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Pectin Aggregation Number by Light Scattering and Reducing End-group Analysis

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ABSTRACT

Pectin determination by the copper–arsenomolybdate method is effectively a count of the total number of polymer molecules in a molar mass, ultimately yielding a number average molecular weight ($\bar{M}_n$)*. Light-scattering measurements yield a weight average* ($\bar{M}_w$) *of the number of light-scattering species that may be polymer molecules or aggregates of molecules, depending on solution (dispersion) conditions. Hence, the ratio* $\bar{M}_w/\bar{M}_n$*, may be interpreted as an aggregation number directly quantifying the molecules of pectin in the weight-average molar mass, under normal conditions of use of this polymer.*

INTRODUCTION

Pectin is the trivial name of the class of polydisperse galacturonans obtained from the action of acid on protopectin, a water-insoluble polymer existing in plants. The primary chemical structure is a linear, 1,4-α-linked D-galactopyranosyl-uronic acid chain (Doner, 1985), indicative of a biocondensation polymerization of galacturonic acid. This biopolymer may therefore be viewed as galacturonic acid substituted in the C-4 position (as the numbering relates to galactose). It is capable of forming gels with water, sugar and acid or calcium. Gels result from an orderly arrangement of the macromolecules relative to each other (large negative entropy) that is exceeded by a tendency toward a condition of minimum energy (large negative enthalpy), usually under cooling or aging.

Pectin molecular weights have been found to be of the order of 10^4, measured by osmometry (Owens *et al.*, 1946; Greenwood, 1952). When dispersed in water, the molecules tend to aggregate in colloidal masses,

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depending on ionic strength, pH, solvent additives, etc. (Sorochan *et al.*, 1971; Berth *et al.*, 1982; Sawayama *et al.*, 1988). Thus the aggregation is a solvent-induced association of molecules with colloidal dimensions and, consequently, the angular dependence of scattered, monochromatic light by pectin dispersions is a function of the micellar size and number of these associations.

Reducing end-group analysis, an extremely useful method of measuring the number-average molecular weight ($\bar{M}_n$) of condensation polymers, is essentially a count of the number of end-groups in a known polymer mass (Young, 1983). In pectin, there is one-to-one correspondence between the free carbonyl group and a single galacturonan molecule. The carbonyl group is located at the first carbon (as the numbering relates to galactose). This method has an upper $\bar{M}_n$ limit in the range of 50 000 (Garmon, 1975) below which $\bar{M}_n$ of commercial pectins is frequently reported.

Light-scattering data ultimately give an average weight, $\bar{M}_w$, of the total number of colloidal-size particles. $\bar{M}_w/\bar{M}_n$ has often been cited as a measure of polydispersity. It may be interpreted as an indication of the presence or absence of aggregation (Sorochan *et al.*, 1971). Fishman *et al.* (1984), using degree of polymerization and osmotic pressure data, identified it as an aggregation number, but they did not discuss its significance. Vold and Vold (1983) do not consider this ratio to provide much useful information about distribution. By reducing end-group analysis, the total number of galacturonan molecules is counted and averaged to give $\bar{M}_n$, and the ratio leads to a redefinition of $\bar{M}_w$ as a number-average function. Alternatively stated, $\bar{M}_w$ is a multiple of $\bar{M}_n$.

Light-scattering data also give the second virial coefficient from which conclusions about polymer–solvent and polymer–polymer interactions may be drawn (Sorochan *et al.*, 1971).

Aggregation phenomena are fundamental to many biological and industrial processes, including food manufacture. In this paper, $\bar{M}_w/\bar{M}_n$ is proposed as a means of characterizing pectins by their inherent tendency to form association colloids (Everett, 1988) in an aqueous medium. The quotient as an aggregation number is made more reliable by the use of reducing end-group analysis instead of osmometry.

EXPERIMENTAL

Pectin dispersions

Water was distilled from, and collected in, laboratory glassware, and allowed to trickle at a rate of 5–10 ml/h through a 24 × 2·5 cm, 0·040–0·063 mm dia., silica-gel column. The silica gel was previously leached with hot concentrated sulfuric acid and rinsed to neutral pH with distilled water. A pectin selection from the 1988 GENU series (Hercules, Inc., Wilmington, Delaware) was dispersed in water at 2·0% (w/v) concentration with mild heat and agitation. For every 100 ml of this dispersion, 300 ml of 95% ethanol was added to effect precipitation of pectin. The precipitated gel was separated from the supernatant liquid, suspended with vigorous agitation in 1:1 water–ethanol, then reprecipitated with two volumes of 95% ethanol. The separated gel was dried *in vacuo* at 75–80°C, then ground to pass a US Standard 60–80 mesh sieve. From this ground pectin, dispersions in 0·04 molar sodium chloride (pH 6·7) and in 0·04 M DL-tartaric acid (pH 2·3) were made to 0·60, 0·50, 0·40, 0·30, 0·20 and 0·10% w/v concentrations. A sample was allowed to trickle dropwise through its own 6·0 × 2·5 cm, o.d., column of silica gel to rid it of undispersed pectin. The first aliquots were discarded until the influent and effluent concentrations, determined by single-point viscometry, were equal. When this condition was met, approximately 45 ml eluate was collected directly in plastic, high-speed, centrifuge tubes which were then covered with a rubber stopper containing inlet and outlet syringe needles. The latter were preset centrally at the middle height of the tubes. The tubes were balanced and spun at 2×10^4 times gravity for 30 min. At the end of the interval, 30-ml samples were withdrawn with a syringe affixed to the outlet needle and emptied directly through a 0·45 nm, hydrophilic acrodisc filter (Gelman Sciences, Inc., Ann Arbor, Michigan) into the light-scattering cell (D101).

Light-scattering photometry

A Brice-Phoenix universal light-scattering photometer (Billmeyer, 1984), assembled with galvanometers, was used with the D101 cell to obtain scatter ratios (R) at angles $\theta = 0°$, 45°, 60°, 75°, 90°, 105°, 120° and 135°. The light source was a mercury vapor lamp from which the 546-nm line (green) was isolated. The operating temperature in the photometer chamber was 32°C. R_θ was calculated, according to the equation of Tomimatsu and Palmer (1963) and outlined step-by-step in the Brice-Phoenix instruction manual. Refractive increments for the calculation of H (Allcock & Lampe, 1981) were measured on a Brice-Phoenix differential refractometer, and the function KC/R_θ (Allcock & Lampe, 1981) was computed. The molecular weight, $\bar{M}_w$, was determined by the double extrapolation technique and corrected for depolarization (Stacey, 1956; Brice-Phoenix instruction manual).

Reducing end-group analysis

Galacturonic acid reducing end-groups were determined by the copper–arsenomolybdate method (Milner & Avigad, 1967; Keijbets, 1974), using galacturonic acid monohydrate as standard. This compound was heated at 105°C to constant weight to yield the anhydrous form (mol. wt 194·14). The range of concentration of standard solutions was 0·020–0·190 μmol. Absorbance was measured at 750 nm (Keijbets, 1974).

RESULTS AND DISCUSSION

Pectin is a stable compound in acidic media, and tartaric acid is a common food acid. The DL-isomer of tartaric acid is optically inactive. Given the incomplete methylation of the galacturonan chain, this acid provided a common ion (the hydrogen ion) with pectin, thereby enabling the molecules to act as neutral scattering centers. In the absence of the H^+, a degree of order would have been imposed on the polyanions, and the non-random system would then have been subject to errors resulting from a diminution of the intensity of scattered light and a change in its angular distribution (Stacey, 1956). However, within statistical limits, there did not appear to be any difference between the neutralized (Na^+) and the theoretically uncharged (H^+) polyuronide.

All but the first potential carbonyl group in the pectin molecule are bound in the α-1,4-linkages that constitute the polygalacturonic acid chain. Therefore, by comparison with galacturonic acid standards, the number of reducing end-groups in one mole of pectin, i.e., the number of pectin molecules equivalent to the number of galacturonic acid monomers, was determined by the arsenomolybdate method in which the quantitative reactions involving the 4-substituted galacturonic acid (the oligogalacturonides) are identical to those involving the monomer (Milner & Avigad, 1967).

The $\bar{M}_n$ derived from osmometry is generally considered to have its origin in molecularly dispersed solute, so that each polymer molecule is a molar mass. Doty *et al.* (1947) are of the opinion that the only reason polymer molecules in dilute solution are believed to be molecularly dispersed is because refuting evidence is lacking and, moreover, because the assumption is attractively simple.

Fishman *et al.* (1983) found the $\bar{M}_n$ from osmometry data to be two-to-four times greater than $\bar{M}_n$ obtained from end-group analysis. They suggested that the former property was an aggregation number, and the latter was the true polymer molecular weight. It should be remembered that osmotic pressure data are colligative properties, not necessarily arising from complete molecular dispersion. Hence $\bar{M}_n$ from osmometry is weighted in favor of the low molecular-weight fractions in a given polymolecular mass of pectin. Consequently, $\bar{M}_w/\bar{M}_n$ may sometimes be an erroneous indicator of distribution. $\bar{M}_n$, obtained by reducing end-group analysis, obviates this problem. In the latter context, the number of moles of unaggregated pectin molecules constituting a weight-average, molar mass of aggregates can be quantified as $\bar{M}_w/\bar{M}_n$, where $\bar{M}_n$ is derived from reducing end-group analysis, and therefore depends exclusively on the sum of the individual molecules.

Fishman and Pepper (1985) subsequently identified the role of aging and heating in the equilibrium events that enabled osmometry data to approach end-group titration data. Under such conditions, neither the speed of dynamic osmometry (with measurements after intervals of minutes) nor the duration of static osmometry (with equilibration time in 2–4 days) would appear to provide valid data for rationalizing $\bar{M}_w$ and $\bar{M}_n$. The principle of reducing end-group analysis, in contrast with the colligative principle of osmometry, also obviates the error arising from the measurement interval.

It is not uncommon for the second virial coefficient of galacturonan dispersions to be zero or negative (Sorochan, *et al.*, 1971; Berth *et al.*, 1982), as the slope of the $\theta = 0$ line (Figs 1 and 2) evidences. Aggregation in aqueous pectin dispersions, preceding deposition, is therefore a spontaneous process whereby energy content of the dispersed solute is minimized by reducing the surface area, notwithstanding macromolecular organization and its negative entropy effects. The addition of hydrogen-bond breakers to effect molecular dispersion (Sorochan, *et al.*, 1971) amounts to intervention in an equilibrium process.

$\bar{M}_w$ was $2{\cdot}67 \times 10^7$ in sodium chloride (Fig. 1) and $2{\cdot}24 \times 10^7$ in DL-tartaric acid (Fig. 2) (Cabannes factor = 0·96). The proximity of the two numbers, in spite of a wide pH difference, indicated the absence of any determinate error resulting from microglobules or dust. $\bar{M}_n$ representing the average of a triplicate analysis was $3{\cdot}58 \times 10^4$. As a result, $\bar{M}_w/\bar{M}_n = 6{\cdot}25\text{–}7{\cdot}45 \times 10^2$. Alternatively stated, 600–700 Genu pectin molecules formed the unit colloidal aggregate in acid-to-neutral pH. Such an aggregation number, denoting the unit assembly of molecules, is scaled to the extent of the macromolecular interactions in aqueous media, and is therefore a simple way to study through one physical parameter the effect of treatments, additives, external conditions and temperature on the tendency of pectins to associate with themselves (high probability of precipitation) or with the medium (high dispersion stability). It is note-

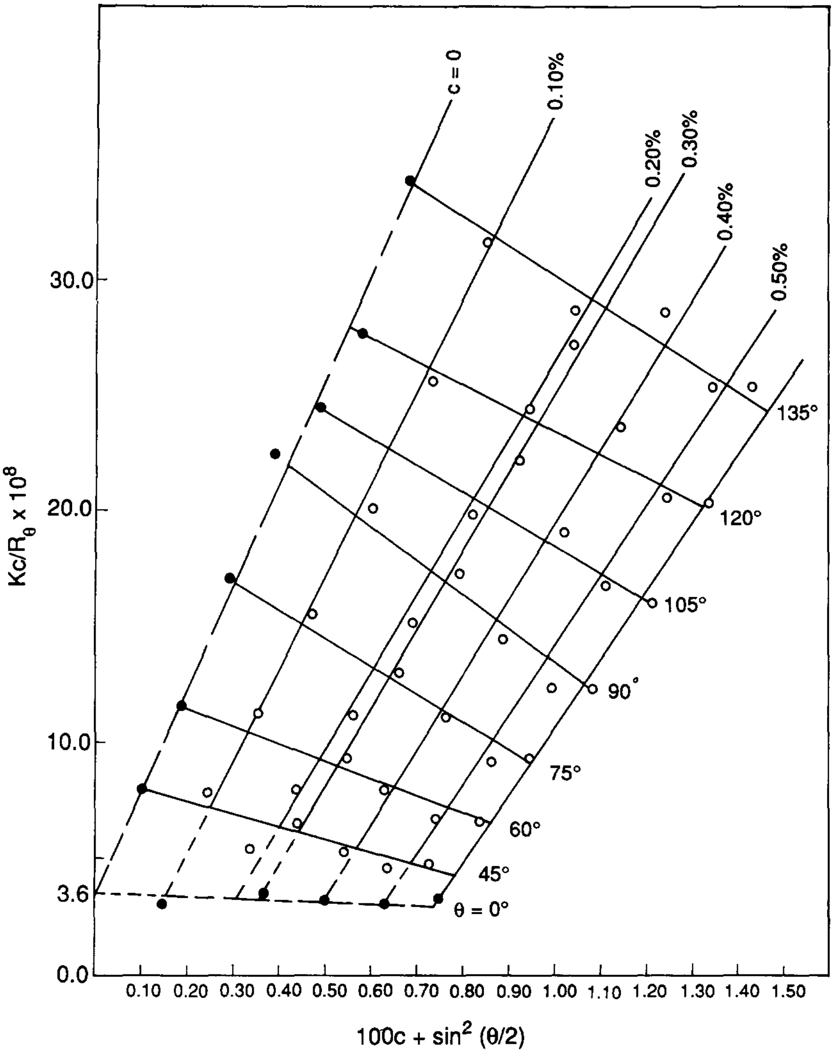

Fig. 1. Zimm diagram at 34°C of a GENU pectin dispersed in 0·04 M NaCl.

worthy that $\bar{M}_w/\bar{M}_n = 267{\cdot}0$ for starch amylopectin, the only other carbohydrate polymer reported to be of the order of magnitude described herein for pectin (Tanford, 1961).

Primary forces that sustain high-methoxyl pectin aggregation are hydrogen and hydrophobic bonding (Oakenfull & Scott, 1984). Notwithstanding the common practice of adding to the solvent chemical compounds that break hydrogen bonds, in order to attempt to obtain macromolecular data in the unperturbed state by light scattering, the ratio, $\bar{M}_w/\bar{M}_n$, computed as outlined, has the advantage of evaluating a

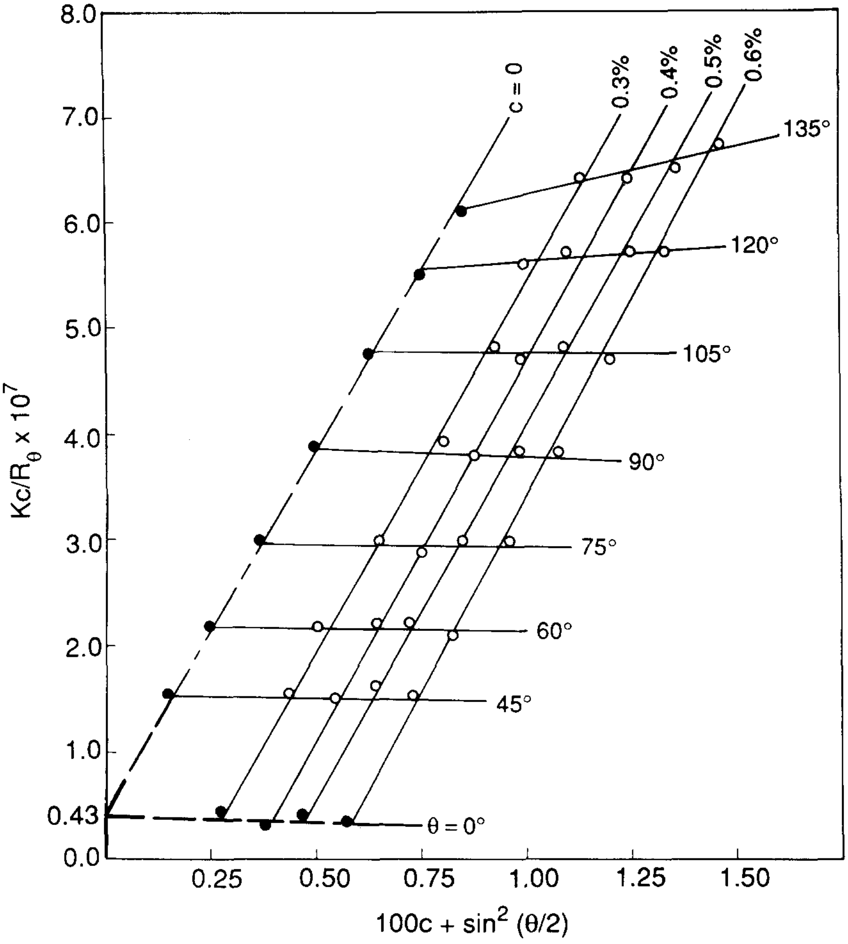

Fig. 2. Zimm diagram at 34°C of a GENU pectin dispersed in 0·04 M DL-tartaric acid.

spontaneous property of pectin and perhaps other polysaccharides, relative to their tendency to form aggregates in their normal dispersion environment.

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