Carbohydrate Research, 165 (1987) 53–68
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

STATIC AND DYNAMIC LIGHT-SCATTERING STUDIES OF PECTIC POLYSACCHARIDES FROM THE MIDDLE LAMELLAE AND PRIMARY CELL WALLS OF CIDER APPLES

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(Received November 7th, 1986; accepted for publication, January 26th, 1987)

ABSTRACT

Mild, selective, and sequential methods have been used to extract pectic polysaccharides from cider apple pomace. The extracts have been characterised by static and dynamic light-scattering. CDTA (cyclohexane-*trans*-1,2-diaminetetraacetate)-soluble pectic polysaccharides from the middle lamellae were characterised as having a broad-molecular-weight distribution ($M_w = 4.2 \times 10^6$) of non-free-draining stiff coils. CDTA-insoluble and Na_2CO_3-soluble pectic polysaccharides ($M_w = 1.1 \times 10^6$) from the primary cell walls showed properties consistent with branched, cross-linked, microgel structures.

INTRODUCTION

Pectic substances are complex mixtures[1–3] of polysaccharides, which can be only partially extracted from the cell walls of soft plant tissues with hot water or hot aqueous solutions of chelating agents. The major constituents of pectic substances are rhamnogalacturonans in which the backbones consist of chains of α-(1→4)-linked D-galactosyluronic acid residues interspersed with (1→2)- and (1→2,4)-linked L-rhamnosyl residues. Attached to the main chain are oligosaccharide side-chains, containing mainly D-galactose and L-arabinose residues, which are attached to position 4 of (1→2,4)-linked L-rhamnosyl residues[3]. Complete or partial methyl esterification of the galacturonic acid residues gives pectins, or pectinates, which usually exist as the calcium salt within the cell wall. Commercially, hot dilute mineral acids are used to extract pectins from lemon peel or apple pomace, and

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these harsh treatments lead to non-selective degradation of the pectic substances. The resulting degraded polysaccharides are complex mixtures that show variations in physical and chemical properties, and are not suitable for studies of the effects of fine structure on the physicochemical properties of pectic polysaccharides.

Recently, mild, selective, chemical extractions have been used to solubilise pectic substances, with minimal degradation, from plant cell walls[4]. By removing Ca^{2+} with cyclohexane-*trans*-1,2-diaminetetra-acetate (CDTA) at room temperature and by hydrolysing ester cross-links under non-degradative conditions at low temperature, it was possible to isolate pectic substances from the middle lamellae and primary cell walls, respectively. We now report on the chemical properties of pectic substances solubilised under mild conditions from cider apple pomace and their physicochemical characterisation by combined static and dynamic light-scattering.

EXPERIMENTAL

General methods. — Neutral sugars were released by Saeman hydrolysis or by using M H_2SO_4, and analysed as their alditol acetates by g.l.c.[5]. Uronic acid was determined colorimetrically by a modification[5] of the method of Blumenkrantz and Asboe-Hansen[6]. The degree of esterification was determined by the method of Bociek and Welti[7].

Preparation of alcohol-insoluble residue (AIR). — Cider apples were a gift from Drs. J. Buckle and C. May of H. P. Bulmer Ltd. (Hereford, U.K.). The cortices from the peeled and cored apples were cut into small pieces (~1 cm^3), frozen in liquid nitrogen, and stored at −40°. The apple pieces were extracted with hot aqueous 80% ethanol to remove intra-cellular compounds of low molecular weight, and the residue was washed with cold distilled water to remove any loosely held material. The insoluble residue was rendered free of starch by extraction with aqueous 90% methyl sulphoxide for 20 h at ambient temperature, washed with distilled water, dialysed exhaustively at ambient temperature to remove traces of Me_2SO, and stored as a frozen suspension at −40°; the final residue is referred to as the alcohol-insoluble residue (AIR). The AIR was kept fully hydrated in order to facilitate rapid dissolution of the pectic polysaccharides.

Sequential extraction of AIR. — The AIR was thawed and stirred with 50mM CDTA (pH 6.5) for 6 h at 20°. The extract was removed by filtration and the residue was further extracted with 50mM CDTA for 24 h at 25°. The extracts (CDTA-1 and CDTA-2, respectively) were concentrated under reduced pressure, and dialysed exhaustively against distilled water. Aliquots were freeze-dried for sugar analysis and the remaining solutions were stored at −40° until required. The CDTA-insoluble residue was cooled to 2° and extracted for 20 h with 50mM Na_2CO_3 containing 20mM $NaBH_4$. After filtration, the residue was further extracted with 50mM Na_2CO_3 containing 20mM $NaBH_4$ at 25° for 20 h. The extracts (Na_2CO_3-1 and Na_2CO_3-2, respectively) were adjusted to pH 5.0 with glacial acetic acid, clarified by filtration, concentrated, dialysed exhaustively against distilled water, and stored at −40°.

Preparation and clarification of pectic polysaccharides. — Light-scattering studies of aqueous pectic polysaccharides are often hampered by the presence of aggregates of high molecular weight[8–11]. These aggregates, although present in small amounts, may make a significant contribution to the intensity of the scattered light, resulting in distorted Zimm plots and zero or negative second osmotic virial coefficients. It is not clear whether these aggregates are natural components of the cell wall or artifacts of the isolation procedure. Such problems have arisen in studies[8–11] of pectic polysaccharides prepared from extracts which had been precipitated with alcohol and/or freeze-dried. In this study, such complications were circumvented by using aqueous extracts of the pectic polysaccharides which had not been subjected to dehydration treatments, and which had undergone minimum degradation during the extraction procedures. The frozen (−40°) pectic polysaccharide samples were thawed, diluted to the required concentration, and filtered through 1.2-μm Millipore filters (prewashed several times with distilled water). Scattering cells were rinsed several times with distilled water and then with a filtered solution of the pectic polysaccharide, before being filled. Filtered, distilled water was used as diluent.

Static light-scattering. — A modified Malvern 4300 spectrometer was used with scintillation vials (2.5-cm diameter) as scattering cells. The light source was a vertically polarised He–Ne (λ = 632.8 nm) laser or a He–Cd (λ = 441.6 nm) laser. Instrument alignment was checked using filtered samples of benzene and found to be satisfactory in the angular range 30–150°. The apparatus was calibrated with benzene. The Rayleigh ratio for benzene was calculated as described[12] by Utiyama to be 64.23×10^{-6} cm^{-1} for vertically polarised light of wavelength 441.6 nm. Depolarisation effects for pectic polysaccharide samples were assumed to be negligible. Scattering data were collected at 25°, over the angular range 30–150°, and analysed by the Zimm method[13]. Sample clarification and instrument calibration procedures were checked by studies of standard samples of polystyrene in toluene.

Dynamic light-scattering. — Dynamic light-scattering studies were made with homodyne detection[14], at wavelengths of 632.8 and/or 441.6 nm, using the modified Malvern spectrometer. Measurements were made at 25° over the angular range 30–120°. Auto-correlation functions were fitted by using weighted third-order polynomials. Instrument calibration was checked by studies of monodisperse suspensions of polystyrene spheres of known diameter.

Refractive index and specific refractive index increment. — The refractive index of pectic polysaccharide solutions was measured using an Abbé refractometer. Specific refractive index increments were determined at λ = 632.8 nm, using a Chromatix KMX-16 laser differential refractometer. The instrument was calibrated at 25° by using aqueous solutions of NaCl. Pectic polysaccharide solutions were equilibrated for 30 min at 25° before taking measurements. Pectic polysaccharide concentrations were determined by weighing freeze-dried and then vacuum-dried aliquots of known volume. The specific refractive index increment *(dn/dc)* was found to be 0.146 $cm^3.g^{-1}$ (λ = 632.8 nm). This value was corrected, as described by Huglin[15], to give a value of 0.15 $cm^3.g^{-1}$ at λ = 441.6 nm. Measurements were made over the concentration range 0–5 $mg.cm^{-3}$.

RESULTS AND DISCUSSION

The starch-free AIR of cider apple pomace was sequentially extracted with CDTA (twice) at 25°, and dilute Na_2CO_3 at 2° and then at 25°, in order to solubilise the pectic polysaccharides. The AIR, instead of the purified cell walls, was used because it is easier to prepare and this is an advantage when relatively large quantities of pectic polysaccharides are required. In earlier studies[4,16], it was shown that sequential extraction of the cell walls with CDTA and dilute Na_2CO_3, as described above, solubilised mainly pectic polysaccharides from the middle lamellae and primary cell walls, respectively. A significant amount of the slightly branched, middle lamella pectins is held within the walls by Ca^{2+} and can be solubilised with the chelating agent CDTA. Intermolecular association *via* the site binding of Ca^{2+} implies the presence within the macromolecules of blocks of polygalacturonic acid greater than 20 residues in length[17]. In order to solubilise the pectic polysaccharides of primary cell walls, it appears that, in addition to removing the bridging Ca^{2+}, the ester cross-links between galacturonic acid residues and hydroxyl groups of sugar residues elsewhere in the cell matrix have to be hydrolysed; the solubilised pectic polysaccharides were virtually free of methyl ester groups. The initial extraction with Na_2CO_3 at 2° was performed to preferentially de-esterify the pectins and thus minimise degradation by β-elimination. In order to prevent formation of artifacts (*e.g.*, aggregates), which may occur when the acidic polysaccharides are in close proximity on an anion-exchange column, no attempt was made to fractionate further the CDTA or Na_2CO_3 extracts. The main aim of the present study was to investigate the properties of pectic polysaccharides that had undergone minimum degradation and were free of aggregates.

The carbohydrate composition and degree of esterification of the pectic polysaccharides are shown in Table I. CDTA-1 and CDTA-2 have similar contents of neutral sugars, but the content of galacturonic acid of the latter is significantly lower. Similar observations have been made with the CDTA-soluble pectic polysaccharides of onions[16]. The Na_2CO_3-extracts had significantly higher levels of arabinose, galactose, and rhamnose compared to those of the CDTA-extracts, but the content of galacturonic acid showed less variation. Most of the light-scattering studies were performed on the pectic polysaccharides present in CDTA-1 and Na_2CO_3-1, but preliminary studies with CDTA-2 and Na_2CO_3-2 gave results similar to those obtained from CDTA-1 and Na_2CO_3-1, respectively. The second CDTA extraction, which yielded CDTA-2, was carried out in order to ensure the efficient removal of chelator-soluble pectic polysaccharides prior to the Na_2CO_3 extraction.

TABLE I

SUGAR COMPOSITION OF AIR AND PECTIN FRACTIONS

Fraction	*D.e.*[a]	*Yield (g/100 g)*	*Sugar composition (µg/mg)*[b]								
			Rha	*Fuc*	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*UA*	*Total*
AIR[c]	—	100	12.4	10.0	155.0	45.4	18.7	74.5	208.4	337.0	861.2
CDTA-1	30–40	6.0	6.7	—	29.5	6.9	1.3	10.7	5.0	775.0	835.1
CDTA-2	30–40	3.5	2.9	—	13.5	3.1	1.2	7.0	8.8	423.3	459.8
Na_2CO_3-1	0	13.6	14.0	—	89.5	6.2	trace	34.8	4.5	736.8	883.3
Na_2CO_3-2	0	4.6	15.3	—	159.1	13.2	trace	62.2	7.5	613.0	870.3
DPAIR[d]	—	72.3	15.1	10.0	147.7	62.5	24.9	90.2	228.6	170.0	749.9

[a]Degree of esterification. [b]"Anhydro-sugar" value after Saeman hydrolysis. [c]Alcohol-insoluble residue. [d]Depectinated alcohol-insoluble residue.

There is increasing evidence[4] to suggest that phenolics are associated with pectic polysaccharides and may serve to cross-link them, but the accurate determination of phenolics is difficult. An estimate of the phenolic content of a pectic polymer may be obtained[18] by dispersing the polymer in 72% H_2SO_4 for 2 h, diluting the slurry to M acid, and then measuring the absorption at λ_{max} in the range λ = 270–300 nm. Based on such studies, Na_2CO_3-1 pectic polysaccharides were shown to have appreciable absorption, whereas the CDTA-1 pectic polysaccharides showed negligible absorption. From these observations, it was tentatively assumed that the Na_2CO_3-1 pectic polysaccharides had more phenolics than those in CDTA-1, and that the associated phenolics were stable under alkaline conditions. The possible significance of this result will be alluded to later.

Static light-scattering data were analysed according to the Zimm method[13], and plots for typical CDTA-1 and Na_2CO_3-1 extracts are shown in Fig. 1. The weight-average molecular weights $\langle M\rangle_w$, z-average square of the radius of gyration $\langle R_g^2\rangle_z$, and the second osmotic virial coefficients are given in Table II. Fig. 2 shows a semi-logarithmic plot of a typical autocorrelation function and the "best fit" third-order polynomial. The initial slope of such a plot characterises the molecular motion within the scattering sample. The effects of internal motions and of particle shape may be eliminated by constructing a "dynamic Zimm plot". Typical dynamic Zimm plots for CDTA-1 and Na_2CO_3-1 extracts are shown in Fig. 3. In the limit of zero angle and zero concentration, the parameter $\langle\Gamma\rangle$ monitors Brownian motion of the centre of mass of the molecules[14]. For polydisperse samples, the initial slope of the "zero concentration" line gives $\langle\Gamma\rangle_z = 2q^2\langle D_T\rangle_z$, where $\langle D_T\rangle_z$ is the z-average translational diffusion coefficient and $q^2 = (4\pi n_0/\lambda)^2\sin^2(\theta/2)$ is the square of the modulus of the scattering vector. The quantitites λ, n_0, and θ are, respectively, the vacuum wavelength of the incident light, the refractive index of the solvent, and the scattering angle. An effective "hydrodynamic radius" $\langle 1/R_H\rangle_z^{-1}$ may be calculated from $\langle D_T\rangle_z$ using Stokes law:

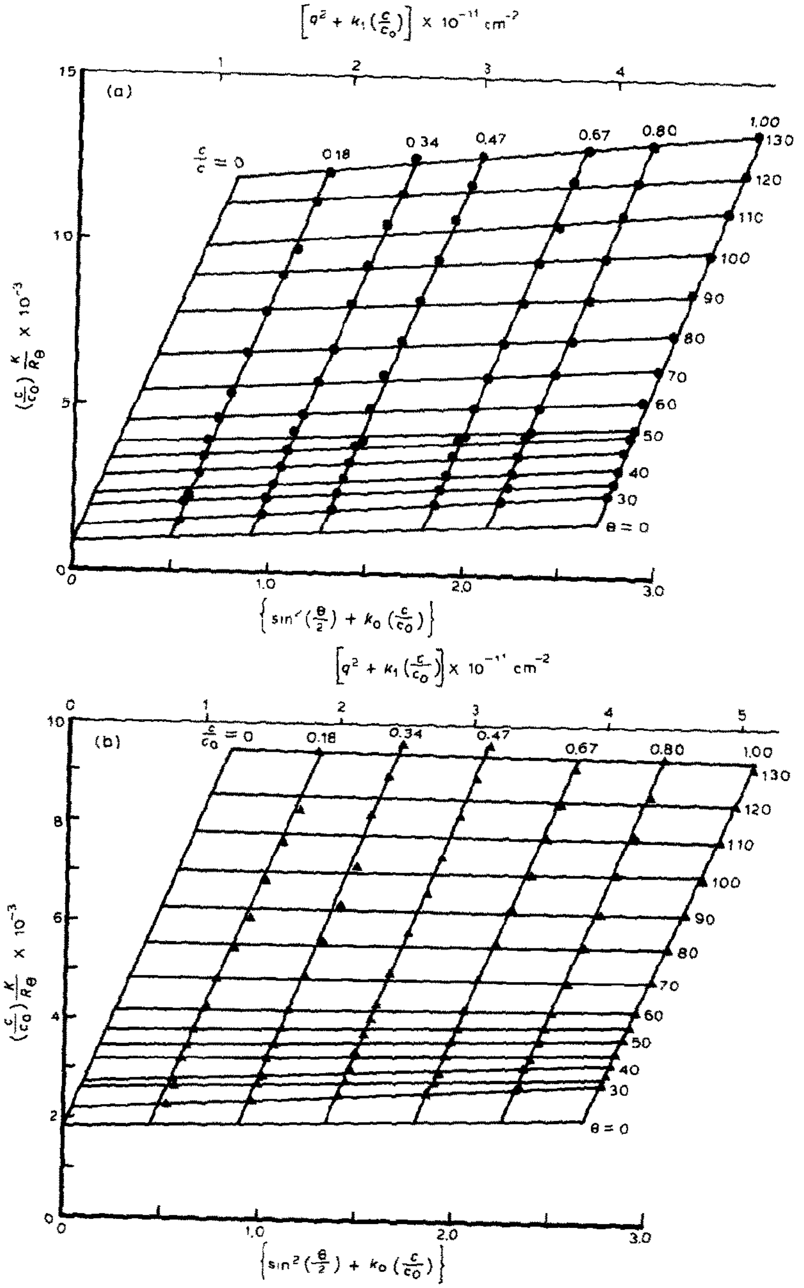

Fig. 1. Static Zimm plots for (*a*) CDTA-1 extract, with $c_0 = 2$ mg.cm^{-3}, $k_0 = 2.68$, and $k_1 = 3.83 \times 10^{11}$ cm^{-2}, (*b*) Na_2CO_3-1 extract, with $c_0 = 0.84$ mg.cm^{-3}, $k_0 = 2.68$, and $k_1 = 3.83 \times 10^{11}$ cm^{-2}. K is the scattering constant and R_θ the Rayleigh ratio.

$$\langle D_{\mathrm{T}}\rangle_z = \frac{kT}{6\pi\eta_0}\left\langle\frac{1}{R_{\mathrm{H}}}\right\rangle_z, \tag{1}$$

where k is the Boltzmann constant, T the absolute temperature, and η_0 the solvent viscosity. The calculated values of $\langle R_g^2\rangle_z$ and $\langle 1/R_{\mathrm{H}}\rangle_z$ may be combined to calculate the dimensionless ρ-parameter[19,20]:

$$\rho = \langle R_g^2\rangle_z^{1/2}\left\langle\frac{1}{R_{\mathrm{H}}}\right\rangle_z. \tag{2}$$

Values of $\langle D_{\mathrm{T}}\rangle_z$, $\langle 1/R_{\mathrm{H}}\rangle_z^{-1}$, and ρ are listed in Table II.

Due to a shortage of material, it was not possible to study the solutions of pectic polysaccharide as a function of ionic strength. The present study is confined to aqueous solutions in the absence of added salt. Due to the polyelectrolyte character of the polysaccharide, it is possible that, upon dilution, the reduced screening could lead to expansion of the chain. However, it can be seen from Fig. 1 that the angular dependence of the scattered light does not change systematically with decreasing concentration of polymer, indicating no detectable expansion of the chain on dilution.

The large molecular weights and radii of gyration found in the present study mean that the limiting phase shifts $U_{\min} = \langle R_g\rangle.q_{\min}$ are larger than normally encountered in studies on biopolymers. For the CDTA-1 and Na_2CO_3-1 extracts, $U_{\min} = 2.34$ and 0.88, respectively. For a reliable linear extrapolation to zero angle, it is

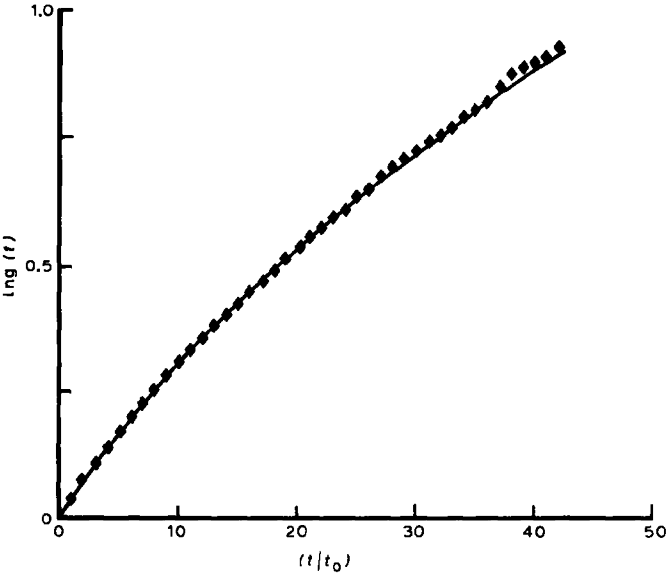

Fig. 2. Semi-logarithmic plot of a typical autocorrelation function obtained for a 2 mg.cm^{-3} CDTA-1 extract: $\lambda = 632.8$ mm, $\theta = 90°$; temperature, 25°; $t_0 = 60$ μs. The full line is the best-fit third-order polynomial.

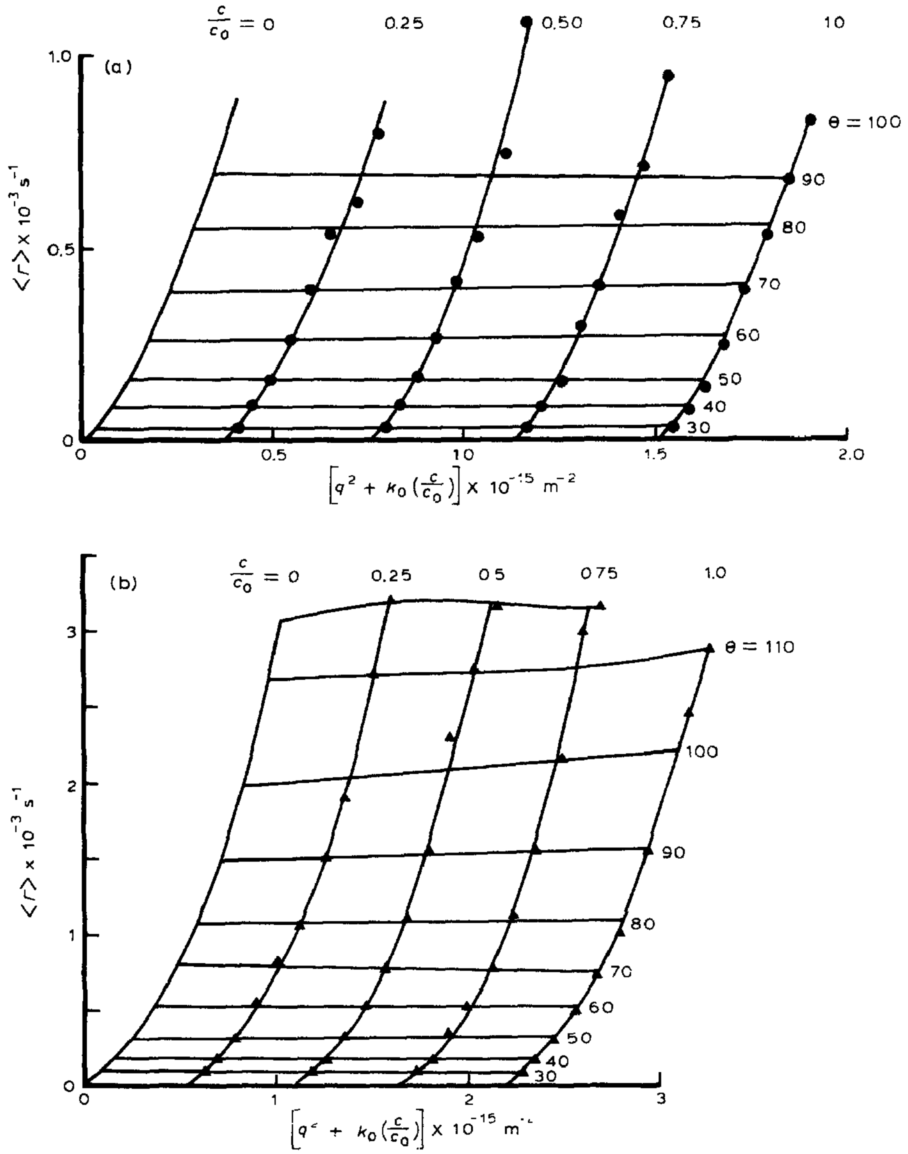

Fig. 3. Dynamic Zimm plots for (*a*) CDTA-1 extract, with $c_0 = 2$ mg.cm^{-3} and $k_0 = 1.5 \times 10^{15}$ m^{-2}; (*b*) Na$_2$CO$_3$-1 extract, with $c_0 = 2.5$ mg.cm^{-3} and $k_0 = 2.2 \times 10^{15}$ m^{-2}.

desirable that $U < 1$. It is not possible experimentally to substantially reduce U_{min}. However, the experimental data can be plotted[20] in the form of a logarithmic "Zimm plot", in which extrapolation of the particle scattering function is carried out as an exponential rather than linear function, thus extending the useful range of phase shifts. The molecular weights calculated using the linear and logarithmic extrapolations agreed within experimental error.

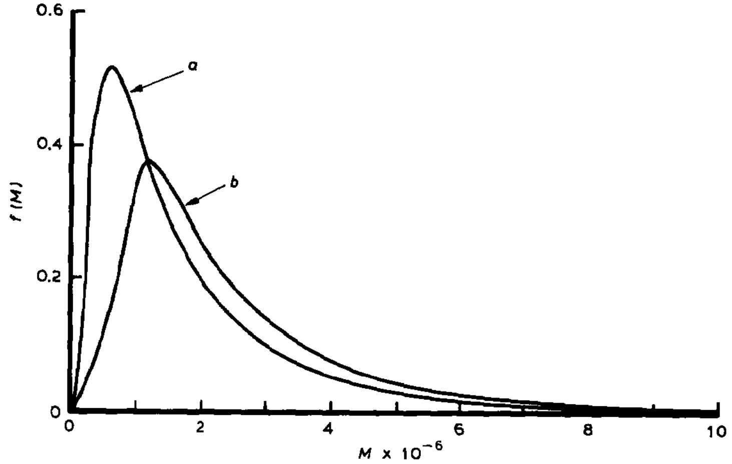

Fig. 4. Calculated molecular weight distribution for CDTA-1 pectic polysaccharide: (*a*) spherical sub-units; (*b*) rod-shaped sub-units.

The present experimental data suggest that the use of mild, selective, and sequential non-degradative extraction procedures, coupled with the absence of steps involving alcohol precipitation, freeze-drying, or evaporation to dryness, results in true solutions free from aggregates. These studies suggest that the aggregates reported by other workers may be artifacts of the isolation procedures and not true macromolecular constituents of the cell wall.

However, the measured molecular weights for the CDTA-1 and Na_2CO_3-1 pectic polysaccharides (Table II) are considerably larger than those previously reported[9,10]. Studies of fruit pectins reported a value of 2×10^5 for commercial citrus pectin[9] and 4–5 × 10^5 for various commercial citrus and apple pectins[10]. The high molecular weights obtained in the present study reflect the mildness of the present extraction procedures.

The data in Table II may be used to estimate the shape of the pectic polysaccharides. From the low content of neutral sugar in the CDTA-1 extract, it can be assumed that the polymers are linear. The molecular weight may be used to calculate a contour length L given by

$$L = (M/M_S)l = nl, \qquad (3)$$

where M_S is the molecular weight, l the extended length, and n the number of sugar residues in the chain. Table III compares the experimental value of $\langle R_g^2 \rangle_z^{1/2}$ with the calculated values for various particle shapes. The extreme models of a rigid rod or a gaussian coil provide equally poor fits to the experimental value. Molecular stiffness may be introduced by picturing the polymer as a worm-like coil. The radius of gyration is given by the expression[21]:

$$R_g^2 = a_0^2 \left\{ \frac{L}{3a_0} - 1 + \frac{2a_0}{L}\left[1 - \exp\left(-\frac{L}{a_0}\right)\right]\right\}, \qquad (4)$$

TABLE II

STATIC AND DYNAMIC LIGHT-SCATTERING DATA

Fraction	$\langle M \rangle_w$	$\langle R_g^2 \rangle_z$ (μm^2)	$\langle A_2 \rangle$ $(cm^3.mol.g^{-2})$	$\langle D_T \rangle_z$ $(cm^2.s^{-1})$	$\{\langle 1/R_H \rangle_z\}^{-1}$ (μm)	ρ
CDTA-1	$(4.2 \pm 0.5) \times 10^6$	0.12 ± 0.01	$(5.0 \pm 0.8) \times 10^{-5}$	$(1.40 \pm 0.2) \times 10^{-8}$	0.17	2.0
Na_2CO_3-1	$(1.1 \pm 0.1) \times 10^6$	0.017 ± 0.002	$(5.0 \pm 0.8) \times 10^{-5}$	$(1.2 \pm 0.2) \times 10^{-8}$	0.22	0.59

TABLE III

THEORETICAL AND EXPERIMENTAL RADII OF GYRATION

Fraction	*Experimental*	*Theoretical*[a]			
		Rod	*Coil*	*Worm-like chain*	*Broken-rod*
CDTA-1	0.35 μm	3.39 μm	0.03 μm	a_0 = 0.031 μm L = 11.7 μm	l_0 = 0.06 μm N_0 = 191
Na_2CO_3-1	0.13 μm	0.87 μm	0.015 μm	a_0 = 0.017 μm L = 3.0 μm	l_0 = 0.034 μm N_0 = 88

[a] The average residue molecular weight (M_s) was assumed to be 160 and the average extended length 4.37 Å; a_0 is the persistence length, and l_0 and N_0 are, respectively, the length and number of broken rods in the chain.

where a_0 is the persistence length. The best fit listed in Table III corresponds to the approximation $L \gg a_0$, for which

$$R_g^2 \simeq a_0 L/3. \tag{5}$$

The concept of a persistence length presupposes a homogeneous chain. More realistic, in view of the chemical structure of the polymer, is a "broken-rod" model[22,23]. For this model,

$$R_g^2 = \frac{l_0^2}{6}\left\{N_0 - 1 + \frac{1}{2N_0}\right\}, \tag{6}$$

where the chain is considered to be composed of N_0 freely jointed stiff rods of length l_0. The stiff rods may be considered to be polygalacturonan blocks interrupted by rhamnose residues. Although the concept of free rotation about the rhamnose linkages is clearly an over-simplification, a broken-rod model is a useful first approximation to the inhomogeneous chemical structure. The best fit values of l_0 and N_0 are given in Table III. Since $N_0 \gg 1$, $l_0 = 2a_0$ (the Kuhn statistical segment length), and equation *6* becomes

$$R_g^2 = \frac{l_0 L}{6} = \frac{N_0 l_0^2}{6}. \tag{7}$$

A value of l_0 = 0.06 μm is equivalent to 137 sugar residues and hence a uronic acid-to-rhamnose ratio of 137:1. The data in Table I suggest a ratio of 87:1. This comparison is difficult to make for at least three reasons: the broken-rod model is an oversimplification, the pectic polysaccharides are polydisperse, and the hydrolysis conditions used to estimate the rhamnose content may result[5] in an underestimate of rhamnose by as much as 40%.

Sample polydispersity may be estimated by comparing the molecular size determined by static and dynamic light-scattering[24]. The pectic polymers may be represented by coils with a radius of gyration given by equation *7*. For $N_0 \gg 1$, one would expect the coils to be non-free draining. For monodisperse non-free-draining coils[25],

$$D_T = \frac{kT}{6\pi\eta_0 \xi R_g}, \tag{8}$$

where ξ is a proportionality constant relating the radius of gyration and the hydrodynamic radius ($R_H = \xi R_g$). For a monodisperse sample, $\rho = \xi^{-1}$, and ξ is given by the expression[25]

$$\xi = \left(\frac{3\pi^{1/2}}{8}\right)\left\{1 + \frac{9\pi^{3/2}\eta_0 l_0}{4\sqrt{6}\,N_0^{1/2}\zeta}\right\}^{-1}, \tag{9}$$

where ζ is the frictional coefficient for the individual segments of the polymer chain. The individual segments are normally pictured as spheres and, for $N_0 \gg 1$, then $\xi \approx (3\pi^{1/2}/8) = 0.665$ and $\rho = 1.50$. Recent attempts to determine ξ experimentally have questioned[26,27] the assumptions used in the derivation of ξ. However, until this matter is resolved, it will be assumed that the theoretical value $\xi = 0.665$ is correct. The interpretation of the results and the estimate of polydispersity are dependent upon this assymption. The experimental value of ρ (ρ_E) will differ from the theoretical value (ρ_T) by an amount which will depend upon sample polydispersity. For polydisperse samples[24],

$$\langle D_T\rangle_z = \frac{kT}{6\pi\eta_0\xi}\left(\frac{6M_s}{l_0^2}\right)^{1/2}\frac{I(3/2)}{I(2)}, \tag{10}$$

$$\langle R_g^2\rangle_z = \frac{l_0^2}{6M_s}\frac{I(3)}{I(2)}, \tag{11}$$

$$\langle M\rangle_w = \frac{I(2)}{I(1)}, \tag{12}$$

where

$$I(j) = \int_0^\infty M^j f(M)\,dM, \tag{13}$$

and $f(M)$ is the number distribution function of molecular weights. In equations *10–12*, it has been assumed that the lower limit of integration may be taken as zero. Clearly, the approximation of a coil will break down below a certain threshold molecular weight (M'). Provided[28]

$$\int_0^{M'} M^j f(M)dM \ll \int_0^{\infty} M^j f(M)dM, \tag{14}$$

then

$$\rho_E^2 = Q\rho_T^2,$$

where

$$Q = \frac{\langle D_T\rangle_z^2 \langle R_g^2\rangle_z}{(kT/6\pi\eta_0\xi)^2} = \frac{I^2(3/2)\, I(3)}{I^3(2)} > 1 \tag{15}$$

If the molecular weight distribution can be approximated by a simple two-parameter skew distribution function, then equations *10–13* and *15* may be used to evaluate $f(M)$. The log-normal distribution[29] is a useful approximation which yields simple analytic expressions for equations *10–13* and *15*. If

$$f(M) = [\sigma M(2\pi)^{1/2}]^{-1} \exp\left\{-\frac{1}{2}\left[\frac{\ln(M/m)}{\delta}\right]^2\right\}, \tag{16}$$

where m is the geometric mean molecular weight and $\exp(\delta)$ is the geometric standard deviation, then

$$I(j) = m^j \exp(j^2\delta^2/2), \tag{17}$$

$$Q = \exp(3\delta^2/4), \tag{18}$$

and

$$M_w = m \exp(3\delta^2/2). \tag{19}$$

Analysis of the experimental data gives $\delta = 0.88$, $m = 1.4 \times 10^6$, and the distribution $f(M)$ is shown in Fig. 4. The degree of polydispersity $\langle M\rangle_w/\langle M\rangle_n = \exp(\delta^2) = 2.2$. Using this distribution, it is possible to obtain a better estimate of l_0 and N_0. From $\langle R_g^2\rangle_z$, the z-average contour length $\langle L\rangle_z = \langle M\rangle_w\,(1/M_s)\exp(\delta^2)$, and equation 7, the values $l_0 = 279$ Å and $N_o = 926$ are obtained. The calculation of ξ and the subsequent determination of m, δ, l_0, and N_0 depend on the assumption that the polymer can be approximated as a string of spherical units. A better approximation would be to replace ζ by the frictional coefficient for a rod of length l_0 and thickness t. An expression for ζ can be obtained by taking the limiting value, at large axial ratios, of the Perrin equation for a prolate ellipsoid[25]:

$$\zeta = 3\pi\eta_0 l_0\{\ln(2\rho_0)\}^{-1}, \tag{20}$$

where $\rho_0 = l_0/t$ is the axial ratio. Assuming $t \simeq 2$ Å and using $l_0 = 279$ Å as a first approximation, it is possible to solve iteratively and refine the values of l_0 and N_0. An iterative solution gives $\delta = 0.73$, $m = 1.93 \times 10^6$, $\xi = 0.611$, $\rho_{\Gamma} = 1.64$, $l_0 = 359$ Å, and $N_0 = 558$. The new molecular weight distribution is also shown in Fig. 4 and corresponds to $\langle M\rangle_w/\langle M\rangle_n = 1.7$. A value of $l_0 = 359$ Å is equivalent to a rhamnose-to-uronic acid ratio of ~1:82 if it is assumed that the rhamnose is evenly distributed along the polymer chain. The data shown in Table I suggest a ratio of 1:87. However, such an analysis in general underestimates[5] the true rhamnose content by ~40%, and the expected ratio is 1:52. Assuming that the chain structure is uniform, the present analysis suggests blocks of polygalacturonan ~80 residues in length interrupted by regions containing, on average, ~2 rhamnose residues. Powell *et al.*[30] have presented evidence for uniform length blocks, based on the isolation of the products of a partial acid hydrolysis, although the estimated block length was ~25 residues.

An alternative, but equivalent, representation of the stiffness of the polymer chain is the characteristic ratio C_∞ defined[31] as

$$C_\infty = \frac{6\langle R_g^2\rangle_w}{\langle n\rangle_w l_0^2} = \frac{6\langle R_g^2\rangle_z}{\langle n\rangle_z l_0^2}. \tag{21}$$

C_∞ is related to the persistence length a_0 by the equation[32]

$$C_\infty = (2a_0/l_0) - 1. \tag{22}$$

The experimental data give $C_\infty = 82$ and $a_0 = 0.018$ μm. The degree of swelling of the polymer coil may be determined by calculating the α-parameter, which monitors segment–segment and segment–solvent interactions, from the measured second osmotic virial coefficient, using the Flory–Orofino equation[32]:

$$\langle A_2\rangle = \frac{16\pi}{(3)^{3/2}} \cdot \frac{N}{M^2} \cdot \langle R_g^2\rangle_z^{3/2} \cdot \ln\left\{1 + \frac{\pi^{1/2}}{2}(\alpha^2 - 1)\right\}, \tag{23}$$

where N is Avogadro's number. Putting $M = \langle M\rangle_w \exp(\delta^2)$, and using the values of $\langle M\rangle_w$, $\langle R_g^2\rangle_z$, and $\langle A_2\rangle$ listed in Table II, gives $\alpha^2 = 1.012$. Thus, the unperturbed characteristic ratio $C'_\infty = C_\infty/\alpha \simeq C_\infty$, and $a'_0 \simeq a_0$.

The static light-scattering data for the Na_2CO_3-1 pectin extract are given in Table II. Analysis of the data on the basis of a linear polymer chain is presented in Table III. Neither a rigid rod nor a gaussian coil provide adequate molecular models. Fitting either a worm-like chain or a broken-rod model suggests a stiff coil with a similar stiffness to the CDTA-1 extract. However, the sugar analysis presented in Table I argues against a linear polymer. The high contents of arabinose and galactose suggest[4,16] neutral sugar side-chains linked to rhamnosyl residues in the polymer backbone. The ρ value is atypical of the values predicted[19,33] for a linear chain or even a randomly branched flexible chain. The experimental value is significantly lower than the value predicted for monodisperse hard spheres of constant density ($\rho = 0.775$). Microgel structures[33,34] or star-shaped molecules[34,35] yield $\rho < 1$, and complex branched structures such as beta-limit dextrin[36] also give low ρ values (0.68). The experimental value $\rho = 0.59$ is similar to that reported[19,33] for polyvinyl alcohol microgels of high molecular weight. Thus, a possible model is a spherical particle with an inhomogeneous density distribution. A cross-linked low-density coat surrounding a high-density central region could explain the disproportionately large hydrodynamic radius compared to the radius of gyration. It is possible that the present mild extraction conditions release complex cross-linked structural units from within the primary cell wall. Possible cross-links could involve direct linkage of rhamnogalacturonan backbones *via* chains of neutral sugars or possibly phenolic cross-links. Selective chemical or enzymic degradations of Na_2CO_3-1 extracts may provide a better picture of the molecular structure.

ACKNOWLEDGMENTS

We thank Mrs. A. V. Hemmant, Miss S. E. King, and Mr. R. G. Wilson for experimental assistance, and VJM thanks Dr. S. G. Ring for discussions concerning the methods and the need for fractionation of pectic polysaccharides. This work was funded, in part, by the Ministry of Agriculture, Fisheries and Food.

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