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Dilute and semi-dilute solution properties of an exopolysaccharide from *Escherichia coli* strain S61

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Abstract

The solution properties of colanic acid, an exopolysaccharide slime isolated from *Escherichia coli* strain 61, have been investigated. Previous chemical analysis (Sutherland, 1969) had shown that this polysaccharide contained glucose, galactose and glucuronic acid, in addition to acetate and pyruvate. Three batches (S61-1, S61-2 and S61-3) of colanic acid were used for rheological measurements. The intrinsic viscosity of S61-1, S61-2 and S61-3 were found to be 20.8 ± 0.4 dl/g (0.2 M NaCl), 22.7 ± 0.6 dl/g (0.2 M NaCl) and 21.1 ± 0.3 dl/g (0.2 M $CaCl_2$) respectively, which indicates that the colanic acid is of high molecular weight. The semi-dilute solution characteristics, investigated by steady shear and dynamic rheometry, suggest that colanic acid acts as a well behaved polymer entanglement system. The viscosity-polymer concentration relationship of colanic acid shows no significant 'critical' breakpoint concentration on passing from a dilute to a semi-dilute solution. Instead, there seems to be a gradual change in slope from the first regime, ~1, to the second regime, ~3.6.

Keywords: *Escherichia coli*; Biofilms; Colanic acid; Exopolysaccharides; Semi-dilute solution; Viscosity; Oscillatory shear rheometry

1. Introduction

Exopolysaccharides are a main component of bacterial biofilms, but their contribution to biofilm structure and function has been examined for only a few organisms. In nature, many bacterial species live attached to each other and to surfaces (Gessey, Mutch, Costerton & Green, 1978), while in industry, biofilms can clog pipes and contaminate food systems, as well as forming on catheters and prostheses causing persistent, antibiotic-resistant infections (Costerton, Stewart & Geesey, 1999). However, biofilms also have beneficial functions, for example by acting as biocontrol agents in preventing fungal infections in certain plants (Geel & Schippers, 1983).

The name colanic acid was given by Goebel (Sutherland, 1969) to an exopolysaccharide slime synthesised by a mucoid *Escherichia coli* strain, which causes the formation of biofilms. Such biofilms can cause major problems, for example, in blocking the filtration stages of sewage processing plants. In vivo, *E. coli* strain 61 is covered in a layer of surface-associated polysaccharide, which has the same sugar components as the material synthesised by several *Salmonella* species (Sutherland, 1969). The perceived high viscosity suggests that it is of high molecular weight and the present work offers a detailed characterisation of the dilute and semi-dilute solution viscosity of the purified polysaccharide. Initial characterisation and preparation was given more than 30 years ago (Sutherland, 1969, 1999), and on the basis of this analysis a repeating hexasaccharide unit of the structure was proposed as seen in Fig. 1.

In the formation of biofilms there are two major mechanisms, initial adhesion to a foreign surface followed by formation of a viscous, macroscopic and three dimensional network-type structure. In the present case, the latter is clearly due to the colanic acid, whereas there appears to be some controversy in the literature about the former aspects. For example Danese, Pratt and Kolter, (2000) have suggested that colanic acid production is not required for surface attachment. Rather, colanic acid is critical for the formation of the complex three-dimensional structure and

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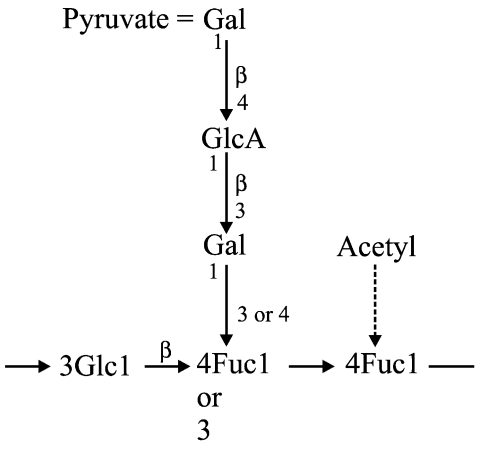

Chemical structure of colanic acid (Sutherland, 1969)

Fig. 1. The chemical structure of colanic acid (Sutherland, 1969).

depth of *E. coli* biofilms, whereas Razatos, Ong, Sharma and Georgiou (1998) who studied the surface aspects of a number of strains using atomic force microscopy seem more equivocal.

In the present work we have investigated the solution properties of colanic acid samples from the dilute to the semi-dilute concentrated range. Clearly this has implications for the biofilm, although we have not investigated through into the concentrated solution regime and the low water, or glassy, film stage.

Colanic acid contains glucuronic acid and, as the name suggests, is a polyelectrolyte. Consequently, since the solution properties of the charged polymer will change in different ionic strength solutions, the effects of different counterions (Na^+ and Ca^{2+}) on dilute solution viscosity were also investigated.

2. Materials and methods

2.1. Materials

The purified colanic acid sample was prepared at the Institute of Cell and Molecular Biology at the University of Edinburgh, and the purity of the colanic acid sample used in the current project is about 97% (w/w) on a dry weight basis. Three different batches were examined, the moisture content of the S61-1 (first batch) was found to be ~7.5% (w/w), the S61-2 (second batch) ~6.8% and S61-3 (the third batch) ~7.2%. The S61-1 and S61-2 were extracted with sodium chloride, and S61-3 was extracted with calcium chloride, to give single counterion forms.

2.2. Methods

2.2.1. Rheological methods

The concentration of the colanic acid sample was calculated on a dry weight basis and on the polysaccharide content. The solution was prepared by dissolving the colanic acid in the appropriate aqueous salt for 10 min at 80 °C with stirring, followed by stirring at room temperature overnight. The colanic acid sample was prepared, as required, by dissolving it in 0.2 M NaCl or 0.2 M $CaCl_2$ solution, and then dialysing the sample (relative molecular mass-RMM-cut-off ~5,000) using the same solvent. For dilute solution measurements, aggregates of the polymer and other insoluble material were removed by filtering the solutions with 0.2 μm and 0.8 μm Millipore filters (Gelman Sciences). The filtered solutions were then used to measure the intrinsic viscosity. Intrinsic viscosity measurements were also performed on unfiltered samples for comparative purposes.

The intrinsic viscosity of colanic acid was measured with a glass viscometer (Ubbelohde), using an appropriate range of concentrations, in this case 0.0143–0.054% w/w, and by dissolving it in 0.2 M NaCl or 0.2 M $CaCl_2$ solution. Steady and dynamic shear experiments on the colanic acid solution were carried out using a Rheometrics Fluids Spectrometer (RFS-II, Rheometric Scientific Ltd, Epsom, UK). These measurements were made over a wide range of concentrations from 0.019–1.5% (w/w) again using 0.2 M NaCl or 0.2 M $CaCl_2$ as solvent. In the lower concentration region a couette geometry was used (cup diameter 34 mm, bob diameter 32 mm and length 35 mm), whereas cone and plate geometry (diameter 25 mm, cone angle 0.02 radians) was used in the higher range.

3. Results

3.1. Intrinsic viscosity

In the range of concentrations used, the viscosity relative to that of the solvent (0.2 M NaCl and 0.2 M $CaCl_2$) lay in the acceptable range $1.2 < \eta_r < 2.0$. For example, intrinsic viscosity of the S61-2 (Batch 2) was determined by

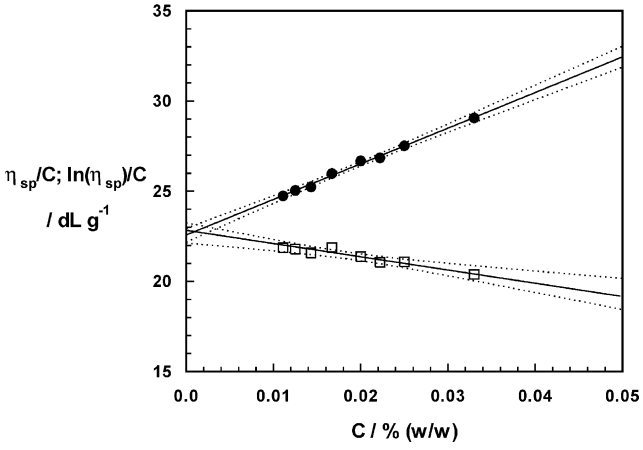

Fig. 2. The intrinsic viscosity of colanic acid (S61-2) dissolved in 0.2 M NaCl solution. Dotted lines indicate 95% confidence intervals, ● = η_{sp}/C, □ = $\ln(\eta_r)/C$.

Table 1
The intrinsic viscosity of S61-2 at different NaCl concentrations

Ionic strength/mol l^{-1}	0.2	0.02	0.002
$[\eta]$/dl/g	22.7 ± 0.62	25.2 ± 0.65	47.5 ± 1.5

The values (±95% confidence limits) were estimated from the intrinsic viscosity plot as seen in Fig. 2.

Huggins–Kramer extrapolation of the regression line and was found to be ~22.7 ± 0.6 dl/g (Fig. 2). At lower NaCl concentrations, the intrinsic viscosity of S61-2 was significantly higher than this (Table 1).

Fig. 3 shows the intrinsic viscosity of colanic acid (S61-2) as a function of the reciprocal of the square root of the ionic strength. From the plot, the intrinsic viscosity was ~19.8 dl/g at 'infinite' ionic strength.

3.2. Intra-and inter-batch variations

Intrinsic viscosity measurements of batches S61-1 and S61-3 were as carried out as above. The values found for S61-1 (also Na^+ ion form in NaCl) and S61-3 dl/g (Ca^{2+} ion form in $CaCl_2$) of ~20.8 and ~21.1 dl/g, respectively, differed somewhat from those found for S61-2, but it was not clear whether or not these differences were statistically significant. Consequently repeat measurements were made, and then an intra-(Table 2) and inter-(Table 3) batch comparison carried out using analysis of covariance (ANCOVA; general linear model, Minitab statistical analysis software, Edgbaston, Birmingham, UK). All the data employed for estimating intrinsic viscosity by extrapolation of the regression lines (e.g. see Fig. 2) were used for the ANCOVA, to establish whether the intercept and slope coefficients were statistically significant. ANCOVA showed replicate intrinsic viscosity measurements for the same batch to be in good agreement with each other. For the three different batches however, although the viscosity values at finite concentrations and slopes of the lines appear to be similar, the intercepts at $C \rightarrow 0$ were found to be significantly different ($P < 0.001$). This means that, strictly, these batches cannot be treated as identical samples and

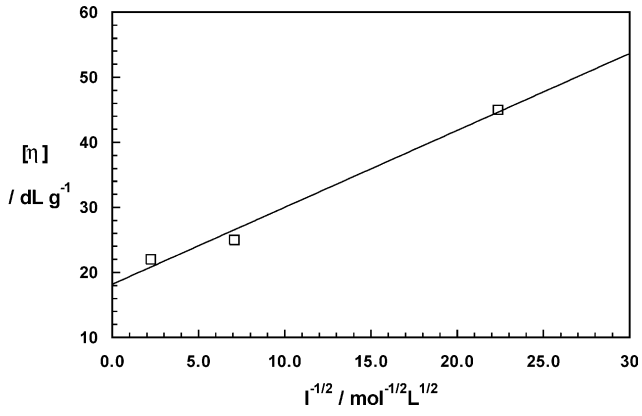

Fig. 3. Intrinsic viscosity $[\eta]$ of S61-2 plotted against the reciprocal square root of NaCl ionic strength.

Table 2
The intra-batch variation in the intrinsic viscosity of S61-2

	Measurement 1	Measurement 2	Measurement 3	Mean
$[\eta]$/dl/g	22.8 ± 0.5	23.2 ± 0.4	22.7 ± 0.62	22.9

The values (± 95% confidence limits) were estimated from the intrinsic viscosity plot (Fig. 2). ANCOVA showed no significant differences in the intercept values used for estimating intrinsic viscosity between the replicates.

Table 3
Intrinsic viscosities for different batches of colanic acid

Sample	S61-1	S61-2	S61-3
$[\eta]$/dl/g	20.8 ± 0.4	22.7 ± 0.6	21.1 ± 0.3

ANCOVA showed that there were significant differences in the intercept values used for estimating intrinsic viscosity between the different batches ($P < 0.001$). Thus, intrinsic viscosity varies between different batches.

suggests that the molecular weight (M_w) varies slightly, but significantly, between batches.

3.3. Steady shear measurements

For S61-2 only the intrinsic viscosity was determined because of the limited amount of sample available, so these measurements were only performed for S61-1 and S61-3. Fig. 4 shows the viscosity versus shear rate in the range of 10^{-2}–10^3 s^{-1} for S61-1. At concentrations lower than 0.054%, viscosity was effectively independent of shear rate. As might be expected, at higher concentrations shear-thinning behaviour becomes more apparent even at lower shear rates, and this became still more pronounced as the polymer concentration increased. The zero shear viscosity at different concentrations was calculated using the Cross equation (1965), and a reasonable fit was found at concentrations greater than 0.11% (w/w). However, at lower concentrations (from 0.054 to 0.019%) there is almost no shear rate dependence.

When data for both S61-1 and S61-3 were plotted, the relationship between log zero-shear specific viscosity, $\eta_{sp,0}$ and log coil overlap parameter $c[\eta]$, was clearly non-linear (Fig. 5). Here the concentration dependence of the zero-shear specific viscosity, $\eta_{sp,o}$, for polysaccharide solutions is presented as a double logarithmic plot against the coil overlap parameter $c[\eta]$. Following the early work of Bueche for synthetic polymers, this approach has been employed by many other workers characterising polysaccharide solutions (Campana, Andrade, Milas & Rinaudo, 1990; Chronakis, Doublier & Piculell, 2000; Morris et al., 1981; Gravanis, Michel, Rinaudo & Tinland, 1987; Kapoor, Milas, Taravel & Rinaudo, 1994; Robinson, Ross-Murphy & Morris, 1982; Stokke, Elgsaeter, Bjornestad & Lund, 1992; Wang, Ellis, Ross-Murphy & Burchard, 1997).

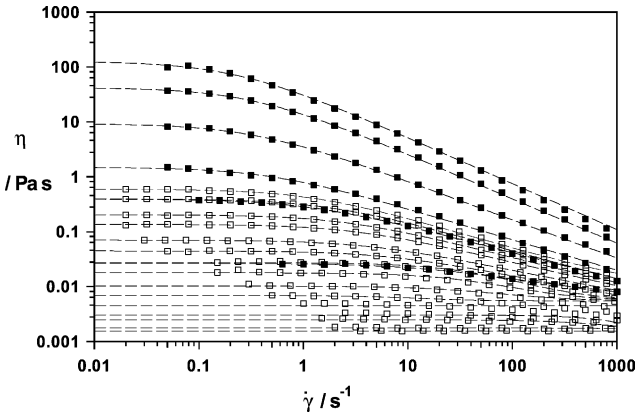

Fig. 4. Shear rate dependence of viscosity for different concentrations of sample S61-1 (■ cone and plate geometry; □ couette geometry). Lines represent the least squares fitted Cross equation.

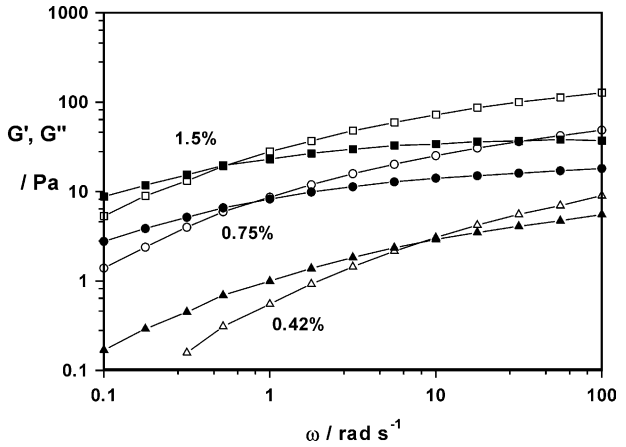

Fig. 6. G' (open symbols) and G'' (closed symbols) for 1.5, 0.75 and 0.42% w/w solutions of sample S61-3.

3.4. Dynamic measurements

In dynamic measurements, both strain and frequency are very important parameters, because only at lower strain limits will G^* and η^* be independent of strain. For these colanic acid solutions, G^* values were found to be reasonably independent of strain from 0.25 to > 25%, and so 25% strain was used in subsequent frequency sweep experiments.

Fig. 6 shows the storage modulus G′ and loss modulus G'' plotted against frequency ω. When ω is lower than 0.6 rad s^{-1}, 1.5, 0.75 and 0.42% w/w colanic acid solutions were predominantly liquid-like. When the frequency increased, the storage modulus G' increased faster than the loss modulus G'', as expected for viscoelastic fluids. There was a cross-over between G' and G'' $\omega \sim 7.5$, 0.8, and 0.42 rad. s^{-1} for solution concentrations 0.42, 0.75 and 1.5%, respectively. Again, as expected, this frequency decreases with increasing concentration.

For many polymer solutions, the frequency dependence of η^* and the shear rate dependence of η are found to be similar, when the same numerical values of ω and $\dot{\gamma}$ are compared. This empirical correlation is known as the Cox–Merz rule (Lapasin & Pricl, 1995; Ross-Murphy, 1994). In the present case, the frequency dependence of η^* and the shear rate dependence of η were found to be virtually identical at different concentrations of colanic acid solution (Fig. 7). The zero-frequency dynamic viscosity was calculated using the Cross equation (1965).

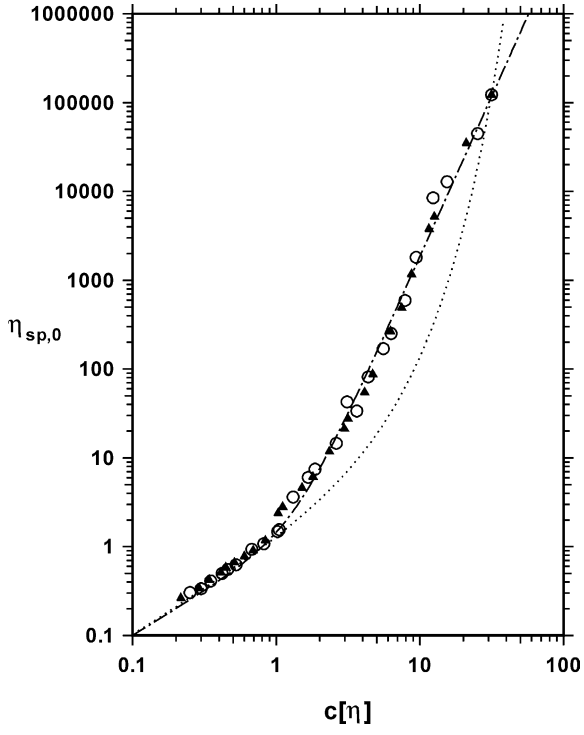

Fig. 5. Concentration dependence of zero-shear specific viscosity for samples S61-1 (▲) and S61-3 (○). Dotted line: Martin model; dash-dot line: modified Kulicke model.

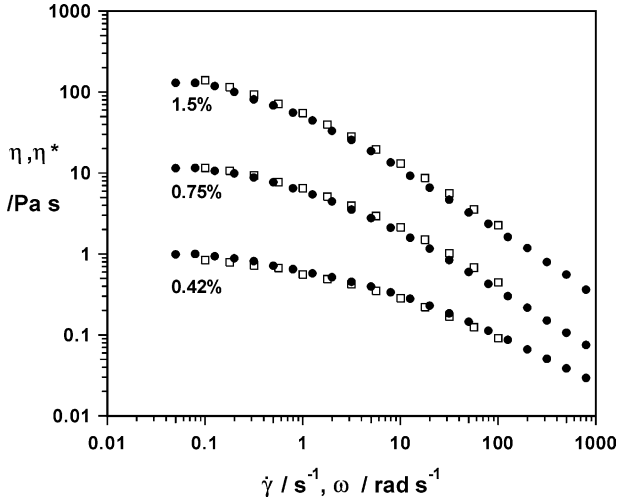

Fig. 7. Cox-Merz plot of steady shear (●) and dynamic (□) viscosities for S61-3, 1.5, 0.75 and 0.42% w/w solutions.

4. Discussion

4.1. Effect of salt on intrinsic viscosity

In Fig. 3, where the intrinsic viscosity $[\eta]$ was plotted against $I^{-0.5}$, a linear dependence was seen. In fact, according to the empirical Smidsrød–Haug method (Launay, Doublier & Cuvelier 1986), the flexibility of a charge-bearing polymer chain is directly proportional to the Smidsrød empirical stiffness parameter, B, defined according to the equation:

$$B = S/([\eta]_{0.1})^{1.3} \quad (1)$$

where S is the slope of the $[\eta]$ versus $I^{-0.5}$ plot. From this equation, $S = 1.18$ and $[\eta] = 19.80$ dl/g were used to calculated the B value for colanic acid, which was found to be 0.42. This value was significantly higher than, for example, carboxymethylamylose ($B = 0.20$, Launay et al., 1986), κ-carrageenan ($B = 0.10$, Launay et al., 1986) and the polysaccharide from *Klebsiella pneumoniae* K40 ($B = 0.12$, Cescutti, Toffanin & Kvam, 1993), indicating that the colanic acid was less stiff than these polymers. In terms of the Smidsrød–Haug B parameter, therefore the colanic acid polymer is relatively flexible, although some caution has to be used in employing this method as pointed out, for example, by Tobitani and Ross-Murphy (1997).

4.2. Shear thinning behaviour fitted to Cross model

The shear-thinning behaviour of colanic acid was described above, and the Cross equation (Cross, 1965) was found to fit this behaviour very well (Fig. 4). The Cross equation has also been used to describe shear-thinning behaviour of a number of polysaccharides including detarium xyloglucan (Wang et al., 1997) and guar galactomannan (Rayment, Ross-Murphy & Ellis, 1995). For interest, we compared the fit obtained omitting the lowest four shear rate data. Although the Cross equation could still be used to fit the data, the confidence limits increased compared with the results from Fig. 4, but the estimated η_0 was within these bounds. Although other methods have been employed to estimate η_0, when there is a paucity of low shear rate data (Morris, 1990) we feel the Cross method is the most appropriate. Elsewhere (Ren, 2000) we give a more detailed comparison to justify this.

4.3. Viscosity-polymer concentration relationship

Examination of Fig. 5 suggests there is no significant 'critical' breakpoint concentration in the experimental data on going from a dilute to a semi-dilute solution. Instead, there seems to be a gradual change in slope from the first regime ~1 to the second regime ~3.6. As mentioned above, it is customary to refer to the generalised (so-called Bueche) representation with co-ordinates $\eta_{sp,0}$ and $C[\eta]$. The dilute and semi-dilute regime can be fitted with separate exponents, where the exponent for the first concentration regime is ~1.0–1.5 and for the second concentration regime is ~3.5–4.5. Many polysaccharides such as guar gum, locust bean gum, dextran, carboxymethylamylose, and λ-carageenan can be fitted to this form of master curve (Morris et al., 1981; Lapasin & Pricl, 1995).

Several equations can be used to fit such data. In our case, the first of these is that attributed to Martin (Graessley, 1974):

$$\eta_{sp,0} = c[\eta]_0 \exp(k_m c[\eta]_0) \quad (2)$$

where k_m is the Martin polymer–polymer interaction parameter. For xanthan gum, a good fit of experimental data to the Martin equation has been obtained (Launay et al., 1986). Our colanic acid data was also fitted to the Martin equation (Fig. 5, dotted line), but it is clear this model does not fit particularly well.

Another equation, due to Kulicke and Keniewske (1984), is written as follows:

$$\eta_{sp,0} = c[\eta]_0 + k_1(c[\eta]_0)^2 + k_2(c[\eta]_0)^n \quad (3)$$

where n is an exponent, and k_1 and k_2 are coefficients. This model has been fitted successfully to data for solutions of succinoglycan (Gravanis, Milas, Rinaudo & Clarke-Sturman, 1990), welan (Campana et al., 1990) and the seed galactomannan from *Cassia nodosa* (Kapoor et al., 1994), although the first two appear to be structured liquids (sometimes called 'weak gels') and only the last is comparable rheologically with the present system.

This equation was used to fit data obtained from colanic acid (S61-1 and S61-3) and seemed to fit this model well. We also employed a simplified two parameter version of the equation above (i.e. setting the coefficient $k_1 = 0$) giving:

$$\eta_{sp,0} = c[\eta]_0 + a(c[\eta]_0)^{n'} \quad (4)$$

Table 4 illustrates the best fit values of these parameters, minimising the sum of squares of the log ($\eta_{sp,0}$) differences as is usual for data collected over such a wide range. Fig.5 illustrates the different calculated curves. It is clear from this figure that the Martin equation gives a poor fit compared with the Kulicke models. The difference between the two Kulicke models is far less obvious, and is not shown here;

Table 4
Parameters determined by Martin, Kulicke and modified Kulicke equations for solutions of both colanic acid samples S61-1 and S61-3, combined data

Martin model		Kulicke model		Modified Kulicke model	
K_m	0.33	k_1	0.66	a	0.49
–	–	k_2	0.31	–	–
–	–	n	3.77	n'	3.60
Σ^{2a}	1.29	Σ^2	0.38	Σ^2	0.57

[a] Here Σ^2 is the sum of squares of the difference between $\log(\eta)_{measured}$ and $\log(\eta)_{calculated}$ for the various models.

indeed close inspection shows any discrepancies are obvious only when $0.5 < c[\eta] < 2$. This, nevertheless, asks the question of approaches based like the Bueche form, and more sophisticated versions of these, on a number of 'broken' straight lines.

The argument for these is embedded in the expectation that polymer systems behave in a so-called universal fashion. For example scaling models, e.g. de Gennes (1979), predict a sharp viscosity transition between dilute and semi-dilute (and in some cases a third transition to more concentrated) solution behaviour. Two points need to be made, however. Firstly most theories are based not on the transition of polymer solutions, but the analogous, but by no means, identical transition observed for the M_w dependence for polymer melts. Secondly, these so-called scaling law arguments apply (best, and even only) for infinitely long monodisperse polymer chains. It is well known that the effects of finite chain length and M_W distribution tend to 'smear out' such sharp transitions. Finally, an appeal either to statistics, or Occam's razor, makes clear that if a satisfactory fit can be obtained with a two parameter model, the burden of proof is on the proposer of more sophisticated models to justify their alternative *statistically*, rather than the converse. For example, a three broken straight-line model has, at least, four parameters, compared to the two of Eq. (4).

4.4. Dynamic measurements

These are quite consistent with the above picture of 'simple' entanglement solution behaviour. The (relatively) high value of the non-linear strain in the strain sweep experiment, the form of the mechanical spectra and the Cox–Merz superposition all lend weight to this conclusion. All are of the expected entanglement form (Lapasin & Pricl, 1995; Ross-Murphy, 1994) and we do not discuss them further.

5. Conclusions

The overall conclusion from this study is that results from intrinsic viscosity determinations are consistent with those of a high molecular weight semi-flexible polymer. This is deduced both from the absolute value of $[\eta]$, which in 0.2 M NaCl is greater than 20 dl/g, and from its significant increase with decreasing ionic strength. Part of the contribution to the high molecular weight will come, of course, from the high residue mass of the hexasaccharide repeat unit, but some must come from the chain length (or degree of polymerisation) which can be estimated, albeit crudely, to be $> \sim 5$–10,000.

The results from semi-dilute solution measurements support this view, in that they are typical of those for a so-called entangled solution. There is good agreement with the $\eta_{sp,0}$ versus $c[\eta]$ 'master curve' behaviour seen for other so-called random coil polysaccharides. The form of the shear rate dependence reinforces this, with a clear zero-shear rate viscosity plateau being reached at all, except the highest concentrations, and the 'Cox–Merz' superposition of steady and oscillatory shear viscosities at comparable rates.

What then has this to say about the extra-cellular polysaccharide exudate which occurs in vivo? Without being overly anthropomorphic, it would appear to be a biological imperative that a high viscosity is achieved at relatively low concentrations. This indeed is what is seen, and the prevalence of such biofilms is a major problem in disposal of industrial fluid wastes. In this respect the results obtained here are confirmatory of what might have been expected, rather than surprising in themselves. Interestingly, however, the behaviour of the samples was even more 'ideal' than we might have expected, and the quality of the resultant shear rate viscosity trace was agreeably good.

Future work should certainly examine the sample under other conditions. For example we did not investigate the temperature dependence of $[\eta]$ to see if any structural change is seen on heating. The technique of pressure cell heating of dilute solutions as a means to obtaining 'molecular solutions' (Picout, Ross-Murphy, Errington & Harding, 2001; Picout, Ross-Murphy, Jumel & Harding, 2002) could also be employed, to allow static light scattering as a further probe of molecular weight and chain flexibility. Nevertheless as a rheological investigation of the solution properties of colanic acid this work is essentially complete, and we were gratified with the self-consistent nature of the results.

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References

Campana, S., Andrade, C., Milas, M., & Rinaudo, M. (1990). Polyelectrolyte and rheological studies on the polysaccharide welan. *International Journal of Biological Macromolecules*, *2*, 379–384.

Cescutti, P., Toffanin, R., & Kvam, B. J. (1993). Structural determination of the capsular polysaccharide produced by *Klebsiella pneumonae* serotype K40-NMR-studies of the oligosaccharide obtained upon depolymerization of polysaccharide with a bacteriophage-associated endoglycanase. *European Journal of Biochemistry*, *213*(1), 445–453.

Chronakis, I. S., Doublier, J. L., & Piculell, L. (2000). Viscoelastic properties for Kappa-and iota-carrageenan in aqueous NaI from the liquid-like to the solid-like behaviour. *International Journal of Biological Macromolecules*, *28*, 1–14.

Costerton, J. W., Stewart, P. S., & Geesey, G. G. (1999). Bacterial biofilms: a common cause of persistent infections. *Sciences*, *284*, 1318–1322.

Cross, M. M. (1965). Rheology of non-Newtonian fluids: a new flow equation for pseudoplastic systems. *Journal of Colloid Science*, *20*, 417–437.

Danese, P. N., Pratt, L. A., & Kolter, R. (2000). Exopolysaccharide production is required for development of *Escherichia coli* K-12 biofilm architecture. *Journal of Bacteriology*, *182*, 3593–3596.

De Gennes, P.G. (1979). *Scaling concepts in polymer physics*. Ithaca, NY, USA: Cornell University Press.

Geel, F. P., & Schippers, B. (1983). Reduction of yield depressions in high frequency potato cropping soil after seed tuber treatments with antagonistic fluorescent *Pseudomonas* spp. *Phytopathologische Zeitschrift—Journal of Phytopathology*, *108*, 207–214.

Gessey, G. G., Mutch, R., Costerton, J. W., & Green, R. B. (1978). Sessile bacteria: an important component of the microbial population in small mountain streams. *Limnology and Oceanography*, *23*, 1214–1223.

Graessley, W. W. (1974). The entanglement concept in polymer rheology. *Advances in Polymer Science*, *16*, 1–179.

Gravanis, G., Michel, M., Rinaudo, M., & Tinland, B. (1987). Comparison of the bacterial polysaccharides xanthan and succinoglycan. *Carbohydrate Research*, *160*, 259–265.

Gravanis, G., Milas, M., Rinaudo, M., & Clarke-Sturman, A. J. (1990). Rheological behaviour of a succinoglycan polysaccharide in dilute and semi-dilute solutions. *International Journal of Biological Macromolecules*, *6*, 201–206.

Kapoor, V. P., Milas, M., Taravel, F. R., & Rinaudo, M. (1994). Rheological properties of seed galactomannan from *Cassia nodosa* Buchheim. *Carbohydrate Polymers*, *25*, 79–84.

Kulicke, W. M., & Keniewske, R. (1984). The shear viscosity dependence on concentration, molecular weight and shear rate of polystyrene solutions. *Rheologica Acta*, *23*, 75–83.

Lapasin, R., & Pricl, S. (1995). *Rheology of industrial polysaccharides: Theory and applications*. Glasgow, UK: Blackie Academic and Professional.

Launay, B., Doublier, J. L., & Cuvelier, G. (1986). Flow properties of aqueous solutions and dispersions of polysaccharides. In J. R. Mitchell, & D. A. Ledward (Eds.), *Functional properties of food macromolecules* (pp. 1–78). London: Elsevier Applied Science Publishers.

Morris, E. R. (1990). Shear-thinning of random coil polysaccharides: Characterisation by two parameters from a simple linear plot. *Carbohydrate Polymers*, *13*, 85–96.

Morris, E. R., Cutler, A. N., Ross-Murphy, S. B., Rees, D. A., & Price, J. (1981). Concentration and shear rate dependence of viscosity in random coil polysaccharide solutions. *Carbohydrate Polymers*, *1*, 5–21.

Picout, D. R., Ross-Murphy, S. B., Errington, N., & Harding, S. E. (2001). Pressure cell assisted solution characterisation of polysaccharides. 1. Guar gum. *Biomacromolecules*, *2*, 1301–1309.

Picout, D. R., Ross-Murphy, S. B., Jumel, K., & Harding, S. E. (2002). Pressure cell assisted solution characterisation of polysaccharides 2: Locust bean gum and tara gum. *Biomacromolecules*, *3*, 761–767.

Rayment, P., Ross-Murphy, S. B., & Ellis, P. R. (1995). Rheological properties of guar galactomannan and rich starch mixtures. I. Steady shear measurements. *Carbohydrate Polymers*, *28*, 121–130.

Razatos, A., Ong, Y. L., Sharma, M. M., & Georgiou, G. (1998). Molecular determinants of bacterial adhesion monitored by atomic force microscopy. *Proceedings of the National Academy of Sciences*, *95*, 11059–11064.

Ren, Y (2000). Rheological and structural studies on novel microbial and plant polysaccharides. PhD Thesis, Division of Life Sciences, King's College London, University of London, UK.

Robinson, G., Ross-Murphy, S. B., & Morris, E. R. (1982). Viscosity-molecular weight relationships, intrinsic chain flexibility and dynamic solution properties of guar galactomannan. *Carbohydrate Research*, *107*, 17–32.

Ross-Murphy, S. B. (1994). Rheological methods. In S. B. Ross-Murphy (Ed.), *Physical techniques for the study of food biopolymers* (pp. 343–392). Glasgow, UK: Blackie Academic and Professional.

Stokke, B. T., Elgsaeter, A., Bjornestad, E.Ø., & Lund, T. (1992). Rheology of xanthan and schleroglucan in synthetic seawater. *Carbohydrate Polymers*, *17*, 209.

Sutherland, I. W. (1969). Structural studies on the colanic acid, the common exopolysaccharide found in the enterobacteriaceae, by partial acid hydrolysis; oligosaccharides from colanic acid. *Biochemical Journal*, *155*, 935–948.

Sutherland, I. W. (1999). Polysaccharases for microbial exopolysaccharides. *Carbohydrate Polymers*, *38*, 319–328.

Tobitani, A., & Ross-Murphy, S. B. (1997). The intrinsic viscosity of polyelectrolytes revisited. *Polymer International*, *44*, 338–347.

Wang, Q., Ellis, P. R., Ross-Murphy, S. B., & Burchard, W. (1997). Solution characteristics of the xyloglucan extracted from Detarium senegalense Gmelin. *Carbohydrate Polymers*, *33*, 114–124.