Carbohydrate Polymers **14** (1991) 281–294

The Estimation of Junction Zone Size from Geltime Measurements

Simon B. Ross-Murphy*

Cavendish Laboratory, University of Cambridge, Madingley Road, Cambridge CB3 0HE, UK

(Received 28 June 1989; revised version received 10 November 1989; accepted 13 November 1989)

ABSTRACT

The model recently proposed to estimate the 'molecularity parameter' n *in the junction zone model of biopolymer gelation from the slope of a log–log plot of reciprocal geltime versus concentration is discussed. In contradiction to the predictions of this simple model, the slope of the latter plot is shown not to be constant but to diverge at the critical concentration* C_0*. Moreover, even in the high concentration regime where the slope approaches linearity, the calculated slope is a more complex function of* n *than previously assumed. A more rigorous model is derived from polymer network theory, and compared with previously published experimental data for carrageenan and serum albumen gels.*

INTRODUCTION

A recent series of papers by Oakenfull and co-workers (Oakenfull & Scott, 1986, 1988; Oakenfull & Morris, 1987) proposes an attractively simple method for the determination of the molecularity parameter n for the formation of physical gels from cross-links involving intermolecular 'junction zones'. Such junction zones are now accepted to be involved in many gels formed from biopolymers, including those where the junction zones are formed from multiple helices, e.g. double helices ($n=2$)

*Permanent address: Unilever Research, Colworth House, Sharnbrook, Bedford MK44 1LQ, UK.

Carbohydrate Polymers 0144-8617/90/$03.50

(carrageenans, agarose) and triple helices ($n = 3$) (gelatin, curdlan) (Clark & Ross-Murphy, 1987). We show in the present paper, however, that the exponent n' extracted from the plot proposed by Oakenfull, viz. log of 'gelation rate' against log concentration is not in general equal to n, nor is it constant. Values cited in the above papers, although consistent with current molecular understanding (Morris, 1986), must rather be regarded with some trepidation. In particular, and contrary to the expectation of this simple model, more accepted treatments of gelation do not lead to a simple n; rather they suggest that the apparent value n' should increase as the critical gel concentration C_0 is approached, and become infinite at C_0 (Clark & Ross-Murphy, 1985, 1987).

THE GELATION KINETICS METHOD

The details of the Oakenfull gelation kinetics method are as follows: it is proposed that the time required to form a gel of small, but precisely determined, rigidity is a measure of the rate of gelation, and that the setting time (geltime) is inversely proportional to the initial rate. By confining measurements to the very early stages of the gelation process, it is then proposed that, since each potential cross-linking locus (L) along a chain acts as an independent species in solution, when n of these form a junction zone (J) then

$$nL \rightarrow J$$

and the rate of gelation v is given by

$$v = \mathrm{d}[J]/\mathrm{d}t = kC^{\mathrm{n}} \qquad (1)$$

where k is a rate constant, $[J]$ is the concentration of J units and C is the polymer concentration. Following this argument, the slope of log (rate of gelation) versus log (concentration) gives a 'reaction order', which is assumed to be the number of polymer chains participating in the formation of a junction zone.

In the above treatment, the gelation rate is assessed by pouring a fixed amount of pre-gel solution into a series of small vials held in a thermostatted waterbath. These vials are then inverted sequentially, and the time, t_c, required to form a gel just strong enough to remain held in position is recorded, for each of a series of concentrations. The rate of gelation is then assumed to be proportional to $1/t_c$, a procedure equivalent to estimating the rate constant from the initial rate method of chemical kinetics.

Analysing data for ι- and κ-carrageenan by this method, n was found to be $4{\cdot}5 \pm 0{\cdot}5$ for ι-carrageenan, and $12{\cdot}5 \pm 0{\cdot}9$ for the κ sample. In Fig.

1a we reproduce the original data (Oakenfull & Scott, 1988), whilst Fig. 1b (inset) shows this same data plotted as $\log(1/t_c)$ against a linear concentration axis, as proposed by Richardson and Ross-Murphy (1981). The values for n noted above were argued to be reasonable, in view of the 'domain model' of carrageenan gelation proposed by Morris *et al.* (1980), in which the ion-mediated gelation is assumed to involve

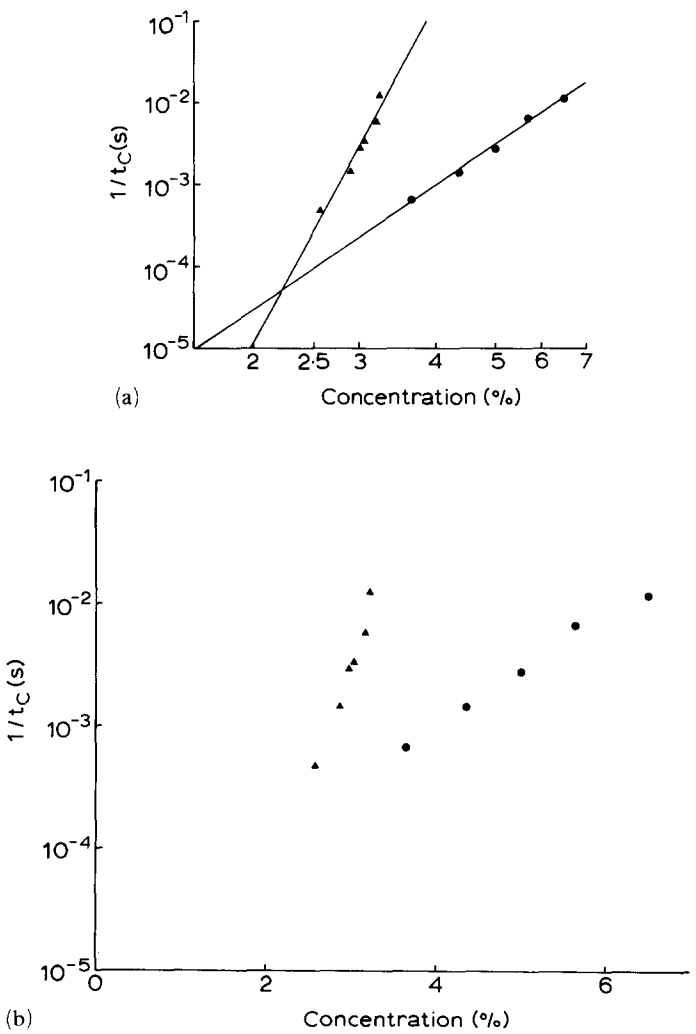

Fig. 1. (a) The data of Oakenfull and Scott (1984) for ι- (●) and κ-carrageenan (▲). Gelation rate is determined as described in this and by Oakenfull and Morris (1987); (b) the same data is plotted against concentration on a linear axis as in Richardson and Ross-Murphy (1981).

the stacking of double helices, into a fringed micellar type structure. In other words, from the above plot it was suggested that ι-carrageenan junction zones involve two to three, and κ-carrageenan around six, such associated double helices.

CRITICISMS OF THE KINETIC MODEL

Number of chains involved in a junction zone

The first, and perhaps most direct, argument against the kinetic approach is that it requires that n-fold collisions of polymer occur, and that these n polymer chains are simultaneously involved in the nucleation of the junction zones. Binary collisions are, of course, very common, and even ternary collisions can occur (although with a much lower probability than binary), but values of $n > 3$ seem to be of progressively much decreased likelihood. In fact some recent work on gelatin, where n might be asserted to be equal to three has confirmed that the reaction order of gelatin renaturation (monitored by the usual optical rotation technique) is equal to one at low concentrations, increasing to two at higher concentrations (Busnel *et al.*, 1988, 1989).

The first-order reaction rate is, in this case, associated with intramolecular helix formation, which 'wastes' cross-links. In other words, not all of the observed helix renaturation results in elastically effective junction zones since, in the absence of 'trapped entanglements' (Langley, 1968; Ferry, 1980), these can only involve intermolecular helices. The second-order process occurring at higher concentrations is assumed to involve an anti-parallel, triple-stranded, double-chain helix. This model reduces the topological complexities of triple helix, but also illustrates how efforts are being made to rationalise the mechanism of gelatin renaturation in terms of $n = 2$ models. Of course, subsequent side-by-side helix aggregation could then occur; but for $n > 3$ this implies a much more cooperative process than is currently accepted. It also suggests a system which is in a thermodynamically extremely poor solvent resulting in, at best, a biphasic gel.Certainly structural and mechanistic studies of the carrageenans and of gelatin are not consistent with a non-nucleated spinodal decomposition mechanism for gelation suggested for some other systems (Miller *et al.*, 1974).

Absence of a critical gel concentration

The kinetic scheme of eqn (1) requires that gelation will always occur, at any concentration provided that a long-enough time has elapsed. This

follows because $[J]$ is a monotonically increasing function of time for $C>0$ and $n>0$. However, a critical concentration must exist below which gelation can never occur. This concentration may be defined theoretically, and also measured, although in a more pragmatic way. There are arguments in the literature which suggest that this concentration, here denoted C_0, may be related to the C^* overlap, in particular suggesting that $C_0 = C^*$, but there are certainly many counter-examples — in some cases $C_0 < C^*$ and in other cases $C_0 > C^*$ — and a prolonged discussion of this issue is given by Clark and Ross-Murphy (1987). However, the presence of such a concentration is not disputed, and is related theoretically to the Flory (1941*a*, *b*) gelpoint requirement, i.e. that a critical number of cross-links (junction zones) per primary polymer chain are required to produce a continuous gel network. Further aspects of this requirement are discussed below, but the crucial point is that v, the gelation rate defined in eqn (1) will always be zero for concentrations less than C_0. What this requires is that there must be (at least) one other term in the kinetic scheme of eqn (1), either a back reaction (equilibration) of the form of the Ostwald dilution law, as assumed by other workers (Hermans, 1965; Clark & Ross-Murphy, 1985, 1987; Gordon, 1986) and explicitly stated in the Oakenfull–Scott calculation of the gel modulus (Oakenfull, 1984; Oakenfull & Scott, 1984), or a wastage term, involving intramolecular reaction steps (Chatellier *et al.*, 1985; Durand *et al.*, 1985), sometimes known as cyclisation (Ross-Murphy & Stepto, 1986). Both of these lead naturally to a more physical model of the gelation rate.

Relationship between gel modulus and [*J*]

The assumption that a gel is self-supporting when the modulus becomes greater than a certain value (say G_c) is probably not unreasonable. Of course, inverting any particular vial is not a simple rheological experiment, since both shear and tensile components may be present, it is by definition a large deformation experiment (at least for the samples which are judged not to have gelled) and the deformation rate is unknown. It also requires that the adhesion of the gels is not itself a strong function of concentration. However, for a series of samples of a given polymer made under identical conditions, these factors may not be dissimilar. Nevertheless, even accepting that there is a sharp distinction between ungelled (with $G < G_c$) and gelled ($G > G_c$) samples, the assumption that G_c is proportional to $[J]$, the concentration of junction zones, is a very poor one. In particular, following the argument above, at and below C_0 the

equilibrium modulus G is zero, and becomes finite only for $C > C_0$. There is a corresponding critical value of $[J]$, here denoted $[J_c]$. The modulus is therefore *not dependent simply upon* $[J]$ *but upon the difference between* $[J]$ *and* $[J_c]$. It is usual to write this dependence in the form (Stauffer *et al.*, 1982):

$$G \approx (([J]/[J_c]) - 1)^p \tag{2}$$

for small values of $[J]/[J_c]$. Here the 'critical exponent' $p = 3$ in the classical theory of percolation on a tree-like or Bethe lattice (Gordon & Ross-Murphy, 1975) and around 1·8 for percolation on a cubic lattice (Stauffer *et al.*, 1982). These exponents are strictly only appropriate extremely close to the gel point, i.e. $[J]/[J_c] \approx 1$; at higher degrees of cross-linking there is a 'wastage' effect, whereby not all of the cross-links lead to the formation of elastically effective chains. In other words, if we are to apply such a formula for $[J]/[J_c] > 1$, the measured exponent will be less than this 'critical value'.

For the moment the actual value of the exponent is not important. What is significant is that for low-modulus gel $[J]$ is only a little greater than $[J_c]$, so that the term $(([J]/[J_c]) - 1)$ is less than 1, and the dependence of G on $[J]$ becomes very non-linear as is illustrated in Fig. 2. The slope of a $\log G$ versus $\log[J]$ plot is proportional to $[J_c]/([J] - [J_c])$ and this behaves as expected, the slope increasing and becoming infinite as $[J] \to [J_c]$ from above. In later calculations we will use the value $p = 2$, which is not far above the percolation exponent and sufficiently below the classical exponent to include implicitly some wastage effects.

FEATURES OF A MORE REALISTIC MODEL

A more realistic model than that assumed by Oakenfull and co-workers must still predict very high slopes in the plot of log (gelation rate) versus log (concentration) for gels since, apart from Oakenfull's own work, these have repeatedly been reported for a wide range of systems. For example, Bisschops (1955) noted that the slope of modulus growth, dG/dt, was proportional to C^{22} for physical gels of poly(acrylonitrile) in DMF, and we have reported that for heat set globular protein gels this slope is $\propto C^{27}$ at low concentrations, and even at higher concentrations ($> 3C_0$), it is $\propto C^6$ (Richardson & Ross-Murphy, 1981). However, the slope is neither constant, nor can it be related to the junction zone size, since for the globular protein gels the gelation mechanism is fundamentally different.

It is possible to reproduce qualitatively some features of the expected behaviour by including a minimal number of assumptions, and we illustrate this below in a series of steps. This calculation is only semi-quantitative, since we are ignoring a number of important factors. Nevertheless, we hope it will predict the correct curvature, and that the parameters obtained will be reliable. In other words, it should retain the character of the overall behaviour.

1. *Assume d[J]/dt is proportional to C^2 — a second-order mechanism implying a binary intermolecular complex in the activated complex*

From assumption 1 we have:

$$-\mathrm{d}[L]/\mathrm{d}t = \mathrm{d}[J]/\mathrm{d}t = kC^2 \text{ (or more generally} = k_n C^n) \qquad (3)$$

Arguments against high exponents for C were given above. Here there is no specific wastage mechanism to give a critical concentration. This will be incorporated by choosing an arbitrary $[J_c]$

2. *Assume that the geltime corresponds to a fixed low-modulus system, and that the relationship between modulus and [J] is calculated using our adopted value ($p = 2$)*

(Much more sophisticated, and realistic solutions to this problem are available, including that by Peniche-Covas *et al.* (1974) specific to the junction zone model, but are not used here.)

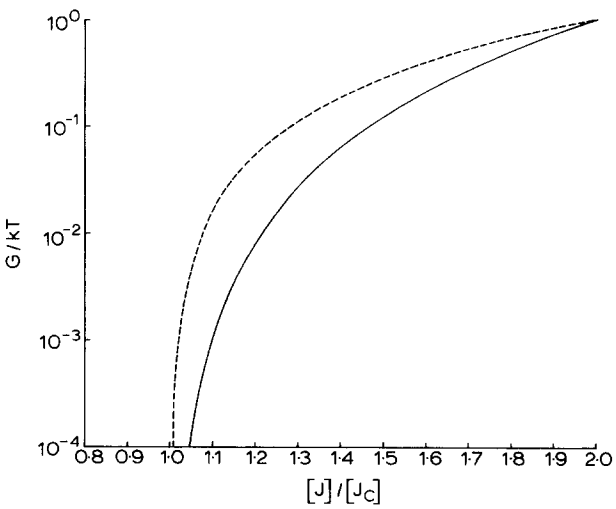

Fig. 2. $[J]/[J_c]$ plotted against reduced modulus (G/kT) — here $p = 3$ (solid line) and $p = 1{\cdot}8$ (dotted line).

To apply assumption 2 fully we need to consider a number of factors. In particular, the gel point will be defined in terms of $[J_c]$, and so the geltime t_c will be given by the time when $[J]=[J_c]$. The geltime could, in principle, be calculated from any of the kinetic schemes discussed above, but the simplest way to examine the dependence of geltime on concentration is to say that the measured gelpoint corresponds to the sample having a small, but finite modulus, G_c. Thus from any equations of the form of eqn (2), this modulus will correspond to a small, but constant ratio (J_r) of $[J]/[J_c]$ a little greater than one. As explained earlier, for gelation to occur, for concentrations less than the critical concentration C_0, the ratio J_r must always be less than one, however long is the time. However, in the absence of another process in the kinetic scheme (either a back reaction leading to an equilibrium state, or an alternative 'wastage' mechanism), this is necessarily a somewhat artificial constraint.

Regardless of this, the simplest way of calculating J_r actually does not require us to solve these kinetic equations at all. Instead when we assume that J_r is very small the ratio $[J]/[J_c]$ is very well approximated by the differential ratio $d[J]/d[J_c]$. Since the concentration corresponding to $[J_c]$ is the critical concentration C_0, we can use eqn (3) and say

$$J_r \approx d[J]/d[J_c] \approx (d[J]/dt)/(d[J_c]/dt) = kC^2/kC_0^2$$

and more generally we have the result that

$$J_r \approx (C/C_0)^n \tag{4}$$

CONCLUSIONS

If we substitute eqn (4) into eqn (2) we can then say

$$G \approx [(C/C_0)^n - 1)^p]$$

and finally since as $C \rightarrow C_0$, then $G \rightarrow 0$ and $t_c \rightarrow \infty$, we now assume that $G \approx 1/t_c$, so that

$$t_c \approx K/[((C/C_0)^n - 1)^p] \tag{5}$$

where K is a proportionality constant. In practice, the inverse relationship between G and t_c is quite well obeyed (Richardson & Ross-Murphy, 1981). Equation (5) has, of course, been obtained by employing a number of approximations, but despite this we feel it will behave correctly, at least for small C/C_0; what is of interest is the values of K and p (assuming that $n=2$). For illustration the function is plotted double logarithmically (Fig. 3) for the simplest case when $n=2$, and $p=2$. The

major features of this curve are of the form expected, but in no region can we locate an actual exponent, n' of 2; much higher exponents would be extracted from gradients drawn at any point, the slope n' of the plot of log (gelation rate) against $\log(C/C_0)$ being given by

$$n' = \mathrm{d}\log(1/t_c)/\mathrm{d}\log(C/C_0) = np(C/C_0)^n/\{(C/C_0)^n - 1\} \qquad (6)$$

so that the limiting value of n' at high C/C_0 will tend towards 4, as then the slope should be well approximated by np. In this particular case we can calculate the slope of the graph for different values of C/C_0. For $C/C_0 = 1{\cdot}2$, the slope is ≈ 13, for $C/C_0 = 2$ it is $\approx 5{\cdot}5$ and for $C/C_0 = 4$ it is beginning to approach the limiting value, i.e. the slope is $\approx 4{\cdot}26$. Clearly, if the value of our parameter p were to be equal to one then the original Oakenfull analysis could still be used. However, under any circumstances where this was the case (say $C > 10C_0$), most of the earlier approximations would already have failed. We do not feel that the original analysis would be made any more valid under these circumstances.

We have attempted to least squares fit eqn (5) to Oakenfull's own data, but with limited success. Fitting was carried out by minimising the sum of $\{(\log(1/t_c)_{\text{measured}} - \log(1/t_c)_{\text{calculated}}\}^2$ values for each data point using the generalised simplex method (Nedler & Mead, 1965). The advantages of

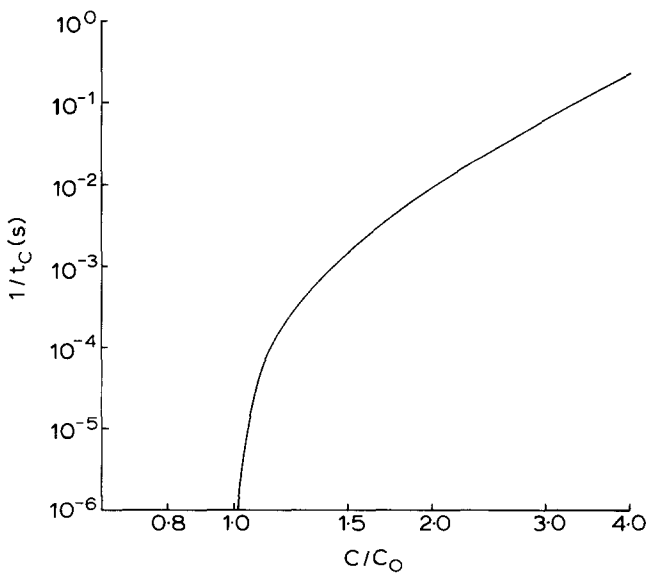

Fig. 3. Reciprocal geltime plotted against log reduced concentration for $n = 2$ and $p = 2$ (the proportionality constant $K = 1000$).

the weighting implied by minimising the sum of log values rather than the values themselves has been discussed recently (Clark *et al.*, 1990).

For the ι-carrageenan, a satisfactory fit is indeed obtained and is illustrated in Fig. 4. Of course we are fitting three parameters to five points so this is not surprising. However, the value of p obtained (2·28) is close to two, in the range of our original estimate, and C_0 is reasonable. In fact the quality of the fit is not really impaired if we constrain these not to vary at all. Nevertheless, it is clear that there is only a little curvature in his data in this regime. For our model curve the equivalent slope to his value of 4·5 corresponds to $C/C_0 \approx 3$. The fit to the κ-carrageenan data is really not so convincing; although there are six points in this dataset the curvature is almost the opposite to that expected from our model. In this case the best overall fit corresponds to $p = 1{\cdot}75$, again not so far from our estimate of two. Although the fit of the curve to the data looks rather unrealistic, we are actually using no more parameters than in the 'log–log is linear' model — again if we were to constrain p to be equal to two, the present model would actually fit adequately with one less parameter. What seems clear is that, from this data, the critical concentration for the ι-carrageenan sample is lower than for the κ sample; this is confirmed by the least-squares fit. In our opinion the behaviour observed in the latter data is not really physically reasonable — we

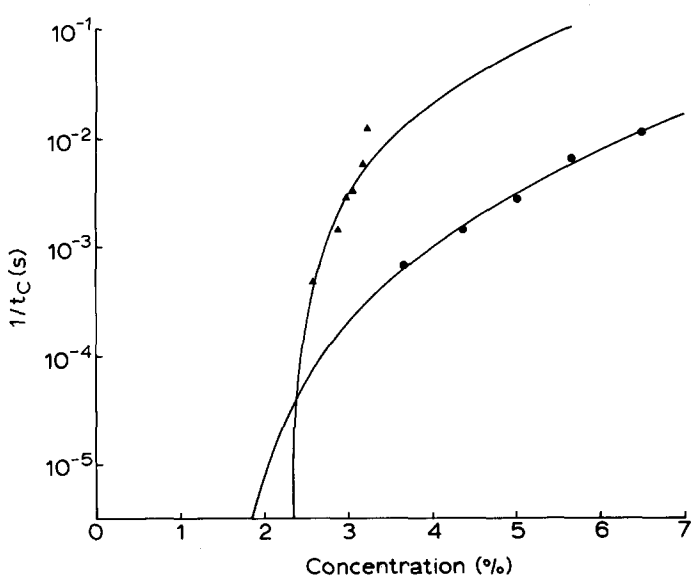

Fig. 4. Data of Oakenfull and Scott fitted to eqn (5) — symbols as Fig. 1; for ι-carrageenan the best fit corresponds to $p = 2{\cdot}28$, $C_0 = 1{\cdot}52$; for κ-carrageenan $p = 1{\cdot}75$, $C_0 = 2{\cdot}34$.

can only attribute this to experimental factors. We note, nevertheless, that the quoted n' slope of $\approx 12{\cdot}5$ corresponds in our model calculations to values of C/C_0 of around 1·2.

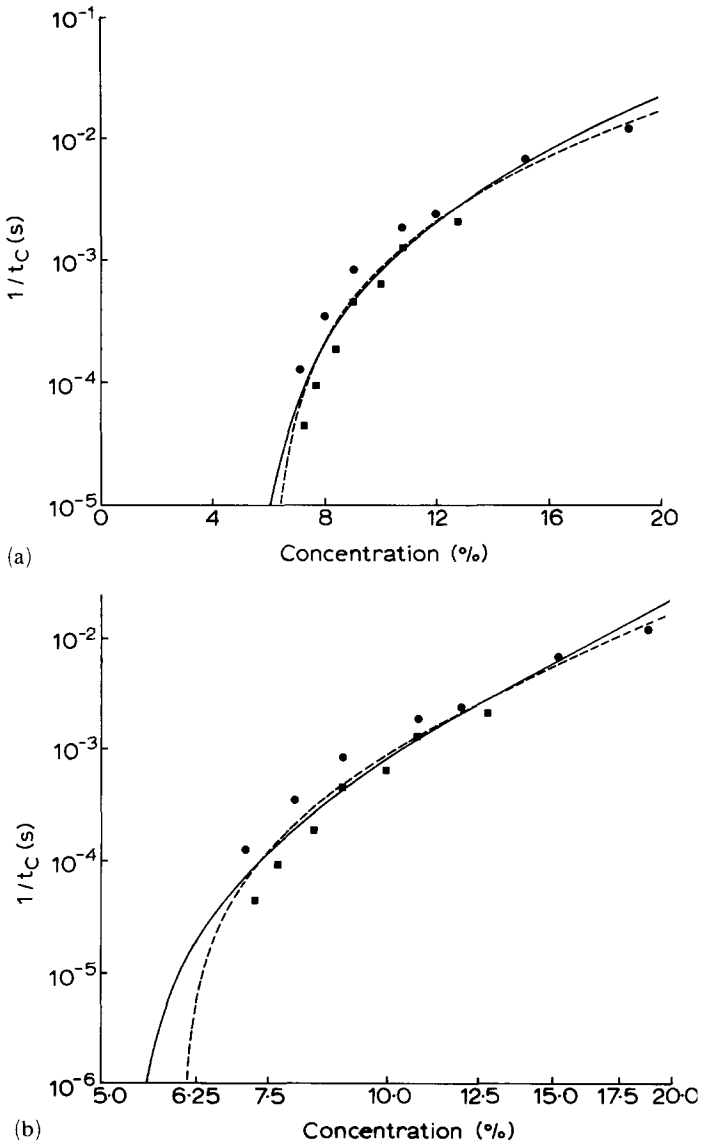

Fig. 5. (a) Reciprocal geltime plotted against concentration for bovine serum albumin gels fitted as discussed in text. Data correspond to measurements with a torsion pendulum (low strain, high strain rate), (●) and a falling sphere viscometer (high strain, low strain rate), (■) — details as in Richardson and Ross-Murphy (1981); the best fit (dotted line) corresponds to $p = 1{\cdot}70$, $C_0 = 6{\cdot}04$; when $p = 2$, $C_0 = 5{\cdot}36$ (solid line); (b) as Fig. 5a, but plotted against log concentration.

In view of the limited agreement mentioned above, we have reproduced some of our own previously published data for heat-set protein gels (bovine serum albumin). In this dataset there are a total of 13 points collected using two different techniques — for details see Richardson and Ross-Murphy (1981). It is clear that this data does behave in the manner predicted, and Figs 5a and 5b illustrate the fits obtained firstly by constraining p to be equal to two and secondly by allowing the three parameters K, p and C_0 to float independently. The best fit is actually obtained for $p \approx 1{\cdot}70$. Figure 5a is on a linear concentration scale, whereas Fig. 5b is double logarithmic. On this latter plot and even with the present data, one might be inclined to assume that the behaviour observed in the slope of the log–log plot was linear; nevertheless, as we have shown above, the statistics of the gelation clarify that this is not really the case. Overall we cannot accept that there is any requirement to adopt the highly cooperative n' parameters suggested by Oakenfull, since satisfactory agreement can be obtained with the present model by assuming only binary collisions. A quite similar conclusion has been reached in a recent publication of ours, which has reexamined gel modulus–concentration relationships (Clark *et al.*, 1990) by comparing cooperative and non-cooperative branching models.

Finally, we repeat our observation that the overall curvature of a plot such as Fig. 4 can actually be well represented by mapping the behaviour of the function of gel modulus against concentrations such as those proposed by Oakenfull himself (Oakenfull, 1984; Oakenfull & Scott, 1984), by Hermans (1965), or by Clark and Ross-Murphy (Clark & Ross-Murphy, 1985; Clark *et al.*, 1990) and assuming that modulus scales directly to the reciprocal geltime. Some of the approximations involved are not quite the same as those described above, but have been discussed in more detail in the previous work on serum albumen gels.

ACKNOWLEDGEMENTS

The author would like to thank Dr Allan Clark, Professor Ed Morris and Dr David Oakenfull for invaluable comments and discussions. The author is also grateful to Mrs Lesley Linger for preparing the figures.

REFERENCES

Bisschops, J. (1955). Gelation of concentrated polyacrylonitrile solutions. *J. Polym. Sci.*, **17**, 89–98.

Busnel, J. P., Clegg, S. M. & Morris, E. R. (1988). Melting behaviour of gelatin gels: origin and control. In *Gums and Stabilisers for the Food Industry* — 4, ed. G. O. Phillips, D. J. Wedlock & P. A. Williams. IRL Press, Oxford, pp. 105–15.

Busnel, J. P., Morris, E. R. & Ross-Murphy, S. B. (1989). Interpretation of the renaturation kinetics of gelatin solutions. *Int. J. Biol. Macromol.*, **11**, 119–25.

Chatellier, J. Y., Durand, D. & Emery, J. R. (1985). Critical helix content in gelatin gels. *Int. J. Biol. Macromol.*, 7, 311–14.

Clark, A. H. & Ross-Murphy, S. B. (1985). The concentration dependence of biopolymer gel modulus. *Brit. Polymer J.*, **17**, 164–8.

Clark, A. H. & Ross-Murphy, S. B. (1987). Structural and mechanical properties of biopolymer gels. *Adv. Polym. Sci.*, **83**, 57–192.

Clark, A. H., Ross-Murphy, S. B., Nishinari, K. & Watase, M. (1990). Shear modulus–concentration relationships for biopolymer gels. Comparison of independent and cooperative crosslink descriptions. In *Physical Networks: Polymers and Gels*, ed. W. Burchard & S. B. Ross-Murphy. Elsevier Applied Science, London, pp. 209–30.

Durand, D., Emery, J. R. & Chatellier, J. Y. (1985). Investigation of renaturation in gelatin gels. *Int. J. Biol. Macromol.*, 7, 315–9.

Ferry, J. D. (1980). *Viscoelastic Properties of Polymers*, 3rd edn. John Wiley, New York, pp. 408–11.

Flory, P. J. (1941*a*). Molecular size distribution in three dimensional polymers. I. Gelation. *J. Am. Chem. Soc.*, **63**, 3083–90.

Flory, P. J. (1941*b*). Molecular size distribution in three dimensional polymers II. Trifunctional branch units. *J. Am. Chem. Soc.*, **63**, 3091–6.

Gordon, M. (1986). Thermodynamics of casein gels and the universality of network theories. In *Integration of Fundamental Polymer Science and Technology*, ed. L. A. Kleintjens & P. J. Lemstra. Elsevier Applied Science, London, pp. 167–76.

Gordon, M. & Ross-Murphy, S. B. (1975). The structure and properties of molecular trees and networks. *Pure Appl. Chem.*, **43**, 1–26.

Hermans, J. R. (1965). Investigation of the elastic properties of the particle network in gelled solutions of hydrocolloids I. Carboxymethyl cellulose. *J. Polym. Sci. Part A.*, **3**, 1859–68.

Langley, N. R. (1968). Elastically effective strand density in polymer networks. *Macromolecules*, **1**, 348–52.

Miller, W. G., Wu, L. L., Wee, E. L., Santee, G. L., Rai, J. H. & Goebel, K. G. (1974). Thermodynamics and dynamics of polypeptide liquid crystals. *Pure Appl. Chem.*, **38**, 37–58.

Morris, E. R. (1986). Molecular origin of hydrocolloid functionality. In *Gums and Stabilisers for the Food Industry* — 3, ed. G. O. Phillips, D. J. Wedlock & P. A. Williams. Elsevier Applied Science, London, pp. 3–16.

Morris, E. R., Rees, D. A. & Robinson, G. (1980). Cation-specific aggregation of carrageenan helixes: domain model of polymer gel structure. *J. Mol. Biol.*, **138**, 349–62.

Nelder, J. A. & Mead, R. (1965). A simplex method for function minimization. *Comput. J.*, 7, 308–13.

Oakenfull, D. (1984). A method for using measurements of shear modulus to estimate the size and thermodynamic stability of junction zones in non-covalently crosslinked gels. *J. Food Sci.*, **49**, 1103–4, 1110.

Oakenfull, D. G. & Morris, V. J. (1987). A kinetic investigation of the extent of polymer aggregation in carrageenan and furcellaran gels. *Chem. Ind.*, 201–2.

Oakenfull, D. & Scott, A. (1984). Hydrophobic interaction in the gelation of high methoxyl pectins. *J. Food Sci.*, **49**, 1093–8.

Oakenfull, D. G. & Scott, A. (1986). New approaches to the investigation of food gels. In *Gums and Stabilisers for the Food Industry — 3*, ed. G. O. Phillips, D. J. Wedlock & P. A. Williams. Elsevier Applied Science, London, pp. 465–75.

Oakenfull, D. G. & Scott, A. (1988). Size and stability of the junction zones in gels of iota and kappa carrageenan. In *Gums and Stabilisers for the Food Industry — 4*, ed. G. O. Phillips, D. J. Wedlock & P. A. Williams. IRL Press, Oxford, pp. 127–34.

Peniche-Covas, C. A. L., Dev, S. B., Gordon, M., Judd, M. & Kajiwara, K. (1974). The critically branched state in a covalent synthetic system and in the reversible gelation of gelatin. *Farad. Discuss. Chem. Soc.*, **57**, 165–80.

Richardson, R. K. & Ross-Murphy, S. B. (1981). Mechanical properties of globular protein gels: 1. Incipient gelation behaviour. *Int. J. Biol. Macromol.*, **3**, 315–22.

Ross-Murphy, S. B. & Stepto, R. F. T. (1986). Cyclization, gelation and network formation. In *Cyclic Polymers*, ed. J. A. Semlyen. Elsevier Applied Science, London, pp. 349–79.

Stauffer, D., Coniglio, A. & Adam, M. (1982). Gelation and critical phenomena. *Adv. Polym. Sci.*, **44**, 103–5.