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STRUCTURAL STUDY OF THE SOLUTIONS OF ACIDIC POLYSACCHARIDES. II. STUDY OF SOME THERMODYNAMIC PROPERTIES OF THE DILUTE PECTIN SOLUTIONS WITH DIFFERENT DEGREES OF ESTERIFICATION†

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ABSTRACT

Light-scattering and surface tension measurements have been used to study the structure and thermodynamic properties of aqueous-salt solutions of pectins, with the degree of esterification (DE) varying from 5 to 100%. Pectin macromolecules were found in the form of aggregates with $\bar{M}_w = 5 \times 10^5$ *to* $1{\cdot}1 \times 10^6$ *D. The second virial coefficient of pectin was negative in these poor solvents and independent of the DE. Pectins exhibited surface activity at the air–water interface, the magnitude of this activity also being independent of the DE.*

INTRODUCTION

The ability of highly esterified pectin to form gels as the activity coefficient of the solvent (water) is lowered is apparently associated with the establishment of the optimal thermodynamic affinity of the polymer for the solvent. It is known that the gel-forming ability depends on the degree of esterification (DE) of pectin, its maximum occurring at a DE of 50% (Deuel *et al.*, 1950). It is believed that differences in

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the gel-forming abiliy of pectins with dissimilar DEs (at the same charge) reflect differences in the thermodynamic affinity of the polymer for the solvent. These differences may exist even in aqueous solutions, or may appear after the introduction of a cosolvent (such as sucrose or glycerol) decreasing the activity coefficient of water. The first possibility is consistent with the hypothesis of the hydrophobic nature of the network cross-links of highly esterified pectin gels (Owens *et al.*, 1954).

The literature contains no systematic data on the thermodynamic properties of pectin solutions with different DEs. According to Bettelheim (1970), aqueous solutions of both highly esterified pectin and sodium pectate are almost ideal in their behaviour. A similar result has been obtained from measurements using the light-scattering method in 0·5 M NaCl and other media (Jordan & Brant, 1978). Finally, the same method when applied to the solutions of a number of pectin samples of different origin (DEs of 35·5 to 75·6%) in 0·3 M acetate buffer has also shown almost ideal solution behaviour (Smith & Stainsby, 1977).

The purpose of this work was to study the states of pectin macromolecules in aqueous solutions. In particular, it was of interest to examine how the DE affects the thermodynamic affinity of the polymer for the solvent. Two methods were adopted for this purpose, viz. light-scattering and the determination of surface tension. As is already known, the surface activity of a substance is a function of its affinity for the solvent (Ueberreiter & Yamaura, 1977; Roberts & Thomas, 1978).

EXPERIMENTAL

Citrus pectin supplied by Koch-Light (Great Britain) was purified by a three-fold reprecipitation from aqueous solution using 0·4 M hydrochloric acid and propan-2-ol of increasing concentration (48, 60 and 96%), followed by dialysis against distilled water until a negative reaction was obtained for chloride ions.

To minimise the effect of differences in the molecular mass and molecular mass distribution on the properties under study, the pectin samples of different DEs were prepared by methylation of pectic acid with diazomethane. According to Volmert (1950) and Berth *et al.* (1980), the degradation of the polyuronide chain in these conditions is minimal. For this purpose the purified sample was subjected to saponification by treating with NaOH at pH 12 for 1 h at room temperature. The pectic acid thus obtained was esterified by using varying amounts of diazomethane at −20°C.

The uronide content in the samples under study, determined titrimetrically, was 87 ± 0·5% while the content of neutral sugars found by a combination of gas chromatography and mass spectrometry (Stromeyer & Linow, 1979) was as follows: rhamnose, 3–7%; arabinose, 4–6%; xylose, less than 1%. The DEs of the samples, also found titrimetrically to an accuracy of ±3%, are shown in Tables 1 and 2.

A sample of sodium alginate supplied by British Drug Houses (Great Britain) was purified as described above for pectin. The content of the blocks was as determined

TABLE 1
Physico-chemical characteristics of pectins

Degree of esterification (%)	$\bar{M}_w \times 10^3$		$\sqrt{\langle \bar{S}^2 \rangle_z}$ (Å)		$(\sqrt{\langle \bar{S}^2 \rangle_z})_{0.1\,M} / (\sqrt{\langle \bar{S}^2 \rangle_z})_{1\cdot01\,M}$	$\sqrt{\langle \bar{S}^2 \rangle_z}/\sqrt{\bar{M}_w} \times 10^{-3}$	
	I[a]	*II*[b]	*I*	*II*		*I*	*II*
5	520	540	480	410	0·86	21·0	17·7
10	570	560	500	440	0·87	20·9	18·6
50	740	660	590	–	–	21·7	–
69	1000	910	770	610	0·84	23·2	20·2
75	1100	1100	760	590	0·78	22·9	17·8
100	640	680	–	390	–	–	–

[a] I = 0·009 M NaCl + 0·001 M NaF.
[b] II = 0·09 M NaCl + 0·01 M NaF.

TABLE 2
Thermodynamic characteristics of pectin solutions

Degree of esterification (%)	$A_2 \times 10^3$ (*mol. m³ kg⁻²*)		*Surface tension (γ) of 2% sodium alginate solution* ($\times 10^{-3}$ *N/m*)
	I	*II*	*II*
5	−0·5	−0·5	51·0
10	−0·4	−0·5	51·1
50	−0·3	−0·5	50·2
69	−0·6	−0·4	49·2
75	–	−0·4	49·5
100	−0·4	−0·6	49·0

[a] I = 0·009 M NaCl + 0·001 M NaF.
[b] II = 0·09 M NaCl + 0·01 M NaF.

by the method of Penman & Sanderson (1972): polyguluronic acid, 47%; polymannuronic acid, 33%; alternating hexuronide residues, 20%. The limiting viscosity number measured in 0·1 M NaCl at 25°C was 695 cm^3/g.

All the polyuronide samples were studied in the form of sodium salts (pH of aqueous solutions 7·2–7·5) and stored at 6°C.

For light-scattering measurements the pectin samples were dissolved at room temperature in aqueous solutions containing 0·009 M NaCl and 0·001 M NaF or 0·09 M NaCl and 0·01 M NaF. Diluting the starting solution containing 1 mg/ml of pectin gave a set of four to five samples that were filtered under pressure (to remove dust particles) into dust-free cuvettes through a 0·45 μm PVC filter (Sartorius GmbH, FRG). Light-scattering measurements were made by means of a Sofica photogoniometer (Fica, France) at 25°C and a wavelength (λ) of 546 nm. The results were processed by the method of double extrapolation according to Zimm (1948). The Zimm diagram is shown in Figure 1. The refractive index increment was 0·151 cm^3/g

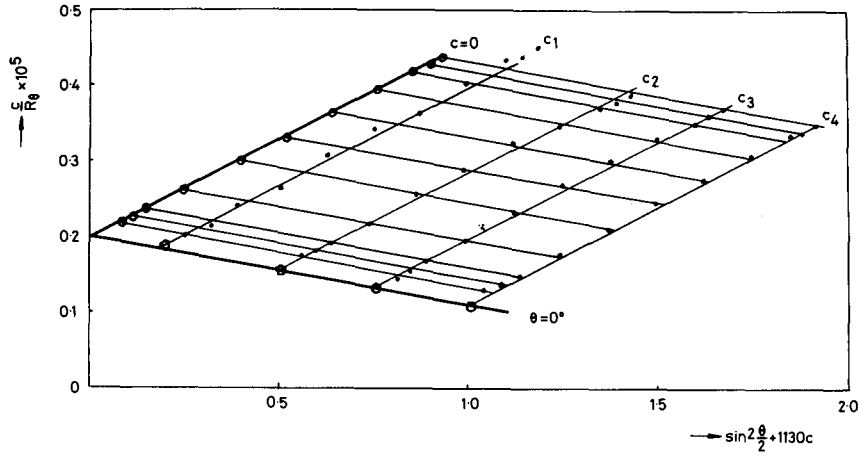

Fig. 1. Zimm diagram of pectin solution with a DE of 5% in a solution of 0·09 M NaCl and 0·01 M NaF.

for a pectin with a DE of 50%, and it was taken to be 0·15 cm^3/g for the remaining samples. The optical constant $K = 1{\cdot}856 \times 10^{-7}$. The light-scattering and refractive index increment measurement methods are detailed elsewhere (Berth *et al.*, 1977). The second virial coefficient A_2 was calculated according to the equation

$$\left(\frac{K \cdot c}{R_\theta}\right)_{\substack{c \to 0 \\ \theta \to 0}} = \frac{1}{\bar{M}_w} + 2A_2 c.$$

where θ is the scattering angle and R_θ is the reduced scattering intensity which depends on the ratio of incident and scattered light but is independent of θ.

The surface tension of the polysaccharide solutions was measured using the Wilhelmy method by following the immersion depth of a glass plate into the solution by means of an analytical balance, as described by Feinerman *et al.* (1970). To maximise the wetting, the plate (18 x 18 x 0·015 mm) was kept for several minutes in an HF-containing atmosphere. Immediately before measurements the plate was cleaned by boiling in a chromic acid mixture with subsequent washing with double-distilled water. The advantages of the Wilhelmy method for the measurement of the surface tension of polymer solutions are discussed elsewhere (Pchelin, 1971). A constant surface tension value was attained within 24 h. Accordingly, all the data presented were obtained for the conditions in which the surface layer was formed during 24 h.

Sample preparation and surface tension measurements were made at the A. N. Nesmeyanov Institute of Organoelement Compounds (Moscow, USSR), while light-scattering measurements and the determination of the neutral sugar contents were carried out at the Central Institute of Nutrition (Potsdam-Rehbrücke, GDR).

RESULTS AND DISCUSSION

The results of the light-scattering measurements of pectins in aqueous-salt solutions with ionic strengths of 0·01 and 0·1 M are summarised in Tables 1 and 2. These results are averages of no less than three parallel measurements. The accuracy of measurement of $\bar{M}_w$ and also $\sqrt{\langle \bar{S}^2 \rangle_z}$ was 10% on average. It is substantially higher than the accuracy attained in the light-scattering measurements of an entire sample of apple pectin containing a centrifugally removable mixture with a high content of neutral sugars (Berth *et al.*, 1977). The good reproducibility of the results obtained by the present authors can be attributed to the fact that the admixtures that might be present in the starting commercial sample of citrus pectin were removed by purification.

As can be seen from Table 1, pectin is present in the solutions in the form of particles with $\bar{M}_w$ of 5×10^5 to $1{\cdot}1 \times 10^6$ D. These results agree qualitiatively with those obtained earlier with studies of pectin samples of different origin (Sorochan *et al.*, 1971; Berth *et al.*, 1977; Kawabata & Sawayama, 1977; Smith & Stainsby, 1977; Jordan & Brant, 1978). The molecular mass of the particles is independent of the ionic strength of the solution, increasing with DEs from 5 to 75%.

The values of $\bar{M}_w$ correspond to an aggregated state of the pectin macromolcules in the solutions under study. This is favoured by the negative values of the second virial coefficients (Table 2) as well as by the type of $\bar{M}_w$-DE dependence, since the methylation reaction of pectin cannot involve a two-fold increase in the molecular mass. The effect of aggregation in pectin solutions has been established by Jordan & Brant (1978) and Sorochan *et al.* (1971). The linear dependence of $c/R_{\theta=0}$ on concentration (Fig. 1) points to the irreversible nature of the aggregation.* This phenomenon is typical of polymer solutions near the phase separation region (Doty *et al.*, 1947). According to Yu. A. Antonov (private communication, 1980), the position of the critical point in the phase diagram shows only a slight dependence on the ionic strength of pectin solutions.

It has been shown by preliminary light-scattering measurements of solutions subjected to centrifugation (for 5 h, 2×10^5 g) that, as reported by some other authors (Smith & Stainsby, 1977), the state of the solutions remains close to ideal.

As distinct from the molecular mass, the radius of gyration of the pectin particles decreases with the ionic strength of the solution. The ratio between the radius of gyration at two values of the ionic strength is independent of the DE (despite differing charge densities of the macromolecules) and for DEs of 6–75% it is $0{\cdot}84 \pm 0{\cdot}04$. The $\langle \bar{S}^2 \rangle_z^{1/2}/\bar{M}_w^{1/2}$ ratio is independent of the DE of pectin and, accordingly, on the $\bar{M}_w$ of the aggregates. This means that the pectin aggregates can be regarded formally as random coils (without allowance for polydispersity).

* The systematic deviations of the experimental points, corresponding to the lowest concentrations studied (roughly 0·02%), from the linear plots on the Zimm diagrams (see Fig. 1) may point to the dissociation of macromolecule aggregates in these highly diluted solutions. These deviations were not taken into account, considering the low experimental precision in this concentration range.

As can be seen from the values of the second virial coefficients (Table 2), the two solvents studied are poor ones for pectin. The affinity of the polymer for the solvent does not depend on the ionic strength of the solvent or on the DE of the pectin. The negative values of the second virial coefficients of pectins in aqueous solutions of different salt composition have been established elsewhere (Sorochan *et al.*, 1971; Jordan & Brant, 1978).

The low affinity of the polymer for the solvent is clearly evident in the surface activity of pectins in aqueous-salt solutions too. Figure 2 gives adsorption isotherms for pectins with DEs of 10, 50 and 100%. For all the remaining samples the adsorption isotherms are similar. The limiting adsorption is attained in the case where a higher concentration of pectin is coupled with its lower DE. In no case, however, does this concentration exceed 2%.

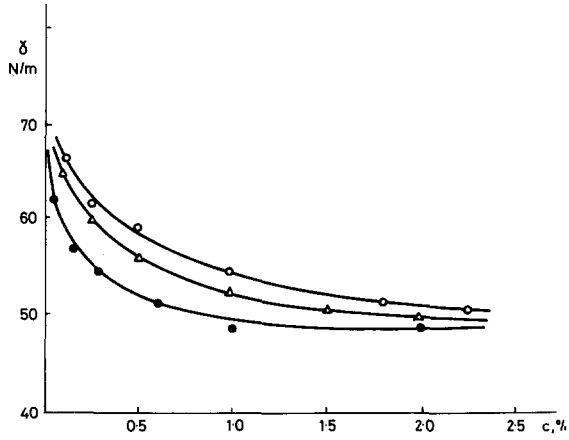

Fig. 2. Adsorption isotherms of pectins with DEs of 10, 50 and 100% in a solution of 0·09 M NaCl and 0·01 M NaF. The surface layer is formed during 24 h.

The surface tensions of 2% pectin solutions are shown in Table 2. It can be seen that the surface activity of pectin, as well as the second virial coefficient, is independent of its DE. Thus the light-scattering and surface tension measurement data suggest that for these aqueous-salt pectin solutions the thermodynamic affinity of the polymer for the solvent does not depend on the pectin's DE.

The regular change of $\bar{M}_w$ as DE increases from 5 to 75% may, however, reflect a slight dependence of the polymer-solvent affinity on DE. Moreover, the decrease in the radius of gyration with increase in ionic strength, whatever the degree of esterification of the pectin may also suggest a change in the thermodynamic affinity for the solvent which is independent of electrostatic interactions. However, the precision, or sensitivity, of these methods to determine thermodynamic properties of solutions is insufficient to follow such weak effects.

The surface activity of pectins in aqueous solutions at the interface with less polar liquids has been established by Mukerjee & Shukla (1967). However, the problem of the nature of molecular fragments controlling the surface activity of pectins remains to be solved. It is obvious that the surface activity is independent of nonuronide components and of the presence of methoxyl groups on the macromolecule. This is, particularly, supported by the fact that the surface tensions of 2% pectin solutions, with different DEs, coincides practically with that for a 2% sodium alginate solution in similar conditions. Alginate represents a pure nonesterified polyuronide (Percival & McDowell, 1967).

Thus the results of studies on the thermodynamic properties of pectin solutions with different DEs point to the absence of hydrophobic interactions which would be governed by the presence of methoxyl groups on the macromolecule (Dubin & Strauss, 1975). Studies of the properties for alternating copolymers of maleic acid with alkylvinyl ethers have also indicated that the methoxyl group gives rise to no hydrophobic interactions. The ability of the methyl group to cause such interactions may depend heavily on the local chemical structure. Thus, as distinct from the methoxyl group (differing views on the nature of methyl cellulose gelling have been discussed elsewhere (Savage, 1971; Whistler, 1973)), the methyl group in the molecule of polymethacrylic acid induces the ability of the macromolecule to undergo conformational coil–globule transition due to hydrophobic interactions (Anufriyeva *et al.*, 1968).

The absence of any DE-dependence of $(\langle \bar{S}^2 \rangle_z^{1/2})_{0.1\,M}/(\langle \bar{S}^2 \rangle_z^{1/2})_{0.01\,M}$ and $\langle \bar{S}^2 \rangle_z^{1/2}/\bar{M}_w^{1/2}$, and also of the second virial coefficient and surface tension of the solutions point to the fact that the DE produces no effect on the conformation of pectin macromolecules. This is consistent with earlier theoretical and experimental results (Palmer & Hartzog, 1945; Rees & Wight, 1971; Plashchina *et al.*, 1978).

CONCLUSIONS

1. Pectin macromolecules in aqueous-salt solutions can be found in the form of aggregates with $\bar{M}_w = 5 \times 10^5$ to $1{\cdot}1 \times 10^6$ D.

2. The second virial coefficient of pectin in aqueous-salt solutions is independent of its degree of esterification.

3. Pectin exhibits surface activity at the aqueous-salt solution – air interface. The magnitude of this activity does not depend on the degree of esterification of the pectin.

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