

# Rheology of okra (*Hibiscus esculentus* L.) and dika nut (*Irvingia gabonensis*) polysaccharides

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Polysaccharide extracts were prepared from two traditional food thickeners with extensive domestic use in central and western parts of Africa: okra (*Hibiscus esculentus* L.) and the seed kernel from 'dika nut' (*Irvingia gabonensis*). Both demonstrated typical polyelectrolyte behaviour in solution, and were therefore studied under fixed ionic conditions (0.1 M NaCl), yielding intrinsic viscosities of  $[\eta] = 7.6 \text{ dl g}^{-1}$  for okra and  $[\eta] = 4.4 \text{ dl g}^{-1}$  for dika. Concentrated solutions gave mechanical spectra typical of entangled networks, with close Cox–Merz superposition of  $\eta(\omega)$  and  $\eta(\dot{\gamma})$ . The variation of 'zero-shear' specific viscosity with degree of space-occupancy ( $c[\eta]$ ) was also broadly similar to the general form observed for most disordered polysaccharides, but with greater separation of  $c^*$  and  $c^{**}$  and steeper slope of  $\log \eta_{sp}$  vs.  $\log c$  above  $c^*$  ( $\sim 4.0$  for okra and  $\sim 4.6$  for dika, in comparison with the usual value of  $\sim 3.3$ ). As found for normal disordered polysaccharides, the shear-thinning behaviour of dika gum could be reduced to a single 'master-curve' for all concentrations above  $c^*$ , but the absolute value of the terminal slope of  $\log(\eta - \eta_s)$  vs.  $\log \dot{\gamma}$  was unusually low ( $\sim 0.58$ , in comparison with the normal value of  $\sim 0.76$ ). Terminal slopes for okra gum were also unusually low, and varied systematically with polymer concentration. These departures from normal solution properties are tentatively ascribed to compact macromolecular structures, coupled, in the case of okra gum, with a strong tendency to self-association. Copyright © 1996 Elsevier Science Limited.

## INTRODUCTION

Many of the polysaccharides currently available as 'industrial gums' were first used in an empirical way in domestic cookery. Obvious examples include pectin as the setting-agent in jam, carrageenan in milk desserts, and starch as a thickener in soups and sauces. There are many others, however, that are not yet exploited commercially, but are used extensively in traditional local recipes, particularly in the less industrialised regions of the world. These materials usually come from plants that grow wild or are cultivated only on a very limited scale, and their functional properties as food hydrocolloids remain largely unexplored.

In Africa, the main culinary use of indigenous hydrocolloids is in thickening soups and stews, and the plants used include okra (*Hibiscus esculentus*), dika nut (*Irvingia gabonensis*), mbol (*Belschmiedia zenkeri*) and

kelekelin (*Triumfetta cordifolia*). The first two of these are the most common in domestic cooking in the central and western parts of Africa. We report here a rheological investigation of the polysaccharide extracts derived from them.

Okra (*Hibiscus esculentus*, Linn) is a bulky annual plant, native to Africa, but now cultivated throughout the tropical and subtropical areas of the world, particularly in the southern part of the USA, India, and West and Central Africa. The plant, also called 'gumbo' (in Africa and the USA) or 'bhindi' (in India), gives fruits which are green pods of various shapes (slender and cylindrical or short and fat; smooth or ribbed). Traditionally, okra is either used fresh or is dried and ground to thicken and flavour soups. The immature fruit is also used in folk medicine as a diuretic and for treatment of dental disease (FAO, 1988). The pods contain a thick, slimy mucilage which can be extracted as a viscous gum.

Okra gum is not produced commercially at present, but it has been evaluated for a number of food and non-food applications, as reviewed recently by BeMiller *et al.* (1993). These include use as a whipping agent for

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reconstituted egg-white, as a 'brightening agent' in electrodeposition of metals, as an additive in the formulation of flour-based adhesives, as a deflocculant in production of papers and fabrics, and for reduction of friction in pipe-flow. It has also been used in India for clarifying sugar-cane juice.

Chemical analyses of okra gum have yielded widely differing results (BeMiller *et al.*, 1993), perhaps due to differences in the extraction procedures used. Tomoda *et al.* (1980) concluded that the major polysaccharide component has a linear disaccharide repeating sequence of:  $\rightarrow 4\text{-}\alpha\text{-GalpA-(1}\rightarrow 2\text{)-}\alpha\text{-L-Rhap-(1}\rightarrow$  with disaccharide sidechains of:  $\beta\text{-Galp-(1}\rightarrow 4\text{)-Galp-(1}\rightarrow$  attached to O(4) of half the L-rhamnosyl residues. Subsequent work by Bhat and Tharanathan (1986, 1987), however, suggested a more complex composition that could be separated into fractions differing widely in the molar ratios of galactose, galacturonic acid and rhamnose, and with some fractions containing substantial proportions of other sugars (glucose, mannose, arabinose and xylose). There have also been reports of a high mineral content ( $\sim 11.9\%$  ash from proximate analysis; Kelkar *et al.*, 1962), and of an appreciable protein component ( $\sim 10\%$ ; Woolfe *et al.*, 1977; Tomoda *et al.*, 1980), although it remains to be established whether or not the protein is covalently linked to the polysaccharide.

In contrast to okra gum, which has received some scientific attention, albeit limited, dika gum has not been studied at all. The plant from which it is derived, *Irvingia gabonensis*, is also known as 'bush mango', although it is not related botanically to the true mango (*Mangifera indica* L.). The tree occurs widely in the humid forests of Central and West Africa, and can reach a height of 35 m (Thaikul, 1983). The fruit is oblong, with diameter 4–7 cm, and has a strong, bulky core surrounded by edible, fibrous flesh. The core comprises two almond-like seeds, which are also edible. The maturation and harvesting period runs from April to July or September, according to region. Before consumption, the core is dried, dehulled, and ground; it is then used to thicken and flavour soups and stews. Preliminary chemical analysis of dika gum is in progress, and will be reported elsewhere. The present investigation is confined to characterisation of the rheological properties of the polysaccharide constituent, in comparison with those of okra gum and of commercial hydrocolloids.

## MATERIALS AND METHODS

Okra pods and dika kernels were purchased locally in Cameroon (N'gaoundere market). Okra seeds (which have a high lipid content) were removed, and the remaining pods (or skin) were milled to a particle size of 400–500  $\mu\text{m}$ . The dika kernels were milled using a domestic coffee-grinder (Moulinex), and oil was

removed by soxhlet extraction with hexane. The delipidated material was then dried, and ground to a final particle size of 400–500  $\mu\text{m}$ . A crude polysaccharide extract was prepared from each powder by the following procedure.

Flour (10 g) was mixed with distilled water (250 ml) in a round-bottomed flask, and heated under reflux in a boiling water bath for 1 h. The resulting mixture was then centrifuged (2000 g; 30 min) and the supernatant was collected. The residue was extracted with two further 250 ml aliquots of distilled water, following the same procedure. The final residue was discarded, and the supernatant fractions were combined, filtered through fine-mesh nylon, and concentrated to about a quarter of their initial volume on a rotary evaporator. The crude polysaccharide was then precipitated with 85% ethanol ( $4\times$  volume), redissolved in deionised water, and purified as follows.

Proteins were removed by digestion (1 h at 55°C, under stirring) with papain (BDH; 2 g), precipitation with 10% trichloroacetic acid, and centrifugation. The resulting supernatant was decolourised by stirring overnight with Amberlite A-27 strong base (type I) anion exchange resin (BDH), dialysed against six changes of deionised water, and freeze-dried.

Concentrated stock solutions of the polysaccharide extracts were prepared by magnetic stirring overnight at ambient temperature in 0.1 M NaCl, with 0.04% sodium azide as preservative, and were dialysed to equilibrium against the same salt solution. The dialysate was then used for all subsequent dilutions to lower concentrations. A few comparative measurements were also made using solutions prepared with deionised water.

Dilute-solution viscosity was measured on a Contraves Low Shear 30 rotational viscometer, using cup-and-bob geometry with inner and outer radii of 5.5 and 6.0 mm, respectively. Viscosity measurements at higher concentrations were made on a Sangamo Viscoelastic Analyser, with cone-and-plate geometry of cone-angle 2° and diameter 5 cm. Oscillatory measurements were made using cone-and-plate geometry (cone-angle 0.05 rad; diameter 5 cm) on a sensitive prototype rheometer designed and constructed by Dr R. K. Richardson (Silsoe College). In all cases, temperature was controlled to  $25\pm 0.5^\circ\text{C}$  using a Haake circulating water bath, and monitored by a thermocouple in contact with the stationary element.

## RESULTS

Figure 1 shows flow curves (variation of viscosity,  $\eta$ , with shear-rate,  $\dot{\gamma}$ ) for a few moderate concentrations of each of the two polysaccharide extracts, in water and in 0.1 M NaCl. As would be expected from the known presence of a substantial proportion of charged (galacturonate) residues in okra gum, its viscosity in salt

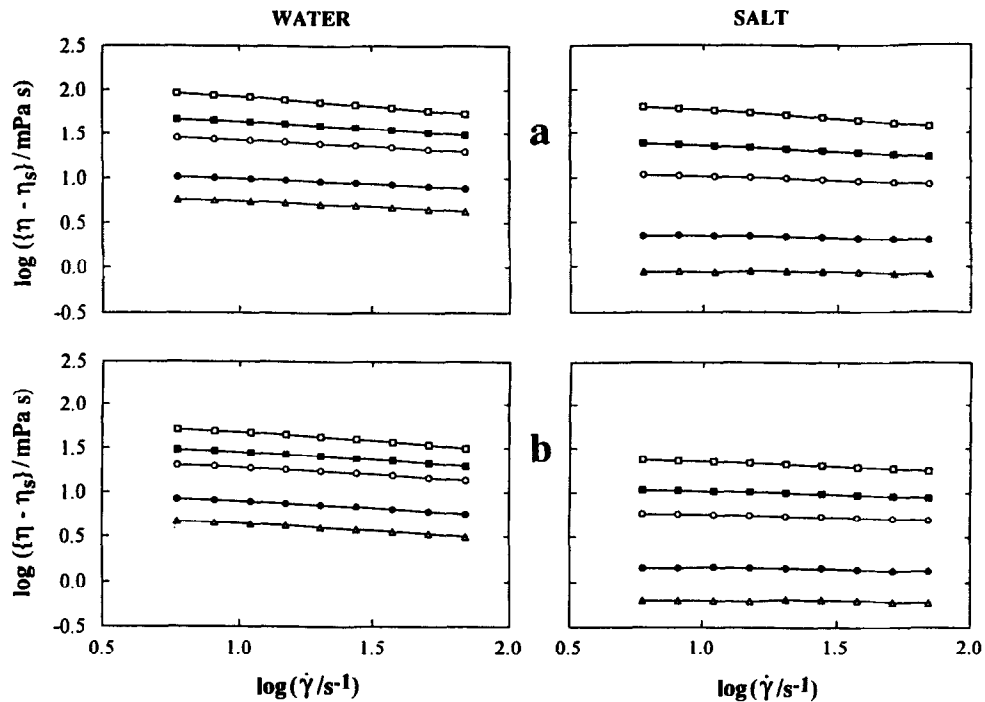


Fig. 1. Shear-rate dependence of viscosity (25°C) for (a) okra gum and (b) dika gum at concentrations (% w/v) of 0.96 (□), 0.67 (■), 0.48 (○), 0.19 (●) and 0.096 (Δ) in water (left) and in 0.1 M NaCl (right).

solution is appreciably lower than in water, and the difference increases systematically with decreasing polymer concentration. Such behaviour is general for disordered polyelectrolytes, and its origin is well understood (see for example Smidsrød and Haug, 1971). In water, the individual coils are expanded by intramolecular electrostatic repulsion. Addition of salt screens the repulsions and allows the coils to contract to a more compact conformation, with consequent reduction in viscosity. The counterions to the charged groups of the polymer act in the same way, so that the extent of coil-expansion in water decreases as the concentration of polymer (and hence the concentration of accompanying counterions) is increased, with a corresponding decrease in the degree of contraction that can occur on addition of extraneous salt. Dika gum behaves in a closely similar way (Fig. 1b) showing that, although the primary structure is not yet known, it is also charged.

To avoid the complications of changes in coil dimensions on varying polymer concentration, all subsequent studies were performed at fixed ionic strength, by dialysing stock solutions against 0.1 M NaCl and using the dialysate for dilution to lower concentrations. The mechanical spectra obtained for the stock solutions ( $\sim 3.4\%$  w/v for okra and  $\sim 4.1\%$  w/v for dika) are shown in Fig. 2. Both polymers give the response characteristic of entangled coils (see for example Ross-Murphy, 1984). At low frequency, where there is sufficient time for substantial disentanglement to occur within the period of oscillation, the predominant response to the imposed deformation is dissipative flow

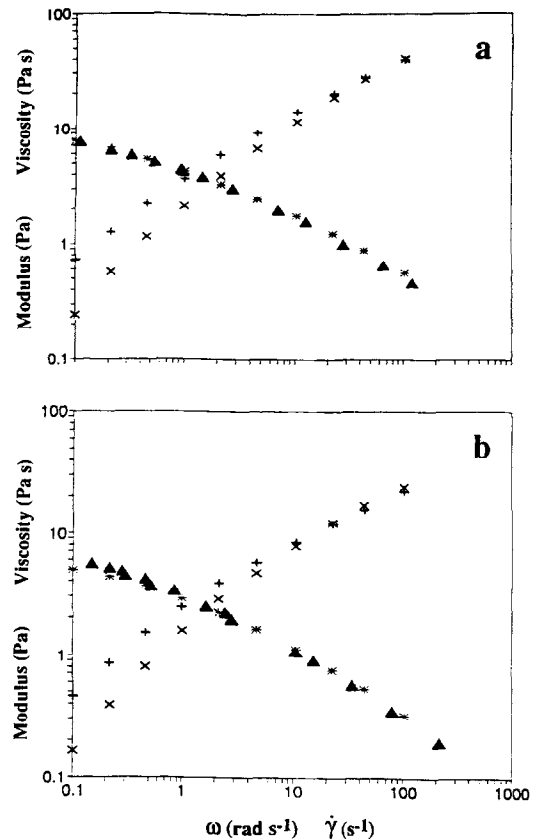


Fig. 2. Frequency-dependence of  $G'$  (×),  $G''$  (+) and  $\eta^*$  (\*) and shear-rate dependence of viscosity (▲) for (a) okra gum (3.36% w/v) and (b) dika gum (4.07% w/v). Both solutions were prepared in 0.1 M NaCl and measured at 25°C. The oscillatory measurements were made at 2% strain.

(characterised by the 'loss modulus',  $G''$ ), but at higher frequencies elastic deformation of the entangled network becomes progressively more significant, with consequent increase in the 'storage modulus',  $G'$ .

Both materials also follow the behaviour of disordered polymer coils in showing close 'Cox-Merz superposition' (Cox and Merz, 1958; Ross-Murphy, 1984) of steady-shear viscosity ( $\eta$ ) and complex dynamic viscosity ( $\eta^* = (G'^2 + G''^2)^{1/2}/\omega$ ) at equivalent values of shear-rate ( $\dot{\gamma}/s^{-1}$ ) and frequency ( $\omega/\text{rad s}^{-1}$ ). Similar results were obtained at lower concentrations.

Flow curves recorded at polymer concentrations above the range shown in Fig. 1 are presented in Fig. 3a (for okra) and Fig. 3b (for dika). In all cases the shear-rate dependence of viscosity could be fitted, to within the experimental error of the measurements, by the relationship:

$$(\eta - \eta_s) = (\eta_o - \eta_s)/[1 + (\dot{\gamma}/\dot{\gamma}_{1/2})^p] \quad (1)$$

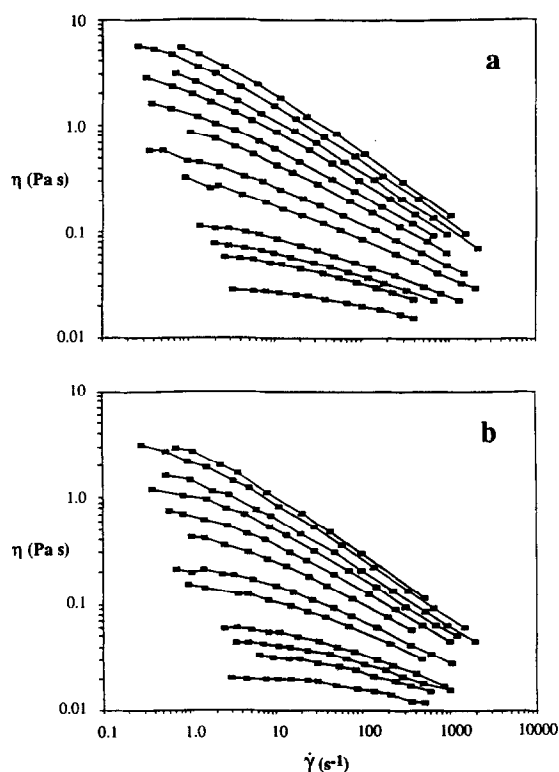
where  $\eta_s$  is the viscosity of the solvent,  $\eta_o$  is the maximum 'Newtonian' viscosity at low shear-rate,  $\dot{\gamma}_{1/2}$  is the shear-rate required to reduce  $(\eta - \eta_s)$  to  $(\eta_o - \eta_s)/2$  and  $p$  corresponds to the absolute value of the terminal slope of  $\log(\eta - \eta_s)$  vs.  $\log \dot{\gamma}$  at high shear-rate (Morris, 1990). The best-fitting values of  $\eta_o$ ,  $\dot{\gamma}_{1/2}$  and  $p$  were obtained by using a simple computer program to mini-

mise the root-mean-square difference between observed and calculated values of  $\log(\eta - \eta_s)$ . At low concentrations (below the range shown in Fig. 1) the flow curves were essentially Newtonian, giving  $\eta_o$  directly.

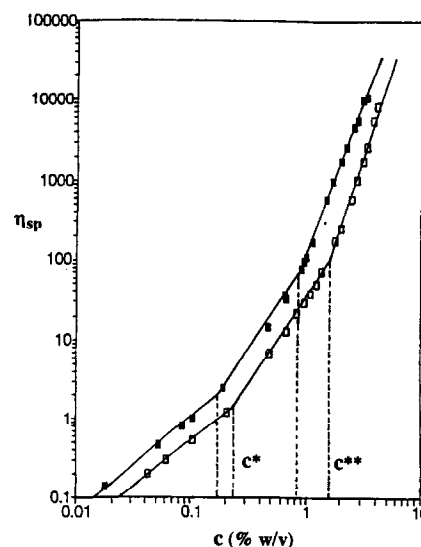
### Concentration-dependence of viscosity

Figure 4 shows the concentration-dependence of  $\eta_o$  for both polymers, plotted in conventional double-logarithmic form with  $\eta_o$  expressed as specific viscosity ( $\eta_{sp} = (\eta_o - \eta_s)/\eta_s$ ). The measured viscosities for the four lowest concentrations of each material were used to derive intrinsic viscosity,  $[\eta]$ , by combined Huggins, Kraemer and 'single-point' extrapolation to infinite dilution (see Morris, 1984). In both cases the Huggins plot of  $\eta_{sp}/c$  (where  $c$  is concentration), Kraemer plot of  $\ln(\eta_{rel})/c$  (where  $\eta_{rel}$  is the 'relative viscosity',  $\eta/\eta_s$ ) and single-point plot of  $[2(\eta_{sp} - \ln \eta_{rel})]^{1/2}/c$  are acceptably linear, and show satisfactory convergence at  $c=0$  (Fig. 5), yielding  $[\eta] = 4.4 \text{ dl g}^{-1}$  for dika and  $[\eta] = 7.6 \text{ dl g}^{-1}$  for okra.

Intrinsic viscosity provides a convenient measure of the hydrodynamic volume of individual polymer coils, and when multiplied by concentration gives an index of the total degree of space-occupancy. Double-logarithmic plots of  $\eta_{sp}$  vs.  $c[\eta]$  for most disordered polysaccharides superimpose closely (Morris *et al.*, 1981) and fall into two linear regions with a change of slope from  $\sim 1.4$  to  $\sim 3.3$  at  $c[\eta] \approx 4$  and  $\eta_{sp} \approx 10$ . As shown in Fig. 6, the results obtained for the polysaccharide extracts from dika kernels and okra comply reasonably well with this generalisation, giving slopes of  $\sim 1.4$  and  $\sim 3.7$  and a break at  $c[\eta] \approx 3.5$  and  $\eta_{sp} \approx 10$ . There is, however, obvious curvature around the 'breakpoint', and significant divergence of values for the two polymers at high  $c[\eta]$ .



**Fig. 3.** Experimental flow curves (25°C; 0.1 M NaCl) for (a) okra gum at concentrations of 3.63, 3.15, 2.80, 2.58, 2.26, 2.02, 1.69, 1.48, 1.14, 1.01, 0.91 and 0.68% w/v and (b) dika gum at concentrations of 4.07, 3.80, 3.39, 3.12, 2.71, 2.44, 2.03, 1.77, 1.37, 1.22, 1.08 and 0.82% w/v.



**Fig. 4.** Concentration-dependence of 'zero shear' specific viscosity (25°C) for okra gum (■) and dika gum (□).

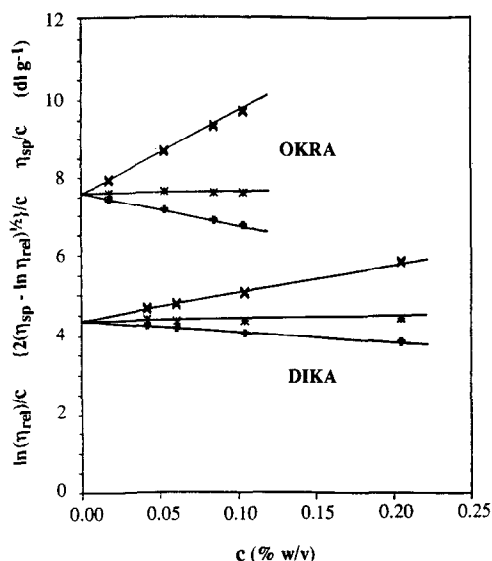


Fig. 5. Determination of intrinsic viscosity (25°C; 0.1 M NaCl) for okra gum and dika gum by combined Huggins (x), Kraemer (+) and single-point (\*) extrapolation to zero concentration.

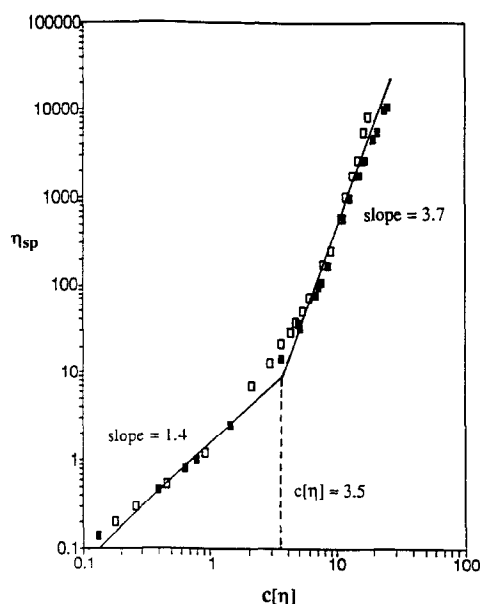


Fig. 6. Variation of 'zero-shear' specific viscosity (25°C) with degree of space-occupancy ( $c[\eta]$ ) for okra gum (■) and dika gum (□) in 0.1 M NaCl.

Inspection of the individual plots of concentration-dependence in Fig. 4 indicates that they can be split more validly into three linear portions, with the slopes and intercepts listed in Table 1. Three regimes of concentration-dependence have been observed previously for other polymer systems, such as ordered xanthan (Launay *et al.*, 1984; Table 1), and are particularly evident by techniques such as dynamic light scattering (e.g. Southwick *et al.*, 1979) or fluorescence-bleaching measurements of self-diffusion (Tinland *et al.*, 1990)

Table 1. Concentration-dependence of 'zero-shear' viscosity

	Okra	Dika	Levan <sup>a</sup>	Xanthan <sup>b</sup>
$c^*$ (% w/v)	0.15	0.21	4.4	0.024
$c^{**}$ (% w/v)	0.83	1.55	21	0.092
$[\eta]$ (dl g <sup>-1</sup> )	7.6	4.4	0.17	46.5
$c^*[\eta]$	1.14	0.92	0.75	1.1
$c^{**}[\eta]$	6.3	6.8	3.6	4.3
Slope 1 (below $c^*$ ) <sup>c</sup>	1.17	1.17	1.4	1.25
Slope 2 (from $c^*$ to $c^{**}$ )	2.1	2.1	2.2	1.94
Slope 3 (above $c^{**}$ )	4.0	4.6	9.3	3.87

<sup>a</sup>Densely branched bacterial levan; from Kasapis *et al.* (1994)

<sup>b</sup>In 0.1 M NaCl; from Launay *et al.* (1984)

<sup>c</sup>Slopes of  $\log(\eta_0 - \eta_s)$  vs.  $\log c$  (Fig. 4)

which are more sensitive than viscosity to changes in molecular mobility.

The first transition (at concentration  $c^*$ ) is attributed to initial contact between the individual coils when, in aggregate, their swept-out volume becomes equal to the total volume of the solution, and normally occurs when the degree of space-occupancy reaches  $c[\eta] \approx 1$  (i.e.  $c^* \approx 1/[\eta]$ ). The two regions of higher concentration-dependence correspond, respectively, to initial compression and subsequent interpenetration of the polymer coils in response to increasing space-occupancy, and the concentration at their point of intersection is denoted as  $c^{**}$  (see for example Launay *et al.*, 1984; Milas *et al.*, 1990). Measured values of  $c^*[\eta]$  vary widely with system and technique, from around 2 to over 10. Both materials studied in the present work give values (Table 1) in good agreement with these generalities:  $c^*[\eta] \approx 1$  and  $c^{**}[\eta] \approx 6.5$ .

### Shear-thinning behaviour

The experimental flow curves for dika gum (Figs 1b and 3b) could be reduced to a single 'master curve' (Fig. 7) by dividing measured values of  $\eta - \eta_s$  for each solution by the maximum 'Newtonian' value ( $\eta_0 - \eta_s$ ), and by scaling applied shear-rates to  $\dot{\gamma}_{1/2}$ . The entire curve (spanning almost two decades of viscosity and more than five decades of shear-rate) can be matched, to within the experimental error of the measurements, by eqn (1), with  $p = 0.58$ .

As illustrated in Fig. 8, however, no such convergence could be achieved for okra gum: the individual curves differ in shape, and cannot therefore be superimposed by transposition along the viscosity and shear-rate axes. The variation in curvature with varying polymer concentration is indicated by the span of ' $p$ ' values (eqn (1)) required to fit the experimental flow curves. The best-fitting value of  $p$  decreases systematically (Fig. 9) on initial dilution from the stock solution ( $p \approx 0.62$  at  $\sim 3.4\%$  w/v), reaches a minimum ( $p \approx 0.43$ ) at around 1.3% w/v, and then increases again at lower concentrations.

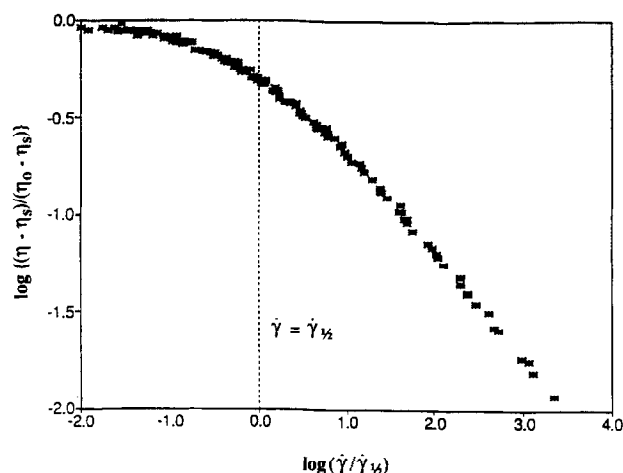


Fig. 7. Generalised shear-thinning curve for dika gum, obtained from the experimental flow curves in Fig. 3b by scaling measured values of  $\eta$  to the maximum 'zero shear' viscosity ( $\eta_0$ ) for each solution, and similarly scaling  $\dot{\gamma}$  to the shear-rate ( $\dot{\gamma}_{1/2}$ ) required to reduce  $(\eta - \eta_s)$  to  $(\eta_0 - \eta_s)/2$ .

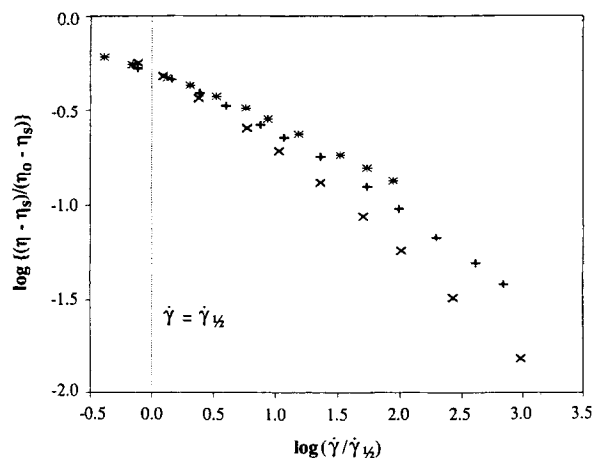


Fig. 8. Shear-thinning curves (25°C; Fig. 3a) for okra gum at concentrations (% w/v in 0.1 M NaCl) of 3.36 (x), 2.02 (+) and 1.15 (\*), re-scaled as in Fig. 7.

## DISCUSSION

As illustrated in Fig. 2, solutions of okra and dika gums resemble those of other polysaccharides in showing normal solution-like mechanical spectra and close Cox-Merz superposition of  $\eta(\dot{\gamma})$  and  $\eta^*(\omega)$ . The dependence of 'zero-shear' specific viscosity on degree of space-occupancy ( $c[\eta]$ ) is also broadly similar to the behaviour typical of disordered polysaccharide coils (Morris *et al.*, 1981), but the separation of  $c^*$  and  $c^{**}$  for both materials is unusually pronounced (Fig. 4), and the slope of  $\log \eta_{sp}$  vs.  $\log c$  at high concentrations is substantially steeper (Table 1) than the 'normal' value of  $\sim 3.3$ . Both effects are particularly evident for dika gum. Wide separation of  $c^*$  and  $c^{**}$  (Table 1) and extremely steep concentration-dependence of viscosity above  $c^{**}$  have been reported recently (Kasapis *et al.*, 1994) for bacter-

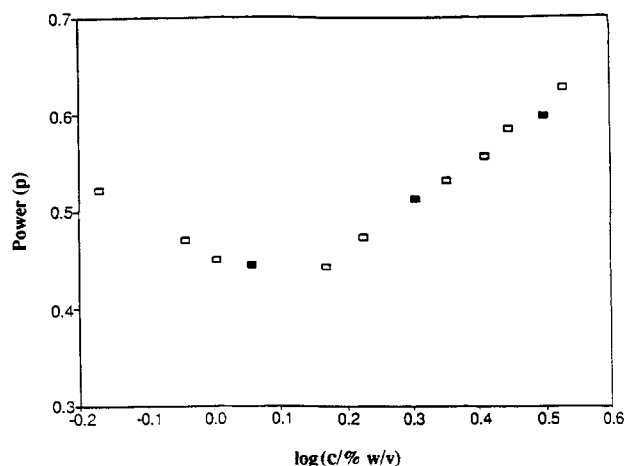


Fig. 9. Concentration-dependence of the values of  $p$  (eqn (1)) required to fit the experimental flow curves shown in Fig. 3a for okra gum in 0.1 M NaCl. Filled symbols indicate the concentrations used in Fig. 8 to illustrate the progressive change in curvature of the individual flow curves.

ial levan, which has a densely-packed branched structure. The similar (though less extreme) behaviour of dika gum, coupled with its rather low intrinsic viscosity ( $[\eta] \approx 4.4 \text{ dl g}^{-1}$  in 0.1 M NaCl), would therefore be consistent with it too having compact molecular geometry.

Dika gum is also unusual in its shear-thinning behaviour. The shear-rate dependence of viscosity for conformationally-disordered linear polysaccharides with a wide distribution of chainlength (as in normal commercial materials) appears to have a general form that is independent of molecular weight and concentration (above  $c^{**}$ ), and which can be matched accurately by eqn (1) using a constant value of  $p = 0.76$  (Morris, 1990). The variation of viscosity with shear-rate for dika gum is also independent of concentration above  $c^{**}$ , but the value of  $p$  required to fit the experimental data is substantially lower ( $\sim 0.58$ ). Closely similar behaviour ( $p \approx 0.56$ ) has been reported recently for methylcellulose (Haque and Morris, 1993) and hydroxypropyl-methylcellulose (Haque *et al.*, 1993), both of which appear, from other evidence, to exist in solution as compact aggregates (whose partial dissociation is a necessary precursor to thermal gelation at higher temperatures), again suggesting that dika gum has a compact macromolecular structure rather than an expanded 'random coil' conformation.

Shear-thinning of okra gum is particularly unusual. The value of  $p$  required to match experimental flow curves is no longer constant, but varies widely with concentration (Fig. 9), showing a minimum ( $p \approx 0.43$ ) at  $\sim 1.3\%$  w/v and increasing steeply at higher and lower concentrations. A possible explanation is that the polysaccharide has a strong tendency to self-association, with the extent of aggregation increasing with increasing concentration. The initial effect of intermolecular asso-

ciation is to form compact 'bundles' with, as proposed for dika gum and commercial methylcelluloses, an accompanying reduction in  $p$ . At higher concentrations, the 'bundles' associate further into larger assemblies with greater overall flexibility and consequent increase in  $p$  towards the value of 0.76 found for disordered coils. Although highly speculative, this interpretation is consistent with electron-microscopy evidence of large, branched aggregates ( $>4\ \mu\text{m}$ ) in dispersions of okra gum (see BeMiller *et al.*, 1993).

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