Carbohydrate Polymers 4 (1984) 283–297

Viscometric and Potentiometric Study of High-methoxyl Pectins in the Presence of Sucrose

F. Michel, J.-F. Thibault and J.-L. Doublier

Institut National de la Recherche Agronomique, Centre de Recherches
Agro-Alimentaires, Laboratoire de Biochimie et Technologie des Glucides,
Rue de la Géraudière, 44072 Nantes Cedex, France

(Received: 27 September 1983)

SUMMARY

The polyelectrolytic behaviour of high-methoxyl pectins (degree of esterification = 72·9) was studied in the presence of sucrose. Potentiometric measurements were carried out on the acidic form by titration with NaOH. Variations of the apparent dissociation constant pK_a *as a function of the degree of dissociation* α *were studied in salt-free solutions on a range of sucrose contents (0–60% w/v). Experimental plots could be described by Lifson-Katchalsky's (LK) treatment up to a 20% sucrose content. The intrinsic apparent dissociation constant* pK_0 *value was 3·1 ± 0·1 in agreement with previous data on pectinic acid. Above the 20% sucrose content, the LK theory was not appropriate owing to aggregation phenomena of macromolecular chains. Viscometric measurement were performed on the sodium form (*$\alpha = 1$*) and for three sucrose contents (0%, 40% and 60%). By carrying out isoionic dilutions, the effect of ionic strength (*I_t*) on the intrinsic viscosity [*η*] was studied. The same infinite limit value* $[\eta]_\infty$ *was obtained for each sucrose content and linear variations of* $[\eta]$ *as a function of* $I_t^{-1/2}$ *allowed an estimate of the relative stiffness of the macromolecular chain. This stiffness was slightly increased by sucrose addition.*

From these data, it could be concluded that a high sucrose content did not significantly change the macromolecular size (for $\alpha = 1$*), as shown by viscometry. However, when* α *decreased, potentiometric investigations with sucrose showed a higher value of* pK_a *and a down-curvature of* $pK_a(\alpha)$ *which could be interpreted by assuming aggregation. These occurred at about 40% sucrose and above which was a much lower concentration than*

Carbohydrate Polymers 0144-8617/84/$03.00 –

the gelling conditions (about 60%). Therefore aggregation phenomena could be present for relatively low sucrose contents if $\alpha < 0{\cdot}5$. *It was postulated that interchain associations could occur progressively (from 40 to 60%) and involve aggregate formation with a critical size for gelation.*

INTRODUCTION

Pectins are plant polysaccharides composed mainly of a backbone of 1,4-linked α-D-galacturonic acid (or its methyl ester) with some interruptions by L-rhamnose residues. Some neutral sugars, mainly arabinose and galactose, are covalently linked to this backbone forming side-chains (Pilnik & Voragen, 1970). Commercial pectins are extracted from by-products such as apple pomace or citrus peels (Nelson *et al.*, 1977) and are used in the food industry for their gelling properties. Pectins with low ester content ('low methoxyl pectins') or with high ester content ('high methoxyl pectins') can be prepared and the rheological properties of gels as well as the gelation mechanisms are different. The former with a degree of esterification (DE) < 45–50% gel with calcium ions in a system similar to the 'egg-box' model proposed for alginates (Morris *et al.*, 1978*a*; Ravanat & Rinaudo, 1980). The latter with a $DE > 55\%$ form gels in an acid medium (pH 2·2–3·5) with a high polyol content (sucrose) (Nelson *et al.*, 1977; Pilnik & Zwiker, 1970). The main factors which govern the gelling properties, pectin concentration, pH, nature and concentration of the polyol, degree of esterification, have been described especially on the basis of the rheological properties of the gels (Hinton, 1940; Harvey, 1950; Taufel & Berschneider, 1959; Kawabata, 1977). Some work has also been done by viscometry but with a low sucrose content (Chen & Joslyn, 1967). Presumably, the low pH value minimizes intermolecular electrostatic repulsions and the high polyol concentration decreases water activity leading to interchain interactions (Owens *et al.*, 1954). More recently, Morris *et al.* (1980) showed that the optimum DE for gelation is about 70%, that the gel formation is not competitively inhibited by addition of short polygalacturonic chains (degree of polymerization (DP) ~ 25) and that addition of urea leads to weakened gels. These results suggest that the network is formed through aggregates of variable size rather than through junction zones and that interchain interactions are stabilized by non-covalent (hydrogen) bonds. In the present paper, it is proposed to devote attention to the behaviour of pectin macromolecules in the presence of high concentrations of sucrose. This study was carried out on very dilute solutions of pectins by viscometry and potentiometry in order to quantify the role of sucrose molecules on the polyelectrolyte behaviour of high methoxyl pectins.

MATERIALS AND METHODS

Materials

Galacturonic acid was purchased from Baker and sucrose from Merck. Commercial apple pectin ('RS pectin', from Unipectine, Redon, France) was purified by precipitation with cupric ions (Jones & Stoodley, 1965; Michel *et al.*, 1981). The anhydrogalacturonic acid content of the purified sample was 62·9%, the content of associated neutral sugars was 13·2% and the *DE* was 72·9 (Michel, 1982). The number-average molecular weight was 27 000 determined by end-group titration, the viscosity-average molecular weight was 90 000 from viscometric measurements in 0·155 M NaCl with the equation proposed by Owens *et al.* (1946) and the weight-average molecular weight was 155 000 determined by light-scattering (Michel, 1982).

Solution preparation

To obtain the acidic form of the polymer (pectinic acid), freeze-dried pectin (at pH 4–4·25) was washed with ethanol (80% v/v) containing hydrochloric acid (1% v/v). The sodium form (sodium pectinate) was prepared by exact neutralization of the polymer solution with N/100 NaOH. Solutions containing sucrose (concentration range 10–60% w/v) were stirred for at least 3 h before use.

Potentiometry

Galacturonic acid and pectinic acid (with and without sucrose) were titrated with N/100 NaOH at 25·0 ± 0·1°C in solutions containing about 1 meq [COOH] litre^{-1} (unless otherwise stated) using a TS 80 Tacussel potentiometer. The apparent dissociation constant (pK_a) was calculated as a function of the degree of dissociation (α) according to eqn (1)

$$\mathrm{pK_a} = \mathrm{pH} + \log(1-\alpha)/\alpha \tag{1}$$

The treatment of experimental data was deduced from the model of Lifson & Katchalsky (1954). The structural charge density ($\bar{\lambda}$) was calculated from eqn (2):

$$\bar{\lambda} = \frac{\epsilon^2}{bDkT} \cdot \frac{100-DE}{100} \tag{2}$$

where ϵ is the electron charge, kT the Boltzmann term, b the length of the monomer and D the dielectric constant. b was taken to be equal to 4·35 Å (Rees & Wight, 1971). D for water was taken as 80 while values were calculated for sucrose/water systems from data given by Malmberg & Maryott (1950). From $\bar{\lambda}$, the effective charge density was calculated from eqn (3) and λ values were compared to the critical value λ_0 given by eqn (4):

$$\lambda = \alpha\bar{\lambda} \tag{3}$$

$$\lambda_0 = \frac{\log(R/a)}{1+\log(R/a)} \tag{4}$$

where R, the radius of the cylindrical subvolume, is calculated from the concentration and a is the minimum distance of approach taken as 6 Å (Rinaudo, 1974). When $\lambda < \lambda_0$, the electrostatic potential at the minimum distance of approach, a, is calculated from eqns (5) and (6), where β is an integration constant:

$$\epsilon\frac{\Delta\psi(a)}{kT} = \log\left(\frac{a^2}{R^2} \times \frac{1-\beta^2}{(\lambda-1)^2-\beta^2}\right) \tag{5}$$

$$\lambda = \frac{1-\beta^2}{1+\beta\coth(\beta\log(R/a))} \tag{6}$$

From the function $\lambda = \mathrm{f}(\beta)$ a theoretical variation $\Delta\mathrm{pK}(\alpha)$ can be obtained from eqn (7):

$$\Delta\mathrm{pK}(\alpha) = -0{\cdot}434\,\frac{\epsilon\Delta\psi(a)}{kT} = \mathrm{pK_a} - \mathrm{pK_0} \tag{7}$$

and the intrinsic dissociation constant ($\mathrm{pK_0}$) can be determined as described by Rinaudo *et al.* (1971).

Viscometry

Viscosities of sodium pectinates ($\alpha = 1$) were measured at 25·00 ± 0·05°C with a Low-Shear 30 viscometer. Variations of reduced viscosities as a function of the ionic strength were studied by the isoionic dilution procedure as described by Pals & Hermans (1952). The total ionic strength (I_t) of the solutions was kept constant according to eqn (8):

$$I_t = \phi C_p + I_s \qquad (8)$$

where C_p is the sodium pectinate concentration in eq litre^{-1}, ϕ the osmotic coefficient (taken as 0·85) and I_s the ionic strength arising from the added electrolyte (NaCl). When I_t is greater than 5×10^{-3}, the contribution of the polymer is neglected. Intrinsic viscosities were extrapolated using the relation of Huggins (1942)

$$\eta_{red} = [\eta] + k[\eta]^2 c \qquad (9)$$

RESULTS AND DISCUSSION

Potentiometric studies

The determination of the pK$_a$ of galacturonic acid at 25°C gives a value of 3·50 (data not shown) independent of the degree of dissociation and of the concentration in the range tested (1–10 meq litre^{-1}). Comparable results have been reported: 3·42 at 27°C (Speiser *et al.*, 1945), 3·47 at 20°C (Holvik & Hoiland, 1977), 3·51 at 20°C (Kohn & Kovac, 1978), 3·52 at 25°C (Cesaro *et al.*, 1982) and 3·36 at 25°C for a methyl-α-D-galacturonopyranosic acid (Ravanat & Rinaudo, 1980).

Due to the high-methoxyl content of the pectin sample, it is possible to prepare perfectly clear solutions of pectinic acid, which remain clear upon sucrose addition, by washing the pectin with acidic ethanol. These solutions were titrated by N/100 NaOH. The presence of sucrose induces a decrease in the dielectric constant of the solution (Malmberg & Maryott, 1950) and therefore increases the structural charge parameter $\bar{\lambda}$ of the pectinic acid, varying from 0·436 in pure water to 0·541 in 60% (w/v) sucrose/water medium. Nevertheless in the present conditions of polymer concentration, these values are smaller than the

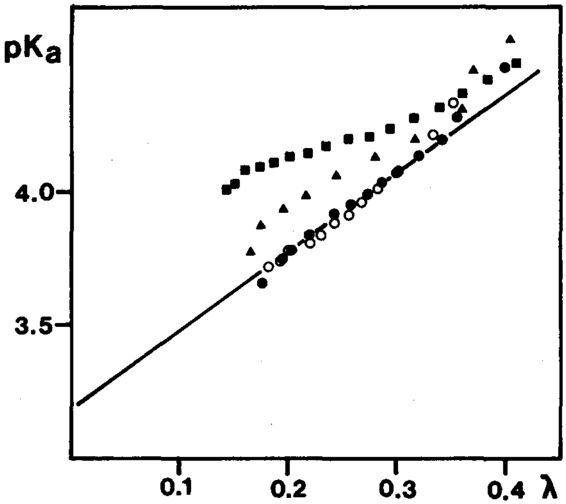

Fig. 1. Variations in pK_a of pectinic acid with the effective charge density parameter λ in aqueous and sucrose/water solutions. (○) 0% sucrose; (●) 20% sucrose; (▲) 40% sucrose; (■) 60% sucrose. The straight line is the theoretical $\Delta pK(\lambda)$ function.

critical value λ_0 (cf. 'Materials and Methods'). Therefore, in order to standardize the experimental curves, pK_a values were plotted versus the effective charge density parameter λ ($\lambda = \alpha\bar{\lambda}$) (Fig. 1). The intrinsic pK value, pK_0, was obtained by superimposing the experimental curve $pK_a = f(\lambda)$ (Rinaudo *et al.*, 1971) on the theoretical curve $\Delta pK = f(\lambda)$ calculated from the electrostatic treatment (Lifson & Katchalsky, 1954).

The pK_a values of pectinic acid without sucrose increased almost linearly with the degree of dissociation (up to 0·8) or with the effective charge density parameter λ. Experimental points were fitted satisfactorily by the theoretical curve and a pK_0 value of 3·1 ± 0·1 was obtained whatever the concentration of the polymer (in the range 1-35 meq litre^{-1}). This value is close to previous data reported for pectinic acids of *DE* varying from 10 to 90 (Katchalsky *et al.*, 1954; Rinaudo & Milas, 1974). It is likely that the pK_0 value of pectin substances is independent of the concentration, the *DE* and the *DP* (Ravanat & Rinaudo, 1980). It is known that carboxymethylcelluloses, whatever the degree of substitution may be, have the same pK_0 value of 3 ± 0·2 (Rinaudo *et al.*, 1971). Furthermore, the position of the carboxylic group on the anhydrosugar unit or the nature of the monomer unit carrying the carboxylic group do not have a pronounced influence on the pK_0 value of the carboxylic polysaccharides (Rinaudo, 1974; Gekko & Noguchi, 1975; Rinaudo & Milas, 1978; Cleland *et al.*, 1982).

The pK_0 value derived from pectinic acid is 0·3–0·5 pK units lower than that of the monomeric unit, D-galacturonic acid. A similar feature was observed by Cleland *et al.* (1982) between hyaluronic acid and the monomeric analogue. This decrease could be ascribed to the effects of substitutions at carbons 2, 3 or 4 of the anhydrouronic units of the polymer (Kohn & Kovac, 1978).

The curves $pK_a = f(\lambda)$ of the pectinic acid with up to 20% sucrose are superposed to the one obtained without sucrose addition (Fig. 1). On increasing the sucrose content, the experimental data do not concur with the theory of Lifson–Katchalsky. The pK_a value is not linearly related to the effective charge density parameter and extrapolations to a zero charge density are impossible. For a given value of the dissociation constant (or for a given value of the effective charge density), the pK_a value is increased in the presence of sucrose, especially in the range of low dissociation constant (or of low effective charge parameter) values and, apparently, the electrostatic interchains repulsions are minimized. Therefore, polymer/polymer interactions are promoted by increasing the content in sucrose. These effects could lead to aggregation phenomena of pectinic acid molecules.

Viscometry

Viscosity measurements of the fully ionized pectinate were carried out in order to study the effect of sucrose on the conformation of the polymer. Variations in the reduced viscosity with sodium pectinate concentration for different ionic strengths and for two concentrations (40% and 60%) in sucrose were obtained by the isoionic dilution procedure. The contribution due to the polymer was neglected for ionic strengths higher than 5×10^{-3}. Solutions were always Newtonian. Straight lines of $\eta_{red} = f(c)$ were obtained and intrinsic viscosity could be extrapolated to zero polymer concentration. A typical plot of reduced viscosity versus polymer concentration is shown in Fig. 2 and

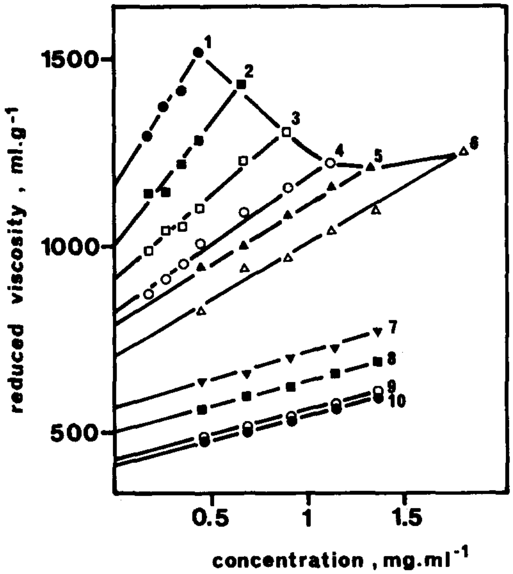

Fig. 2. Variations of the reduced viscosity of sodium pectinate with polymer concentration in 40% sucrose solution. Total ionic strength: 1, $0{\cdot}4 \times 10^{-3}$; 2, $0{\cdot}6 \times 10^{-3}$; 3, $0{\cdot}8 \times 10^{-3}$; 4, 10^{-3}; 5, $1{\cdot}2 \times 10^{-3}$; 6, $1{\cdot}6 \times 10^{-3}$; 7, 5×10^{-3}; 8, 10^{-2}; 9, 10^{-1}; 10, 5×10^{-1}.

values of intrinsic viscosity and Huggins coefficient are listed in Table 1 as a function of the ionic strength and of the sucrose content. Results show that at a given sucrose content, an increase in ionic strength leads to a decrease in intrinsic viscosity. The effect of sucrose on the intrinsic viscosities of sodium pectinate is pronounced when the ionic strength of the solution is low and is weak at a high salt concentration. The influence of ionic strength on the intrinsic viscosity is generally ascribed to chain expansion owing to intrachain electrostatic repulsions (Moan & Wolff, 1974). The effect of sucrose can be attributed either to a solvent effect or to specific interactions between pectinate and sucrose molecules.

The intrinsic viscosities were plotted versus the reciprocal of the square-root of the ionic strength in Fig. 3. A linear relationship was obtained as previously reported for many synthetic or natural polyelectrolytes when the ionic strength of the solution was greater than 10^{-3} (Pals & Hermans, 1952; Smidsrød & Haug, 1971; Moan & Wolff,

TABLE 1

Values of Intrinsic Viscosities ($[\eta]$) and Huggins Coefficient (k) Obtained with the Isoionic Dilution Procedure for Sodium Pectinate in Solutions of Different Ionic Strength (I_t) and of Different Sucrose Content

I_t	*0% sucrose*		*40% sucrose*		*60% sucrose*	
	$[\eta]$, ml g^{-1}	*k*	*$[\eta]$, ml g^{-1}*	*k*	*$[\eta]$, ml g^{-1}*	*k*
$0{\cdot}32\times10^{-3}$	1480	0·09				
$0{\cdot}40\times10^{-3}$			1150	0·57	910	1·22
$0{\cdot}48\times10^{-3}$	1380	0·11				
$0{\cdot}60\times10^{-3}$			1000	0·61	885	0·76
$0{\cdot}64\times10^{-3}$	1335	0·08				
$0{\cdot}80\times10^{-3}$			910	0·53	855	0·58
10^{-3}	1210	0·07	825	0·53	815	0·46
$1{\cdot}2\times10^{-2}$			790	0·52	735	0·63
$1{\cdot}28\times10^{-2}$	1090	0·11				
$1{\cdot}6\times10^{-2}$			710	0·58	660	0·67
5×10^{-3}	590	0·31	570	0·45	535	0·49
10^{-2}	520	0·56	500	0·55	500	0·57
10^{-1}	435	0·65	435	0·65	450	0·69
5×10^{-1}	420	0·61	415	0·78	405	0·98

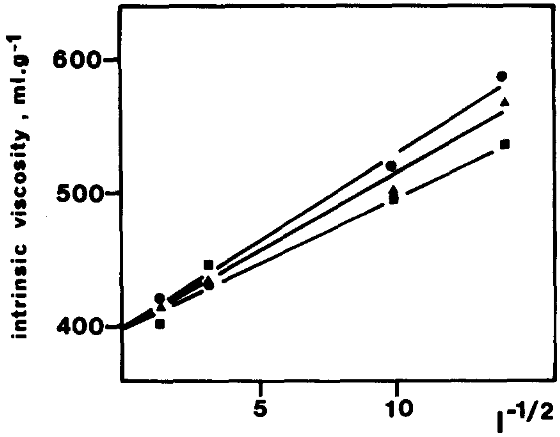

Fig. 3. Determination of intrinsic viscosity at infinite ionic strength of sodium pectinate as a function of sucrose content. (●) 0%; (▲) 40%; (■) 60%.

1974; Chien *et al.*, 1976; Vreeman *et al.*, 1980). In this way it is possible to extrapolate the intrinsic viscosity to infinite ionic strength, $[\eta]_\infty$, a value presumably close to that of intrinsic viscosity in a theta solvent (Tan & Gasper, 1974). The data in Table 2 show that the $[\eta]_\infty$ value is 400 ml g^{-1} and that a sucrose addition leads to the same value, but the slopes of the straight lines are decreased in the presence of sucrose. The constancy of $[\eta]_\infty$ shows that the limiting dimensions are not affected by the sucrose content. Hence, the possibility of specific interactions between pectinate and sucrose is eliminated. A similar effect has been observed by Smidsrød *et al.* (1973) for alginate in 20% aqueous ethanol.

On the basis of the theory of Fixman (1964), Smidsrød & Haug (1971) used the linear relationship between intrinsic viscosity and the reciprocal of the square-root of the ionic strength for the estimation of the relative stiffness of polyelectrolytes. Since the slope (S) of this straight line depends on the flexibility as well as on the molecular weight of the polymer, the flexibility parameter (B) can be calculated from eqn (10):

$$B = (S/[\eta]_{0\cdot 1})^{1\cdot 3} \tag{10}$$

Values of B obtained for sodium pectinate with different sucrose concentrations are indicated in Table 2. The value of 0·02 for sodium pectinate in sucrose-free solution is close to that reported by Smidsrød & Haug (1971) for a sodium pectinate with a comparable *DE*. This value is low in comparison with those obtained for other polyelectro-

TABLE 2

Values of Intrinsic Viscosity Extrapolated at Zero, $[\eta]_0$, and Infinite, $[\eta]_\infty$, Ionic Strength and Values of the Flexibility Parameter (B)

Sucrose content (%)	$[\eta]_\infty$ *ml* g^{-1}	$[\eta]_0$ *ml* g^{-1} [a]	$[\eta]_0$ *ml* g^{-1} [b]	B
0	400	1745	2115	0·020
40	400	1775	2615	0·018
60	400	970	1930	0·015

[a] Value obtained by the equation of Moan & Wolff (1974).

[b] Value obtained by the equation of Fuoss (1948).

lytes: *c.* 0·1 for κ-carrageenans (Vreeman *et al.*, 1980); 0·065 for carboxymethylcellulose of degree of substitution of 1·06 (Smidsrød & Haug, 1971); or 0·05 for λ-carrageenans (Morris *et al.*, 1978*b*). It can be only compared to that obtained (0·031) for an alginate sample rich in guluronic acid residues (Smidsrød *et al.*, 1973). Therefore one can consider sodium pectinates as highly inflexible macromolecules. Furthermore, addition of sucrose leads to a decrease in this value ($B = 0{\cdot}015$ in 60% sucrose solution) and consequently makes the sodium pectinate less sensitive to changes in ionic strength. On the basis of the theory of Fixman generalized to polyelectrolytes (Noda *et al.*, 1970; Smidsrød & Haug, 1971), $[\eta]_I$ variations can be expressed as:

$$[\eta]_I = [\eta]_\infty + k_1 I^{-1/2} \bar{M}_w^{1/2} \tag{11}$$

with

$$k_1 = k_2 b_\theta^{-1} \frac{D_0}{D} D^{1/2}$$

and D_0 = 'effective' dielectric constant operating near the polyionic chain; D = dielectric constant of the solution; $b_\theta = (\bar{r}_\theta^2)^{1/2}/n^{1/2}$ = effective bond length in theta solvent; $(\bar{r}_\theta^2)^{1/2}$ = root-mean-square end-to-end distance in theta solution; and $n = DP$.

Assuming D_0/D is constant whatever the sucrose content is, k_1 and consequently S would decrease with $D^{1/2}$. Then, the sucrose dependence of S and B could be directly related to variations in the dielectric constant. This stiffening of pectinate chains on sucrose addition can also be related to increases in Huggins coefficients (cf. Table 1). High values of this coefficient such as 0·98 or 1·22 in 60% sucrose solution may be interpreted by a stiffening of the polymer molecules (Moan & Wolff, 1974).

Another interesting macromolecular parameter concerns the intrinsic viscosity extrapolated to zero ionic strength. Two procedures based on empirical relations were used for this purpose, namely the one proposed by Fuoss (1948) and the one proposed by Moan & Wolff (1974). Results are shown in Table 2. The equation of Fuoss leads to overestimated values of intrinsic viscosity at zero ionic strength; this fact has been observed by other workers (Kurucsev, 1964; Moan & Wolff, 1974). As shown in Fig. 4, with the equation of Moan & Wolff, linear plots are obtained for sodium pectinate in sucrose-free solution and

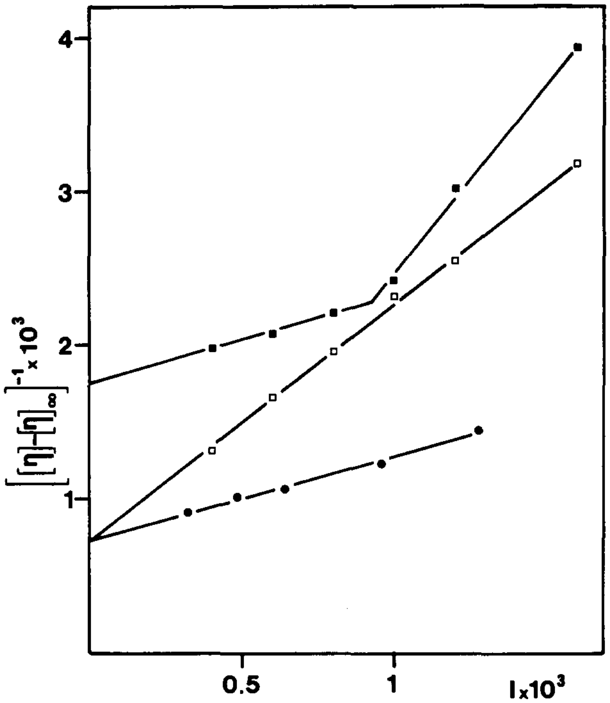

Fig. 4. Determination of intrinsic viscosity at zero ionic strength of sodium pectinate as a function of sucrose content. (●) 0%; (□) 40%; (■) 60%.

with 40% sucrose content. The extrapolated value $[\eta]_0$ is the same (about 1 750 ml g^{-1}) but the slope value is increased by the presence of sucrose. The plot obtained with 60% is peculiar since two consecutive straight lines are obtained with a breaking for an ionic strength of *c.* 10^{-2}. The value of intrinsic viscosity extrapolated to zero ionic strength is 970 ml g^{-1}. Such data could be interpreted in terms of changes in the hydrodynamic volume of the macromolecules in salt-free solutions and this characteristic parameter would be decreased by about one half with a 60% sucrose content. However, in the low ionic strength range, electroviscous effects can perturb the hydrodynamic behaviour of the sodium pectinate molecules and the variations of intrinsic viscosities may not be related to conformational changes (Domard, 1976).

CONCLUSION

As shown by potentiometry and viscometry, the presence of sucrose has two main effects on the polyelectrolyte behaviour of high methoxyl pectins, namely, promotion of aggregation phenomena and the stiffening of macromolecular chains. When the sucrose concentration is above 20%, potentiometric data are in disagreement with the theoretical treatment of Lifson–Katchalsky. As the presence of sucrose decreases the dielectric constant value, the carboxylic groups are less dissociated and, consequently, interchain electrostatic repulsions between these groups are minimized. Chen & Joslyn (1967) came to similar conclusions using viscometric data in the presence of 20% sucrose. This effect combined with the strong dehydrating action of sucrose decreasing the amount of water available for pectins could result in aggregate formation of pectin chains especially when the pH is lowered to pH 3. It must be pointed out that apparently these aggregation phenomena occur for sucrose contents which are relatively low (about 40%) compared with the usual gelation conditions (above 60%). From viscometry, the constancy of $[\eta]_\infty$ shows that the limiting dimensions of the polymer at high ionic strength are not changed upon addition of sucrose. This result can be interpreted as a sign of nonspecific interactions between pectins and sucrose. Then, sucrose molecules play mainly the role of a poor solvent, involving a stiffening of pectinate macromolecules which can more easily aggregate when the net charge of the polyelectrolyte is lowered. Therefore, it can be postulated that interchain associations occur progressively from 40 to 60% sucrose and the gelation is allowed only when aggregates lead to a critical size. This interpretation seems to be in agreement with data reported by Morris *et al.* (1980) on the non-competitive inhibition of pectin gelation by short polygalacturonic chains.

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