Carbohydrate Polymers 5 (1985) 241–250

Gel Permeation Chromatography of Sunflower Pectin

H. Anger and G. Berth

Central Institute of Nutrition, GDR Academy of Sciences,
Potsdam-Rehbrücke, German Democratic Republic

(Received: 17 September 1983)

SUMMARY

Sunflower pectin has been fractionated on Sepharose 2B/Sepharose 4B. Molecular weights were measured within the eluate by light scattering and intrinsic viscosities to establish a universal calibration line, e.g. a plot of the logarithm of the product of the weight-average molecular weight and the intrinsic viscosity against the elution volume. It was found that the universal calibration line of pectin differs, if only modestly, from those of dextran and dextran sulphate. Intrinsic viscosities and molecular weights do not correlate in the region of high molecular weights concerning about 15% of the sample. In most instances with molecular weights below 100 000 a Mark-Houwink relation of $[\eta] = 0{\cdot}0851\bar{M}_w^{0{\cdot}68}$ *is valid.*

INTRODUCTION

Macromolecules are fractionated by gel permeation chromatography (GPC) according to their hydrodynamic volume (Grubisic *et al.*, 1967). Plotting the logarithm of the product of the intrinsic viscosity $[\eta]$ and the molecular weight $\bar{M}_w$ of any polymer against the elution volume V_e results in a calibration line, the so-called universal calibration curve, which is valid for linear and branched molecules independently of their chemical nature and therefore also includes microgels (Hoffmann, 1974). This method was used for the neutral polysaccharides pullulan (Kato *et al.*, 1982) and dextran (Spatorico & Beyer, 1975). Rod-like molecules, such as xanthan, represent an exception to the universal function according to Lambert *et al.* (1982) because their equilibrium distribution is different from that of coils or spheres.

Carbohydrate Polymers 0144-8617/85/$03.30

The question is whether pectin can be included in the universal calibration. Up to now GPC has been used only to describe changes in the relative molecular weight distribution (Barrett & Northcote, 1965; Bock *et al.*, 1977; Barth, 1980; Barbier & Thibault, 1982). The aim of this publication is to check the utility of the universal calibration method for pectins by measurements of $[\eta]$ and $\bar{M}_w$ within the GPC eluate and to determine simultaneously the indices of the Mark–Houwink equation.

The determination of $[\eta]$ is possible without problem, whereas the evaluation of molecular weights gives some difficulties. Thus some authors discovered no (Devine, 1974; Smith & Stainsby, 1977) or only a small, reliable correlation (Kawabata & Sawayama, 1977) between $[\eta]$ and $\bar{M}_w$. The published indices of the Mark–Houwink equation differ considerably from each other (Owens *et al.*, 1946; Glikman & Orlow, 1950; Fritsche *et al.*, 1977). Previous investigations on apple pectins (Berth *et al.*, 1977) have shown that the light scattering method for the determination of the molecular weight is disturbed by a fraction of the pectin which has a high molecular weight and is rich in neutral sugars. The presence of this fraction has only a minor influence on the intrinsic viscosity, but its separation is a precondition for the reproducible establishment of the $[\eta]$–$\bar{M}_w$ relation.

Because of this problem sunflower pectin was used as the raw material for these investigations. Sunflower pectin is a low-esterified pectin (degree of esterification is about 30%) and is simple to isolate at a high purity (Zitko & Bishop, 1965). Galacturonic acid contents near 95% (Painter, 1982) and the absence of starch should favour the accurate determination of the average molecular weight by light scattering and the determination of $[\eta]$, respectively.

The results obtained were evaluated by comparing them with those of other water-soluble polysaccharides. Two dextran samples of different average molecular weights were used as representatives for electrically neutral polymers. Dextran sulphate served as a model for branched polyelectrolytes.

EXPERIMENTAL

Sunflower pectin from the flower head with blockwise arrangement of free carboxyl groups was isolated by Dr Kirtschev, College of Food Industry, Plovdiv, Bulgaria. The sugar composition was analysed after

TABLE 1
Average Molecular Weights $\bar{M}_w$ and Intrinsic Viscosities of the Samples

	$\bar{M}_w \times 10^{-3}$	$[\eta]$ (*ml/g*)
Dextran T 2000	1600	53
Dextran T 500	424	50
Dextran sulphate	625	90
Sunflower pectin	400-1200	142

methanolysis by gas chromatographic separation of the trimethylsilyl derivatives (Stromeyer & Linow, 1978) by Dr M. Petrzika, Central Institute of Nutrition. The degree of esterification was determined titrimetrically (Kertesz, 1951) to be 30%.

Dextran T 2000, dextran T 500 and dextran sulphate were commercially available samples (Pharmacia, Sweden). According to the supplier the sulphate content of the dextran sulphate amounted to 17%. Further details concerning the molecular weight by the light scattering method and the intrinsic viscosity are given in Table 1.

The polysaccharide solutions (about 2 mg/ml) used for the gel chomatographic separation were prepared at room temperature and purified by membrane filtration (pore size 0·45 μm; Sartorius GmbH, FRG). Dust-free aqueous salt solution (0·09 mol/litre sodium chloride, 0·01 mol/litre sodium fluoride and 0·001 mol/litre Na_2EDTA) adjusted to pH 4·0 was utilized as solvent and eluent; 15 ml of the sample solution were used for one run. About 400 ml Sepharose 2B/Sepharose 4B (Pharmacia, Sweden) were eluted at 12 ml/h. After passage through a refractive index detector (Knauer, FRG) for continuous determination of the concentration, the polysaccharide was collected in about 20 fractions of 12 ml each. (The accurate volume was determined gravimetrically.)

The intrinsic viscosities of these fractions were determined using a capillary viscometer from FICA, France, and, after repeated filtration through membrane filters with a pore size of 0·20 μm, the molecular weights were found using a light scattering photometer (Sofica, FICA, France). For both methods the concentration dependence was neglected and no extrapolation to zero concentration was used. This seemed to be

justified since dilution to half of the original polysaccharide concentration (up to 0·3 mg/ml) did not yield significant differences.

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°. After correction for the blanks the asymmetry function Z was calculated as the quotient of the light scattering intensities $R_{45°}$ and $R_{135°}$. Knowledge of Z is necessary to determine the reciprocal value of the scattering function $1/P(90°)$. At the beginning of the polysaccharide elution the Z values were about 1·6–1·8 but decreased continuously and became $Z = 1$ at an elution volume of about 240 ml. $\bar{M}_w$ is calculated from the expression

$$\bar{M}_w = \frac{R_{90°} \cdot 1/P(90°)}{Kc}$$

where $R_{90°}$ means the difference in light scattering intensities between solution and solvent at 90° and c is the polymer concentration in g/ml. K is given by

$$K = \frac{4\pi^2 n_0^2 (\partial n/\partial c)^2}{R_B N_L \lambda_0^4}$$

where λ is the wavelength, n_0 the refractive index of the solvent, $\partial n/\partial c$ the refractive index increment of pectin after equilibrium dialysis [$\partial n/\partial c = 0{\cdot}150$ ml/g] and N_L the Avogadro number. The value $R_B = 12{\cdot}0 \times 10^{-6}$ cm^{-1} is taken from Millaud & Strazielle (1979).

RESULTS AND DISCUSSION

Dextran T 2000, dextran T 500 and dextran sulphate are fractionated on the separation system Sepharose 2B/Sepharose 4B in different ways (Fig. 1). The elution of dextran T 2000 and dextran sulphate starts with the exclusion volume ($V_0 = 125$ ml), whereas dextran T 500 does not appear before $V_e = 160$ ml. This sequence does not correspond with the average molecular weights $\bar{M}_w$ in Table 1. Such behaviour is to be expected because it is not possible to compare directly the elution curves of different polymers. They are only comparable if one takes into account the hydrodynamic volume (Grubisic *et al.*, 1967).

For this purpose the logarithm of the product of the intrinsic viscosity $[\eta]$ and the molecular weight $\bar{M}_w$ is plotted against the elution

volume. Figure 1 shows the results obtained for a neutral (dextran) and a charged (dextran sulphate) polysaccharide. The two dextran samples differ in their average molecular weight. The measured points scatter around a uniform straight line. This demonstrates that in accordance with the hydrodynamic volume a universal calibration is possible for neutral and acidic polysaccharides. Consequently, it can be assumed that it is valid for pectin, too.

The used sample of sunflower pectin which was used consisted of 92% galacturonic acid, 4% rhamnose and 4% other sugars. The elution curve and the $\log([\eta]\,\bar{M}_w)$ values are plotted against the elution volume in Fig. 2. The elution of pectin starts at $V_e = 130$ ml. At the commencement the slope of the curve is very flat. Not more than 20% of the whole amount of pectin is eluted in the first 245 ml. Then the concentration increases rapidly to reach its maximal value at 300 ml. The logarithm of the product of $[\eta]$ and $\bar{M}_w$ depends linearly on the elution volume for large ranges just like that for the different polysaccharides

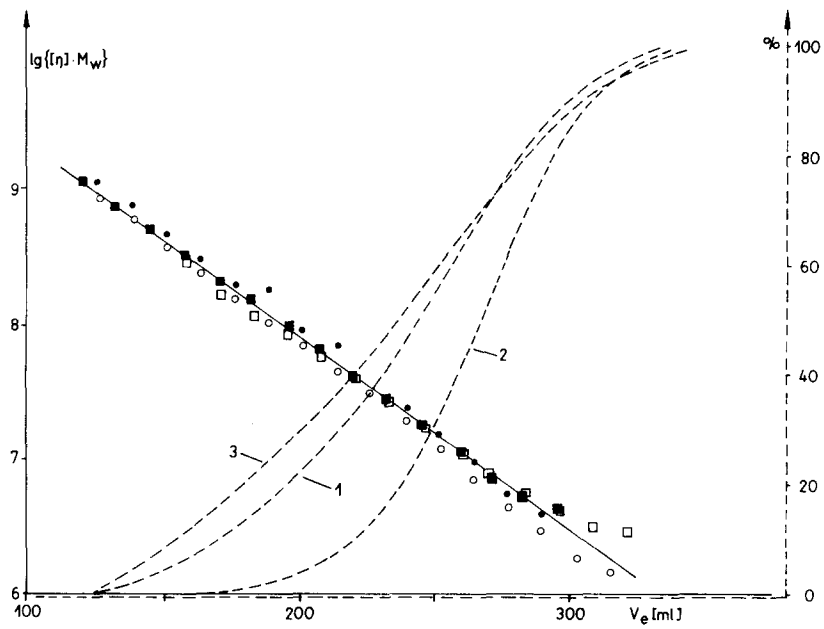

Fig. 1. Integral elution curve and universal calibration line of GPC. Dextran T 2000 (1, ■, ●); dextran T 500 (2, □); dextran sulphate (3, ○). The % scale refers to the integral part of the whole pectin mass.

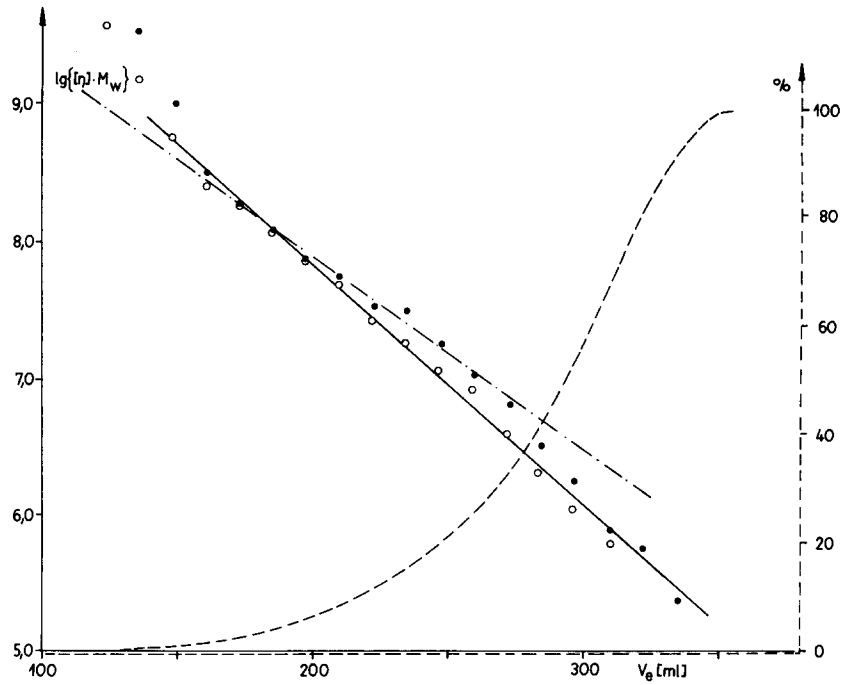

Fig. 2. Integral elution curve and universal calibration line of GPC of sunflower pectin (twofold determination) (——) in comparison with the calibration line from Fig. 1 (– · –). The % scale refers to the integral part of the whole pectin mass.

in Fig. 1. Comparing carefully both of the universal calibration lines it is evident that they do not agree in detail; significant differences occur especially above elution volumes of 270 ml. Nevertheless, the linear connection between the logarithm of the hydrodynamic volume and the elution volume within three orders of magnitude and the similarity to the calibration line of dextran and dextran sulphate, respectively, is interpreted as proving the reliability of the results measured in the pectin fractions.

In order to discuss the individual results it is desirable to study the connection between molecular weight and intrinsic viscosity. Plotting both these values on a logarithmic scale (Fig. 3), a straight line is obtained in the case of a polymer homologous series (Mark–Houwink relation). A linear connection occurs in the molecular weight region

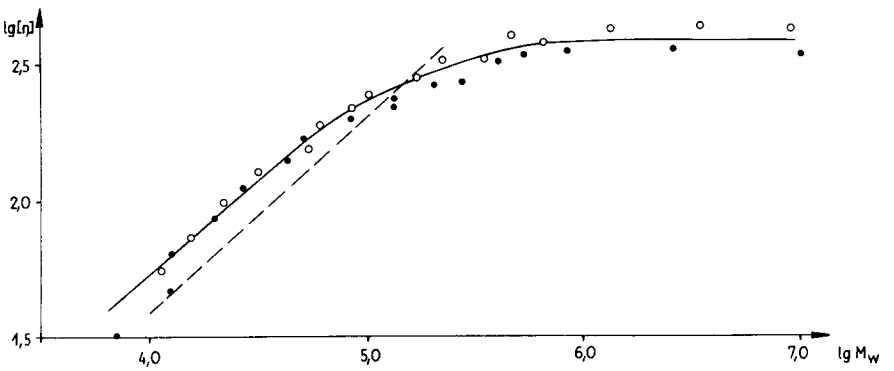

Fig. 3. Correlation between molecular weight $\bar{M}_w$ and intrinsic viscosity $[\eta]$. ——, Sunflower pectin (twofold determination); – – –, apple pectin (Berth *et al.*, 1977).

from 10^4 up to 10^5. This region is represented by the following Mark–Houwink relation

$$[\eta] = 0{\cdot}0851\bar{M}_w^{0{\cdot}68}$$

Above this region the intrinsic viscosity remains almost constant whereas the molecular weight increases by two orders of magnitude. This non-linear $[\eta]$–$\bar{M}_w$ relation, i.e. the great increase of the molecular weights for only slightly changed intrinsic viscosities, indicates the occurrence of particles which are built up in another way. These may be either branched molecules or microgels. High molecular weight, branched compounds have been proved to be present in sycamore pectin by McNeil *et al.* (1980), in apple pectin by De Vries *et al.* (1982) and in cherry pectin by Barbier & Thibault (1982). Microgels as a result of an incomplete dissolution were assumed by Smith & Stainsby (1977), Berth *et al.* (1977) and Jordan & Brant (1978).

Although the $[\eta]$–$\bar{M}_w$ curve is divided into two different regions a straight line can be obtained over the whole range in the case of a universal calibration plot. This result verifies once more the universality of the calibration according to the hydrodynamic volume; the heterogeneity in the shape of the particles is compensated by this plotting technique.

The procedure described above, consisting of the determination of the molecular weight and the intrinsic viscosity after GPC, yields new knowledge on the composition of the sample. First of all, the connection between the elution curve of sunflower pectin and the non-linear course of the $[\eta]$–$\bar{M}_w$ relation is to be examined. If $\bar{M}_w$ attains a value of 10^5, $\log[\eta]$ remains constant at 2·4. This means that the proportionality between $[\eta]$ and $\bar{M}_w$ is valid to $\log([\eta]\,\bar{M}_w) = 7{\cdot}4$. The elution volume which belongs to this value is 245 ml. At this point only 14% of the pectin is eluted. In other words, the linear region in Fig. 3 with molecular weights below 100 000 represents 86% of the sample.

This explains the difficulty in establishing the Mark-Houwink relation from a series of different samples. The intrinsic viscosity depends only weakly on the high molecular weight material whereas $\bar{M}_w$ is essentially influenced by it. Small changes in the high molecular weight part during dissolution of the sample and the subsequent purification of the solution cause enormous shifts in $\bar{M}_w$ but do not disturb the determination of $[\eta]$. This also explains why the treatment of apple pectins by ultracentrifugation or ion exchange chromatography (Berth *et al.*, 1977) reduces the average molecular weights much more than the intrinsic viscosities.

The main part of the sample (86%) consists of molecules with molecular weights below 100 000. Its average molecular weight amounts to 32 000; the concentration maximum is constituted by molecules with a molecular weight of 15 000. For this part representing pectin in the normal sense, the Mark-Houwink relation is valid with an α value of 0·68, thus confirming the relative stiffness of the glucosidic bond between $\alpha(1 \rightarrow 4)$ linked galacturonic acid units.

The average molecular weight which was calculated from the elution curve to be about 600 000 or was found by the light scattering method to be $(0{\cdot}4\text{–}1{\cdot}2) \times 10^6$ characterizes the pectin only imperfectly because it is mainly caused by a small (14%) fraction. The intrinsic viscosity of the unfractionated sample seems to be more suitable to characterize the main part of the pectin. Using the above relation and $[\eta] = 142$ ml/g, $\bar{M}_w = 54\,000$ is obtained instead of $\bar{M}_w = 32\,000$ from GPC fractions with V_e greater than 245 ml.

If the results of sunflower pectin are compared with the former ones on mechanically degraded apple pectin purified by ultracentrifugation or ion exchange chromatography (Fig. 3), one can observe that for identical intrinsic viscosities the molecular weights of apple pectin are

higher than those of sunflower pectin. The factor is 1·4–2. Differences in the source and uniformity or in the degree of esterification can be introduced to explain this phenomenon. Moreover, it cannot be excluded that the former values of the light scattering molecular weights were somewhat too high because of an incomplete separation of material high in molecular weight.

ACKNOWLEDGEMENT

The authors thank Mr D. Lexow and Miss E. Wroz for their careful technical assistance.

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