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Influence of storage on the molecular weight of tetramethylammonium-type gellan gums

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The number average molecular weights $\bar{M}_n$ and the second virial coefficients A_2 for tetramethylammonium-type gellan gums (TMA-gellans) were obtained in aqueous tetramethylammonium-chloride at 40°C by osmometry. The TMA-gellans were prepared from two types of gellan gum (gellan-1 and gellan-2), supplied from different companies. $\bar{M}_n$ and A_2 obtained from the TMA-gellan sample, prepared from gellan-1 in 1992, almost agree with those obtained from the sample prepared from the same gellan-1 in 1994, suggesting that gellan-1 has not deteriorated during the two-year period. Even if the values of $\bar{M}_n$ of the two TMA-gellans prepared from two types of the gellan gums are very close, their aqueous solutions have different A_2 values at the same added salt concentrations. This could be interpreted as a partial difference in the chemical structure, e.g. the number of charged carboxyl groups in the two types of gellan gum. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Gellan gum is an extracellular microbial polysaccharide produced by *Pseudomonas elodea*, which has potential applications in the food and biotechnology industry. The chemical structure of gellan gum was reported as a linear anionic heteropolysaccharide consisting of β-D-glucose, β-D-glucuronic acid, β-D-glucose and α-L-rhamnose, (Fig. 1, Jansson *et al.*, 1983 and O'Neill *et al.*, 1983).

The physicochemical properties of gellan gum have been studied by many research groups. However, as is often the case with biopolymers, the interpretation of results is complicated because of differences in materials and the possibility of deterioration during storage.

To determine whether gellan shows changes during storage from 1992 to 1994, we measured the number average molecular weights $\bar{M}_n$ and the second virial coefficients A_2 for tetramethylammonium-type gellan gums (TMA-gellans) in aqueous tetramethylammonium-chloride (TMACl) by osmometry. TMA-gellans were prepared from starting materials, gellan-1 and gellan-2.

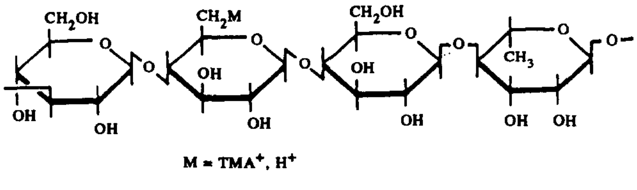

Fig. 1. Repeat unit of gellan gum.

The superscripts (92), (93) and (94) are used to designate the time when the TMA-gellan was prepared or the data were obtained, e.g. TMA-gellan$^{(92)}$ is the sample prepared in 1992 and $\bar{M}_n^{(92)}$ the value obtained in 1992 using the TMA-gellan$^{(92)}$ and $[\bar{M}_n]^{(92)}$ the average value of $\bar{M}_n^{(92)}$.

EXPERIMENTAL

Materials

The TMA-gellans were prepared from the two gellan gums, gellan-1 and gellan-2 (the starting materials). Gellan-1 is a deacetylated gellan gum kindly supplied by the San-Ei Kagaku Co., (lot 62058A). Gellan-2 is a sodium-type deacetylated gellan gum kindly supplied by the Kelco Division of Merck & Co. Inc. Using these gellan gums, TMA-gellans were prepared by the following method (Ogawa, 1993). A 0.5g sample of gellan-1 or gellan-2 was swelled in 0.5dm^3 of deionized water at 40°C overnight. The mixtures were stirred for 8h at 105°C for gellan-1 and at 90°C for gellan-2, in order to dissolve the mixtures completely. The solutions were filtered through a 0.45μm Milliporefilter at 105° and 90°C for gellan-1 and gellan-2, respectively, and then passed through a column packed with TMA-type

Table 1. Metal contents in the gellan gum samples

Sample	Na	K	Ca	Mg(μg/g)
Gellan-1^a	1350	29000	3870	1550
TMA-gellan$^{(92)}$	420	4200	42	84
TMA-gellan$^{(94)}$	285	125	100	ND
Gellan-2^b	33000	3320	1420	110
TMA-gellan$^{(93)}$	170	70	8	2

ND; not detected. a and b; measured in 1992 and 1993, respectively. Metal contents were measured by flame spectrometry (Na, K) and flame atomic absorption spectrometry (Ca, Mg). (Perkin Elmer Model 3100).

Table 2. The experimental plan

Starting Material	Time	Experiment
Gellan-1	1992	Preparation of TMA-gellan$^{(92)}$ Osmometry
	1994	Preparation of TMA-gellan$^{(94)}$ and H-gellan$^{(94)}$ Osmometry, Titration
Gellan-2	1993	Preparation of TMA-gellan$^{(93)}$ and H-gellan$^{(93)}$ Osmometry, Titration

ion exchange resin. The pH of the effluents was adjusted to 7 with TMA-hydroxide. After neutralization the effluents were concentrated by evaporation under reduced pressure and poured into 2-propanol to precipitate the TMA-gellan. Finally, the precipitate was dried for 48h at 40°C under a vacuum. The conversion to TMA-salts was checked by measuring the ion contents of the TMA-gellan samples (Table 1). The TMA-gellan was dissolved in TMACl solution and stirred for 2h at 60°C. This solution was dialyzed (for 2–4 days at 40°C) against TMACl solution and diluted with this TMACl solution. Concentration of the solutions was determined using a Union Giken Differential Refractometer Model RM-102.

H-type gellan gum (H-gellan) was prepared by the same method using H-type ion exchange column.

Measurements

A Hewlett-Packard High Speed Membrane Osmometer Model 503, with a special type of glass tube, was employed for the osmometry (Ogawa, 1993).

The equivalent weights (the weight of polyelectrolyte equivalent to 1 mol of monovalent acid) of the two gellan gums were determined by potentiometric titration with standard NaOH solutions using the H-gellans.

RESULTS AND DISCUSSION

The schedule of the experiments are shown in Table 2. Using the TMA-gellan prepared from gellan-1, osmotic pressure measurements were made in 1992 and 1994. First, in 1992, TMA-gellan$^{(92)}$ was prepared, and the osmotic pressure for aqueous TMA-nitrate solutions of TMA-gellan$^{(92)}$ was measured at 40°C. (In this case, TMA-nitrate solutions were used in place of TMACl solutions, but no difference was observed between these two osmotic pressures). Gellan-1 was stored in a refrigerator, and in 1994, TMA-gellan$^{(94)}$ was prepared and osmotic pressure measurements were made in TMACl solutions at 40°C. The results of the two sets of measurements are shown in Fig. 2a–c. π is the osmotic pressure and c the polymer concentration. From Fig. 2, it is noted that the plots of the data obtained in 1992 coincide with those obtained in 1994.

It is known that the osmotic pressure for the polymer solutions is expressed by the following equation with appropriate value of g (Yamakawa, 1971).

$$\pi/c = (RT/\bar{M}_n)[1 + A_2\bar{M}_n c + g(A_2\bar{M}_n)^2c^2] \tag{1}$$

Here R is the gas constant and T the absolute temperature. The parameter g in equation 1 relates to the third virial coefficient A_3, $g = A_3/A_2M$. To diminish the third virial contribution, $g = 1/4$ is often used. However, this g value is an empirical and its applicability to polyelectrolyte aqueous solutions is not yet fully established. Previously we evaluated A_2 and g separately using the Stockmayer-Casassa procedure, and showed that g values, obtained at 40°C from the osmometry of TMA-gellan gum in aqueous salt solutions over a wide salt concentration range, were close to 1/4 (Ogawa, 1995). By assuming $g = 1/4$, eqn. 1 can be rewritten as in the following form (Yamakawa, 1971).

$$(\pi/c)^{1/2} = (RT/\bar{M}_n)^{1/2}(1 + \bar{M}_n A_2 c/2). \tag{2}$$

As shown in Fig. 3, the data fitted this equation well. $\bar{M}_n$ and A_2 were determined from the intercepts and slopes of these lines, respectively, and listed in Table 3. It is found that the values of $\bar{M}_n^{(92)}$ and $\bar{M}_n^{(94)}$, at different salt concentrations (C_s), correlate with each other within experimental error, and the value of $[\bar{M}_n]^{(92)}$ correlates well with that of $[\bar{M}_n]^{(94)}$. The second virial coefficients of $A_2^{(92)}$ or $A_2^{(94)}$ decrease with increasing salt concentrations. It is found that the values of $A_2^{(92)}$ and $A_2^{(94)}$ at the same ionic strength correlate well. These results suggest that gellan-1 did not change over a two-year period from 1992 to 1994. It has been said that the natural products such as gellan gum should not be stored for long periods due to deterioration. From these results it can be said that well-purified gellan gum, used for the measurements of the molecular weight and the second virial coefficient, did not change over a two-year period.

Osmotic pressure measurements of TMACl solutions of TMA-gellan$^{(93)}$, prepared from gellan-2, were carried out at 40°C in 1993. Results are shown in Figs 2a, 2b, 2c

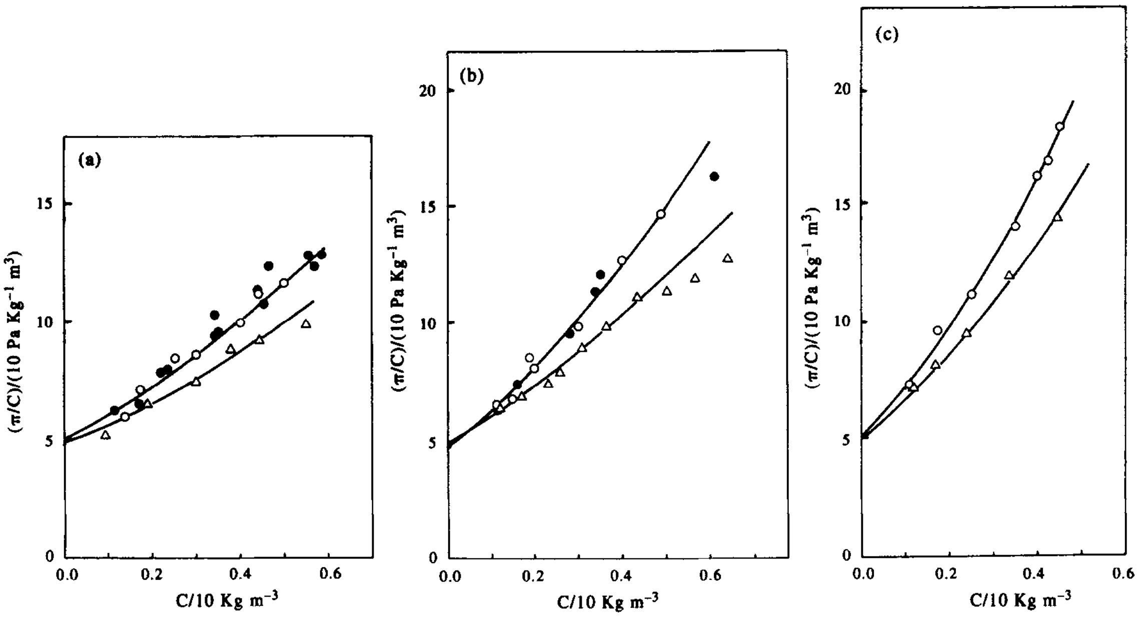

Fig. 2. a, b and c, Plots of π/c vs. for the TMA-gellan solutions of $C_s=0.075$ (a), 0.05 (b) and 0.025 (c). Data obtained in 1992 (●, C_s:TMA-nitrate concentration), in 1994 (○, C_s: TMACL concentration), in 1993 (△, C_s: TMACL concentration).The solid curves denote the values calculated from eqn. 2 using the values of $\bar{M}_n^{(94)}$ and $A_2^{(94)}$ or $\bar{M}_n^{(93)}$ and $A_2^{(93)}$ as shown in Table 3.

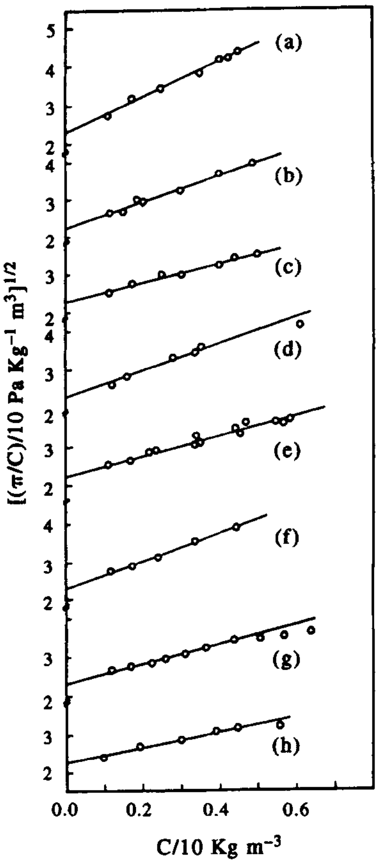

Fig. 3. Plots of $(\pi/c)^{1/2}$ vs. c for the TMA-gellan solutions at 40°C. Data obtained in 1994 at TMACL concentration $C_s=0.025$ (a), $C_s=0.05$ (b), $C_s=0.075$ (c), in 1992 at TMA-nitrate concentration $C_s=0.05$ (d), $C_s=0.075$(e), in 1993 at TMACL concentration $C_s=0.025$ (f), $C_s=0.05$ (g), $C_s=0.075$ (moldm^{-3}) (h).

and Fig 3, together with the data of TMA-gellan$^{(92)}$ and TMA-gellan$^{(94)}$ prepared from gellan-1. The values of $\bar{M}_n^{(93)}$ and $A_2^{(93)}$ are also shown in Table 3. It is found that $\bar{M}_n^{(93)}$ at different C_s agrees with each other and $A_2^{(93)}$ decreases with increasing salt concentration. It can be seen that the value of $[\bar{M}_n]^{(93)}$ is very close to the values of $[\bar{M}_n]^{(92)}$ or $[\bar{M}_n]^{(94)}$, while the value of $A_2^{(93)}$ is rather smaller than those of $A_2^{(92)}$ or $A_2^{(94)}$ at the same ionic strength.

It is well known that the second virial coefficient for an ideal solution can be expressed as (Tanford, 1961);

$$A_2 = 10^3 Z^2/4M^2 C_s \tag{3}$$

indicating that the second virial coefficient is proportional to the square of the charge (z) on the macroion.

The theoretical expression for A_2 for a uniformly charged rod-like molecule of diameter d and length L can be written as follows (Nicolai & Mandel, 1989; Kawakami & Norisye, 1991);

$$A_2 = (\pi N_A/4M_L^2)[d + \kappa^{-1}(\ln Y + 0.7703)] \tag{4}$$

Table 3. Number average molecular weights and second virial coefficients for the TMA-gellan solutions at 40°C

Cs/mol dm^{-3}	$\bar{M}_n \times 10^{-4}$			$A_2 \times 10^3$/molKg^{-2}m^3		
	$\bar{M}_n^{(92)}$	$\bar{M}_n^{(94)}$	$\bar{M}_n^{(93)}$	$A_2^{(92)}$	$A_2^{(94)}$	$A_2^{(93)}$
0.025		4.9_3	5.0_2		8.0_0	6.3_6
0.05	5.0_2	5.2_6	4.8_9	6.0_2	5.9_0	4.4_0
0.075	5.2_0	5.0_0	5.2_0	4.3_5	4.2_0	3.4_0
(average)	5.1_6	5.0_8	5.0_1			

Table 4. Equivalent weights of the gellan gum samples

Samples	M_{eq}/g[a]
H-gellan$^{(94)}$	656
H-gellan$^{(93)}$	700
(theoretical)	(646)

[a]; weight of polyelectrolytes equivalent to 1 mol of monovalent acid.

$$Y = 2\pi v_{eff}^{2} Q\kappa^{-1}\exp(-\kappa d)$$

$$v_{eff} = [(Q\kappa d/2)K_1(\kappa d/2)]^{-1}$$

Here N_A is Avogadro's number, M_L (= M/L) the molecular weight per contour length, v_{eff} the effective charge density, Q the Bjerrum length, k^{-1} the Debye length ($k^2 = 8TLN_AQC_s/1000$), K_1 the first-order modified Bessel function. The parameters M_L and d relate to the chemical structure and molecular conformation, and changing these parameters results in a change in the A_2 value, e.g. with increasing extension of polyelectrolyte chains in solution, M_L decreases and A_2 increases as shown in eqn. 4. It was reported that the gellan gum molecule shows an order (helix)-disorder (coil) conformational transition with increasing temperature, and the transition temperature is around 32°C (Milas *et al.*, 1990). We showed previously that at 40°C, the polyelectrolyte TMA-gellan should take a coiled conformation at high ionic strength, however, as the ionic strength is decreased, the TMA-gellan coil is extended due to the electrostatic repulsions, consequently, below C_s = ca. 0.075 mol dm^{-3} the A_2 can be expressed by eqn. 4 (Ogawa, 1995).

The carboxyl group contents were determined for the two H-gellans prepared from gellan-1 and gellan-2 (Table 4). It was found that the value of equivalent weights obtained for H-gellan$^{(94)}$ and H-gellan$^{(93)}$ were very close to the theoretical value calculated from the chemical structure of gellan gum shown in Fig. 1. It is noted, however, that the value for H-gellan$^{(93)}$, prepared from gellan-2, is slightly larger than that for H-gellan$^{(94)}$, prepared from gellan-1, which means that the contents of the charged carboxyl group in gellan-1 is slightly higher than that in gellan-2.

From these results together with eqns 3 and 4, it may be considered that the difference between the A_2 values for the two sample solutions, TMA-gellan$^{(93)}$ and TMA-gellan$^{(92)}$ (or TMA-gellan$^{(94)}$), is due to the partial difference of the chemical structure of the starting materials, i.e. gellan-1 contains a slightly larger number of charged carboxyl groups than gellan-2, and the gellan-1 coil may be more extended due to electrostatic repulsive interactions.

It is concluded that, at least for the well-purified gellan gum used for the measurements, the values of $\bar{M}_n$ and A_2 do not change over a 2-year period and that although the values of $\bar{M}_n$ for the two samples such as TMA-gellan$^{(93)}$ and TMA-gellan$^{(92)}$ (or TMA-gellan$^{(94)}$) are very close, their aqueous solutions have different A_2 values at the same C_s, suggesting a difference in the number of charged carboxyl groups between the two starting materials of gellan-1 and gellan-2.

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REFERENCES

Jansson, P., Linberg, B. & Stanford, P.A. (1983). Structural studies of gellan gum, an extracellular polysaccharide elaborated by *Pseudomonas elodea*. *Carbohydr. Res.*, **124**, 135–139

Kawakami, K. & Norisye, T. (1991). Second virial coefficient for charged rods. *Sodium Macromolecules*, **24**, 4898–4903.

Milas, M., Shi, X. & Rinaudo, M. (1990). On the physico-chemical properties of gellan gum. *Biopolymers*, **30**, 451–464.

Nicolai, T. & Mandel, M. (1989). Ionic strength dependence of the second virial coefficient of low molar mass and fragments. *fragments in aqueous solutions. Macromolecules*, **22**, 438–444.

Ogawa, E. (1993). Osmotic pressure measurements for gellan gum aqueous solutions. *Food hydrocolloids*, **7**, 397–405.

Ogawa, E. (1995). Conformational Properties of Tetramethylammonium Gellan Gum in Aqueous Solutions, *Polym. J.*, **27**, 567–574.

O'Neill, M.A., Selvendran, R.R. & Morris, V.J. (1983). Structure of the acidic extracellular gelling polysaccharide produced by *Pseudomonas elodea*. *Carbohydr. Res.*, **124**, 123–133.

Tanford, C. (1961). *Physical Chemistry of Macromolecules*. Willey, New York.

Yamakawa, H. (1971). *Modern Theory of Polymer Solutions*. Harper & Row, New York.