Note

Preparation and properties of cellulose spherical particles and their ion exchangers

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(Received May 10th, 1984)

Some natural polysaccharides are available as packing materials for gel chromatography in an aqueous medium. Most of these, *e.g.*, galactomannan[1], starch[2] and agar[3], are now of no practical use. The well known hydrophilic gels are dextran derivative and agarose marketed by Pharmacia under the trade names Sephadex and Sepharose, respectively. Sephadex gels[4,5] are prepared from dextrans produced during the growth of *Leuconostoc mesenteroides* on sucrose by subsequent cross-linking with epichlorohydrin. Sepharose gels are prepared from agarose in bead form. Sepharose gels are widely used, especially in biochemical applications. Nevertheless, they have some disadvantages, *e.g.*, they are so soft that they are susceptible to deformation and exhibit a high resistance to the flow-through of fluids.

Cellulose has been used in fibrous or microgranular form as the raw material of ion exchangers. However, these forms are not suitable, as a spherical form is essential for packing materials in chromatography.

The first proposal for preparing spherical cellulose particles was made by O'Neill and Reichardt[6] in 1951. Spherical cellulose derivatives were made available by Reanal (Hungary) and Pharmacia (Sweden) several years ago. Stamberg *et al.*[7] proposed a simple and reproducible process for preparing spherical cellulose particles in 1979.

Our process for preparing cellulose spherical particles[8,9] is unique and the particles obtained have a variety of pore sizes and the prospect of wide applicability.

EXPERIMENTAL AND RESULTS

Porous cellulose spherical particles

A solution of cellulose triacetate in a mixed solvent consisting of dichloromethane and a diluent was suspended in an aqueous medium to form droplets, then the dichloromethane in the droplets was removed by evaporation at about 38°C (*i.e.*, slightly below the boiling point of dichloromethane) to obtain cellulose triacetate beads containing the diluent. The beads thus obtained were saponified and the diluent was subsequently removed to give porous cellulose beads.

Fig. 1 shows a optical micrograph of the porous cellulose spherical particles. The average diameter of the particles is about 100 μm. Table I shows the effect of the diluent on the properties of the porous cellulose spherical particles.
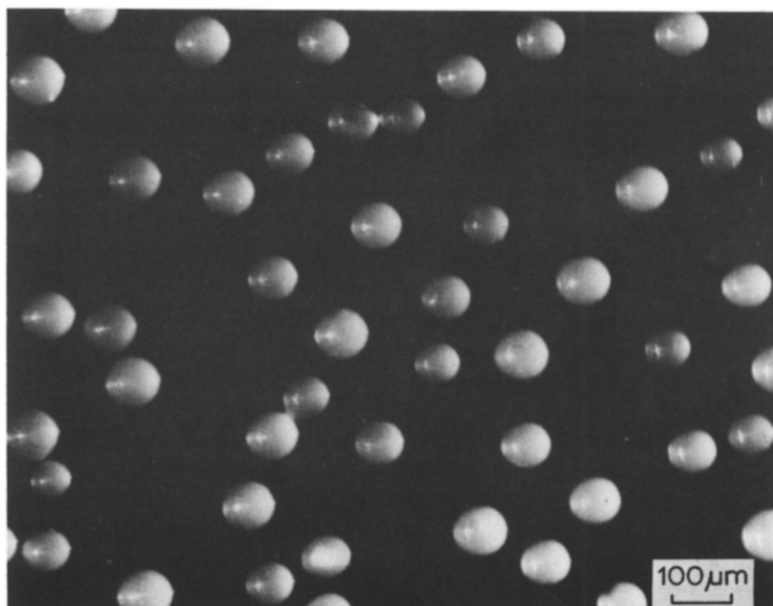
Fig. 1. Optical micrograph of porous cellulose spherical particles.

Diluents were used in order to control the pore size of the spherical cellulose particles. The exclusion limit, M_{lim}, corresponds to the molecular weight of the smallest substance that does not penetrate the gel phase during chromatography.

Here, the degree of swelling and water regain are expressed as (wet gel bed in ml)/(dry gel in g) and (water in g)/(dry gel in g), respectively. In this instance, 17 vol.-% of the diluent was used.

TABLE I

PROPERTIES OF CELLULOSE GELS PREPARED FROM CELLULOSE TRIACETATE SOLUTION IN DICHLOROMETHANE BY USE OF DILUENTS

Diluent*	M_{lim}	Degree of swelling**	Water regain***
None	1900	2.3	0.9
1-Pentanol	10,000	7.0	3.2
1-Hexanol	12,000	5.3	3.0
1-Heptanol	12,000	6.0	3.2
1-Octanol	19,000	5.5	3.1
1-Nonanol	15,000	8.2	4.1
1-Decanol	17,000	6.9	4.1
1-Undecanol	14,000	6.0	3.0
1-Dodecanol	10,400	6.7	3.6
1-Tetradecanol	3600	4.7	2.2

    * All gels were prepared by use of 17 vol.-% of diluent in dichloromethane.
    ** Wet gel bed (ml)/dry gel (g).
    *** H_2O (g)/dry gel (g).

TABLE II

PROPERTIES OF CELLULOSE GELS PREPARED FROM CELLULOSE TRIACETATE SOLU-
TION IN DICHLOROMETHANE BY USE OF 1-HEPTANOL, 1-OCTANOL AND 2-OCTANOL AS
DILUENTS

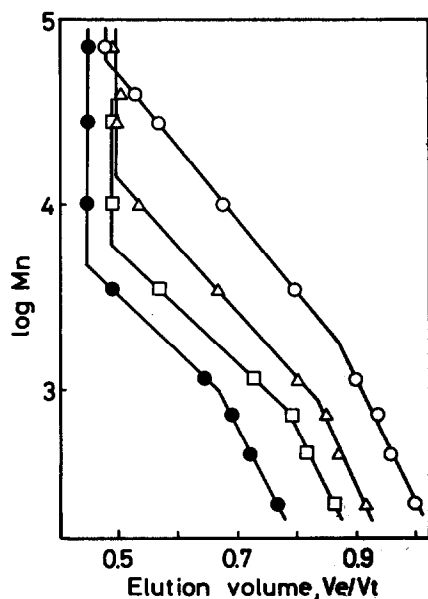| Diluent	Amount of diluent in dichloromethane (vol.-%)	M_{lim}	Degree of swelling*	Water regain*
1-Heptanol	5	4700	4.1	2.5
	10	6400	6.5	3.1
	15	10,800	6.8	3.3
	20	14,000	6.2	3.4
	25	25,000	7.8	4.4
	30	62,000	8.8	5.6
1-Octanol	5	4600	4.2	2.5
	10	10,000	5.6	3.2
	15	18,000	6.2	3.1
	20	23,000	5.9	3.6
	25	25,000	7.0	3.9
	30	30,000	7.8	4.3
	35	48,000	10.5	7.4
2-Octanol	10	5700	5.4	3.2
	15	13,000	6.4	3.2
	20	15,000	6.8	3.3
	25	24,000	7.5	4.3

* See footnote to Table I.

Fig. 2. Calibration graphs for porous cellulose spherical particles prepared by use of 1-heptanol as a diluent.

TABLE III

TYPES AND FRACTIONAL RANGES OF CELLULOFINE

Type*	Polyoxyethylene	Protein
GC-15-m	< 1500	
GC-100-m	<10,000	10,000– 60,000
GC-200-m	<20,000	10,000–120,000
GC-700-m	<70,000	10,000–400,000
GCL-25-m	< 2500	
GCL-90-m	< 9000	<35,000
GH-25-c	< 2500	
GH-25-m	< 2500	

* Diameter of beads: m, 45–105 μm; c, 105–210 μm.

Table II shows the effect of the amount of the diluent on the properties of the gels.

Fig. 2 shows some examples of calibration graphs for our porous cellulose spherical particles. The exclusion limits in the molecular weight of poly(oxyethylene) were 1500–70,000. The particle diameters of the porous spherical cellulose were in the range 50–200 μm.

Several kinds of porous cellulose spherical particles and their ion exchangers have been marketed by Chisso since 1983 under the trade name Cellulofine. Table 3 shows the types and fractional ranges of Cellulofine. The numbers in the "Type" column corresponds to the upper limit of the fractional range of Cellulofine for poly(oxyethylene). GC-15-m to GC-700-m are standard Cellulofine.
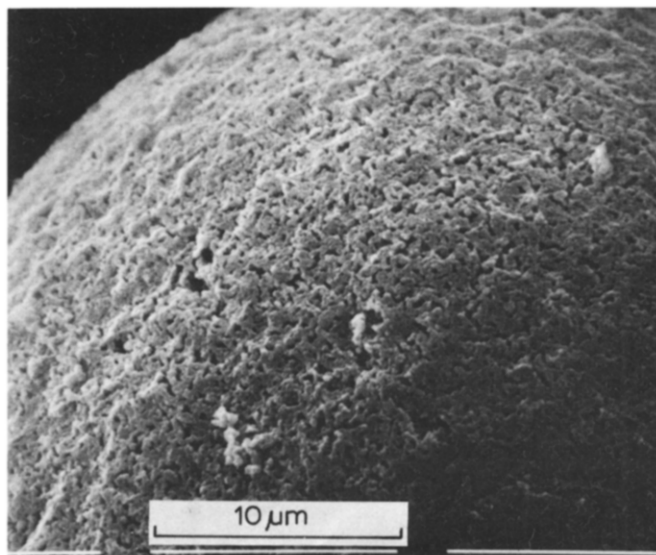

Fig. 3. Surface of macroporous cellulose spherical particle.

Macroporous cellulose spherical particles

Composite beads were prepared by suspending a dichloromethane solution of cellulose ester and poly(vinyl acetate) mixture in an aqueous solution of poly(vinyl alcohol) and then evaporating the solvent at about 38°C. Macroporous cellulose spherical particles were obtained from the composite beads by saponification and subsequent removal of poly(vinyl alcohol) produced by saponification of poly(vinyl acetate).

In order to change the states of phase separation of the two components in the particles, the type of cellulose ester, the ratio of the components and the temperature of suspension were varied.

Excluded critical molecular weights of the macroporous cellulose spherical particles prepared from poly(vinyl acetate) and cellulose triacetate or acetate butylate of high viscosity were 2400–80,000 for poly(oxyethylene).

Fig. 3 shows an electron micrograph of the macroporous cellulose spherical particles. Table IV shows the effect of the polymer content in the composite beads on the excluded molecular weights of the macroporous cellulose spherical particles. CAB-L denotes cellulose acetate butylate of low viscosity, CAB-H cellulose acetate butylate of high viscosity, TAC cellulose triacetate, PVAc poly(vinyl acetate) and M_{lim} the exclusion limit. Fig. 4 shows some examples of calibration graphs for the macroporous cellulose spherical particles. Here, M_n denotes the number-average molecular weight of the substance used as the sample.

The excluded critical molecular weights of the macroporous spherical cellulose particles prepared from poly(vinyl acetate) and cellulose acetate butylate of low viscosity were in the range 10^4–10^7 for poly(oxyethylene). The particle diameters of macroporous spherical cellulose were in the range 50–200 μm.

Cellulose ion exchangers

Cellulose ion exchangers were prepared from the porous or macroporous cel-

TABLE IV

POLYMER CONTENTS IN COMPOSITE BEADS AND EXCLUDED MOLECULAR WEIGHTS

See text for definitions of abbreviations.

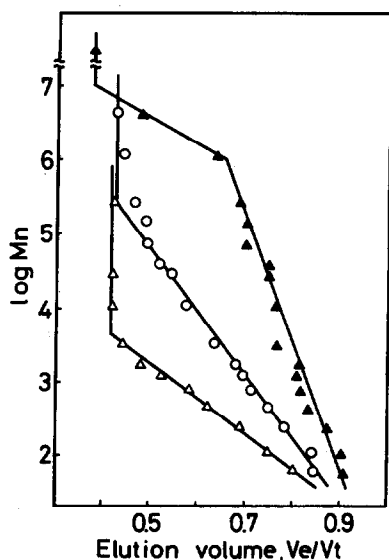| Composition (wt.-%)				Excluded molecular weight
CAB-L	CAB-H	TAC	PVAc	
100	0	0	0	4300
80	0	0	20	560,000
70	0	0	30	>4,500,000
60	0	0	40	>4,500,000
0	100	0	0	2100
0	95	0	5	2400
0	90	0	10	2500
0	80	0	20	4500
0	70	0	30	25,000
0	60	0	40	38,000
0	50	0	50	80,000
0	40	0	60	38,000
0	0	80	20	2800

Fig. 4. Calibration graphs for macroporous cellulose spherical particles.

lulose spherical particles via cross-linked cellulose spherical particles. If non-cross-linked cellulose spherical particles were used as an intermediate material, the ion exchangers obtained would dissolve in aqueous media. After the porous or macroporous cellulose spherical particles had been immersed in 4 mol/l sodium hydroxide solution, they were cross-linked by treatment with epichlorohydrin in kerosene. Cross-linked DEAE-cellulose exchangers were prepared from alkali-containing cross-linked cellulose spherical particles by treatment with β-diethylaminoethyl chloride.

Cross-linked cellulose carboxyl exchangers were prepared from aqueous alkali-containing cross-linked cellulose spherical particles by treatment with chloroacetic acid in the same way as cross-linked DEAE-cellulose exchangers.

Table V shows the types and ion-exchange capacities of Cellulofine ion exchangers marketed by Chisso. Here, AL denotes anion low capacity, AM anion medium capacity, AH anion high capacity, CL cation low capacity, CM cation medium capacity and CH cation high capacity.

TABLE V

TYPES AND ION-EXCHANGE CAPACITIES OF CELLULOFINE ION EXCHANGERS

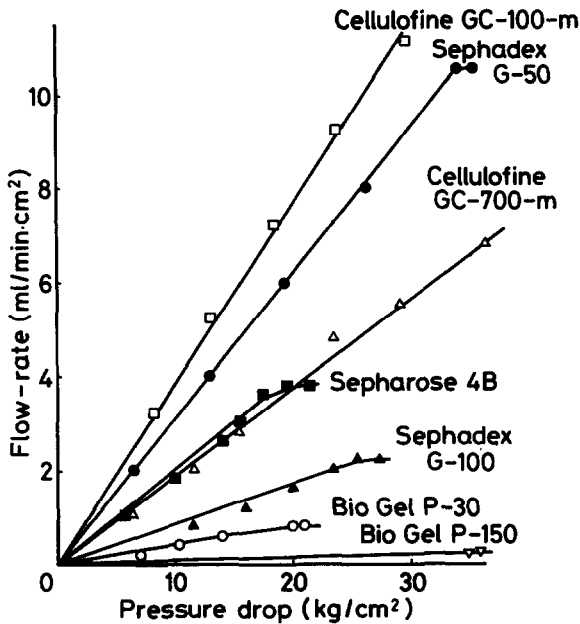| Type	Degree of swelling (ml/g)	Ion-exchange capacity		Protein (mg/ml)	
		Mequiv./g	Mequiv./ml	Albumin	γ-Globulin
DEAE-Cellulofine AL	3–5	0.6	0.15	45– 55	—
DEAE-Cellulofine AM	4–6	0.6	0.12	60– 80	—
DEAE-Cellulofine AH	8–10	1.0	0.11	120–140	—
CM-Cellulofine CL	2–4	0.6	0.2	—	6– 8
CM-Cellulofine CM	3–5	0.6	0.15	—	60– 70
CM-Cellulofine CH	9–11	1.0	0.1	—	180–200

Fig. 5. Relationship between flow-rate and pressure drop. All measurements were made by use of a 150 × 8 mm I.D. metal column packed with gels ranging from 44 to 105 μm in diameter.

Fig. 5 shows the relationship between pressure drop and flow-rate for various gels. The straight lines indicate that these cellulose spherical particles are very rigid and suitable as packing materials for high-performance liquid chromatography.

Some applications of the porous or macroporous cellulose spherical particles and their ion exchangers are as follows. Fig. 6 shows the desalting of bovine serum albumin by use of Cellulofine GH-25 as a packing material, Fig. 7 shows the fractionation of proteins by use of Cellulofine GC-500, Fig. 8 shows the fractionation of the hydrolysed product of chitin by use of Cellulofine GCL-25-m and Fig. 9 shows the fractionation of human serum by use of DEAE-Cellulofine AH.

Both the porous and macroporous cellulose spherical particles are so rigid that
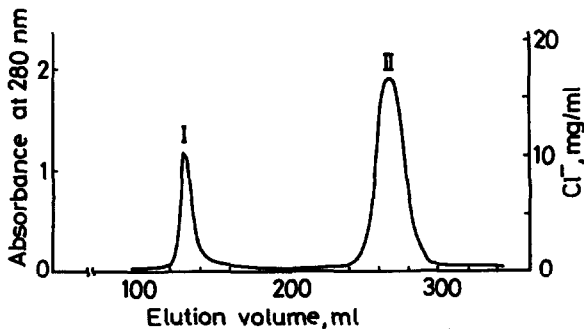

Fig. 6. Desalting of albumin. Column, Cellulofine GH-25 (60 × 2.6 cm I.D.). Eluent, 0.05 mol/l ammonium formate. Detector, UV spectrophotometer. Flow-rate, 36 ml/h (6.8 ml/h · cm²). Samples: I, bovine serum albumin (30 mg); II, NaCl (150 mg).
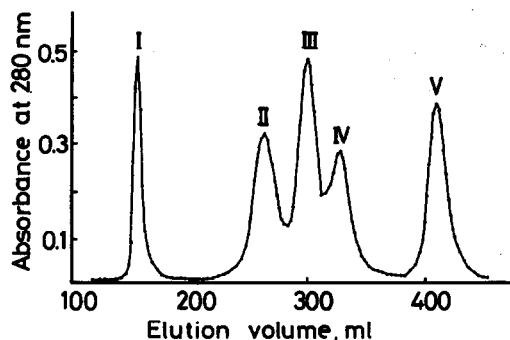
Fig. 7. Fractionation of proteins. Column, Cellulofine GC-500 (66.6 × 2.6 cm I.D.). Eluent, 0.05 mol/l phosphate buffer (pH 7.0) + 0.2 mol/l NaCl. Flow-rate, 17 ml/h (3.2 ml/h · cm^2). Samples: I, blue dextran (2 mg); II, albumin (12 mg); III, chymotrypsinogen-A (5 mg); IV, ribonuclease (10 mg); V, ε-DNP-lysine (0.5 mg).

they are stable to deformation and exhibit only a low resistance to the flow-through of fluids. They have excellent permeability over a wide range of molecular weights of permeable substances. Further, they do not have substantial adsorption or exclusion towards many substances.

These porous and macroporous cellulose spherical particles may be advantageous not only for packing materials in gel chromatography but also as raw materials for ion exchangers or carriers in affinity chromatography, with wide applications in biochemistry.
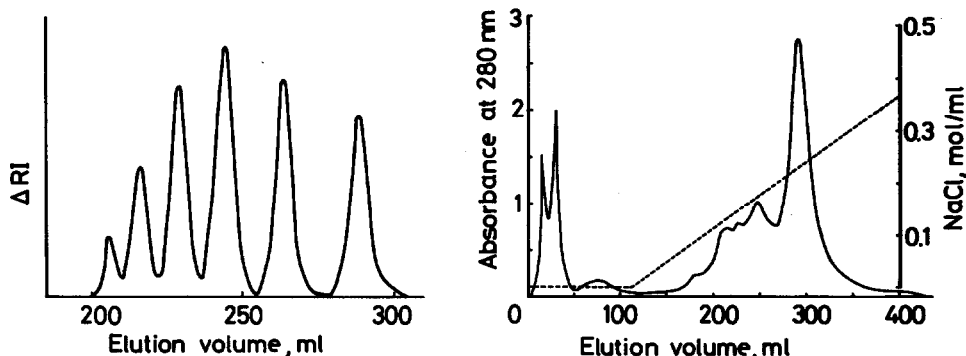

Fig. 8. Fractionation of hydrolysed products of chitin. Column, Cellulofine GCL-25-m (75 × 2.6 cm I.D.). Eluent, distilled water. Flow-rate, 42 ml/h (8 ml/h · cm^2). Detector, differential refractometer.

Fig. 9. Fractionation of human serum. Column, DEAE-Cellulofine AH (25 × 1.5 cm I.D.). Flow-rate, 40 ml/h (22.6 ml/h · cm^2). Eluent, 0.05 mol/l Tris–HCl buffer (pH 8.6); 0–0.5 mol/l NaCl gradient.

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