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Gel Permeation Chromatography and the Mark–Houwink Relation for Pectins with Different Degrees of Esterification

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SUMMARY

Citrus pectins with degrees of esterification between 30 and 95%, apple pectin and sunflower pectin were fractionated on Sepharose 2B/Sepharose 4B. The intrinsic viscosities and the molecular weights by light scattering of the fractions were determined. It was found that a universal calibration plot of the logarithm of the product of the intrinsic viscosity and the weight average molecular weight vs. the elution volume held for all the samples. The majority of the samples had molecular weights below 100000 and obeyed the Mark–Houwink relation of $[\eta] = 9{\cdot}55 \times 10^{-2} \bar{M}_w^{0{\cdot}73}$ *irrespective of the degree of esterification and the source of the pectin; however, the intrinsic viscosity of high molecular weight fractions* ($\bar{M}_w$ 10^5 *to* 10^7) *did not correlate with molecular weight.*

INTRODUCTION

During biosynthesis pectins in their polymer form are esterified partially or completely with methanol. De-esterification takes place to some extent in plant cell walls by pectin esterases or during isolation by alkaline solvents. Solubility, gel forming capacity and enzymatic degradation depend on the degree of esterification (DE) (Smit & Bryant, 1967; Dongowski *et al.*, 1983), but its influence on the con-

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formation of the pectin macromolecules in salt containing aqueous solutions has not been satisfactorily established. Some contradictory conclusions have been presented. Vollmert (1950) carried out viscosity measurements on pectin solutions at different concentrations of sodium chloride using pectins with DEs between 0 and 100%. He reported that the intrinsic viscosity decreases with increasing DE. Smidsrød & Haug (1971) investigated the effect of the variation of ionic strength on intrinsic viscosity, and they concluded that the stiffness of the polymer chains increases with increasing DE. This result is confirmed by Michel *et al.* (1982) for two pectins with DEs of 60 and 73%. Fishman *et al.* (1984) concluded, from high-performance size exclusion chromatography (HPSEC) measurements on the radius of gyration of pectins, that the degree of aggregation depended on DE.

In addition to the DE the degree of dissociation of pectinic acid could also effect the conformation of the macroion by changing the charge density. Decreasing dissociation of pectic acid as a result of decreasing pH should be comparable to increasing the DE of pectins under conditions which allow their complete dissociation. Cesàro *et al.* (1982) reported that the viscosity of pectic acid increases with increasing dissociation. The effect is more marked with pure water as a solvent than in 0·05 M sodium perchlorate. In interpreting these results Cesàro *et al.* (1982) argued that a more charged and extended chain can be more flexible than a relatively compact sequence of rigid segments.

According to the theoretical considerations of Burton & Brant (1969) the conformation of the pectin molecules in solution is determined primarily by the lack of flexibility of the α-1,4 glycosidic bond between two galacturonic acid residues due to the axial–axial linkage.

In this paper we attempt to obtain information about the dependence of the conformation on the DE by determining the parameters in the Mark–Houwink equation for pectins with different DEs. For this purpose citrus pectins with DEs in the range of 30–95% were used. They were prepared by different procedures and have a statistical distribution of methoxyl groups (Anger & Dongowski, 1984). In addition, commercially produced citrus and apple pectin, and a laboratory-made high DE sunflower pectin, were included in these studies.

The approach used was that previously employed for the study of low methoxyl sunflower pectin (Anger & Berth, 1985). It consists of

the fractionation of the carbohydrate on Sepharose 2B/Sepharose 4B followed by measurements of intrinsic viscosities and molecular weights by light scattering.

EXPERIMENTAL

The pectin samples used are listed in Table 1. Citrus pectin was esterified by treatment with methanol in a sulphuric acid medium (sample 1). The other samples were derived from this material by enzymatic de-esterification with pectin esterase from Pectinol D (samples 2 and 7) and *Aspergillus* sp. (samples 4 and 6), respectively, or by alkaline saponification (sample 5). A detailed description of these materials is given elsewhere (Anger & Dongowski, 1984) together with the evidence for the narrow distribution of the DE and the statistical arrangement of the ester groups. Sample 3 is an exception and was prepared by reaction of diazomethane with pectic acid.

Genu pectin type A was a commercially produced sample used without further purification. Apple pectin (Herbstreith, FRG) was purified prior to gel chromatography by ultracentrifugation (ultracentrifuge VAC 602, MLW, GDR) for 4 h at 40 000 rpm.

TABLE 1
Degree of Esterification (DE) and Content of Anhydrous Galacturonic Acid (AGU content) on a Dry Weight Basis

	Code	*DE* (%)	*AGU content* (%)
1	Citrus pectins prepared as described in the text	95·0	76·8
2		73·7	80·2
3		58·0	~90
4		48·9	66·8
5		48·1	70·2
6		39·2	66·3
7		31·4	75·8
8	Genu type A	62·2	~50[a]
9	Apple pectin	66·4	~62[a]
10	Sunflower pectin	60·0	~90[a]

[a] These samples were not dried and contain about 10% moisture.

Sunflower pectin was extracted from the alcohol insoluble substances of the sunflower head with 2% ammonium oxalate solution at 80°C. After this it was purified by fractionation on DEAE Sephacell in a similar manner to previous investigations on apple pectins (Berth *et al.*, 1977). The degree of esterification of the last three samples was determined titrimetrically.

Pectin samples were dissolved (~ 2 mg ml^{-1}) at room temperature in an aqueous solution of 0·09 M sodium chloride, 0·01 M sodium fluoride and 0·001 M Na_2 EDTA at a pH of 6·5. (The pH was adjusted again to 6·5 if it was necessary.) This solvent was also used as eluent after filtration through membrane filters of pore size 0·45 μm (Sartorius, FRG). Fifteen millilitres of the dust-free pectin solution were fractionated on about 400 ml Sepharose 2B/Sepharose 4B. The polysaccharide concentration within the eluate was continuously monitored using a refractive index detector (Knauer, FRG) which was calibrated both with purified pectins of known composition and with dextran. Recoveries from the column were in the range 90–105%. Volume fractions of about 10 ml each (an accurate elution volume determination was carried out gravimetrically) were used to determine intrinsic viscosities at 25°C using a capillary viscometer from FICA, France, and molecular weights using a light scattering photometer (Sofica, FICA, France). The light scattering photometer was equipped with a helium–neon laser light source of $\lambda = 632$ nm (Zeiss Jena, GDR). Light scattering intensities were recorded at 45, 90 and 135°. Solutions were subjected to repeated filtration through membrane filters (pore size 0·2 μm) prior to measurement. Further details have been given elsewhere (Anger & Berth, 1985).

RESULTS AND DISCUSSION

A representative example of the elution profiles obtained is shown in Fig. 1 for sample 3 (DE 58%). The elution of the polysaccharide starts close to the exclusion volume (125 ml), and the maximum in concentration is reached at an elution volume (V_e) of 300 ml. Plotting the logarithmic product of intrinsic viscosity and molecular weight of the fractions against V_e according to the universal calibration principle developed by Benoit (Grubisic *et al.*, 1967) gives a straight line (Fig. 1) similar to that found for low methoxyl sunflower pectin (Anger &

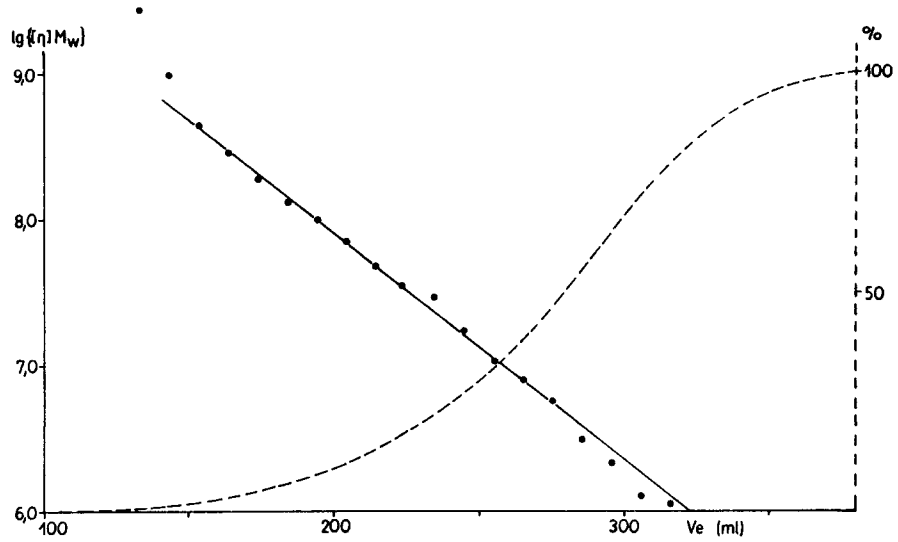

Fig. 1. The elution curve (– – –) and universal calibration plot (——) for citrus pectin with a DE of 58% (GPC on Sepharose 2B/Sepharose 4B).

Berth, 1985). When plotted in this way all the pectin samples listed in Table 1 gave the same relationship. This gives support to the validity of the single measurements of both intrinsic viscosity and molecular weight.

A logarithmic plot of the intrinsic viscosity against the molecular weight of fractions separated from pectin (sample 3) is shown in Fig. 2. This plot is similar to that found for sunflower pectin (Anger & Berth, 1985). The curve is divided into two sections: in the range of molecular weights 10 000–100 000 a linear relationship exists between the logarithm of intrinsic viscosity and the logarithm of molecular weight (the Mark–Houwink relation) as would be expected for a polymer homologous series. Molecular weights in this range correspond to about 82% of the total weight of pectin and this material is believed to be the molecularly dispersed part of the dissolved polymer. Polymer fractions with molecular weights higher than 100 000 are characterized by an almost constant value of the intrinsic viscosity as the molecular weight increases by two orders of magnitude. Only 18% of the polysaccharide is included in this region. Figure 3 displays the Mark–Houwink plots for samples 1–7. The same type of relationship

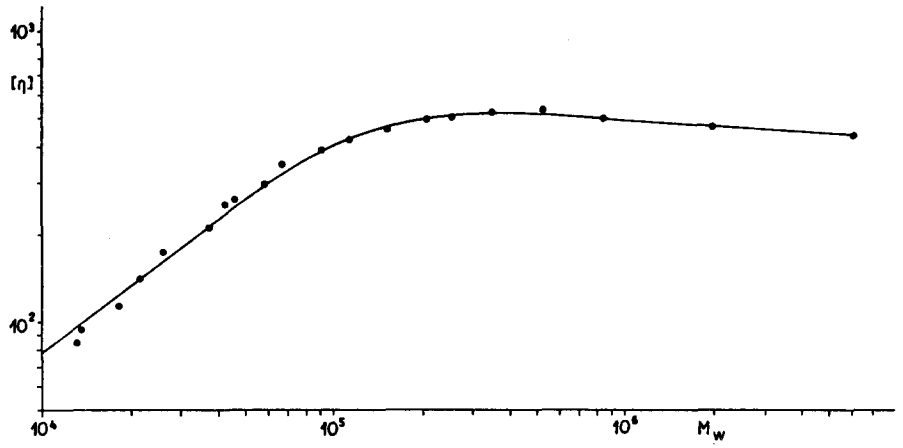

Fig. 2. Relation between intrinsic viscosities $[\eta]$ and molecular weights M_w from GPC fractions of pectin with a DE of 58% (sample 3, Table 1).

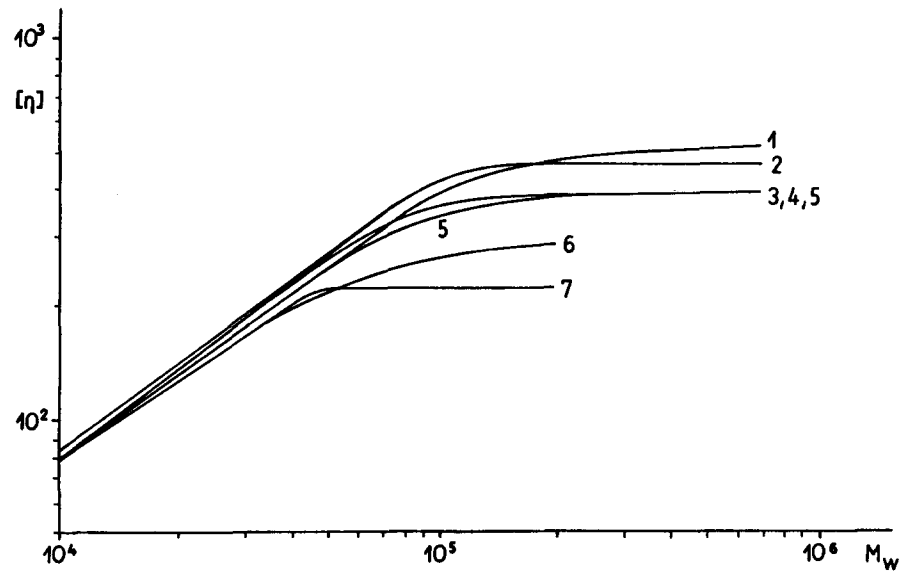

Fig. 3. Relation between intrinsic viscosities $[\eta]$ and molecular weights M_w from GPC fractions of pectins with different DEs (samples 1–7, Table 1).

is found for all the samples, though differences occur in the position of the kinking point. The initial linear part of Fig. 3 is described by the equation:

$$[\eta] = 9{\cdot}55 \times 10^{-2} M_w^{0{\cdot}73}$$

This means that the DE does not influence the intrinsic viscosity in the range between 30 and 95%. This relationship is valid in the case of a statistical distribution of the methoxyl groups along the polymer chain which results from all the methods of de-esterification/esterification used (Anger & Dongowski, 1984). The insertion of methoxyl groups and the resultant modification of charge density does not influence the conformation of the pectin at least in solutions of ionic strength 0·1. This result confirms experimentally the dominant influence of the glycosidic bond on the molecular conformation.

It is important to consider whether pectins from different sources satisfy the same $[\eta]$–M relation. Figure 4 shows the Mark–Houwink

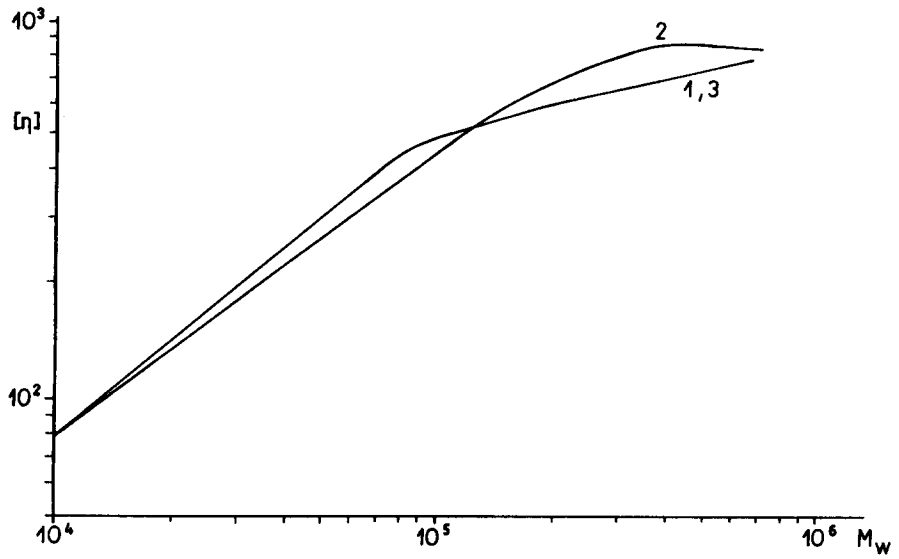

Fig. 4. Relation between intrinsic viscosities $[\eta]$ and molecular weights M_w from GPC fractions of pectins from different sources (samples 8–10, Table 1): 1, sunflower pectin (sample 10); 2, apple pectin (sample 9); 3, citrus pectin (sample 8).

plot for fractions obtained by chromatography of high DE sunflower pectin, apple pectin and commercially produced citrus pectin (Table 1). The good agreement with the plot in Fig. 3 is obvious so it appears that the conclusion presented above for citrus pectins can be extended to pectins from other sources. It should be mentioned that pectic acid (Anger & Berth, unpublished work) or low methoxyl sunflower pectin with a blockwise arrangement of free carboxyl groups (Anger & Berth, 1985) show different $[\eta]$–M relationships. Further work is necessary to explain this.

The independence on DE of the $[\eta]$–M relation has also been reported by Owens *et al.* (1946). The constants in the Mark–Houwink

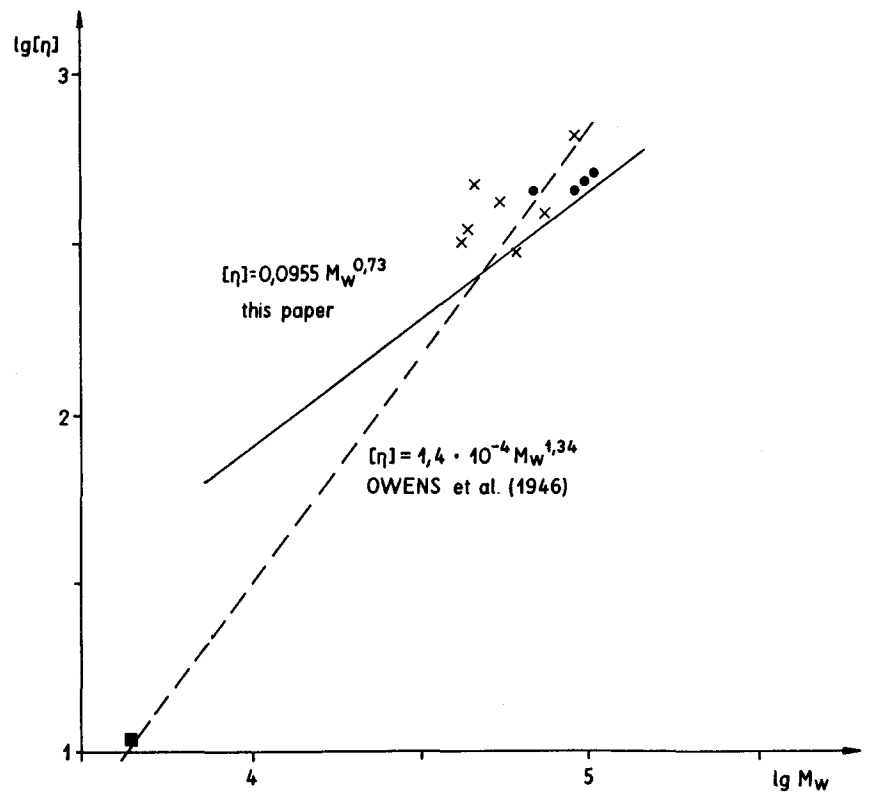

Fig. 5. Comparison of different $[\eta]$–M relationships: ×, Säverborn (1945); ■, Owens *et al.* (1946); ●, Devine (1974).

relationship differ considerably from ours. In Fig. 5 the values used by Owens *et al.* (1946) are reproduced. A problem with this earlier work is that viscosity and sedimentation data taken from Säverborn (1945) (symbol ×) are combined with a result (symbol ■) obtained from 'a sample of methyl glycoside of polygalacturonic acid methyl ester... which has an average molecular weight of 4400 and an intrinsic viscosity of 0·11 dl/g' (Owens *et al.*, 1946) to establish the $[\eta]$–M relation.

This last point disagrees completely with our curve, whereas Säverborn's data are in its neighbourhood. Sedimentation/viscosity measurements of Devine (1974) (symbol ●, Fig. 5) support our relationship. Although other authors have observed a dependence of intrinsic viscosity on DE, it is possible that inappropriate techniques have been used. For example, Vollmert (1950) assumed that the molecular weights of his samples were constant but did not control them during his viscosity measurements at pH 7–8. The approach used in this paper has two important advantages: first, the preparation of fractions with a relatively narrow molecular weight distribution and, secondly, the control of the single measurements (intrinsic viscosity, molecular weight and also pectin concentration) by the principle of universal calibration.

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