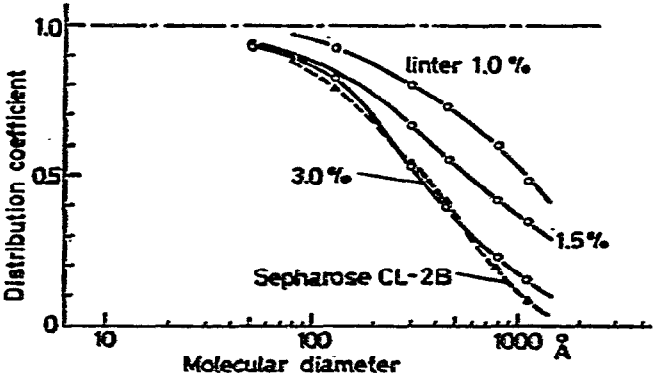

Fig. 8. Calibration curves for cotton linter cellulose gels.

since the curves can be drawn smoothly on the plots for dextran and poly(ethylene oxide) fractions. (In Figs. 7 and 8 the points at above 300 Å correspond to the poly (ethylene oxide) fractions; the others to the dextran fractions.

These curves can be regarded as normalized pore size distributions of the gels provided that partition equilibrium is established instantaneously between the inside and outside of the gel particles. The calibration curve of the cellulose gel changes systematically with cellulose concentration. The mean pore size increases with decrease in the cellulose concentration. It also depends on the kind of cellulose material or its DP. Cotton linter gave a smaller pore size than CF-1 at the same cellulose concentration. This is probably due to the fact that longer cellulose molecules cause greater shrinking of the gel structure when cellulose is regenerated, and is in agreement with previous measurements of the water content of the gels[4].

CF-1:9% gel, which has the smallest pore size, nevertheless has larger pores than the commercial cellulose packings tested. Although the pore size of the cellulose gel would be further reduced on increasing the initial cellulose concentration, it was difficult to dissolve large amounts of cellulose because of the high viscosity of the solution. The upper limit of cellulose concentration was about 10% for CF-1 and 5% for cotton linter under the present dissolution conditions.

The gels of low cellulose concentration have very large pores. CF-1:6% and cotton linter:3% gels gave calibration curves almost equal to that of Sepharose CL-2B, which has one of the largest pore sizes so far available. Gels having the lowest cellulose concentrations, which showed wider fractionation ranges for large molecules, will be advantageous for separation of ultra-high-molecular-weight solutes such as native dextran, viruses or DNA.

No adsorption of solutes on the cellulose gel was detected except for a weak aromatic sorption effect as in the case of Sephadex. The ion-exchange capacity of CF-1:6% gel was estimated by pH-metric titration to be less than 10^{-5} equiv./g dry cellulose, which is of the same order as that of Sephadex.

This cellulose gel probably consists of semi-crystalline cellulose microfibrils instead of single molecular chains or aggregates of several molecular chains as in dextran or agarose gels, thus explaining the enhanced rigidity of the gel. For the same reason, the imbided water can be replaced by any polar or non-polar liquid without significant contraction of the gel. In addition, the gel is thermally stable and contains densely distributed hydroxyl groups. These features are highly advantageous for chemical modification of the gel for the purposes of ion-exchange or affinity chromatography.

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