LIGHT-SCATTERING STUDIES OF PECTIC SUBSTANCES IN AQUEOUS SOLUTION

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ABSTRACT

The behaviour of pectic substances in aqueous solution has been studied by the light-scattering technique. Pectic substances were found to be present as high molecular aggregates having strong, intermolecular interactions, and their behaviour depends upon the nature of the carbohydrate chain. Additives can cause dissociation of the polysaccharide aggregates, resulting, in some cases, in particles having similar weight-average ($\overline{M}_w$) and number-average ($\overline{M}_n$) molecular weights.

INTRODUCTION

Light-scattering studies of aqueous solutions of polysaccharides containing various additives represent a convenient method for elucidation of the structures and physico-chemical and biological properties of these biopolymers.

Recently, we attempted[1] to determine the true molecular weight of some pectic substances by a light-scattering method. Simultaneously, the great susceptibility of pectic substances to aggregation and polyelectrolyte effects was detected. Polysaccharide behaviour in aqueous solution is significantly affected by additives[2-6]. Light scattering permits an evaluation of the effect of additives in terms of changes of weight-average molecular weight, particle size, and the second virial coefficient and plot pattern.

The present work reports a light-scattering study of the effect of various factors upon the behaviour of pectic substances in aqueous solution.

EXPERIMENTAL

Materials. — Zosterine was isolated[7] from *Zosteraceae* plants, and the corresponding galacturonan (ZGU) and apiogalacturonan (AGU) were prepared[8,9] from zosterine. *Panax ginseng* C. A. Mey pectin (panaxan) and its galacturonan (PGU) were prepared as described previously[10]. A commercial sample of dextran (Pharmacia, Uppsala, Sweden) having a molecular weight 1.5×10^4–2×10^4 was also used. Some characteristic data for the above polysaccharides are given in Table I.

TABLE I

ANALYTICAL DATA FOR THE POLYSACCHARIDES

Polysaccharide	*Uronic acid content* (%)	*Methoxyl groups* (%)	$\overline{M}_n \times 10^{-3\,a}$
Zosterine	60	1.5	78
Apiogalacturonan (AGU)	39.6	1.52	22
Galacturonan of zosterine (ZGU)	98.3	1.7	18–22
Panaxan	60	5.0	25–29
Galacturonan of panaxan (PGU)	100	5.9	15–20

[a]Determined as described in Ref. 1.

The solutions were prepared directly in the light-scattering cell (50 cm^3 in volume) by the consecutive dilution procedure. Low concentrations of polysaccharide (1×10^{-5}–2×10^{-3} g/ml) were employed in twice-distilled water. Sodium chloride, ammonium hydroxide, urea, and Tween-20 (Schuchardt, München) were employed as additives. The polysaccharides were completely dissolved by stirring at 20°, and the solutions were clarified by three-fold filtration through millipore filters having pore diameters of 1.2, 0.6–0.9, and 0.1–0.3 μm.

Light scattering. — The measurements of Rayleigh ratios were performed in the photocurrent light-scattering apparatus FPS-2M (U.S.S.R.). Relative light intensities were obtained by direct measurement of photocurrents with an electrophotometric circuit that compensated for the fluctuations of incident light and registered the signals automatically. Benzene was used as the scattering standard. The refractive index increment was determined in monochromatic light, using ITR-2 and a differential refractometer with a thermostatic cell. The refractive index was determined with an IRF-23 refractometer.

DISCUSSION

According to the particle type as determined by depolarization characteristics[11], the light-scattering data were treated by a single extrapolation and the method of Zimm[12]. An example of a Zimm plot in this work is given in Fig. 1, in which $Kc/R(\Theta)$ is plotted against $\sin^2(\Theta)+kc$, where $R(\Theta)$ is the Rayleigh ratio (referred to a benzene standard) of the light scattered at angle (Θ), K is the turbidimetric constant, c is the polysaccharide concentration, and k is an arbitrary constant. The weight-average molecular weight was calculated by the Debye equation[13] (1),

$$Kc/R(\Theta) = 1/\overline{M}_w \times P(\Theta) + 2A_2C \times Q(\Theta) \tag{1}$$

where $\overline{M}_w$ is the weight-average molecular weight, A_2 is the second virial coefficient, $P(\Theta)$ and $Q(\Theta)$ are functions of intramolecular interference which approach unity as $\Theta \to 0$. The correction for the preferable behaviour of the biopolymer with respect to the additive was introduced into the K-value. The particle size was estimated by equation (2),

$$\langle S^2\rangle^{\frac{1}{2}} = \left(\frac{3S_0\,\lambda}{A_0\,16\pi^2}\right)^{\frac{1}{2}} \tag{2}$$

where $\langle S^2\rangle^{\frac{1}{2}}$ is the average value of the mean-square radius (inertia radius), S_0 is the initial slope of the plot $Kc/R(\Theta)$ at $c=0$ and $\Theta=0$, A_0 is the initial ordinate of a double extrapolation plot, and λ is the wavelength of the incident light.

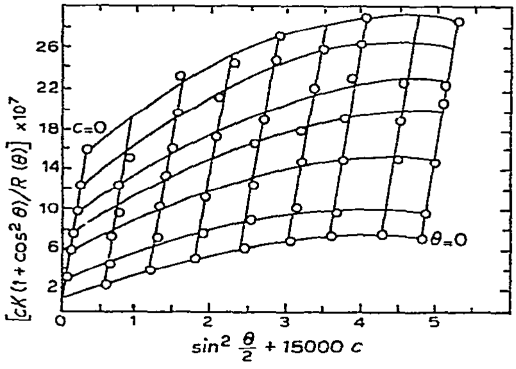

Fig. 1. Zimm pattern obtained for aqueous solutions of ZGU.

The data obtained are presented in Table II and show that the behaviour of polysaccharides in solution is greatly dependent on the nature of the carbohydrate chain and especially on the content of uronic acid and methoxyl groups which affect both the solubility and the pattern of intermolecular interaction.

In aqueous solutions at 20°, the polysaccharides as a rule are in the form of aggregates of high molecular weight. Increase of temperature leads to dissociation and to a decrease in the size and molecular weight of the particles.

Effect of sodium chloride (Fig. 2). — Zosterine and AGU are poorly soluble in aqueous sodium chloride and are precipitated even at very small concentrations of the salt. Thus, it is not possible to investigate the effect of sodium chloride upon the behaviour of these polysaccharides in solution. However, ZGU is soluble in 10 mM sodium chloride, which makes possible a study of its behaviour in solution (Fig. 2). From the average values of the mean-square radius of ZGU in aqueous and salt solutions (Table II), it is seen that in the salt medium a decrease of particle size is observed, although a comparison of $\overline{M}_w$ with the $\overline{M}_n$ values determined earlier shows that ZGU is still in the form of aggregates. The zero value of the second virial coefficient for ZGU in salt solution indicates weak, intermolecular interaction and the absence of further aggregation or dissociation. Unfortunately, ZGU is poorly soluble in more concentrated solutions of sodium chloride and further study of its behaviour is impossible.

Panaxan and PGU contain a higher percentage of methoxyl groups (Table I) which leads[14] to increased solubility. In 0.1 and 0.3M sodium chloride (Fig. 2 and

TABLE II

LIGHT-SCATTERING DATA FOR AQUEOUS SOLUTIONS OF POLYSACCHARIDES WITH VARIOUS ADDITIVES

Solvent		*Temperature (degrees)*	A_2	$\langle S^2\rangle^{\frac{1}{2}}$, *Å*	$\overline{M}_w$
Zosterine					
Water		20	2.2×10^{-4}	3000	3.0×10^{8}
Water		85	6.0×10^{-4}	2000	1.0×10^{7}
Urea,	0.02M	20	2.4×10^{-5}	3000	3.0×10^{8}
	M	20	1.9×10^{-4}		3.0×10^{8}
	0.3M	85	—		1.0×10^{6}
Tween	0.002%	85	-0.6×10^{-3}		1.9×10^{5}
			-4.5×10^{-3}[a]		1.1×10^{5}[a]
	0.004%	85	-6.8×10^{-3}		1.2×10^{5}
			-1.0×10^{-2}[a]		6.3×10^{4}[a]
	0.005%	85	-2.7×10^{-2}		—
AGU					
Water		20	7.5×10^{-2}		1.8×10^{5}
Urea,	0.2M	20	7.6×10^{-2}		1.8×10^{5}
	0.2M	70	-1.6×10^{-2}		2.1×10^{4}
ZGU					
Water		20	4.0×10^{-5}	2800	6.2×10^{7}
NaCl,	0.01M	20	0	2400	3.1×10^{7}
NH_4OH,	0.19M	20	-5.2×10^{-3}		1.3×10^{5}
0.17M NH_4OH + 0.02M NH_4Cl		20	0		1.8×10^{4}
NH_4OH,	0.4M	20	9.1×10^{-4}	2000	1.1×10^{7}
	0.7M	20	—		1.2×10^{7}
	1.9M	20	1.0×10^{-3}		1.3×10^{7}
Urea,	0.01M	20	-1.9×10^{-3}		1.3×10^{5}
Panaxan					
NaCl	0.3M	20	0.8×10^{-2}		9.0×10^{5}
	2M	20			3.3×10^{4}
PGU					
NaCl,	0.1M	20	1.4×10^{-2}		1.0×10^{6}
	2M	20	-5.0×10^{-3}		1.9×10^{5}
NH_4OH,	0.19M	20	—		1.7×10^{4}
Dextran					
Water		20	4.5×10^{-4}		1.2×10^{5}
NaCl,	0.2M	20	-2.1×10^{-4}		5.4×10^{4}
			-4.1×10^{-3}[a]		2.2×10^{4}[a]

[a] At very low concentrations of polysaccharide in solutions.

Table II), each polysaccharide is in the form of aggregates of high molecular weight, with considerable intermolecular interaction probably associated with the Donnan effect[15]. The dissociation observed in 2M sodium chloride leads to a considerable decrease in molecular weight and particle size. Moreover, for panaxan, complete dissociation occurs as assessed from the similar values of $\overline{M}_w$ and $\overline{M}_n$ (Tables I and II). The behaviour of dextran was studied for comparison. In aqueous solution

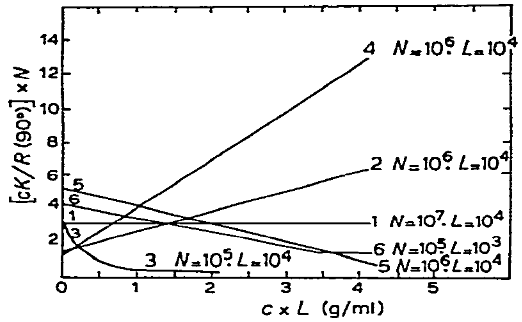

Fig. 2. Effect of sodium chloride on the behaviour of polysaccharides in solutions at 20°. 1, ZGU in 0.01M NaCl; 2, Panaxan in 0.3M NaCl; 3, Panaxan in 2M NaCl; 4, PGU in 0.1M NaCl; 5, PGU in 2M NaCl; 6, Dextran in 0.2M NaCl.

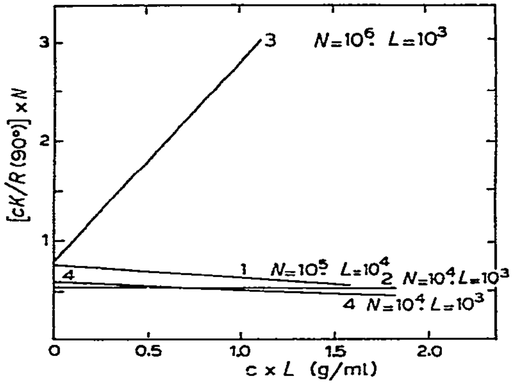

Fig. 3. The behavior of galacturonans in solutions of ammonium hydroxide at 20°. 1, ZGU in 0.19M NH_4OH; 2, ZGU in 0.17M NH_4OH+0.02M NH_4Cl; 3, ZGU in 1.9M NH_4OH; 4, PGU in 0.19M NH_4OH.

(Table II), dextran is in the form of aggregates of high molecular weight with significant intermolecular interaction ($A_2 = 4.5 \times 10^{-4}$). Dissociation (Fig. 2) is observed in 0.2M sodium chloride and, in the region of low concentration, this process is more extensive and leads to particles having similar values of $\overline{M}_w$ and $\overline{M}_n$ (Tables I and II).

Thus, in all the cases studied, the addition of sodium chloride to aqueous solutions of polysaccharides leads to dissociation of aggregates.

Effect of ammonium hydroxide (*Fig. 3*). — It is well known[16,17] that pectic substances are susceptible to degradation in alkaline media. In order to eliminate degradation, we used ammonia solutions of low concentration at room temperature, and measurements were obtained as quickly as possible.

Zosterine, AGU, and panaxan dissolve in aqueous ammonia to form colloidal solutions which were not studied in the present work. Galacturonans are readily soluble in very dilute ammonia. In 0.19M ammonium hydroxide, PGU forms particles having $\overline{M}_w = 1.7 \times 10^4$, aggregation and dissociation being practically absent (Fig. 3).

ZGU is dissociated under the same conditions and particles having $\overline{M}_w = 1.3 \times 10^5$ are formed. In aqueous ammonia containing a small amount of ammonium chloride, complete dissociation of ZGU is observed to furnish particles having $\overline{M}_w = 1.8 \times 10^4$.

It is noteworthy that aggregation of polysaccharide molecules to form colloidal solutions is observed at concentrations of ammonia which exceeded 0.4M.

Effect of urea (*Fig. 4*).— Zosterine is more soluble in water containing urea. However, the value of the second virial coefficient (Table II) demonstrates the occurrence of the same type of aggregation as in water, which leads to the formation of particles of similar weight-average molecular weight (3.0×10^8). This phenomenon is also observed for solutions of AGU in 0.2M urea. When a solution of zosterine in 0.3M urea is heated up to 95°, dissociation is incomplete, and the heating of a solution of AGU in 0.2M urea up to 70° results in considerable dissociation of aggregates to give particles having similar $\overline{M}_w$ and $\overline{M}_n$ values (Tables I and II). It is interesting that, for ZGU, dissociation is observed in 0.01M urea at 20°.

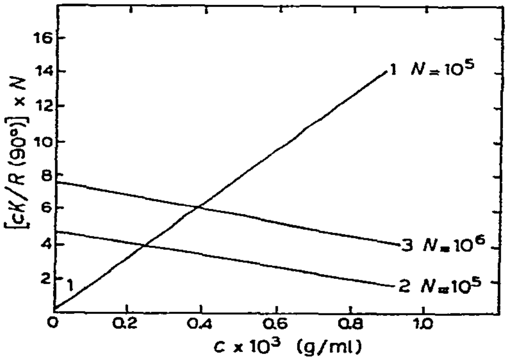

Fig. 4. The behavior of pectins in urea solutions. 1, AGU in 0.2M urea at 20°; 2, AGU in 0.2M urea at 70°; 3, ZGU in 0.01M urea at 20°.

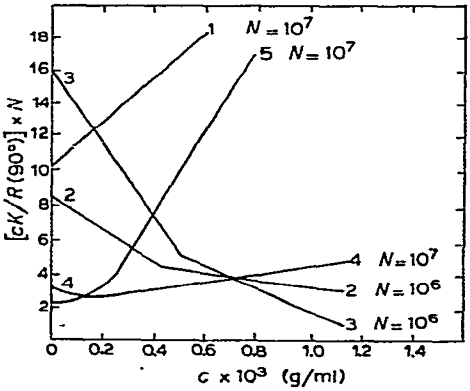

Fig. 5. The behaviour of pectins in water and Tween-20 solutions at 85°. 1, Zosterine in water; 2, zosterine in 0.002% Tween; 3, zosterine in 0.004% Tween; 4, AGU in 0.001% Tween; 5, ZGU in 0.001% Tween.

Effect of Tween-20 (Fig. 5). — The effect of Tween additives upon the behaviour of zosterine, AGU, and ZGU in aqueous solutions was investigated at 85° because of the higher solubility of the polysaccharides at this temperature.

Dissociation was observed even at 0.002% concentration of Tween-20 (Table II Fig. 5). This process is intensified with an increase of concentration of Tween and leads to the formation of particles having approximately equal values of $\overline{M}_w$ and $\overline{M}_n$. Thus, by variation of the additive, it is possible to influence considerably the behaviour of pectic substances in aqueous solutions.

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