Rheology and Aggregation of Cactus (Opuntia ficus-indica) Mucilage in Solution

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ABSTRACT

The mucilage obtained from cactus is commonly described as water-soluble pectin-like polysaccharide. Although few potential uses of this material have been described, it is not an industrial hydrocolloid. The objective of this study was to evaluate the rheological behavior of the polysaccharide isolated from the cladodes of *Opuntia ficus-indica*. The polymer had a weight average molecular mass (M_w) of 3×10^6 and a number average molecular mass (M_n) of 2.4×10^6, with a polydispersity index (M_w/M_n) of 1.4, as assessed by SEC-HPLC. Both M_w and M_n values exceeded those previously reported for the polysaccharide from *O. ficus-indica*. This overestimation of molecular mass may indicate extensive formation of large macromolecular aggregates in solution. The viscoelastic behavior of concentrated mucilage solutions (0.4% to 5.8% w/w; NaCl 0.1 M at 20°C) was investigated under sensitive dynamic mechanical spectroscopy and steady-shear rheological tests. Frequency (ω) dependence of the mechanical storage (G') and loss (G") mechanical moduli described spectra characteristic of an entangled network system where the polysaccharide is in the ``random coil'' fully disordered conformational state. The viscous (G") response predominating over the elastic (G') one at low ω, which, at higher ω, behave like an elastic solid (G' > G"). However, under steady-shear deformation tests, shear- thinning curves deviated from the typical random-coil behavior well documented for many polysaccharides used as industrial thickeners. This behavior is rationalized also as a consequence of the formation of colloidal aggregates in agreement with SEC-HPLC evidence. The formation of these large aggregates may underlie some of the special functional properties already identified for the use of cactus mucilage in food and other products.

INTRODUCTION

Sustainable plant and marine natural resources of biomass that exist in many regions of the world may be used as less expensive alternatives for producing added-value industrial polysaccharide gums. Among resources currently being sought for this purpose, indigenous plants from arid lands deserve special attention due to their agronomic advantages, such as the low input of water and energy needed for their commercial exploitation. Besides, desertification in arid regions has become a major concern worldwide, and encouragement of the sustainable use of the native flora of these regions may contribute to revert this problem.

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Along with its direct use as fruit or vegetable, cactus (Opuntia spp.) has been considered a potential source of an industrial hydrocolloid gum. Although the gum it is not yet

commercially available, there is a genuine interest among companies to start producing it on a large scale. It is well known to everyone familiar with handling of cactus cladodes (known in Mexico as nopalitos) or prickly-pear fruit in the kitchen, that when cut, both secrete a characteristic slime. The main constituent of this secreted fluid is a polysaccharide mucilage. The carbohydrate composition of mucilages from several Opuntia species has been well established by different chromatographic techniques (Amin et al., 1970; Mindt et al., 1975; Paulsen and Lund, 1979, Trachtenberg and Mayer, 1981, McGarvie and Parolis, 1981, 1981b; Forni et al., 1994). In general, they contain varying proportions of L-arabinose (pyranose and furanose forms), D-galactose, L-rhamnose, and D-xylose as the major neutral sugar units as well as D-galacturonic acid. The suggested primary structure describes the molecule as a linear repeating core chain of (1   4)- linked β-D-galacturonic acid and α(1   2)-linked L-rhamnose with trisaccharide side chains of β(1   6)-linked D-galactose attached at O(4) of L-rhamnose residues (McGravie and Parolis, 1981a). The galactose side residues present further branching in either O(3) or both O(3) and O(4) positions. The composition of these acid-labile peripheral chains is complex; at least 20 different types of oligosaccharides (mostly as disaccharides and trisaccharides) have been identified (McGravie and Parolis, 1981b). These invariably containing L-arabinose residues present as (1   5)-linked residues and possibly as branch points (McGravie and Parolis, 1981b) and single D-xylose groups occur as end groups, to give a xylose-to-arabinose ratio of approximately 1:2. This chemical composition is similar to that of the highly branched regions (hairy regions) of cell- wall pectins, particularly to the rhamnogalacturonan I (RG-I) fraction (Voragen et al., 1995; Pellerin et al., 1996). Hence, cactus mucilage is often referred to as a pectin polysaccharide. Recently, Forni et al. (1994), reported a methoxyl degree and acetyl degree of 10% and 10.4%, respectively, in the pectin extract from the peel of prickly pear of *Opuntia ficus-indica*.

Cactaceae are well adapted to arid and hot drylands, where the plants have a marked capacity to withstand prolonged drought. The ability of cactaceae to retain water under such unfavorable climatic conditions is due in part, at least, to the water-binding capacity of mucilage (Mindt et al., 1975). The mucilage biosynthesis takes place in specialized cells that excrete it into the apoplast, where it helps regulate the cellular water content during the initial phase of dehydration (Nobel et al., 1992). It has also been suggested that the mucilage has a predominant role in the Ca^{+2} economy of the plant (Trachtenberg and Mayer, 1982).

Although cactus mucilage physiological role in the plant and chemistry have been studied in detail, little is known about the molecular and rheological characteristics underlying specialized functional properties as an additive for food and other industries. Therefore, the objective of this investigation was to study the rheological properties of the mucilage of Opuntia ficus-indica in salt solution using sensitive dynamic and steady-flow deformation tests.

MATERIALS AND METHODS

Extraction Procedure

A batch of fresh cladodes (pencas) of Opuntia ficus-indica was a gift from a local producer (Mr.Vicente Sánchez) in San Pedro Hermosillo, Sonora. The pencas were diced and immediately afterwards boiled in 80% ethanol to inactivate any enzymatic activity. The diced sample was kept in this solution until further extracted. The polysaccharide mucilage was

extracted by blending the diced cladodes in water, filtered through a cloth and the filtrate resuspended in water and filtered (×3). The filtrate was centrifuged at 5,000 G and the pellet collected, suspended, and centrifuged again (×3). The extract thus obtained was freeze-dried, suspended in a small amount of water, and trichloroacetic acid (TCA) added to a final concentration of 5%, left to stand and centrifuged at 10,000 G. The precipitate was resuspended and centrifuged again. The TCA- treated supernatant was dialyzed against distilled water for 24 h, precipitated with absolute ethanol, then centrifuged at 15,000 G (×3). The precipitate was freeze-dried (Trachtenberg and Mayer, 1981). The yield of dry mucilage recovered was approximately 0.7g/kg of cladodes.

Molecular Mass Distribution by SEC-HPLC

The purified cactus polysaccharide molecular mass distribution was estimated by size-exclusion chromatography with a series of Ultrahydrogel columns (UG 500 and 2000, Waters), using a conventional HPLC system with a differential refractometer detector (Waters, 410). The eluant was 0.1M $NaNO_3$ at 40°C at a flow rate of 0.9 ml/min. Data were processed with PC-compatible software (Maxima GPC, Waters). The columns were calibrated using pullulans of *Aeurubasidium pullulans* as standards of molecular mass. A 50-µl volume of mucilage solution (1 mg/ml) was injected into the HPLC via a sample loop.

Rheological Determinations

A concentrated stock solution of mucilage was dialyzed to equilibrium against 0.1M NaCl solution with 0.02% sodium azide added to prevent spoilage at room temperature. The dialyzate was used for all subsequent dilutions. Actual polymer concentration was determined by freeze-drying accurately weighed aliquots. All rheological determinations were at 20°C. Low-amplitude oscillatory and steady-shear measurements were recorded using a cone and plate geometry (5-cm diameter; 0.04-rad cone angle) on a strain-controlled fluids spectrometer (Rheometrics RFSII). All mechanical spectra (frequency sweeps) were recorded at constant strain in the linear viscoelastic region.

RESULTS AND DISCUSSION

The yield of recovered mucilage obtained in this study (0.07%) was indeed lower than data on total pectin contents reported for O. ficus-indica of 1.9% (Villarreal et al., 1963). However, recently pectin contents of 0.12% were found in the peel of the fruits of O. ficus-indica (prickly pear) (Forni et al., 1994). Other Opuntia species (e.g., O. robusta) are reported to contain up to 3.3% pectin (Villarreal et al., 1963). Weight average (M_w) and number average molecular mass (M_n) of the cactus mucilage extract were respectively 3.4×10^6 and 2.4×10^6 to yield a polydispersity index (M_w/ M_n) of 1.4. The recorded M_w value is greater than that reported previously for *Opuntia ficus-indica* polysaccharide ($M_w = 1.56 \times 10^6$) using light scattering (Trachtenberg and Mayer, 1982). The greater molecular mass found here for cactus mucilage may be an indication of the formation of large molecular aggregates that are unable to dissociate during permeation through the gel beads in the column and impossible to detect by differential refractometry.

Figure 1 shows the mechanical spectra describing the viscoelastic behavior of three mucilage solutions in the concentrated regime subjected to low-amplitude oscillatory deformation tests. The mechanical response observed is characteristic of an entangled network of disordered

polymer coils (Ferry, 1980). Notice that at low T, there is sufficient time for substantial chain disentanglement and rearrangement within the time-scale of the oscillation period. Hence, the predominant response of the polymer to the imposed deformation is dissipative viscous flow of energy (characterized by the predominance of the loss modulus, G", over the storage modulus, G'). At higher ω, as the oscillation period of the applied deformation decreases, the time needed for chain rearrangements exceeds the time scale of the rate of deformation, hence elastic deformation of the entangled network becomes progressively more significant and, consequently, the two moduli. At such high ω, the system behaves as an elastic solid (G'>G"). The three graphs (Figure 1a-c) also show the Cox-Merz superposition (Cox and Merz, 1958) of steady-shear (0) and complex dynamic viscosity (0*) plotted at equivalent values of shear rate (() and frequency (T). Disordered polymer coils normally meet the Cox-Merz empirical rule where interactions between chains in the entangled network are merely topological.

Steady-shear flow curves of mucilage solutions of varying concentration are shown in Figure 2. All solutions showed non-Newtonian shear-thinning behavior, often described as pseudoplastic, which became progressively more pronounced as the mucilage concentration increased. A nonlinear model (Cross equation) was used to describe the viscosity of the solution at any given shear rate as:

$$\eta - \eta_s = \eta_0 - \eta_s / [1 + (\gamma / \gamma_{0.5})_\rho] \quad (1)$$

where η is measured solution viscosity at a given shear rate (g), η_s is the viscosity of the solvent, η is the maximum Newtonian or "zero-shear" viscosity at low shear-rate, (is the shear-rate $\gamma_{0.5}$ required to reduce $(\eta - \eta_s) = (\eta_0 - \eta_s)/2$, and ρ corresponds to the absolute value of the terminal slope of the log $(\eta - \eta_s)$ *vs.* log (curve (Morris, 1990). To compute the best-fit values of η_0, $\gamma_{0.5}$, and ρ, the root-mean-square difference values between observed and calculated values of $(\eta - \eta_s)$ were minimized using a computer subroutine available in Microsoft Excel 5.0. It is well established that the experimental flow curves of disordered polysaccharides in solution can be reduced to a single "master curve" regardless of the type of polymer and the concentration (Morris et al., 1981). Measured values of (0 - 0) for each solution were divided by the maximum "zero-shear" value η_0, and the corresponding shear-rate values scaled to (to give a family of $\gamma_{0.5}$ shear-thinning curves as shown in Figure 3. This shear-thinning behavior is particularly unusual, because the normalized curves do not converge into a single function as it was expected for typical linear random coil polysaccharide thickeners. Instead they "peel off" in the shear-thinning region on increasing the polymer concentrations. This deviation from the typical behavior of linear polysaccharide coils has been observed before for okra (*Hibiscus esculentus* L.) mucilage solutions (Ndjouenkeu et al., 1996). The interpretation offered to these phenomena in okra mucilage was that a strong tendency to self-association or aggregation increases with increasing concentration. The interpretation offered was consistent with scanning electron microscopy evidence of large, branched aggregates (>4μm) in dispersions of okra gum. Cactus mucilage shares apparently similar macroscopic properties with okra gum, as both form ropy or stringy solutions in water, so the existence of large molecular aggregates may be postulated. Such an hypothesis could be addressed in future studies.

There are a number of potential special applications already identified for okra gum, which include among other: improvement of whipping properties of reconstituted egg white (BeMiller et al. 1993); deflocculant agent; brightening agent for electrodeposition of nickel and other metals. Similar and new uses for cactus mucilage also can be suggested. Cactus mucilage

may find applications in the food, cosmetics, pharmaceutical and other industries. It has indicated that cactus mucilage may work as a clarifying agent for drinking water in the same way that okra gum does (Bobby Crabb, pers. comm.: Cactus List). Also, the culinary properties of cactus mucilage as a fat replacer and a flavor binder have been emphasized (Jay McCarthy, pers. comm.: Cactus List). From the molecular mass distribution and rheological evidences presented here, cactus mucilage forms large molecular aggregates, which underlie its shear-thinning behavior in solution. The specialized functional applications already found in cactus and okra mucilages seem to be related to the capacity of these polysaccharides to form such colloidal dispersions of large molecular aggregates. The influence of the ionic environment as well as the effect of chemical and enzymatic modifications on the overall physicochemical properties, are key aspects that deserve much further attention. Progress in this direction is ongoing in our laboratory.

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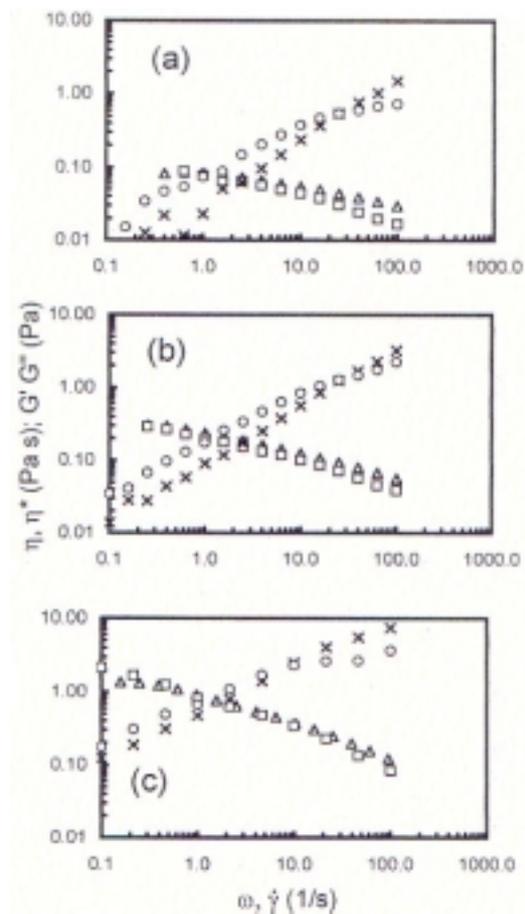
Figure 1. Dependence of storage, G′(Δ), loss, G′′(o) mechanical moduli, and dynamic viscosity, η* (□), on frequency, ω, (strain 0.05) and of steady-shear viscosity, η(◊), on shear rate, γ, for cactus mucilage solutions at concentrations (% w/w; 20°C in 0.1M NaCl) of: a) 1.0; b) 1.6; and c) 2.0.
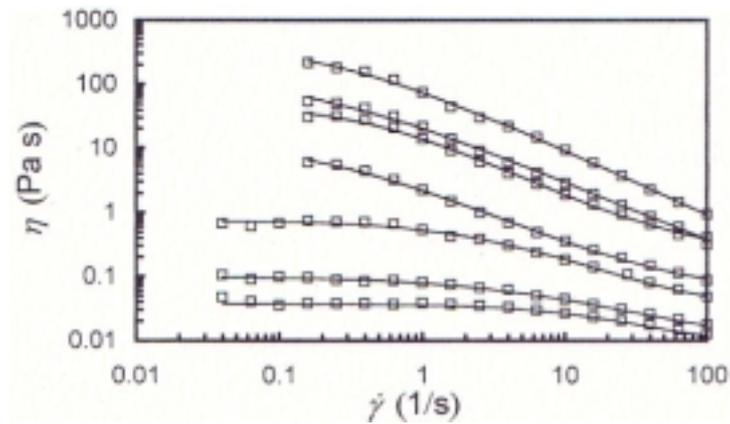
Figure 2. Experimental flow curves (20°C; 0.1M NaCl) for cactus mucilage
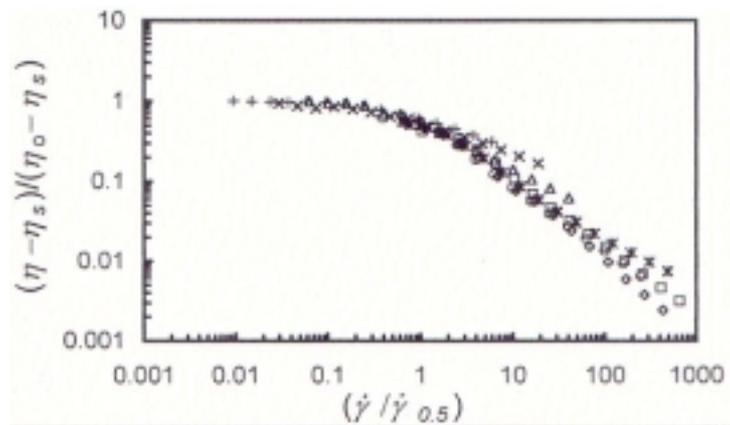solutions at concentrations (% w/w)(from top) of: 5.8, 3.10, 2.9, 1.6, 0.8, 0.5 and
0.4.

Figure 3. Generalized shear-thinning curves (20°C) obtained from the
experimental flow curves in Figure 2 by scaling measured values of 0 to the
maximum "zero-shear" viscosity (η) for each solution, and similarly scaling γ
to the shear-rate required to reduce η to η_0 /2.
Mucilage solutions concentrations (% w/w in 0.1M NaCl) were:
5.8 (◊), 3.10(□), 2.9(o), 1.6(*), 0.8(Δ), 0.5(x), 0.4(+).