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Structural Studies of the Solutions of Anionic Polysaccharides. IV. Study of Pectin Solutions by Light-scattering

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SUMMARY

Aqueous solutions of pectins, with a degree of esterification (DE) varying from 0 to 95%, have been studied by light-scattering. A set of samples of similar molecular sizes were prepared by methylation of sodium pectate with diazomethane. The solutions were subjected to ultracentrifugation to ensure the removal of small amounts of gel fraction. The second virial coefficient of pectin was positive and constant for DE values varying between 43 and 95%, but increased by a factor of three when the DE was reduced to zero. This increase is at least partly due to coulombic electrostatic interactions. All of the pectins investigated behaved as semi-rigid-chain polymers. The chain flexibility was a maximum in the DE range 43 to 58%.

In pectin solutions with a DE higher than 58% attractive forces exist between pectin macromolecules due to the presence of ester groups. It is expected that the interactions between ester groups contribute to both the chain rigidity and the gel-forming ability of pectins.

INTRODUCTION

The results of light-scattering measurements on pectin solutions, with a degree of esterification (DE) varying between 5 and 100%, are discussed by Berth *et al.* (1982). The pectin samples were prepared by methyla-

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tion of sodium pectate with diazomethane; sodium pectate was obtained by alkali saponification of commercial citrus pectin. The $\bar{M}_w$ of the samples studied ranged from 5×10^5 to $1{\cdot}1 \times 10^6$ daltons. The second virial coefficient, A_2, in a 0·01–0·1 M solution of the mixed electrolyte (NaCl : NaF = 9 : 1) was $(-5 \pm 1) \times 10^{-4}$ m^3 mol kg^{-2} and was independent of the pectin DE. This value is close to those reported earlier by other authors (Berth *et al.*, 1977; Smith & Stainsby, 1977; Jordan & Brant, 1978). A light-scattering study of sodium alginate (Smidsrød & Haug, 1968) has shown that negative values of A_2 are due to the presence of a gel-fraction in the solution. The latter was removed by two-stage centrifugation (0·5 h at 28×10^3 ***g*** followed by centrifugation for 2 h at 14×10^4 ***g***).

In the present paper, light-scattering studies of pectin solutions are described that have been made to extend the earlier data obtained. To eliminate any possible contribution from the gel fraction, the solutions were purified under conditions similar to those described by Smidsrød & Haug (1968). The pectin samples were those used by Berth *et al.* (1982) so that a direct comparison could be made with the earlier work.

EXPERIMENTAL

1. Preparation of pectin with different DEs

Pectin samples of different DEs were prepared using commercial citrus pectin manufactured by Koch–Light of Great Britain.

The starting material, a commercial citrus pectin, was dissolved in distilled water at room temperature and centrifuged for 1 h at 15×10^3 ***g***. Pectin was purified by subsequent reprecipitation with 48% isopropanol containing 0·4 M HCl, and then with neutral 60% and 96% isopropanol. The gel obtained was dialyzed against distilled water until a negative reaction was obtained for chloride ions, and then dissolved in distilled water.

The purified pectin was saponified by treating with NaOH at pH 12 for 1 h at room temperature. After neutralization with hydrochloric acid and removal of NaCl by dialysis the sample was lyophilized. The lyophilized sodium pectate was then kept in acetone at 278 K for 2 days to remove the residual water; acetone was decanted after every 3–4 h. Thereafter, acetone was replaced by dry ether. Methylation with diazomethane was carried out at 253 K by the method of Vollmert (1950). The methylated product was washed with chilled dry ether until the yellowish tint disappeared, and then kept in acetone for 2 days. The samples of pectin obtained were dissolved in distilled water, centrifuged for 10 min at $10^3 g$ to remove any possible polymethylene impurity, and lyophilized.

The uronide content in the pectin samples was determined by the modified carbazole method as described by Filippov & Vlasyeva (1973); galacturonic acid (Serva, USA) was used as a standard for calibration. The uronide content obtained was 88 ± 0·5%.

The DE was determined by the calorimetric method of Filippov & Kuz'minov (1971). Calibration curves were obtained using dehydrated methyl alcohol.

The neutral sugar content found from gas chromatography and mass spectrometry (Stromeyer & Linow, 1979) was as follows: rhamnose, 3–7%; arabinose, 4–6%; xylose, less than 1%. The determination was carried out at the Central Institute of Nutrition, Potsdam-Rehbrücke, East Germany.

2. Preparation of solutions

The pectin samples were dissolved in 0·005 M solutions of EDTA at room temperature. The solutions with a pectin concentration of 0·3–0·5% w/v were dialyzed for 3 days against a solvent containing 0·09 M NaCl and 0·01 M NaF. Thereafter, the concentration of pectin in the solution was determined by the phenol–sulphuric acid method (Bath, 1958).

Optically pure solutions were obtained by ultracentrifugation for 4 h at $14 \times 10^4 g$. A decrease of 0·3–0·5% in the pectin in solution was observed after ultracentrifugation. Increasing the centrifugation time from 4 to 8 h did not further diminish the pectin concentration in the solutions or change the intensity of the light scattered at $\theta = 90°$. The content of gel fraction, estimated by the technique of Lange (1970), was found to be negligible.

A set of five or six samples were prepared by diluting the starting solution containing 0·3–0·4 w/v of pectin. The samples were filtered under pressure (to remove dust particles) through a 0·45 μm PVC filter (Selectron, West Germany) into dust-free cuvettes.

3. Determination of the refractive index increment

The refractive index increment, $\partial n/\partial c_2$, was determined at 298·2 K and a wavelength (λ) of 436 nm using a differential refractometer (Shimadsu, Japan). For this purpose, preliminary dialysis of the pectin solutions was carried out against a solvent. The value obtained was $\partial n/\partial c_2 = (146 \pm 3) \times 10^{-6}\ \mathrm{m^3\,kg^{-1}}$ at a significance level of 0·95. The increment was found to be independent of the DE.

4. Light-scattering measurements

The light-scattering measurements were made with a Soviet-produced nephelometer (Central Design Bureau for Unique Apparatus, USSR Academy of Sciences) at 298·2 K and a wavelength (of unpolarized light) of 436 nm in the range 30–150°. The Rayleigh constant of dust-free benzene ($R_{\mathrm{u}\,90^\circ}$) used as a standard was $43{\cdot}2 \times 10^{-8}\ \mathrm{m^{-1}}$.

The results were processed by the double extrapolation method of Zimm. To simplify the construction of the Zimm diagrams, negative values were assigned to the constant k appearing in the function $\sin^2(\theta/2) + kc_2$.

RESULTS

Figures 1–7 show Zimm diagrams of pectins with different DEs. The values of the weight-average molecular weight ($\bar{M}_{\mathrm{w}}$), the second virial coefficient (A_2), and the z-average root mean square radius of gyration $\langle \bar{R}_{\mathrm{g}}^2 \rangle_z^{1/2}$ are summarized in Table 1, which also contains the values of the function

$$X = 16\pi^2 \frac{\langle \bar{R}_{\mathrm{g}}^2 \rangle_z}{\lambda^2} \sin^2 \frac{\theta}{2}$$

conforming to the average value of $\theta = 90°$. The range of X_{aver} conforms to the region of curvilinear dependence $P^{-1}(\theta) = f(X)$ (for mono-

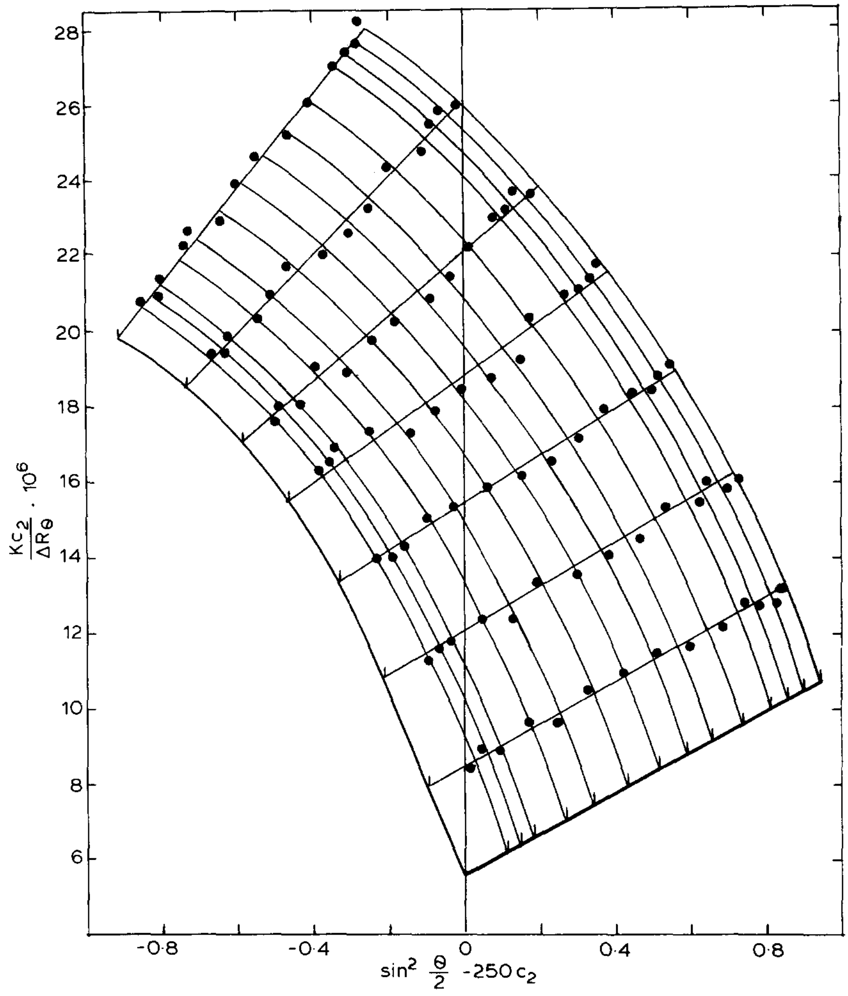

Fig. 1. Zimm diagram of sodium pectate solution.

disperse Gaussian coils). This region covers the values $0{\cdot}5 < X_{\text{aver}} < 6$ (Eskin, 1973). The procedure for introducing a correction in calculating the radius of gyration is described by Eskin (1973). The corrected values of the radius $[\langle \bar{R}_g^2 \rangle_z^{1/2}]^*$ are also given in Table 1.

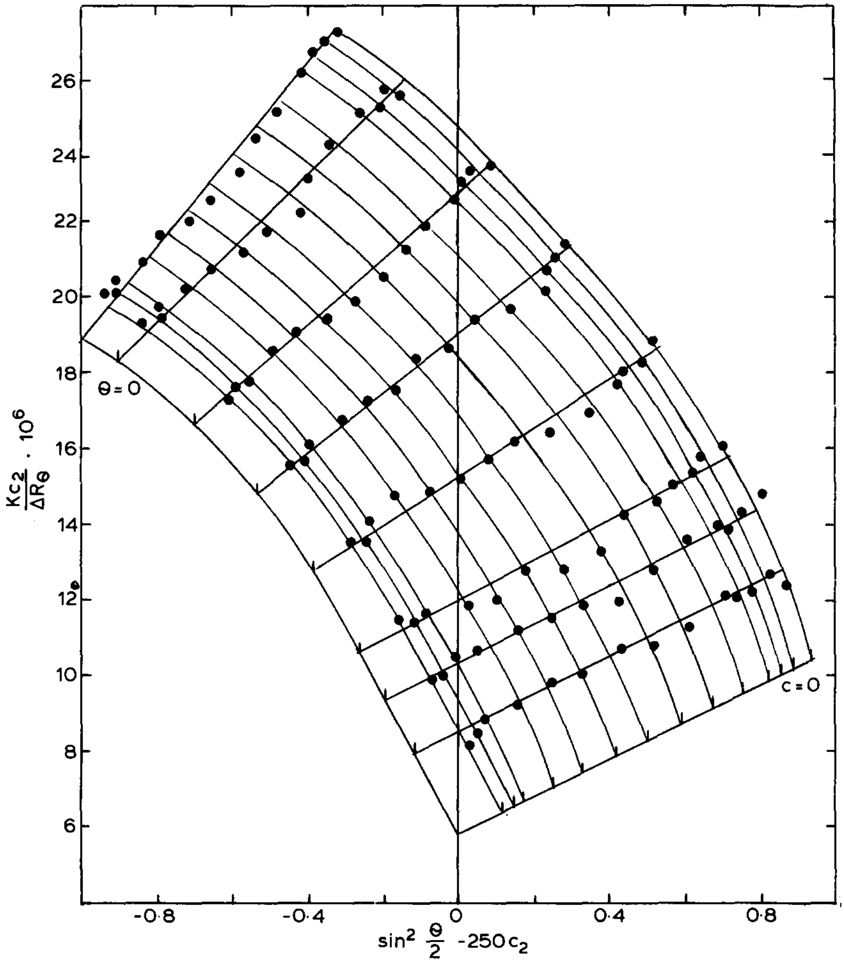

Fig. 2. Zimm diagram of pectin solution with a DE of 14%.

DISCUSSION

1. Effect of the gel fraction

A comparison of our results with those of Berth *et al.* (1982) reveals that the presence of a gel fraction which precipitates upon ultracentrifugation has a pronounced effect on the light-scattering characteristics

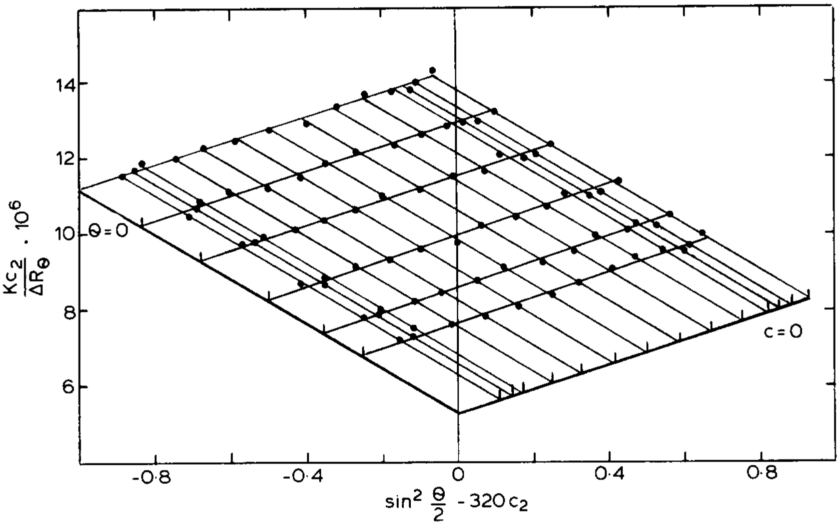

Fig. 3. Zimm diagram of pectin solution with a DE of 43%.

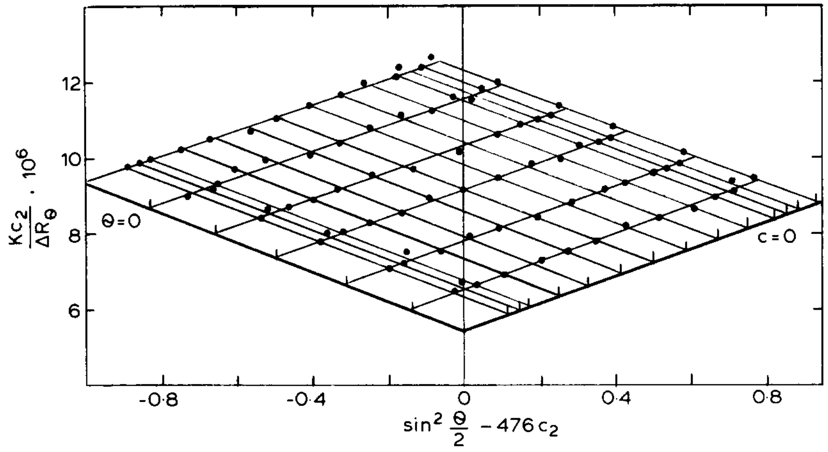

Fig. 4. Zimm diagram of pectin solution with a DE of 58%.

of pectin. On removing the gel fraction a 3–5-fold decrease in $\bar{M}_w$ is observed and the values of A_2 become positive. A similar effect was noticed by Smidsrød & Haug (1968). The presence of the gel fraction has little effect on $\langle \bar{R}_g^2 \rangle_z^{1/2}$, although in these experiments a decrease

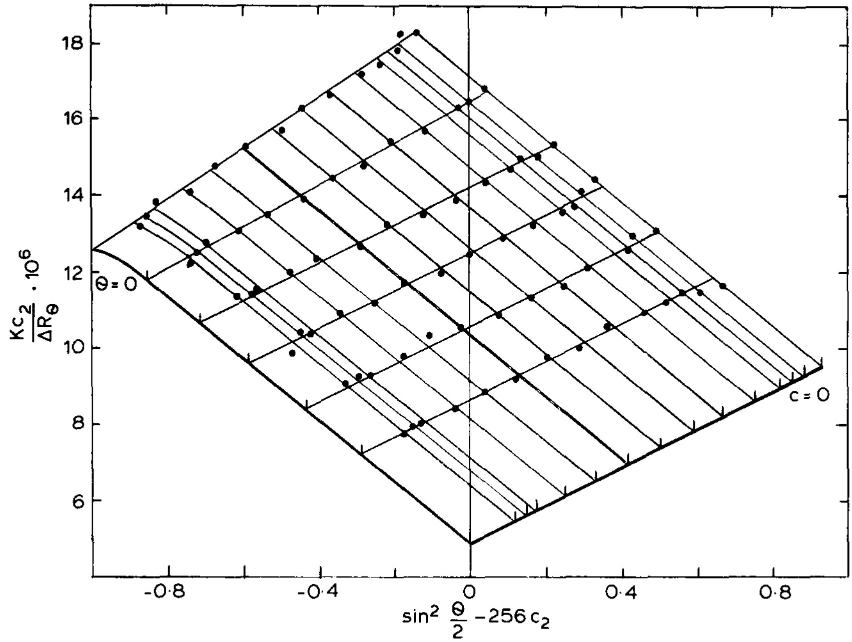

Fig. 5. Zimm diagram of pectin solution with a DE of 69%.

in $\langle \bar{R}_g^2 \rangle_z^{1/2}$ was not observed with completely esterified pectin as previously found (Berth *et al.*, 1982). The gel fraction appears to consist of compact particles which on removal from the solution by centrifugation do not change the angular dependence of the intensity of the scattered light. Thus, failure to remove the gel fraction leads to anomalously high values of $\bar{M}_w$ and incorrect conclusions about the nature of the polymer–solvent interaction.

2. Degree of polymerization of pectins with different DEs

The degree of polymerization of the pectin samples remains practically constant on increasing the DE from 0 to 95% (Table 2). This supports earlier data (Vollmert, 1950; Berth *et al.*, 1980) that show that methylation of pectins with diazomethane at temperatures below 278 K (in our case at 253 K) does not cause appreciable degradation.

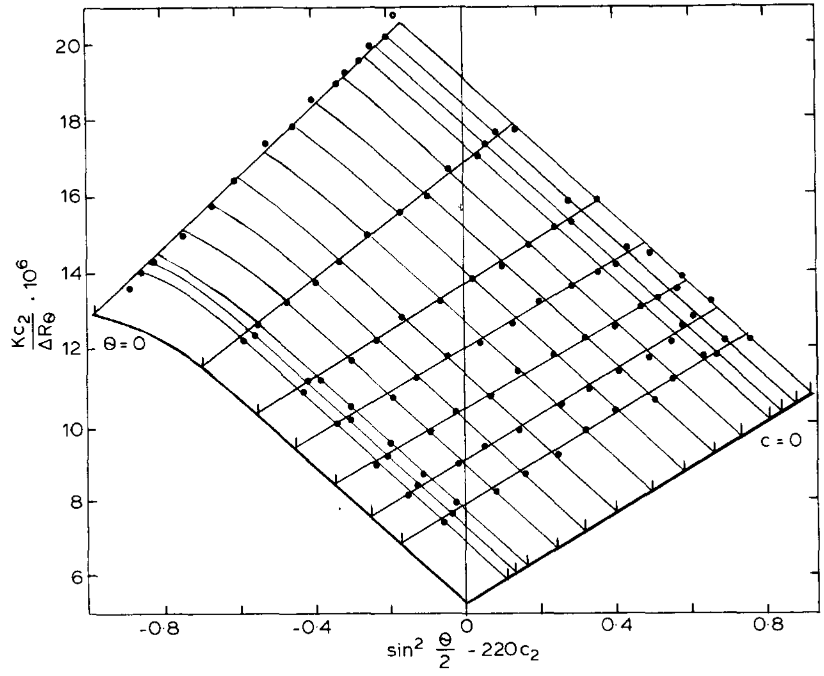

Fig. 6. Zimm diagram of pectin solution with a DE of 78%.

3. Dependence of the second virial coefficient on the DE of pectin

The second virial coefficient of pectins in aqueous solutions is constant in the DE range 43 to 95% (Table 1), but increases by a factor of three when the DE is reduced to zero (sodium pectate).

For solutions of polyelectrolytes this coefficient is determined from the sum of the terms characterizing the effect of the excluded volume, the coulombic electrostatic interaction between macro-ions, Donnan equilibrium, and the interaction of small ions both with themselves and with macro-ions (Tanford, 1961). The contribution of the last two terms may increase with decreasing DE.

The method of calculating the coulombic component of the second virial coefficient, A_2^{C}, appearing in the relationship between the osmotic pressure and the concentration of the polyelectrolyte solution, is given in the Appendix. The values of the excluded volume component, A_2^{EV},

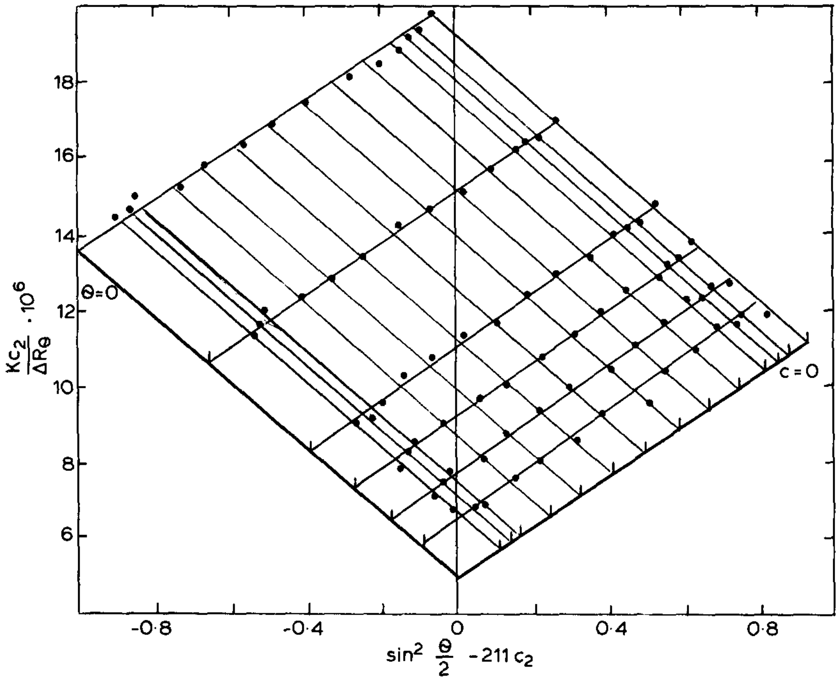

Fig. 7. Zimm diagram of pectin solution with a DE of 95%.

have been calculated as the difference between A_2 and A_2^C. As can be seen from Table 1, the increase in the value of A_2^{EV} at low degrees of esterification is less pronounced than that of A_2.

It must be noted that the calculated values of A_2^{EV} are very approximate because in polydispersed systems the method is strictly valid for data obtained using osmometry. The use of results obtained from light-scattering instead ($\bar{M}_w$ rather than $\bar{M}_n$, and the corresponding value of A_2) in these calculations underestimates the contribution of the excluded volume to osmotic pressure. At a constant molecular size distribution the error is larger as the DE decreases.

In a number of papers (Davis *et al.*, 1980; Yuryev *et al.*, 1981) it has been reported that aggregates of pectin macromolecules exist in aqueous and salt solutions. In addition, we have established (Berth *et al.*, 1982) that pectins are surface-active in salt solutions and are, therefore, amphiphilic in nature. As can be seen from the Zimm diagram (Figs 1–7) the dependence of $Kc_2/\Delta R_\theta$ on $(c_2)_{\theta=0}$ is linear at least for $c_2 \leqslant 0{\cdot}25\%$. The linear nature of the Zimm diagrams and

TABLE 1
Light-scattering Data and Other Parameters of Pectin Solutions

Degree of esterification (%)	$\bar{M}_w \times 10^{-3}$ *(Daltons)*	*Weight-average degree of polymerization,*[a] $\bar{P}_w$	*Second virial coefficient and its components* $(10^3\ m^3\ mol\ kg^{-2})$			$\langle \bar{R}_g^2 \rangle_z^{1/2}$ *(nm)*	X $(\theta = 90°)$[b]	$[\langle \bar{R}_g^2 \rangle_z^{1/2}]^*$ *(nm)*
			A_2	A_2^C	A_2^{EV}			
0	180	910	3·0	1·1	1·9	59	1·4	54
14	170	870	2·3	0·8	1·5	55	1·0	51
43	190	970	1·2	0·2	1·0	46	0·9	43
58	180	940	0·9	0·0	0·9	48	1·0	45
69	200	1060	1·0	0·0	1·0	61	1·5	55
78	190	980	1·0	0·0	1·0	62	1·6	56
95	200	1050	0·9	0·0	0·9	68	1·9	60

[a] The degree of polymerization was computed with due regard for counter-ion bonding.

[b] Calculated from $X = 16\pi^2 \frac{\langle R_g^2 \rangle_z^{1/2}}{\lambda^2} \sin^2(\theta/2)$.

TABLE 2
Conformational Characteristics of the Pectin Chain

Degree of esterification (%)	$\Psi(\alpha)$	$\Psi^*(\alpha)$	*Total contour length* $L = 4{\cdot}37\bar{P}_w$ *(nm)*	*Worm-like chain parameters*				*Zigzag-chain parameters (nm)*		
				a_0 *(nm)*	L/a_0	a_0^* *(nm)*	L/a_0^*	$\bar{l}$	$\bar{l}^*$	$\bar{l}_{theor}$
0	0·022	0·029	400	34	12	27	15	62	50	28
14	0·026	0·032	380	30	13	25	15	54	46	27
43	0·027	0·033	420	17	24	15	30	34	28	30
58	0·020	0·024	410	20	21	16	25	38	32	29
69	0·014	0·019	460	30	16	23	24	54	44	32
78	0·011	0·015	430	34	13	26	16	64	50	30
95	0·009	0·012	460	39	12	28	16	72	54	32

high values of A_2 are indicative of the absence of association under given conditions or point to the fact that association is irreversible. In calculating the total contour length of the macromolecules the possibility of irreversible association was not taken into account.

4. Pectin chain rigidity and its dependence on the DE

As a preliminary step in calculating the rigidity parameters for unperturbed pectin macromolecules the chain swelling coefficients (α) were determined. To do this, the function

$$\Psi(\alpha) = 0{\cdot}75 \times 10^{-25} A_2^{\mathrm{EV}} \bar{M}_{\mathrm{w}}^2 \langle \bar{R}_{\mathrm{g}}^2 \rangle_z^{-3/2}$$

was computed and the swelling coefficient was determined from the $\Psi(\alpha)$ versus α curves for semi-rigid-chain polymers (Yamakawa & Stockmeyer, 1972). Calculations were performed for both $\langle \bar{R}_{\mathrm{g}}^2 \rangle_z^{1/2}$ and $[\langle \bar{R}_{\mathrm{g}}^2 \rangle_z^{1/2}]$,* and $\Psi(\alpha)$ and $\Psi^*(\alpha)$, respectively, were obtained.

The values of $\Psi(\alpha)$ and $\Psi^*(\alpha)$ are summarized in Table 2. These are very small so the calculated swelling coefficients are very close to unity (irrespective of the chosen value of the reduced chain diameter). Analogous results are obtained if the $\Psi(\alpha)$–α dependence for coils is used (Tsvetkov *et al.*, 1964). Evidently the errors introduced by neglecting the curvature of $P^{-1}(\theta) = f(X)$ curves and also by ignoring polydispersity (i.e. by using $\langle \bar{R}_{\mathrm{g}}^2 \rangle_z^{1/2}$ instead of $\langle \bar{R}_{\mathrm{g}}^2 \rangle_{\mathrm{w}}^{1/2})$ in calculating $\Psi(\alpha)$, are insignificant in this case. Thus, the unperturbed dimensions of pectin macromolecules can be computed directly from light-scattering data.

The low values of the swelling coefficient are due to the small contribution from excluded volume effects to the pectin chain conformation, despite large values of the second virial coefficients. This is typical of semi-rigid-chain polymers (Schulz & Penzel, 1968; Tsvetkov, 1969; Yamakawa & Stockmeyer, 1972).

The conformational characteristics of semi-rigid-chain polymers can be evaluated using the worm-like-chain model (Burchard, 1965; Tsvetkov, 1969). The persistence lengths of pectins, a_0, were calculated by using the equation

$$\langle \bar{R}_{\mathrm{g}}^2 \rangle_z = a_0^2 \left\{ \frac{L}{3\alpha_0} - 1 + \frac{2a_0}{L} \left[1 - \frac{a_0}{L} \left(1 - \mathrm{e}^{-L/a_0}\right) \right] \right\}^*$$

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* Strictly, $\langle \bar{R}_{\mathrm{g}}^2 \rangle_{\mathrm{w}}$ should be used in calculating a_0.

in which the total contour chain length $L = \bar{P}_w h'$, where $\bar{P}_w$ is the weight-average degree of polymerization (Table 2) and h' is the projection of the monomer unit on the macromolecule axis.

The distance h' was taken as 4·37 Å in accordance with the X-ray diffraction data of Palmer & Hartzog (1945). These authors found that pectins with a DE varying between 0 and 70% have a helical conformation with a three-fold screw axis and a fibre axis identity period of 13·1 Å. Gidley *et al.* (1979) have suggested that calcium pectate in solution takes a conformation similar to that of the polyguluronic acid salts for which a value for h' of 4·35 Å has been reported (Mackie, 1971). Plashchina *et al.* (1978) have shown that the conformation of pectins in salt solutions remains unchanged in the DE range from 0 to 100%. Thus, $h' = 4{\cdot}37$ Å satisfies both the alternative models and may be used for calculating the persistence length of pectin with any DE.

As can be seen from Table 2, the pectin chain is very rigid, the persistence length approaching that of polymers such as aromatic polyamides ($a_0 = 100$–265 Å) (Tsvetkov, 1979; Iovleva *et al.*, 1983), the tricarbanilates of cellulose ($a_0 = 300$ Å) and amylose ($a_0 = 392$ Å) (Burchard, 1965). As the ratio of total contour chain length to the persistence length, L/a_0, is less than 20, pectins with a low or high DE cannot be considered to behave as Gaussian coils at these values of $\bar{P}_w$.

Rees & Wight (1971) have shown theoretically that 1,2-bonded residues of L-rhamnose, in the chain backbone, act as kinks causing a change in chain direction. The calculations of Hallman & Whittington (1973) have shown that an increase in the content of L-rhamnose residues in the galacturonan chain causes a large decrease in the characteristic ratio (C_∞). This effect is particularly prominent when the concentration of L-rhamnose residues is low (less than 10–15%). Rees (1982) has shown for three pectin samples, including citrus pectin, that L-rhamnose residues are roughly uniformly distributed along the chain and the chain fragment between the neighbouring L-rhamnose residues is about 25 monosaccharide units.

Based on the above ideas regarding the function of L-rhamnose residues and their close-to-uniform distribution in the chain, an attempt was made to describe the pectin chain conformation using a model consisting of N freely-jointed rods of length comparable to the wavelength of scattered light. The values of the rod length $\bar{l}$ calculated using the equation

$$\langle R^2 \rangle_z = \frac{\bar{l}^2}{6}\left(N - 1 + \frac{1}{2N}\right)$$

(Hermans and Hermans, 1958) are given in Table 2. Also listed in Table 2 are the theoretical values of the rod length, $\bar{l}_{\text{theor}}$, computed using the formula

$$\bar{l}_{\text{theor}} = \frac{\bar{P}_{\text{w}} c h'}{100}$$

where c stands for the concentration of L-rhamnose residues (mol%). For all pectin samples the value of c was taken as 7·1%; this corresponds to 5·3% w/v. It is obvious that $\bar{l} > \bar{l}_{\text{theor}}$. The discrepancy may be related to the deviations from uniform distribution of L-rhamnose residues in the chain as well as to the increased rigidity of the molecule. Actually, the model of a free-linked zigzag chain forms the basis of calculations, but the rotation of L-rhamnose residues around 1,2-glycosidic bonds can be assumed to be hindered. The increase in the barrier to rotation should result in a decrease in the effective rod length for a given radius of gyration.

The chain rigidity parameters $(a_0, \bar{l})$ show a minimum at intermediate DEs, that is to say pectins with a DE of between 43 and 58% exhibit maximum flexibility.

This conclusion is in good agreement with the results of Smidsrød & Haug (1971), who estimated the flexibility of pectins with different DEs from the dependence of intrinsic viscosity on ionic strength, and also of Yuryev *et al.* (1981), who determined the flexibility of pectins with different DEs by measuring the counterion activity coefficient in aqueous solutions. A similar relationship between chain flexibility and the DE of pectin is observed in the absence (Yuryev *et al.*, 1981) and presence of salt (Smidsrød & Haug, 1971; and this work).

The nature of the dependence shows that free carboxyl as well as esterified groups contribute to the chain rigidity. The effects are cooperative but different in nature.

The effect of carboxyl groups is due to an increase in the energy barrier to rotation associated with electrostatic repulsion. The increase in chain rigidity at high DEs may be due to (i) the increase in steric hindrance to rotation about the glycosidic bond and (ii) the additional conformational contribution of secondary interactions requiring the participation of methoxyl groups.

5. Gel-formation of high-DE pectins

Pectins with a DE of more than 40% are known to form gels in an acid medium in the presence of certain substances that lower the activity coefficient of water (sucrose, glycerol, ammonium sulphate, etc.). The consequent worsening of solvent quality aids the development of pectin–pectin interaction. The increase in chain rigidity as the DE increases from 40% suggests that interactions in which ester groups participate contribute to the chain rigidity. It can be assumed that such interactions also play a role in the gel network formation of high-DE pectins. This assumption is supported by the fact that at constant charge density the strength of gels obtained for a pectin with a DE of 72% is higher than for gels formed from pectin with a DE of 38% (Morris *et al.*, 1980).

The decrease in the parameter γ is indicative of the intensification of mutual attraction between pectin macromolecules at high DEs. This parameter is the ratio of the radius of the equivalent solid sphere to the radius of gyration of the macromolecule (Tanford, 1961), i.e.

$$\gamma = \frac{R_e}{\langle R_g^2 \rangle_z^{1/2}}$$

where

$$R_e = \left(\frac{3}{16\pi N_A}\right)^{1/3} (A_2^{EV})^{1/3} M_2^{2/3}$$

Thus

$$\gamma = \left(\frac{3}{16\pi N_A}\right)^{1/3} \frac{(A_2^{EV})^{1/3} M_2^{2/3}}{\langle R_g^2 \rangle_z^{1/2}}$$

The standard deviation of γ was calculated using the following values for measurement error (Eskin, 1973): 10%, 10%, and 5% respectively for $\bar{M}_w$, A_2, and $\langle \bar{R}_g^2 \rangle_z^{1/2}$. In doing so, the three parameters were assumed to be independent.

The dependence of γ on the DE of pectin is shown in Table 3. It is seen that γ does not change up to a DE of 58% but decreases at higher DEs.

TABLE 3
Ratio of the Radius of Equivalent Solid Sphere to the Radius of Gyration of Pectin Macromolecules

Degree of esterification (%)	γ	γ^*
0	0·31 ± 0·03	0·34 ± 0·03
14	0·30 ± 0·03	0·32 ± 0·03
43	0·33 ± 0·03	0·36 ± 0·03
58	0·30 ± 0·03	0·32 ± 0·03
69	0·26 ± 0·02	0·29 ± 0·03
78	0·25 ± 0·02	0·27 ± 0·02
95	0·23 ± 0·02	0·26 ± 0·02

For flexible-chain polymers, $\gamma = 0{\cdot}735$ (Berry & Casassa, 1970). The low values of γ found indicate the high penetrability of the comparatively rigid macromolecules of pectins.

Thus, the light-scattering data show that in aqueous solutions of high-DE pectins there are attractive forces between macromolecules, which are associated with the presence of ester groups. These forces compensate for the entropy contribution to the excluded volume so that the second virial coefficient is constant.

In addition to the intermolecular interaction energy, a significant role in the gel-formation process is played by chain flexibility which influences the number of contacts between macromolecules. It is interesting to note that a negative correlation exists between the mechanical properties of gels of high-DE pectins and the gel-formation rate and chain rigidity. Gels attain maximum hardness and strength at a DE of 52 to 61%. This corresponds to the minimum rate of gel formation (Smith & Bryant, 1968), which as we have shown here is close to the DE at which pectin shows maximum flexibility.

CONCLUSIONS

1. The second virial coefficient of pectins in aqueous solutions is positive and constant in the degree of esterification range 43 to 95%.

2. The pectin chain flexibility passes through a maximum as the degree of esterification is increased.

ACKNOWLEDGEMENTS

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APPENDIX

Method for calculating the coulombic component of the second virial coefficient for polyelectrolyte solutions

If the non-ideality of a polyelectrolyte solution is determined by the Donnan effect only, then, according to Tanford (1961), the osmotic pressure is given by

$$\Pi^{\mathrm{D}} = \frac{RTM_1}{\bar{V}_1}(m_{\mathrm{p}} + m_+ - m'_+ + m_- - m'_-)$$

where M_1 and $\bar{V}_1$ are respectively the molecular mass and partial molar volume of the solvent; m_{p} is the concentration of macro-ions (g-equiv kg^{-1}); m_+ and m_- are respectively the concentration of low-molecular cations and anions in the solution containing macro-ions (g-ion kg^{-1}); and m'_+ and m'_- the concentration of low-molecular cations and anions in the solution not containing macro-ions.

Taking account of the coulombic electrostatic interactions between all ions, according to Nagasawa *et al.* (1959), we have

$$\Pi^{\mathrm{C}} = \frac{RTM_1}{\bar{V}_1}(\gamma_{\mathrm{p}} m_{\mathrm{p}} + \gamma_+ m_+ - \gamma'_+ m'_+ + \gamma_- m_- - \gamma'_- m'_-)$$

$$\Pi^{\mathrm{C}} = \frac{RTM_1}{\bar{V}_1}\Sigma \gamma_i^{\mathrm{K}} m_i^{\mathrm{K}} \tag{1}$$

where γ_i are the appropriate activity coefficients. The virial equation for osmotic pressure of a polyelectrolyte solution, without taking into account the contribution of the excluded volume, can be written as

$$\Pi^C = RTc_2\left(\frac{1}{M_2} + A_2^C c_2 + \ldots\right) \tag{2}$$

where c_2 is the polyelectrolyte concentration (kg m^{-3}), and M_2 stands for the molecular weight of the polyelectrolyte.

Neglecting higher terms in the virial expansion, from eqns (1) and (2) we obtain

$$A_2^C = \frac{1}{c_2}\left(\frac{M_1 \Sigma \gamma_i^K m_i^K}{\bar{V}_1 c_2} - \frac{1}{M_2}\right)$$

Calculation of the concentration of the small ions (Nagasawa *et al.*, 1959)

The concentration of ions in a solution that does not contain macro-anions is assumed to be equal to the analytical concentration 1 : 1 electrolyte in the system (m_s). Clearly,

$$m'_+ = m'_- = m_s \tag{3}$$

For electroneutrality in the presence of a macro-ion

$$m_+ = m_p + m_- \tag{4}$$

The concentration m_- was calculated using the equation

$$\gamma_+\gamma_- m_+ m_- = (\gamma_\pm m_s)^2 \tag{5}$$

in which $\gamma_\pm$ denotes the mean activity coefficient of 1 : 1 electrolyte.

From eqns (3)–(5),

$$m_- = \left[\frac{m_p^2}{4} + \frac{(\gamma_\pm m_s)^2}{\gamma_+\gamma_-}\right]^{1/2} - \frac{m_p}{2}$$

Calculation of ion activity coefficients

Methods of calculating the activity coefficients of macro-ions (γ_p) in a solution containing low-molecular electrolyte are not known to the authors. In this paper we have taken γ_p to be equal to 1. In actual practice the coulombic electrostatic interactions can result in an increase in γ_p and because of this A_2^C will be slightly higher.

The activity coefficients of small ions were calculated on the basis of the assumption of MacInnes (1919). According to this, the activity coefficient of a chloride ion is equal to the mean activity coefficient of KCl in a solution of the same equivalent concentration. Because the accuracy of the calculation is not very high, 1:1 electrolyte was assumed to contain only NaCl.

The values of γ_+ were computed by the modified formulae of Manning, as described in Yuryev *et al.* (1983). In this paper it is shown that for infinitely dilute solutions of sodium pectate and pectin with a DE of 58%, which contain more than 5×10^{-3} g-equiv kg^{-1} NaCl, the experimentally determined values of γ_+ are in good agreement with the theoretical ones: γ_+ exhibits only a weak dependence on polymer concentration at relatively high concentrations of NaCl.

The values of γ_- are practically independent of the presence of macro-anions in solution (Joshi & Kwak, 1978). For this reason γ_- was assumed to be equal to $(\gamma_\pm)_{\mathrm{KCl}}$ in a solution of the same equivalent concentration. It was also assumed that $\gamma'_- = (\gamma_\pm)_{\mathrm{KCl}}$ and $\gamma'_+ = (\gamma_\pm)^2_{\mathrm{NaCl}}/(\gamma_\pm)_{\mathrm{KCl}}$ at a 0·1 g-equiv kg^{-1} concentration of NaCl and KCl.

REFERENCES

Bath, J. A. (1958). *Analyst* **83**, 451.

Berry, G. C. & Casassa, E. F. (1970). *Macromol. Revs* **4**, 1.

Berth, G., Anger, H. & Linow, F. (1977). *Nahrung* **21**, 939.

Berth, G., Anger, H. & Linow, D. (1980). *Nahrung* **24**, 529.

Berth, G., Anger, H., Plashchina, I. G., Braudo, E. E. & Tolstoguzov, V. B. (1982). *Carbohydr. Polym.* **2**,1.

Burchard, W. (1965). *Macromol. Chem.* **88**, 11.

Davis, M. A. F., Gidley, M. J., Morris, E. R., Powell, D. A. & Rees, D. A. (1980). *Intern. J. Biol. Macromol.* **2**, 330.

Eskin, V. A. (1973). *Rasseyanie sveta rastvorami polimerov (Dispersion of light by polymer solutions)* (in Russian), Moscow, Nauka.

Filippov, M. P. & Kuz'minov, V. I. (1971). *Z. Anal. Khim.* **26**, 143.

Filippov, M. P. & Vlasyeva, T. V. (1973). *Prikl. Biokhimiya i Mikrobiologiya* **9**, 134.

Gidley, M. J., Morris, E. R., Murray, E. J., Powell, D. A. & Rees, D. A. (1979). *J. Chem. Soc. Chem. Commun.* 990.

Hallman, G. M. & Whittington, S. G. (1973). *Macromol.* **6**, 386.

Hermans, J. (Jr) & Hermans, J. (1958). *J. Phys. Chem.* **62**, 1543.

Iovleva, M. M., Prozorova, G. E. & Smirnova, V. N. (1983). *Vysokomolek. Soed.* **B25**, 15.

Jordan, R. C. & Brant, D. A. (1978). *Biopolym.* **17**, 2885.

Joshi, Y. M. & Kwak, J. C. T. (1979). *J. Phys. Chem.* **83**, 1978.

Lange, H. (1970). *Koll.-Z. u Z. Polym.* **240**, 747.

MacInnes, D. A. (1919). *J. Amer. Chem. Soc.* **41**, 1086.

Mackie, W. (1971). *Biochem. J.* **125**, 89.

Morris, E. R., Gidley, M. J., Murray, E. J., Powell, D. A. & Rees, D. A. (1980). *Intern. J. Biol. Macrmol.* 2, 327.

Nagasawa, M., Takahashi, A., Izumi, M. & Kagawa, J. (1959). *J. Polym. Sci.* **38**, 213.

Palmer, K. J. & Hartzog, M. B. (1945). *J. Amer. Chem. Soc.* **67**, 2122.

Plaschina, I. G., Braudo, E. E. & Tolstoguzov, V. B. (1978). *Carbohydr. Res.* **60**, 1.

Rees, D. A. (1982). *Carbohydr., Polym.* **2**, 254.

Rees, D. A. & Wight, A. W. (1971). *J. Chem. Soc. B*, 1366.

Schulz, G. V. & Penzel, E. (1968). *Makromol. Chem.* **112**, 260.

Smidsrød, O. & Haug, A. (1968). *Acta Chem. Scand.* **22**, 797.

Smidsrød, O. & Haug, A. (1971). *Biopolym.* **10**, 1213.

Smith, J. B. & Bryant, E. F. (1968). *J. Food Sci.* **33**, 262.

Smith, J. E. & Stainsby, G. (1977). *Brit. Polym. J.* **9**, 284.

Stromeyer, M. & Linow, F. (1979). *Nahrung* **23**, 327.

Tanford, C. (1961). *Physical chemistry of macromolecules*, New York and London, Wiley.

Tsvetkov, V. N. (1969). *Uspekhi Khimii* **38**, 1674.

Tsvetkov, V. N. (1979). *Vysokomolek. Soed.* **A21**, 2606.

Tsvetkov, V. N., Eskin, V. E. & Frenkel, S. Ya. (1964). *Struktura makromolekul v rastvorakh (Structure of macromolecules in solutions)* (in Russian), Moscow, Nauka, p. 342.

Vollmert, B. (1950). *Makromol. Chem.* **5**, 101.

Yamakawa, H. & Stockmeyer, W. H. (1972). *J. Chem. Phys.* **57**, 2843.

Yuryev, V. P., Plashchina, I. G., Braudo, E. E. & Tolstoguzov, V. B. (1981). *Carbohydr. Polym.* **1**, 139.

Yuryev, V. P., Braudo, E. E. & Tolstoguzov, V. B. (1983). *Coll. Polym. Sci.* **261**, 210.