Carbohydrate Polymers **6** (1986) 361–378

Calibration and Application of High-Performance Size Exclusion Columns for Molecular Weight Distribution of Pectins

H. A. Deckers,* C. Olieman

Netherlands Institute for Dairy Research, Kernhemseweg 2, 6718 ZB Ede, The Netherlands

F. M. Rombouts and W. Pilnik

Agricultural University, Department of Food Science, De Dreijen 12, 6703 BC Wageningen, The Netherlands

(Received: 4 September 1985)

SUMMARY

The calibration of TSK-PW columns for pectins is described. Use was made of the monodisperse standard pullulan and polydisperse pectin samples of different molecular weight, obtained after mechanical degradation. Using an iterative calculation procedure, different calibration curves were obtained for high- and low-DE pectin. The following Mark–Houwink constants were obtained for high-DE pectin in the eluent: $a=0{\cdot}79$ and $K=0{\cdot}49\times10^{-3}$. For low-DE pectin the Mark–Houwink constants were dependent on the molecular weight.

Experiments are described showing the influence of the molecular weight distribution and average molecular weight on the stabilising effect of pectin in drinking yogurt. Quantitative analysis of pectin in the serum of drinking yogurt is described. The thermal degradation of pectin in various solutions was analysed.

*Present address: Bergschot Centre for Research (BCO), Bergschot 69, PO 2176, 4800 CD Breda, The Netherlands.

Carbohydrate Polymers 0144-8617/86/$03.50 —

INTRODUCTION

The physical and chemical parameters of pectin determine its use in food processing, for instance its ability to stabilise drinking yogurt. The chemical structure of pectin has been studied by several groups; for a recent review see the thesis by De Vries (1983). Pectins with the same chemical parameters might stabilise foods differently. These differences can be quantified by measuring rheological parameters, such as viscosity. These parameters are related to the chemical nature of the thickening agent and its molecular weight distribution (MWD). It follows that the MWD of a thickening agent can be an important factor in understanding its functional behaviour in foods (Beltman, 1975; Mitchell, 1976; Michel, 1982; Watase & Nishinari, 1982).

The MWD of biopolymers, such as pectin, can be investigated using the following techniques, e.g. endgroup analysis, osmometry, ultracentrifugation, light scattering, viscometry and size exclusion chromatography (SEC). Each of these techniques, with the exception of SEC, gives only one type of molecular weight average. Three different averages are not sufficient to determine unambiguously the MWD (Yau *et al.*, 1979). Size exclusion chromatography generates a complete MWD, assuming that the method is properly calibrated. From this MWD the different averages can be calculated. The determination of individual molecular weight averages for pectin has received limited attention in the literature (Pals *et al.*, 1952*a*, *b*; Albersheim *et al.*, 1960; Barrett & Northcote, 1965; Sorochan *et al.*, 1971). Gel filtration of pectins was studied by Davis *et al.* (1980), and high-performance size exclusion chromatography (HPSEC) experiments were performed by Strubert & Hovermann (1978), Barth (1980) and Fishman *et al.* (1984). These investigators used HPSEC in a more or less qualitative manner, to compare the elution patterns or related data from different pectin samples. Recently, Anger *et al.* (1985) reported SEC of sunflower pectin on a soft gel column, in combination with viscosity and light scattering measurements.

To study the relation between the MWD of pectin and the stabilisation of drinking yogurt or other foods, it is necessary to be able to compare reliable molecular weight data obtained over a long period and in different laboratories, using perhaps different types of size exclusion columns. Therefore, it was necessary to develop a method to calibrate high-performance size exclusion columns for pectins.

METHODS

Pectin preparations

Brown (DE 73%) and violet (DE 40%) ribbon apple pectin (ObiPektin AG, Bischofszell, Switzerland) were used in the protonated form, obtained by a treatment with a mixture of isopropanol/hydrochloric acid (35%)/water (7:2:1). The product was washed with a mixture of isopropanol/water (70:30) until the filtrate was chloride free. After subsequent washing with isopropanol/water (96:4) the residue was air dried and stored at 4°C.

Pectin was mechanically degraded with a ball mill (Swingmühle Vibraton type SMO.6, Siebtechnik GMBH, Mühlheim, FRG) as described by Dongowski (1970) for 8, 20, 34 and 48 h.

The anhydro-uronic acid (AUW, MW 176) content of hydrolysed pectin was determined by an automated method using metahydroxydiphenyl as described by Ahmed & Labavitch (1977).

Free neutral sugars were isolated with a DEAE-Sepharose CL-6B column (10 × 0·7 cm i.d.), eluted with a sodium acetate buffer (0·05 M, pH = 4·8 (Michel, 1982)). Twenty fractions (2 ml) were collected and analysed for neutral sugars as described by Dubois *et al.* (1956).

Viscometry

Pectin (0·2%, m/m) was dissolved in the eluent and treated with Polyclar AT (General Aniline Corp., New York, USA) to remove polyphenolic residues. The solution was centrifuged at 6000*g* for 10 min and/or filtered through a Jena G3 glass filter. The viscosity of the polymer solutions was measured with an Ubbelohde type capillary viscometer at 30°C. The intrinsic viscosity ($[\eta]$) was calculated on the basis of the ash-free dry uronic acid content.

Chemicals

The calibration of the HPSEC columns was carried out using monodisperse pullulan (Polymer Laboratories Ltd, Shropshire, UK), monodisperse polyethyleneoxide (Toya Soda Ltd, Tokyo, Japan) and polydisperse dextran (Pharmacia Fine Chemicals AB, Uppsala, Sweden). Relevant data, supplied by the manufacturer, are given in

TABLE 1
Specifications of Standards Used for HPSEC

Standard	$M_w \times 10^{-3}$	M_w/M_n	$[\eta]$ *(dl g^{-1})*	*Mark–Houwink parameters*	
				K×10^3	*a*
Pullulan				0·224	0·663[a]
PL85	853[a]	1·14[a]	—		
PL38	380[a]	1·12[a]	—		
PL19	186[a]	1·13[a]	—		
PL10	100[a]	1·10[a]	—		
PL5	48[a]	1·07[a]	—		
PL2	23·7[a]	1·06[a]	—		
PL1	12·2[a]	1·06[a]	—		
PL0·6	5·8[a]	1·07[a]	—		
Polyethylene oxide				0·588	0·65[c]
SE 150	1290[b]	1·12[b]	—		
SE 70	723[b]	1·10[b]	—		
SE 30	310[b]	1·05[b]	—		
SE 15	160[b]	1·04[b]	—		
SE 8	79[b]	1·02[b]	—		
SE 2	40[b]	1·03[b]	—		
SE 2	24[b]	1·14[b]	—		
Dextran				1·36	0·47[c]
T2000	2000[d]		0·70[e]		
T500	500[d]	2·77	0·54[e]		
T70	73·1[d]	1·61	0·28[e]		
T40	43·9[d]	1·68	0·22[e]		
T10	10·5[d]	2·06	0·10[e]		

[a] Obtained in water at 25°C.
[b] Obtained with SEC in water at 25°C.
[c] Obtained in 0·1 M sodium chloride at 25°C (Kato *et al.*, 1983) and using M_w instead of M_v.
[d] Obtained by light scattering in water.
[e] Obtained in water at 20°C.

Table 1. Sodium acetate, sodium sulphate and acetic acid were of reagent grade quality. Water for eluents and solutions was double distilled.

HPSEC sample preparation

The polymer was weighed into a glass vial and eluent was added to obtain a final concentration of 0·5–1·0 mg ml^{-1}. After swelling the solution was gently shaken for 0·5–6 h, dependent on the rate of solubilisation. If necessary the solutions were centrifuged at 10000*g* for 5 min to remove undissolved material.

Apparatus for HPSEC

A Waters Model 6000 A pump with a Wisp 710 B automatic sample injector was used in combination with an Erma Optical Works Ltd Model ERC 7510 refractive index detector and a Kipp Analytica 9222 column oven. The oven was modified with an improved temperature regulation unit and a cooling device, connected with a cryostat. These modifications resulted in a long-term stability of 0·1°C and a short-term stability of 0·01°C. The columns were lagged with aluminium foil. Chromatograms were recorded and sliced with a Perkin-Elmer Sigma 15B data system. Data reduction was carried out with the programs GPC3C and POLYDISP, both from Perkin-Elmer. Both programs were improved; the POLYDISP program is suited for calibration with a polydisperse standard. Polynomial calibration curves were calculated with a Hewlett-Packard 9845B desk-top computer using a least squares program. The analyses were performed on Biogel TSK columns (30 × 0·7 cm i.d., Bio-Rad Labs, Richmond, California, USA) type 6000, 5000, 4000 and 3000 PW. The columns were connected with decreasing pore size in the direction of the flow. Two types of precolumn were used: 2 × 0·3 cm i.d., packed with I-125 (Waters Assoc. Inc., Milford, Massachusetts, USA), and a Biogel TSK-PW Guardcolumn (7·5 × 0·75 cm i.d., Bio-Rad Labs).

Chromatographic conditions

One litre of the eluent contained 0·44 mol acetic acid, 0·06 mol sodium acetate, 0·1 mol sodium sulphate and 1 ml propionic acid (to

prevent growth of moulds and yeasts), pH = 3·70. The flow rate was 0.6 ml min^{-1}, the columns were maintained at 30°C (secondary cooling of the oven was 10°C) and the refractive index detector was set at 40°C. Unless otherwise stated, the injection volume was 70 μl of a 0·5–1·0 mg ml^{-1} sample concentration using the low syringe speed of the automatic injector. The data system was programmed to collect 200 data slices, which were stored on cassette tape for subsequent analysis. Chromatograms were recorded with a refractive index of $\times 2$.

Preparation and analysis of drinking yogurt

Drinking yogurts were prepared according to standard methods with varying amounts of citrus pectin (DE 68%) as stabilising agent (Hooydonk *et al.*, 1982). Corrections were made for the presence of ash, moisture and added sugars in the pectins used.

Citrus pectin (DE 68%) was depolymerised by the enzyme polygalacturonase (Hooydonk *et al.*, 1982) in order to investigate the stabilisation of drinking yogurt as a function of the molecular weight.

Drinking yogurts were diluted with the eluent (drinking yogurt: buffer = 1:3 (v/v)) and centrifuged at 10000*g* for 15 min. Calibration samples of pectin were prepared by dissolving pectin (0–3 mg ml^{-1}) in the acetate buffer, diluted with yogurt serum (3:1 (v/v)), followed by centrifugation at 10000*g* for 15 min. Of the supernatants 100 μl aliquots were injected. These conditions eliminate high *g* and long times of centrifugation. Furthermore, the ionic strength (I) and pH were equal for the standard and sample solutions.

RESULTS AND DISCUSSION

Buffer choice

The polyacid nature of pectin requires that electrostatic interaction with the column packing is reduced and that expansion of the pectin molecule is suppressed. Therefore, the use of a buffer with a low pH (pH < p*K* of galacturonic acid) and a high salt concentration is necessary. The acetate buffer (pH = 3·70, I = 1·44 M) used by Barth (1980) caused baseline instabilities with our instrument. Decreasing the ionic

strength to 0·34 M (pH = 3·70) eliminated the baseline fluctuations. According to the Debye–Hückel theory the wall potential is reduced to 60% at about 0·55 nm distance. The average distance between the galacturonic acid residues is of the order of 0·5 nm, which implies that this buffer does not completely suppress electrostatic repulsion. However, the low pH of the eluent minimises the number of ionised carboxylic acid residues. Furthermore, β-elimination is prevented and pectin samples were found stable for several days at room temperature with respect to their MWD and viscosity.

The Mark–Houwink constants K and a for pullulan were obtained by their manufacturer in water at 25°C, from which the intrinsic viscosity could be calculated using the Mark–Houwink equation:

$$[\eta] = KM^a$$

Influence of injection volume and injected amount

The results obtained with pullulan ($M_w = 10^5$ and 8×10^5) are summarised in Table 2. Injection volumes in the range of 10–200 μl have no influence on the polydispersity. The injected amount of material

TABLE 2
Influence of the Injection Volume and the Injected Amount on Polydispersity of Pullulan

Injection volume[a] (μl)	$\Delta M_w/M_n$ (%)	*Injected amount*[b] (μg)	$\Delta M_w/M_n$[c] (%)
10	0·1	21	—
20	—	35	1·1
35	0·2	70	1·8
70	0·2	105	6·4
100	0·2	140	8·6
140	0·5	210	7·8
200	0·5		

The analysis which gave the smallest value for the polydispersity was taken as the reference and is indicated with a dash.
[a] 100 μg of pullulan ($M = 10^5$) were injected.
[b] 70 μl of pullulan solution ($M = 8{\cdot}5 \times 10^5$) were injected.
[c] $\Delta M_w/M_n(\%) = ((M_w/M_n - (M_w/M_n)_{ref})/(M_w/M_n)_{ref}) \times 100$.

should, however, not exceed 70 μl ($M_w/M_n < 2\%$), which depends of course on the viscosity of the solution injected. This experiment was carried out with a high molecular weight pullulan; for lower molecular weight and/or polydisperse samples the situation is more favourable.

Band broadening

The calculated correction factors (Yau *et al.*, 1979) for symmetrical and asymmetrical band broadening are shown in Fig. 1 as a function of log M_w. This figure shows that corrections for band broadening are not necessary. The expected deviations in M_w and M_n, caused by Gaussian dispersion, are given in Table 3. No corrections are necessary, because all values are smaller than 2·9%.

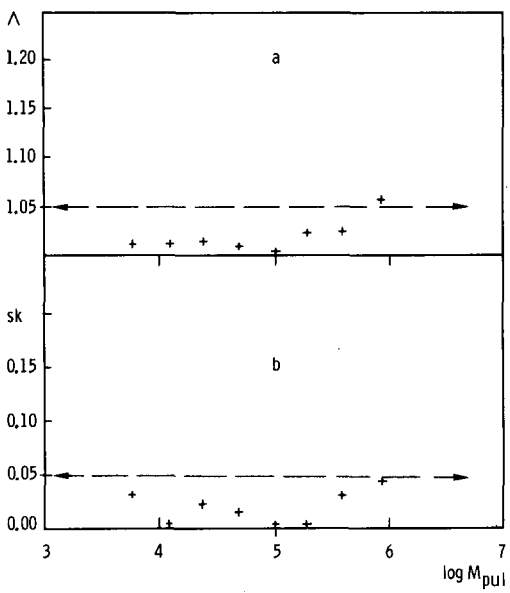

Fig. 1. Molecular weight correction factors for symmetrical (a) (Λ) and asymmetrical (b) (sk) band broadening as a function of the molecular weight of pullulan. The data were obtained with a TSK PW 6000, 5000, 4000 and 3000 column set and 70 μl injections containing 50 μg of pullulan. The dashed line gives the level above which corrections are necessary.

TABLE 3
Corrections for the Weight and Number-Average Molecular Weight Based on a Gaussian Dispersion

Pullulan $M \times 10^{-3}$	*Correction factor* (%)
853	2·9
380	1·7
186	1·3
100	1·0
48	0·8
23·7	1·6
12·2	1·9
5·8	1·7

Calibration of columns

The polymer molecules are separated by SEC on the basis of the size distribution of the internal pores of the packing material and the hydrodynamic volume of the dissolved polymer. Polymers with the same molecular weight can have different hydrodynamic volumes due to different conformations. Consequently, the elution volumes of those polymers are different. Therefore, in general it is not possible to calibrate SEC columns using monodisperse standards which are different from the polymers to be analysed. Figure 2 shows the relation between elution volume and log molecular weight obtained with the monodisperse standards pullulan and polyethyleneoxide, using peak maximums and the polydisperse standard dextran, using the POLYDISP program. The results obtained are similar to those of Kato *et al.* (1983).

The ideal calibration should be performed with well-characterised monodisperse standards of the same polymer to be analysed. This requires, however, a considerable investment of time and labour and is therefore, in most cases, not practical. A useful calibration can be obtained by plotting the logarithm of the product of the molecular weight and the intrinsic viscosity as a function of the elution volume.

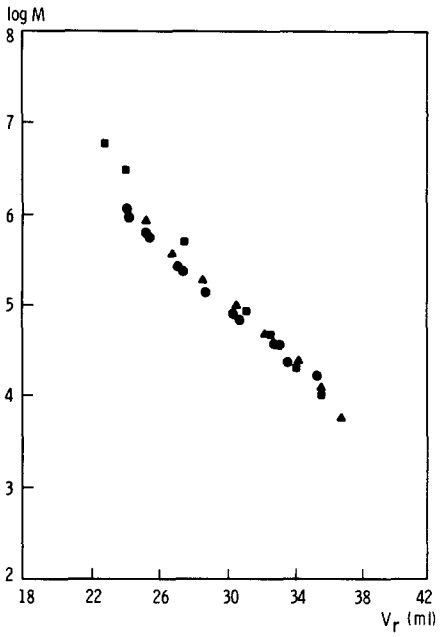

Fig. 2. Calibration of TSK 6000, 5000, 4000 and 3000 column set in combination with a precolumn packed with I-125: ▲, pullulan; ●, polyethyleneoxide; ■, dextran, obtained with the POLYDISP program.

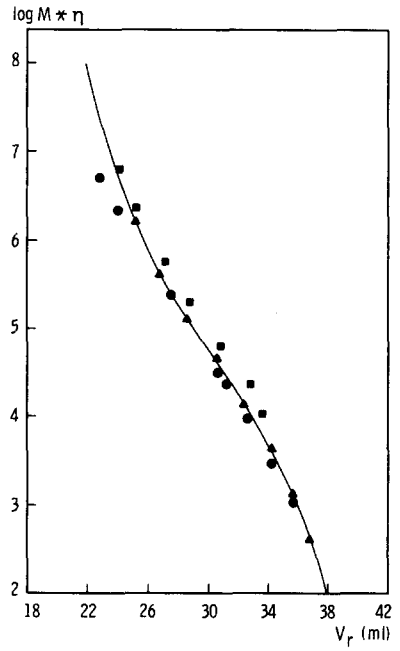

Fig. 3. Universal calibration of the TSK PW column set in combination with a precolumn packed with I-125. Key: ▲, pullulan; ■, polyethyleneoxide; ●, dextran, obtained after combination of the results of each sample with the POLYDISP program. The data points of pullulan are fitted with a least squares polynomial of the third degree (solid line).

This is termed the universal calibration relationship (Fig. 3). The deviation of the results for polyethyleneoxide from the results for the other two polymers is caused by adsorption on the precolumn packed with I-125. Adsorption did not occur on the TSK precolumn.

The data points for pullulan can be fitted satisfactorily by a third-order polynomial using least squares regression analysis (Fig. 3). The deviation of dextran from the polynomial relationship is most probably caused by the linear relationship between log M_w and the elution volume, which is used by the POLYDISP program, or by the inherent

branching of dextran. For the TSK-PW column set in combination with the precolumn packed with I-125, the following polynomial was found:

$$\log M = 44{\cdot}8731 - 3{\cdot}6339\, v_r + 0{\cdot}11446375\, v_r^2 - 0{\cdot}001252086\, v_r^3 \quad (r^2 = 0{\cdot}99978)$$

$$\log M[\eta] = 70{\cdot}31818 - 5{\cdot}98181\, v_r + 0{\cdot}18843016\, v_r^2 - 0{\cdot}002062274\, v_r^3 \quad (r^2 = 0{\cdot}9996), \text{(Fig. 3)}$$

in combination with the TSK precolumn:

$$\log M = 39{\cdot}8333 - 2{\cdot}9802\, v_r + 0{\cdot}08872238\, v_r^2 - 0{\cdot}000925871\, v_r^3 \quad (r^2 = 0{\cdot}99990)$$

$$\log M[\eta] = 59{\cdot}84504 - 4{\cdot}71354\, v_r + 0{\cdot}14033429\, v_r^2 - 0{\cdot}001468836\, v_r^3 \quad (r^2 = 0{\cdot}9997)$$

where v_r is the elution volume in millilitres.

In order to obtain a more adequate calibration for pectin, we have used a method which was applied to the SEC of polylactic acid by van Dijk & Smit (1983). This iterative procedure is based on polydisperse samples with different unknown molecular weights and known intrinsic viscosities ($[\eta]$) in combination with monodisperse standards with known molecular weights and intrinsic viscosities. Therefore, we needed pectin samples with different molecular weights, preferably from a common origin. Mechanical degradation with a ball mill should produce pectin samples with a lower molecular weight. The splitting of molecular bonds is expected to occur at random and to a greater extent if the milling time increases, without affecting the degree of esterification. The intrinsic viscosity and other relevant data of high- and low-DE pectin samples thus obtained are given in Table 4.

The calibration procedure was started with a universal calibration graph, a polynomial of the third degree, obtained with pullulans and their intrinsic viscosities calculated via the Mark–Houwink equation. After measurement of the ball mill degraded pectin samples (Fig. 4), the iterative procedure for the calculation of the Mark–Houwink constants was carried out, according to van Dijk & Smit (1983). The results given in Table 5 show that the Mark–Houwink constants are dependent on the degree of esterification. It was necessary to calculate

TABLE 4
Analytical Data of Pectins Degraded with a Ball Mill

Sample code	*Ash-free dry matter (%)*	*Esterification degree (%)*	*$[\eta]^a$ ($dl\ g^{-1}$)*	*AUA^a (%)*
H0	82·9	72·5	2·37	76·9
H8	80·3	71·7	2·37	76·9
H20	81·4	72·9	1·32	76·2
H34	80·3	72·4	0·83	75·2
H48	76·4	69·6	0·74	74·4
L0	80·8	39·7	3·56	82·9
L8	79·8	39·6	1·53	83·3
L20	77·8	39·9	0·64	81·6
L34	76·9	39·6	0·52	83·8
L48	73·3	36·8	0·38	84·2

[a] Based on ash-free dry matter.

TABLE 5
Mark–Houwink Constants K and a Obtained by the Iterative Calculation for High- and Low-DE Pectin

Iteration no.	*High-DE pectin (H0–H48)[a]*		*Low-DE pectin*					
			(L0–L48)[a]		*(L8–L48)*		*(L0–L8)*	
	a	*$K\times10^3$*	*a*	*$K\times10^3$*	*a*	*$K\times10^3$*	*a*	*$K\times10^3$*
0[b]	0·459	25	0·459	25				
1	0·655	2·61	0·639	2·474				
2	0·758	0·781	0·696	1·076				
3	0·765	0·670	0·728	0·689				
4	0·773	0·591			0·790	0·285	0·588	3·08
5	0·788	0·500			0·845	0·175	0·588	3·39
6	0·788	0·492			0·853	0·166	0·587	3·43

[a] Number indicates milling time (h).
[b] Starting value for the iteration.

separate Mark–Houwink constants for the high and low molecular weight fraction of the low-DE pectin. Possibly, this is caused by a different degree of branching in the higher molecular weight fraction in the material. Similar results were obtained for sunflower pectin, which is a low-DE pectin (Anger & Berth, 1985).

The calibration graph for pectin (Fig. 5) shows that pectin molecules are expanded as compared to pullulan molecules. This might be caused by electrostatic repulsion, which the mobile phase insufficiently suppresses, but the conformation of pectin could also account for this phenomenon. Figure 5 demonstrates also that low-DE pectin has a smaller hydrodynamic volume than high-DE pectin. These results are in agreement with those of Fishman *et al.* (1984) who found that the radius of gyration was a function of the degree of esterification. The following polynomials were obtained for high- and low-DE pectins:

$$\log M = 36{\cdot}8643 - 2{\cdot}77237\, v_r + 0{\cdot}08253451\, v_r^2 - 0{\cdot}000861297\, v_r^3$$

(high-DE pectin)

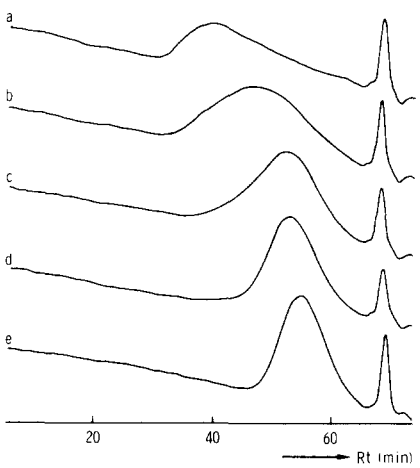

Fig. 4. Chromatograms of high-esterified pectins, which were milled in a ball mill during 0, 8, 20, 34 and 48 h (chromatograms a, b, c, d and e respectively). For experimental conditions see 'Methods'.

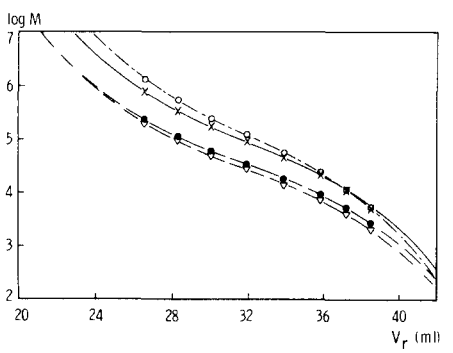

Fig. 5. Calibration curves of a TSK PW column set in combination with a TSK precolumn for pullulan (×), dextran (○), low-DE pectin (high molecular weight part) (●), and high-DE pectin (▽).

$$\log M = 40{\cdot}9711 - 3{\cdot}12097\, v_r + 0{\cdot}09291253\, v_r^2 - 0{\cdot}000969598\, v_r^3$$

(low-DE pectin, $M > 10^4$)

$$\log M = 35{\cdot}8188 - 2{\cdot}67464\, v_r + 0{\cdot}07962511\, v_r^2 - 0{\cdot}000830936\, v_r^3$$

(low-DE pectin, $M < 10^4$)

Analysis of high-DE pectin in drinking yogurt

The influence of the MWD on the stabilising power of pectin in drinking yogurt was investigated by depolymerising a citrus pectin with the enzyme polygalacturonase. The pectin preparations thus obtained were used to prepare drinking yogurts. Table 6 shows that the stability of drinking yogurt decreases with decreasing average molecular weight (Hooydonk *et al.*, 1982).

The quantity of pectin in the serum of drinking yogurt was obtained from the total slice area above the baseline for pectin (Fig. 6). A base-point at 49 min was used, which compensated effectively for the area above the baseline found in yogurt without pectin. A linear relationship was obtained between the concentration of added pectin in the serum in the range 0–3 mg ml^{-1} and the peak area (Fig. 7). This is given by the relationship:

$$\text{area} = 1{\cdot}551 \times \text{amount (mg ml}^{-1}) + 0{\cdot}0154 \qquad (r^2 = 0{\cdot}994)$$

Using this procedure it was demonstrated that for pectin-stabilised drinking yogurt more than 75% of the pectin is in the serum phase of drinking yogurt.

Thermal stability of pectin solutions

In practice, prior to the addition of pectin to yogurt, a concentrated suspension of pectin (6%, m/m) and sugar (36%, m/m) is first heated at 90°C to accelerate solubilisation and then stored for a period. During this treatment degradation of pectin might occur. In a laboratory experiment a pectin suspension of the above described composition was kept at 90°C. Solutions of pectin (DE 68%, 5 mg ml^{-1}) in the eluent buffer and in succinate buffer (0·2 mol $litre^{-1}$, pH 5·51) were also kept at 90°C. Especially at higher pH values β-elimination occurs (Pilnik & Voragen, 1974). The viscosity molecular weight average, determined with SEC, decreases with increasing heating times (Fig. 8).

TABLE 6
Serum Separation in Drinking Yogurt, Prepared with Enzymatically Degraded Pectins

Pectin code	*Esterification (%)*	*Viscosity reduction (%)*	*$10^3 M_w$*	*$10^3 M_n$*	*$10^3 M_z$*	*$10^3 M_v$*	*M_w/M_n*	*$[\eta]$ (dl g^{-1})*	*Serum separation*	
									4 weeks (20°C)	*10 weeks (20°C)*
a	68	0	178	41	542	154	4·38	6·04	6	10
b	69	32	143	35	422	124	4·02	5·11	9	15
c	68	43	128	34	390	111	3·81	4·70	10	25

[a] 0·5% Pectin in 0·1 M tris-succinate (pH = 6·0), measured with an Ubbelohde viscometer.
[b] Measured in a cylindrical bottle (11 × 6·5 cm i.d.).

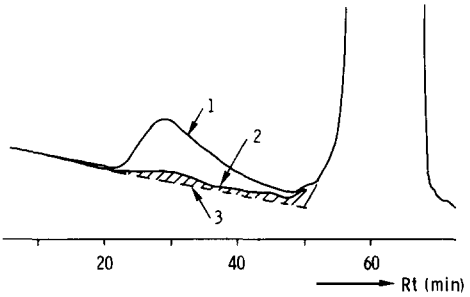

Fig. 6. Analysis of pectin in serum of drinking yogurt. Before injection, serum was diluted with eluent buffer (1:3, v/v); 1, chromatogram of pectin containing yogurt serum; 2, chromatogram of yogurt serum without pectin — the elution pattern indicates possibly the presence of exopolysaccharides secreted by yogurt bacteria; 3, extrapolated baseline.

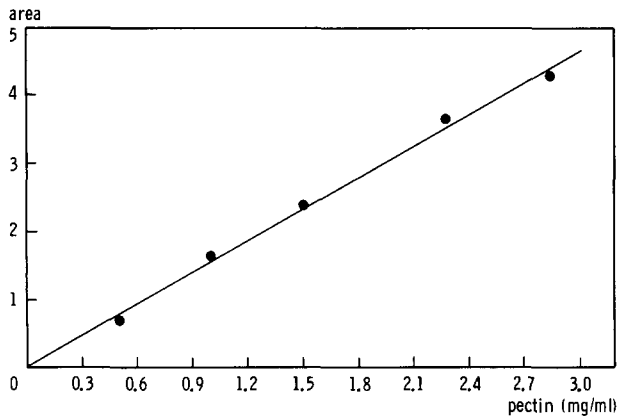

Fig. 7. Calibration graph for pectin in serum of drinking yogurt.

The stability of pectin in the succinate buffer is considerably less than in the other two solvents. The results of this experiment demonstrate that pectin solutions should be stored for as short a time as possible at elevated temperatures.

CONCLUSIONS

The results of this study make it possible to determine the MWD of pectins and its averages, independent of the columns used. Hence

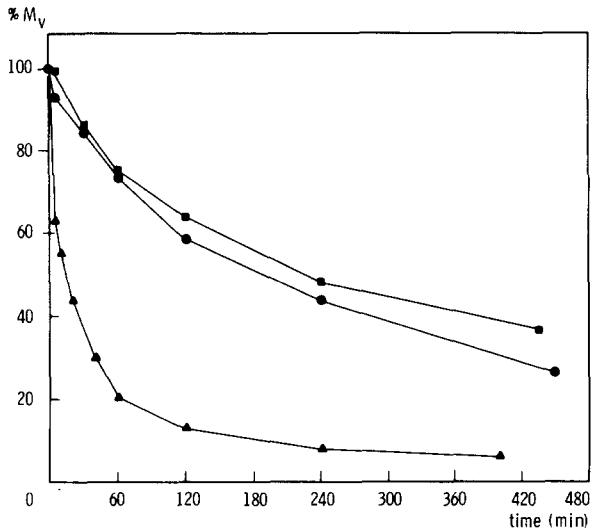

Fig. 8. Thermal decomposition of pectin, expressed as the relative reduction in M_v at 90°C in a concentrated suspension of pectin (6% m/m) and sugar (36% m/m) ●, in the HPSEC buffer (0·44 mol litre^{-1} acetic acid, 0·06 mol litre^{-1} sodium acetate, 0·1 mol litre^{-1} sodium sulfate, and 1 ml litre^{-1} propionic acid) ■, and in the succinate buffer (0·2 mol litre^{-1}, pH = 5·51) (▲).

SEC can be used by producers and used for quality control of pectins. The determination of the Mark–Houwink constants of pectin in the eluent used in this study permits the measurement of the viscosity average molecular weight (M_v) from viscosity data. Pectin displays an excellent stability in the eluent used for SEC. The experiment with drinking yogurt demonstrates that the MWD and average molecular weight influence the stabilising characteristics of pectin, in addition to other parameters such as the degree of esterification and distribution of ester groups.

ACKNOWLEDGEMENTS

We wish to thank Mr A. C. M. van Hooydonk and Dr A. G. J. Voragen for stimulating discussions and Messrs L. Smalbrink and A. Streuper for technical assistance with the drinking yogurt experiments.

REFERENCES

Ahmed, E. R. A. & Labavitch, J. M. (1977). *J. Food Biochem.* **1**,361.
Albersheim, P., Neukom, H. & Deuel, H. (1960). *Arch. Biochem. Biophys.* **90**, 46.
Anger, G. & Berth, G. (1985). *Carbohydr. Polymers* **5**, 241.
Barrett, A. J. & Northcote, D. H. (1965). *Biochem. J.* **94**, 617.
Barth, H. G. J. (1980). *J. Liq. Chromatogr.* **3**, 1481.
Beltman, H. (1975). Thesis, Agricultural University Wageningen, The Netherlands.
Davis, M. A. F., Gidley, N. J., Norris, E. R., Powell, D. A. & Rees, D. A. (1980). *Int. J. Biol. Macromol.* **2**, 330.
De Vries, J. A. (1983). Thesis, Agricultural University, Wageningen, The Netherlands.
Dongowski, G. (1970). Thesis, University of Rostock, GDR.
Dubois, M., Gilles, K. A., Hamilton, K. A., Rebers, P. A. & Smith, F. (1956). *Anal. Chem.* **28**, 350.
Fishman, M. L., Pfeffer, P. E., Barford, R. A. & Doner, L. W. (1984). *J. Agric. Food Chem.* **32**, 372.
Hooydonk, A. C. M. van, Smalbrink, L., Hagedoorn, H. G. & Reitsma, J. C. E. (1982). *Zuivelzicht* **74**, 901; *Voedingsmiddelentechnologie* **15** (20), 25.
Kato, T., Tokuya, T. & Takahashi, A. (1983). *J. Chromatogr.* **256**, 61.
Michel, F. (1982). Thesis, University of Dyon, France.
Mitchell, J. R. (1976). *J. Text. Stud.* **7**, 313.
Pals, D. T. F. & Hermans, J. J. (1952*a*). *Recl. Trav. Chim. Pays-Bas* **71**, 433.
Pals, D. T. F. & Hermans, J. J. (1952*b*). ibid. **71**, 458.
Pilnik, W. & Voragen, A. G. J. (1974). *Voedingsmiddelentechnologie* **7** (26/27)W80; **7** (30/31)W97.
Sorochan, V. D., Dzizenko, A. K., Bodin, N. S. & Ovodov, Y. S. (1971). *Carbohydr. Res.* **20**, 243.
Strubert, W. & Hovermann, W. (1978). *GIT Fachz. Lab.* **22**, 615.
van Dijk, J. A. P. P. & Smit, J. A. M. (1983). *J. Polym. Sci.* **21**, 197.
Watase, M. & Nishinari, K. (1982). *Colloid & Polymer Sci.* **260**, 971.
Yau, W. W., Kirkland, J. J. & Bly, D. D. (1979). *Modern size-exclusion liquid chromatography*, New York, John Wiley & Sons.