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# A hydrodynamic study of the depolymerisation of a high methoxy pectin at elevated temperatures

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**Abstract**

The hydrodynamic properties (intrinsic viscosity, $[\eta]$; infinite dilution sedimentation coefficient, $s^0_{20,w}$; weight average molecular weight, $M_w$ and translational frictional ratio, $f/f_0$) of a high methoxy pectin have been evaluated at various temperatures (20–60°C). A reduction in the value of all four hydrodynamic parameters is indicative of depolymerisation and is in agreement with an earlier study using viscometry [Axelos, M.A.V., & Branger, M., (1993). Food Hydrocolloids, 7, 91–102]. The apparent linearity of the Mark – Houwink plot of $\log[\eta]$ vs $\log M_w$ suggests that the conformation of the pectin molecule does not change significantly over the temperature range studied. The evaluation of the Mark–Houwink viscosity exponent ($a = 0.84$) indicates a moderately extended structure. This then allows the calculation of the number of Kuhn statistical lengths per chain from the adapted 'blob' theory of Dondos [Dondos A. (2001). Polymer, 42, 897–901]. The weight average number of Kuhn statistical lengths per chain is reduced from (170 ± 10) to (125 ± 10) when the temperature is increased from 20–60°C. This may be of significance as many high methoxy pectins are exposed to high temperatures during processing in both the food and pharmaceutical industries. 

*Keywords*: High methoxy pectin; Depolymerisation; $\beta$-elimination; Elevated temperature analytical ultracentrifugation; Adapted 'blob' theory; Kuhn statistical chain length

## 1. Introduction

Pectins are the family of complex polysaccharides, which constitute approximately one-third of the dry weight of higher plant primary cell walls (Walter, 1991). Pectins are particularly prevalent in fruit cell walls, especially citrus fruits and apple pommage. The main pectin chain is composed of $\alpha(1 \rightarrow 4)$ linked D-galacturonic acid residues.

Many of the galacturonic acid residues are in the esterified state as methyl esters. Theoretically the degree of esterification (DE) can range from 0–100%. Pectins with a degree of esterification (DE) >50% are known as high methoxyl (HM) pectins and consequently low methoxyl (LM) pectins have a DE <50% (Walter, 1991). $\alpha(1 \rightarrow 2)$-rhamnose residues are incorporated into the main chain at random intervals, which results in a kink in the otherwise linear chain. Side chains composed of arabinose and galactan residues are also present, either randomly dispersed or in localised 'hairy' regions. The degree of esterification and, therefore, the charge on a pectin molecule is important to the functional properties in the plant cell wall. It also significantly affects their commercial use as gelling and thickening agents (Lapasin & Pricl, 1995).

Relatively little has been published on the ultracentrifuge behaviour of these or many other polysaccharide solutions at elevated temperature (>40°C), although a recent study described the effect of temperature on *low methoxy* pectin (Morris, Butler, Foster, Jumel & Harding, 1999). Axelos and Branger (1993) have applied a single hydrodynamic technique—viscomtery—to the study of the effect of heating at elevated temperature on pectins as a function of degree of esterification under non-buffered saline solution conditions ($I = 0.1$). Those workers reported chain degradation which increased as a function of both temperature of treatment and degree of esterification.

We now report a more complete hydrodynamic study for high methoxy-pectin using size exclusion chromatography coupled to multi-angle laser light scattering (SEC-MALLS) (measurement of weight average molecular weight, $M_w$ and polydispersity), sedimentation velocity analytical ultracentrifugation (measurement of sedimentation coefficient and polydispersity) and viscometry (measurement of intrinsic

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viscosity) as a function of temperature under buffered conditions, and compare these data with a similar study performed for low-methoxy pectin (Morris et al., 1999) and the Axelos and Branger (1993) viscometry study. As we shall we see, we essentially confirm and extend the earlier observations.

## 2. Materials and methods

### 2.1. Materials

The high methoxy pectin (degree of esterification ~70%) of high degree of purity was a gift from Copenhagen Pektin Fabric (Copenhagen, Denmark). The material was solubilised for 24 h in pH 6.8, ionic strength 0.1 M, standard phosphate/chloride buffer (Green, 1933), of the following composition $Na_2HPO_4 \cdot 12H_2O$—4.595 g; $KH_2PO_4$—1.561 g and NaCl—2.923 g all made up to 1litre and then dialysed against this buffer.

### 2.2. Densimetry

The partial specific volume, $\bar{v}$ of the high methoxy pectin molecule was measured with an Anton Paar (Graz, Austria) precision density metre at (25.0 ± 0.1)°C according to the procedure of Kratky, Leopold and Stabinger (1973) (see also Pavlov, Korneeva, Harding & Vichoreva, 1998).

### 2.3. Capillary viscometry

Solutions and reference solvents were analysed using a 2-ml automatic Schott–Geräte Oswald viscometer (Schott–Geräte, Mainz, Germany), under precise temperature control (±0.01°C). The relative, $\eta_{rel}$, and specific, $\eta_{sp}$, viscosities were calculated from the following relations:

$$\frac{\eta}{\eta_0} = \left(\frac{t}{t_0}\right)\left(\frac{\rho}{\rho_0}\right) = \eta_{rel} \tag{1}$$

where $t$ is the flow time for the macromolecular solution, $t_0$ is the flow time for the solvent in seconds. Because of the low concentration used ($\rho/\rho_0$) is usually taken as unity (Harding, 1997). The specific, $\eta_{sp}$ can, therefore, be defined as follows

$$\eta_{sp} = \eta_{rel} - 1 \tag{2}$$

A common method for measuring intrinsic viscosity is to calculate the relative and specific viscosity at one concentration (in this case 2.5 mg/ml) and employ the Solomon/Ciuta approximation [Eq. (3)] (Harding, 1997; Morris, Foster & Harding, 2000; Abel-Azim, Atta, Farahat & Boutros, 1999; Kravtchencko & Pilnik, 1990). According to Kravtchencko and Pilnik (1990), the intrinsic viscosity can be accurately estimated (error 1%) by a single measurement at low concentration. In the same article they achieved good agreement between single point measurements and traditional multi-point extrapolations (Kravtchenko & Pilnik, 1990; Harding, 1997; Morris, 2001) for pectin solutions up to 5 mg/ml.

$$[\eta] \approx \frac{[2\eta_{sp} - 2\ln\eta_{rel}]^{1/2}}{c} \tag{3}$$

### 2.4. Elevated temperature sedimentation velocity (in the analytical ultracentrifuge)

Sedimentation velocity permits measurement of the rate of movement of solute through a solvent in a centrifugal field, and can be represented by the following relation:

$$\frac{M(1 - \bar{v}\rho_0)}{N_A f} = \frac{dr_b/dr}{\omega^2 r} = s \tag{4}$$

where $M$ is the molecular weight in g/mol, $\bar{v}$ is the partial specific volume, $\rho_0$ is the density of the solvent, $N_A$ is Avogadro's number, $f$ is the frictional coefficient (which depends on the shape and size of the macromolecule of interest), $dr_b/dr$ is the effective velocity; the angular velocity, $\omega = 2\pi$ rpm/60, $r$ is the radial position (i.e. distance from the centre of the rotor) and $s$ is the sedimentation coefficient.

The high temperature unit for the Beckman Model E analytical ultracentrifuge allows centrifugation at temperatures up to 125°C, however, for aqueous solutions temperatures >80°C are impractical. Since parallel measurements were being made with SEC/MALLS, and the latter set-up has a high temperature limit of 60°C, we restricted our ultracentrifuge studies to this upper limit for temperature. Both the rotor and sample cells are pre-heated to the required temperature prior to sample injection. Samples were run at 52,640 rpm and Schlieren images were captured semi-automatically onto photographic film. $s_{T,b}$ values were calculated from Schlieren images using a graphics digitising tablet. $s_{20,w}$ values were then calculated from $s_{T,b}$ values [sedimentation coefficients at temperature ($T$) and in buffer ($b$)] using the standard Eq. (5) (see, for example, Pavlov, 1997; Ralston, 1993; Rowe, 1977).

$$s_{20,w} = s_{T,b}\left[\frac{(1 - \bar{v}\rho_{20,w})\eta_{T,b}}{(1 - \bar{v}\rho_{T,b})\eta_{20,w}}\right] \tag{5}$$

where $\eta_{T,b}$, $\eta_{20,w}$ are respectively the viscosities of the solvent at temperature $T$ and water at 20°C, and $\rho_{T,b}$ and $\rho_{20,w}$, are the corresponding solvent densities. This correction can be done before (convention) or after the concentration extrapolation of Eq. (6).

A partial specific volume (for the macromolecular component), $\bar{v}$ of (0.63 ± 0.01) ml/g was calculated from density measurements using the procedure of Kratky et al. (1973) (see Section 3.1) and assumed to be constant with temperature. Apparent sedimentation coefficients, $s_{20,w}$ were calculated at various concentrations from 0.5 to 2.5 mg/ml and extrapolated to zero concentration using the standard

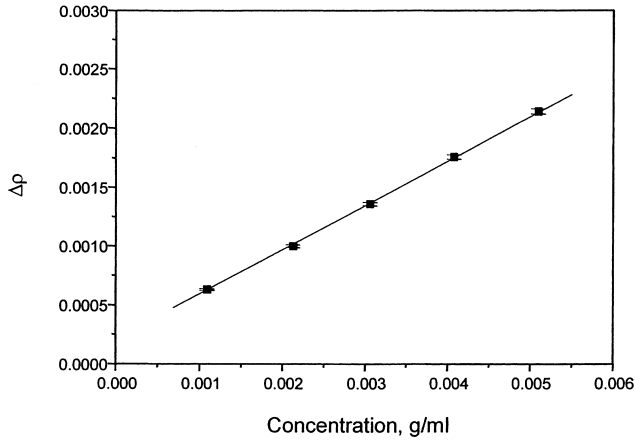


Fig. 1. Dependence of density increment $\Delta\rho = \rho - \rho_0$ (where $\rho$ and $\rho_0$ are the densities of the pectin solution and the buffer respectively) for a high methoxy pectin in standard phosphate chloride buffer (pH = 6.8, $I = 0.1$ M).

equation (see, for example, Ralston, 1993):

$$s = s^0(1 - k_s c) \quad (6)$$

where the Gralén (1944) parameter, $k_s$ is a measure of concentration dependence.

### 2.5. SEC–MALLS

SEC–MALLS (size exclusion chromatography coupled to multi-angle laser light scattering) allows on-line light scattering of a heterogeneous solute fractionated by size exclusion chromatography, permitting the extraction of absolute molecular weights and molecular weight distributions (see, for example, van Holde, 1985; Jumel, Browne & Kennedy, 1992; Jumel, 1994). The Wyatt Technology (Santa Barbara, USA) Dawn F multi-angle laser light scattering photometer (Wyatt, 1992) was coupled to TSK Gel 4000, TSK Gel 5000 and TSK Gel 6000 columns protected by a similarly packed guard column (Anachem Ltd., Luton, UK). The eluent was the standard pH 6.8 $I = 0.1$ buffer and the injection volume was 100 μl. The pectin refractive increment (d$n$/d$c$) of 0.146 ml/g (Theisen, Johann, Deacon & Harding, 2000) was assumed to be independent of temperature.

## 3. Results and discussion

### 3.1. Densimetry

A partial specific volume, $\bar{v}$ of (0.63 ± 0.01) ml/g was calculated from the slope of a plot of $(\rho - \rho_0)$ vs. concentration (Fig. 1). This value is similar to the value of 0.57 ml/g obtained for citrus pectin (Harding, Vårum, Stokke & Smidsrød, 1991; Harding, Berth, Ball, Mitchell & Garcia de la Torre, 1991).

### 3.2. Capillary viscometry

There is a clear decrease in intrinsic viscosity, $[\eta]$ with an increase in temperature up to at least 60°C (Table 1 and Fig. 2). This would be indicative of molecular breakdown and/or a conformational change to a more compact shape, as observed by Morris et al. (1999) in the case of a low methoxy pectin under the same conditions.

### 3.3. Elevated temperature sedimentation velocity (in the analytical ultracentrifuge)

In Fig. 3 we can see a representative plot of $\ln r_b(t)/r_b(t_0)$ vs. $\omega^2 t$ which has been used to calculate the apparent sedimentation coefficient at a temperature of 50°C and a concentration of 2.5 mg/ml. From Fig. 4, we see that increase in temperature does not affect the unimodality of the sedimenting boundary as seen in earlier studies (Harding, Berth et al., 1991). As the temperature is increased there is a slight decrease in sedimentation coefficient, $s^0_{20,w}$ (Table 1 and Fig. 5), which is consistent with a decrease in molecular weight (although as with intrinsic viscosity, conformational effects cannot be ruled out). To obtain an unequivocal demonstration of decrease in molecular weight we need, however, to consider results from SEC–MALLS.

### 3.4. SEC–MALLS

It is clear from the results of the SEC–MALLS experiments (see Table 1 and Fig. 6) that there is a distinct decrease in molecular weight with increasing temperature. This is the contrary to the observations of Morris et al. (1999) in the study of a low methoxy pectin. The molecular weight at 20°C is in good agreement with the molecular weight of 160,000 g/mol obtained previously for this HM

Table 1
Effect of temperature (of measurement) on a high methoxy pectin

| Temperature (°C) | $[\eta]$ (ml/g) | $s^0_{20,w}$ (S) | $M_w$ (g/mol) | $f/f_0$ |
|---|---|---|---|---|
| 20 | 406 ± 2 | 1.83 ± 0.01 | 156,000 ± 10,000 | 8.2 ± 0.4 |
| 30 | 387 ± 4 | 1.81 ± 0.02 | 144,500 ± 10,000 | 7.9 ± 0.3 |
| 40 | 362 ± 4 | 1.79 ± 0.01 | 133,000 ± 10,000 | 7.5 ± 0.3 |
| 50 | 338 ± 3 | 1.77 ± 0.02 | 126,500 ± 10,000 | 7.4 ± 0.5 |
| 60 | 321 ± 8 | 1.76 ± 0.01 | 116,700 ± 10,000 | 6.9 ± 0.4 |

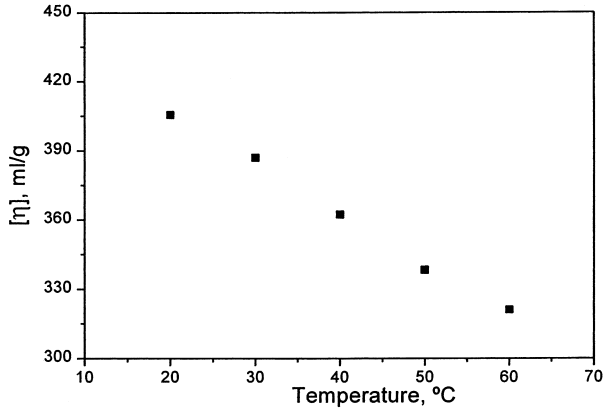


Fig. 2. Effect of increased temperature on the intrinsic viscosity, $[\eta]$ for a high-methoxy pectin in standard phosphate chloride buffer (pH = 6.8, $I = 0.1$ M).

pectin from sedimentation equilibrium (extrapolated to zero concentration to account for thermodynamic non-ideality) in the analytical ultracentrifuge (Morris, unpublished observation).

Knowledge of both molecular weight and sedimentation coefficient allows the calculation of the translational frictional ratio, $f/f_0$ (see Tanford, 1961), where $f_0$ is the frictional coefficient of an anhydrous sphere of the same molecular weight.

$$\frac{f}{f_0} = \frac{M_w(1 - \bar{v}\rho)}{(N_A 6\pi\eta_0 s^0_{20,w})\left(\frac{4\pi N_A}{3\bar{v}M_w}\right)^{-1/3}} \quad (7)$$

The decrease in $f/f_0$ observed with increase in temperature (Table 1 and Fig. 7) indicates a reduction in asymmetry, which is again consistent with a decrease in molecular weight.

The linear relationship appears to exist between $\log M_w$ and $\log[\eta]$ (Fig. 8): the corresponding Mark–Houwink

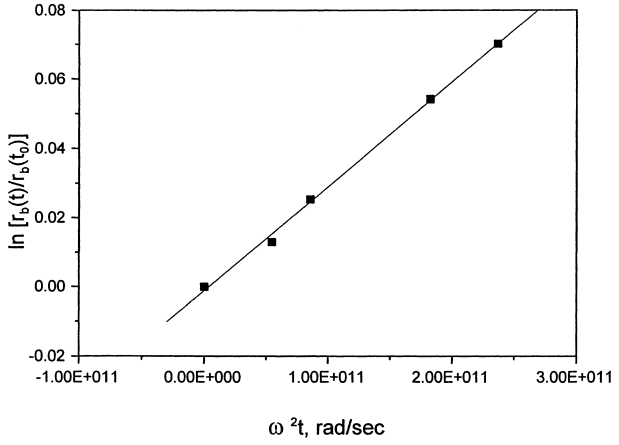


Fig. 3. A plot of $\ln r_b(t)/r_b(t_0)$ vs. $\omega^2 t$ for the high methoxy pectin HR 7021 at 50°C using the Schlieren optical system on a Beckman Model E analytical ultracentrifuge.

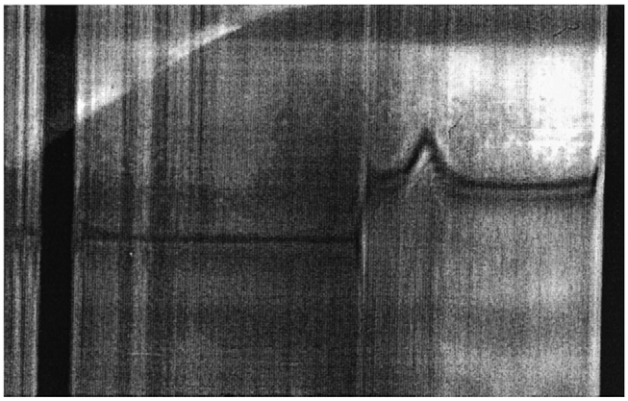

Fig. 4. Representative Schlieren peak for a high methoxy pectin in standard phosphate chloride buffer (pH = 6.8, $I = 0.1$ M), concentration 2.5 mg/ml at 50°C, direction of sedimentation left to right.

(MHKS) viscosity exponent of 0.84 compares with a value of 0.79 obtained by Berth, Anger & Linow (1977). Representative values are ~0 for a compact sphere, 0.5–0.6 for a random coil and up to 1.8 to rigid rods (see, for example, Smidsrod & Andresen, 1979; Jumel, 1994; Tombs & Harding, 1998).

The results obtained clearly indicate that HM pectins, unlike LM pectins (Morris et al., 1999) are strongly susceptible to a loss of structural integrity at elevated temperatures (up to 60°C). This is consistent with the viscometry results obtained in unbuffered saline conditions ($I = 0.10$) by Axelos and Branger (1993). Indeed, HM pectins are susceptible to β-elimination at pH ≥7, even at room temperature, as Pilgrim, Walter and Oakenfull (1991) pointed out earlier. According to those latter workers (Pilgrim et al., 1991), a combination of elevated temperature and long holding times increases the likelihood that aqueous pectin solutions will lose viscosity (molecular weight) through $\beta$-elimination of water. It is also likely that partial de-esterification is at least partly responsible for the loss of molecular integrity. It is,

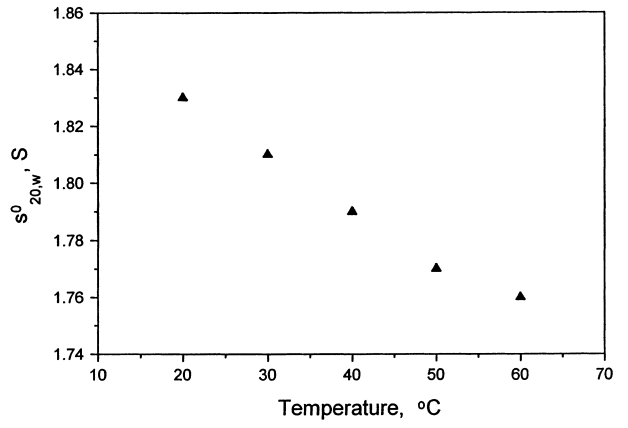


Fig. 5. Effect of increased temperature on sedimentation coefficient, $s^0_{20,w}$ for a high-methoxy pectin in standard phosphate chloride buffer (pH = 6.8, $I = 0.1$ M).

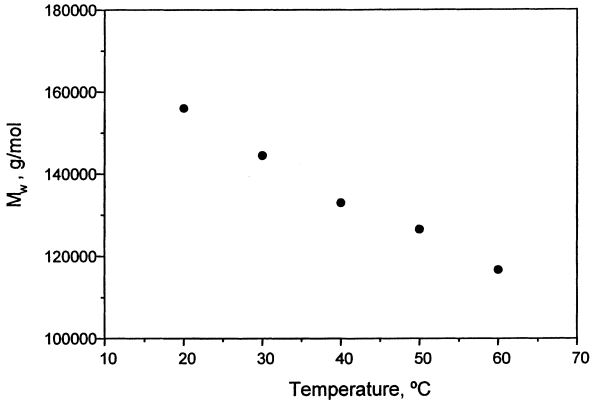


Fig. 6. Effect of increased temperature on the weight average molecular weight, $M_w$ for a high-methoxy pectin in standard phosphate chloride buffer (pH = 6.8, $I = 0.1$ M).

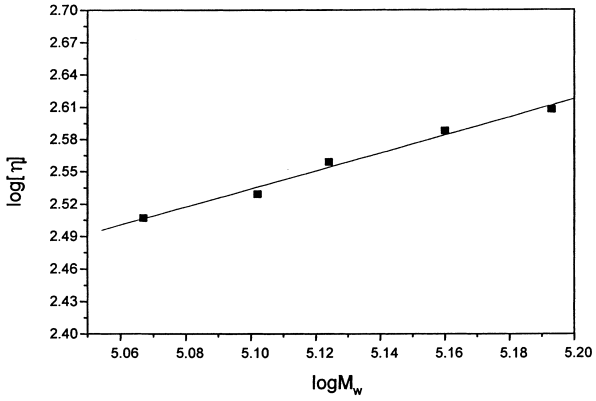


Fig. 8. Mark–Houwink (MHKS) plot for a high-methoxy pectin at different temperatures.

therefore, probable that we are observing (using capillary viscometry, sedimentation velocity and SEC–MALLS) an increase in the rates of these reactions at elevated temperatures.

It is possible to quantify the degree of depolymerisation using the 'blob' model approach of Dondos (2001). This relates the unperturbed chain dimensions to the intrinsic viscosity and Kuhn statistical length as follows

$$a_\eta^3 = C\left(\frac{N}{N_c}\right)^{3v-1} \tag{8}$$

where $v$ is the excluded volume index and is related to the Mark–Houwink–Kuhn–Sakurada viscosity exponent, as follows

$$3v - 1 = a \tag{9}$$

where $N$ is the number of Kuhn statistical segments per chain and $N_c$ is the number of Kuhn statistical segments per 'blob'

$$N = \frac{M}{\lambda^{-1} M_L} \tag{10}$$

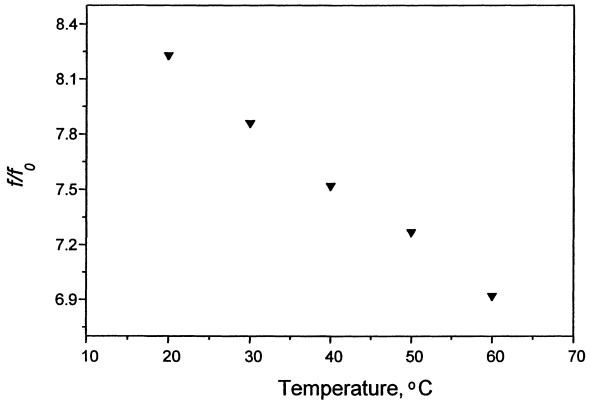


Fig. 7. Effect of increased temperature on the translation frictional ratio, $f/f_0$ for a high-methoxy pectin in standard phosphate chloride buffer (pH = 6.8, $I = 0.1$ M).

$$N_c = 0.3a^{-8} \tag{11}$$

and

$$C = \frac{[4(1-v)(2-v)]}{[(2+v)(1+v)]} \tag{12}$$

Therefore,

$$\frac{[\eta]}{M^{0.5}} = \frac{(\lambda^{-1})^{3v-1}\Phi C}{M_L^{3v} N_c^{3v-1.5}} M^{3v-1.5} \tag{13}$$

and

$$\frac{[\eta]}{M^{0.5}} = K_\theta + 0.51 B \Phi M^{0.5} \tag{14}$$

where $\Phi = 2.6 \times 10^{23}$ and $K_\theta$ in the Stockmayer–Fixman–Burchard equation is equal to

$$K_\theta = \frac{(\lambda^{-1})^{1.5}\Phi}{M_L^{1.5}} \tag{15}$$

Therefore, by plotting graphs of $\eta/M^{0.5}$ vs. $M^{3v-1.5}$ and $\eta/M^{0.5}$ vs. $M^{0.5}$, one can calculate the Kuhn statistical length, the mass per unit length and the persistence length by solving simultaneous equations. Figs. 9 and 10 show the

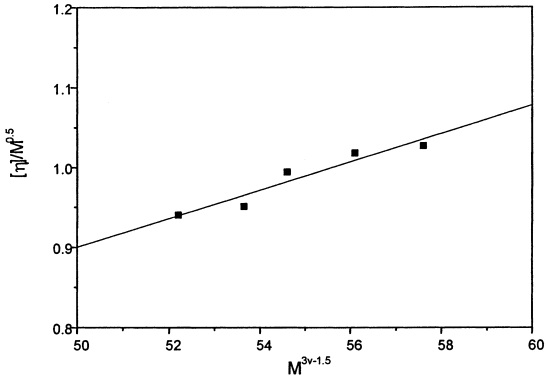


Fig. 9. 'Blob' model plot of $[\eta]/M^{0.5}$ vs. $M^{3v-1.5}$ for a high methoxy pectin sample at elevated temperatures in standard phosphate chloride buffer (pH = 6.8, $I = 0.1$ M).

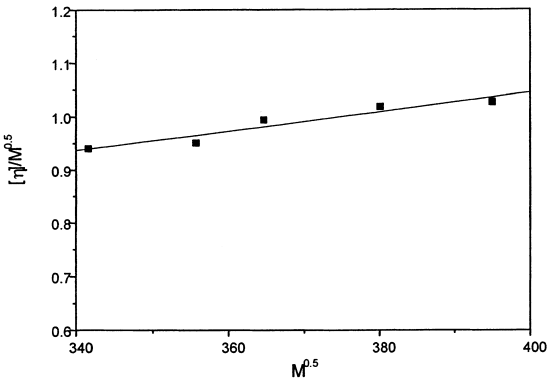


Fig. 10. Stockmayer–Fixman–Burchard plot of $[\eta]/M^{0.5}$ vs. $M^{0.5}$ for a high methoxy pectin sample at elevated temperatures in standard phosphate chloride buffer (pH = 6.8, $I = 0.1$ M).

respective Dondos 'blob' plot and Stockmayer–Fixman–Burchard plots. Although it appears from Table 2 that there is a decrease in segment length with increasing temperature, an average decrease of ten segments per chain is likely to be on or near the limit of sensitivity of this modelling technique.

This decrease in molecular weight (probably due to β-elimination) is clearly important in the processing of products containing HM pectins, as many food and pharmaceutical processes involve at least one heat treatment stage.

## 4. Conclusions

The effect of increased temperature on high methoxy pectin is quite dramatic and significantly different to that on low methoxy pectin (Morris et al., 1999). There is significant decrease in intrinsic viscosity (consistent with observations of Axelos & Branger, 1993), sedimentation coefficient, weight average molecular weight and translational frictional ratio with increasing temperature, which all indicates depolymerisation (Table 1). This depolymerisation is apparently due to β-elimination (Pilgrim et al., 1991; Axelos & Branger, 1993) although demethoxylation and loss of 'hairy' side groups will also result in a in decrease molecular weight. As the $-CH_3$ or ester groups appear to be the major driving force in the β-elimination reaction this would possibly go some way to explaining the differences between high and low methoxy pectins when exposed to high temperature.

Table 2
Results of the 'blob' model approach for calculating the number of Kuhn statistical segments per chain for a high methoxy pectin sample at elevated temperature, from Figs. 9 and 10 above [where $M_L = 2.83 \times 10^9$ g · mol$^{-1}$ · cm$^{-1}$; $\lambda^{-1} = 3.29 \times 10^{-7}$ cm; $L_c = 1.65 \times 10^{-7}$ cm; $v = 0.61$; $C = 0.60$; $N_c = 1.21$ and $K_\theta = 0.33$ (see, for example, Dondos, 2001)]

| Temperature (°C) | $[\eta]$ | $M_w$ | $N$ |
|---|---|---|---|
| 20 | 406 | 156,000 | 170 ± 10 |
| 30 | 387 | 144,500 | 155 ± 10 |
| 40 | 362 | 133,000 | 145 ± 10 |
| 50 | 338 | 126,500 | 135 ± 10 |
| 60 | 321 | 116,700 | 125 ± 10 |

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