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Note

N-Methylenechitosan gels, and some of their properties as media for gel chromatography*

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Recently, we prepared *N*-acyl-[1,2] and *N*-arylidene-chitosan[3] gels from chitosan, a (1→4)-2-amino-2-deoxy-β-D-glucan by reaction with carboxylic anhydrides and arylaldehydes, respectively Brouissignac[5] reported briefly on gel formation by mixing chitosan with formaldehyde Muzzarelli *et al*[6] found that rigid gels were produced by mixing solutions of glutaraldehyde and chitosan acetate, and these

TABLE I

GEL FORMATION BY REACTION OF SOME ALDEHYDES WITH CHITOSAN

Aldehyde	*Mol of aldehyde added/hexosaminyl residue*[a]	*Solvent*[b]	*Products*	
			Gelation[c]	*Color*
Formaldehyde	0 8	A	—	Colorless
	4	A	±	Colorless
	8	A	+	Colorless
Acetaldehyde	5	A	+	Brown
	20	B	—	Colorless
	50	B	+	Brown
Propionaldehyde	32	B	—	Colorless
	88	B	+	Yellow
Acrolein	0 8	A	±	Yellow
	0 8	B	±	Yellow
Glutaraldehyde	0 2	A	±	Yellow
	0 02	B	±	Yellow
	0 08	B	+	Yellow
Glyoxal	0 01	A	±	Colorless
	0 02	A	+	Colorless

[a]The concentration of chitosan was in the range of 1 7–5 0% [b]A, 2% acetic acid B, 2% acetic acid–methanol (1 2, v/v) [c]Key +, gels formed, —, gels did not form

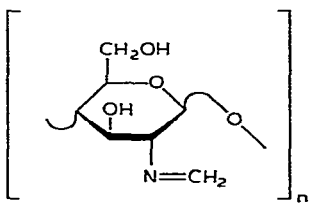
*A preliminary report appeared in Ref 4

gels were utilised for immobilisation of some enzymes However, the detailed structure and properties of the gels were not described.

We now report on the formation of gels by treatment of chitosan with aliphatic aldehydes, the structural analysis of the gels formed from chitosan and formaldehyde, and some of their properties as media for gel chromatography

The minimum amount of aldehydes required for gel formation from chitosan followed the sequence formaldehyde < acetaldehyde < propionaldehyde (Table I) and was in the range 0 02–0 08 mol of aldehyde per hexosaminyl residue

When isolated after treatment of chitosan with 8 mol of formaldehyde at room temperature in aqueous acetic acid, the gels were colourless, transparent, rigid, and did not melt on heating at ~200° for 10 min The gels consisted of *N*-methylenechitosan (1–3%) and solvents (97–99%), and were insoluble and stable towards cold and boiling water, 2M NaOH, formamide, methyl sulphoxide, and common organic solvents The dry gels had ν_{max}^{KBr} 1650 cm^{-1} (C=N of the Schiff base), and the elemental analysis agreed with the *N*-methylenechitosan structure (**1**) The products were degraded by treatment with 0 5M HCl or 10% acetic acid overnight at room temperature, to generate chitosan salts and formaldehyde



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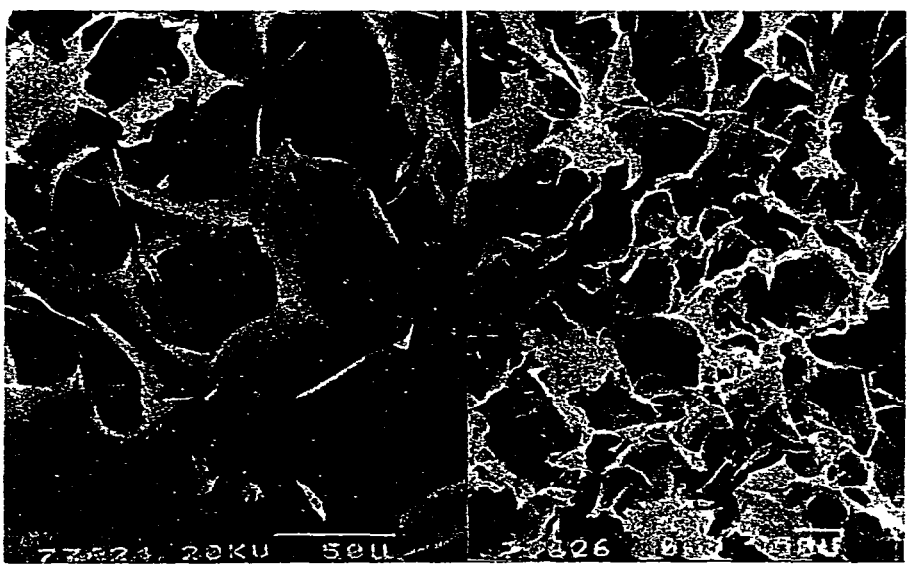

Fig 1 General surface views of *N*-methylenechitosan xerogels These show a polyphasic, microporous structure The pore size is not uniform The bar at the lower right equals 50 μm

TABLE II

THE ULTRASTRUCTURES OF *N*-METHYLENECHITOSAN XEROGELS AND THE XEROGELS OF SOME OTHER ORGANIC GELS, AS EXAMINED BY SCANNING ELECTRON MICROSCOPY

Xerogel[a]	*Pore structure (μm)*		*Ref*
	Dimension	*Wall thickness*	
N-Methylenechitosan	40–70 × 50–100	Very thin	
N-Acetylchitosan	30–50 × 80–300	Very thin	7
Cellulose (Japanese pears)	50–90 × 80–300	Very thin	8
Polyacrylamide	2–10	0 3–1 0	9
Polyvinyl alcohol	0 1–0 3	Very thin	9

[a]Prepared by freeze-drying

The *N*-methylenechitosan xerogels were polyphasic and microporous, as shown by the scanning electron microscope (Fig 1). The pores were surrounded with very thin, membranous walls and had globular shapes of 40–100 μm in diameter The partly broken pores present in the pictures may be due to the formation of ice-crystallites on freezing The range of pore and wall dimensions were compared with those of the other organic xerogels (Table II) The pore dimension was smaller than that of *N*-acetylchitosan xerogels, and no definite number of membranous walls was present at the junction zones of pore assemblies In contrast, *N*-acetylchitosan xerogels had three membranous walls in a junction zone[7]

Each gram of dry *N*-methylenechitosan regained ~23 g of water, to give gels (Table III) that did not synerize and were easily fragmented into small granules (50–350 μm) *N*-Acyl-[1] and *N*-arylidene-chitosan[3] gels synerized, did not regain water, and were difficult to fragment *N*-Methylenechitosan gels were utilised as a new medium for gel chromatography (Table IV) When compared with commercial media, the present gels were similar in properties to Sephadex G-200, but they showed a relatively small inner-volume (Tables III and IV) The gels could not be used for

TABLE III

THE GEL PROPERTIES OF *N*-METHYLENECHITOSAN AND SEPHADEX G-200

Gel	*Fractionation range (mol wt)*	*Water regain (g of water/g of dry gel)*	*Bed volume*[a] *(ml/g of dry gel)*	*Particle size (μm)*
N-Methylenechitosan	~2,900–~200,000	23 ± 2	30–35	50–350
Sephadex G-200[b]	5,000–200,000	20 0 ± 2	30–40	40–120

[a]Volume of 1 g of dry gel after equilibration with excess of water for 18 h [b]A product of Pharmacia, Uppsala, Sweden

TABLE IV

CHROMATOGRAPHIC PROPERTIES[a] OF *N*-METHYLENECHITOSAN GELS AND SEPHADEX G-200

Sample (mol wt)	*N-Methylenechitosan gel (ml)*	*Sephadex G-200 (ml)*[b]
Glycogen (> 10^6)[c]	65	55
Dextran (61,500)	75	95
Amylose (2,900)[d]	135	155
D-Glucose (180)	135	155

[a]For a column (92 × 1 5 cm) eluted with 0 5M NaCl [b]A product of Pharmacia, Uppsala, Sweden [c]V_o [d]V_s

gel chromatography of proteins, because of their absorption Aqueous, neutral or basic solvents could be used, but not acidic solvents which cleaved the Schiff base

EXPERIMENTAL

Chitosan, $[\alpha]_D^{17}$ −10 5° (*c* 1 3, 10% acetic acid), was prepared[1] from chitin (crab shells) by *N*-deacetylation with 40% NaOH Elemental analyses were performed at the Elemental Analysis Center of Kyoto University A Hitachi scanning electron microscope (S-500) was used with an accelerating voltage of 20 kV

The other methods have been described previously[1 2]

N-*Methylenechitosan gels* — Treatment of chitosan (0 5 g) with 2% acetic acid (20 ml) at room temperature gave a viscous solution to which 35% aqueous formaldehyde (2 ml, 8 mol per hexosaminyl residue) was added When the mixture was stored at room temperature, solidification occurred within a few hours The resulting gel (~20 g) was suspended in methanol (~100 ml) for 1 day to remove excess of formaldehyde The process was repeated several times, and the gel was then suspended in ether (~100 ml) and filtered to give colourless, transparent material which was dried in air and then over P_2O_5 *in vacuo* for 8 h at 90° to give a gelatinous product (0 53 g, 99%), decomposition temperature, ~235°, ν_{max}^{KBr} 3500–3300 (OH), 2900 (CH), 1650 (C=N of the Schiff base), 1160–1000 (C–O), and 900 cm^{-1} (β-D configuration)

Anal Calc for $(C_7H_{11}NO_4 \cdot 0.35H_2O)_n$ C, 46 84, H, 6 58, N, 7 81 Found C, 46 86, H, 6 82, N, 7 80

For scanning electron microscopy, a small cube (~1 cm^3) of gel was rapidly frozen at ~−60° and lyophilized to give the xerogel, which was further dried over P_2O_5 at 110° for 5 h *in vacuo*

Acid hydrolysis of N-*methylenechitosan* — A suspension of dry *N*-methylenechitosan gel (20 mg) in 0 5M HCl (~5 ml) was stirred overnight at room temperature, to give a clear solution to which ethanol (100 ml) was added The precipitate was collected by centrifugation, washed with ethanol and then ether, and dried over

P_2O_5 at 90° for 8 h *in vacuo* to give chitosan hydrochloride (20 mg, 88%), $[\alpha]_D^{17}$ −2.6° (*c* 0.8, water). The i.r. spectrum was identical with that of an authentic sample of chitosan hydrochloride prepared from chitosan and 0.5M HCl, and isolated as described above.

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