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Rationalisation of the elastic modulus–molecular weight relationship for kappa-carrageenan gels using cascade theory

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Literature results for the variation of the elastic modulus of kappa-carrageenan gels with molecular weight have been modelled using cascade theory. Excellent fits have been obtained. The critical nature of gelation is used to explain inhibition of gelation below a lower molecular weight threshold, while constancy of modulus at higher molecular weights is attributed to optimum effectiveness of the cross-linking process. Such behaviour can be anticipated both for reversible and irreversible cross-linking mechanisms, but is crucially dependent on there being proportionality between molecular weight and the number of sites for cross-linking per molecule. Parameters of the model, determined by least-squares fitting, are discussed in the light of recent developments in the cascade theory description of biopolymer gelation.

INTRODUCTION

In a recent article Rochas *et al.* (1990) presented Young's modulus versus molecular weight data for a series of molecular weight fractions of kappa-carrageenan, fully gelled, and measured under varying conditions of polymer concentration and amount of added KCl. In all situations, for a constant set of gelling conditions, but for samples of increasing weight-average molecular weight, a critical threshold of molecular weight was found below which the elastic modulus was effectively zero. Beyond this threshold, the modulus rose steeply before plateauing out and remaining constant within the experimental uncertainty. In general, the plateau result was found to depend strongly on conditions of concentration and ionic strength, while the critical threshold seemed less sensitive to these variables (but nonetheless also varied systematically). As Rochas *et al.* (1990) point out, this behaviour is not unique to kappa-carrageenan, but has been observed before for other gelling polysaccharide systems such as alginate (Mitchell, 1976, 1980).

The present paper seeks to obtain a quantitative description of the reported kappa-carrageenan data using a previously developed model for biopolymer gelation (Clark & Ross-Murphy, 1985; Clark, 1987) based on cascade theory (Gordon & Ross-Murphy, 1975). If such a description is possible it should become clear under what conditions behaviour of this kind is to be expected for gelling polysaccharides, and indeed for biopolymer systems generally. In what follows the elements of cascade theory necessary to model modulus–molecular weight data are reviewed, the results of modelling the data of Rochas *et al.* (1990) are described, and conclusions are summarised.

THEORY

As has been reported previously (Clark, 1987) the shear modulus G of a fully-cured biopolymer gel (or equivalently, the Young's modulus E, if $E = 3G$) can be written using the expression:

$$G = [Nf\alpha(1 - v)^2(1 - \beta)/2]\,aRT. \qquad (1)$$

In this equation, the quantity in square brackets is a measure of the number of moles of elastically-active network chains (EANCs) per unit volume, where an EANC is a segment of the network which is deformed as the network is deformed and which contributes to the overall increase in free energy accompanying the deformation. The factor aRT is a measure of the average contribution per mole of EANCs to the free energy increase per unit strain, and hence to the modulus.

As has often been shown (e.g. Flory, 1953) $a = 1$ for an ideal rubber network. For many biopolymer gels, however, particularly at higher concentrations, it appears that a can be substantially greater than this (Clark & Ross-Murphy, 1985). Under these conditions, flexible Gaussian chains, characteristic of rubber networks, are replaced by much less flexible EANC elements, and there is probably then an important enthalpic contribution to G. In this situation a is expected to be temperature dependent (falling as T increases) and to be greater than the constant value of unity anticipated on the basis of the entropic contribution calculated for ideal rubbers.

In eqn (1), the quantity in square brackets, which is crucial to the description of modulus–molecular weight data, contains a number of factors which need explanation. Here N is the number of moles of polymer per unit volume initially present, if the system is treated as equivalent to a monodisperse material with corresponding molecular weight M_w (the weight-average molecular weight); f is the number of sites (or functionalities) along each molecule's length *potentially* available for cross-linking to other sites on other chains; α is the fraction of all such sites which have reacted when the fully-cured gel has formed; and the quantities v and β, which are functions of α and f, and which are key elements of the cascade approach to network formation, are given by the equations:

$$v = (1 - \alpha + \alpha v)^{f-1} \tag{2}$$

and

$$\beta = (f-1)\alpha v/(1 - \alpha + \alpha v). \tag{3}$$

Equation (2), which is a recurrence relation, defines the so-called extinction probability v (Gordon & Ross-Murphy, 1975) which is crucial to the present discussion. The extinction probability measures the probability that a reacted functionality on an arbitrary molecule in the system, becomes 'extinct': that is, does not connect through to the boundaries of the gel (i.e. to infinity) *via* connection to other units. Equation (3) defines the quantity β as a function of v, α and f and this has been formulated (Gordon & Ross-Murphy, 1975) so that the full term in square brackets in eqn (1) measures the number of moles of EANCs per unit volume, the product $f\alpha(1-v)^2(1-\beta)/2$ measuring the average number of EANCs per polymer molecule. In this calculation an EANC is precisely defined (Gordon & Ross-Murphy, 1975) as a network chain segment which is both initiated and terminated by two polymer molecules (active junction points) which have *at least* three independent paths (or ties) to infinity.

From eqn (1), it is clear that when $v = 1$ there are no EANCS and hence zero modulus, while when $v = 0$, β is also zero, and the modulus has the limiting value of $[Nf\alpha/2]\,aRT$, corresponding to all reacted functionalities contributing EANCs.

The question now is, how can eqns (1–3) provide a description of the variation of G with molecular weight and explain the particular type of behaviour found for kappa-carrageenan? These questions are answered using the following argument. For a fully-cured gel, where gelling is reversible, the corresponding final value of α can be thought of as an equilibrium value, achieved at long times, and determined by the polymer concentration, and an appropriate equilibrium constant K for cross-link formation. A previous article (Clark, 1987) has shown that, in this case, α is a function of the product NfK only. Since N is inversely proportional to molecular weight ($N = C/M$, if C is concentration in mass terms), and if (and this is a crucial assumption of the present approach) it is possible to assume for a biopolymer, such as kappa-carrageenan, that f is proportional to M ($f = kM$, say), then α becomes dependent on K and C, and of course the constant k, but not on molecular weight. Similarly in eqn (1), if $f = kM$, then the product Nf is also independent of M and, clearly, in these circumstances, the only dependence of G on molecular weight comes from the behaviour of v and β. Since β is a function of v, it is primarily the behaviour of v at constant α (fixed C and K in the experiment) as f increases (i.e. as M increases) that determines the form of the G (or E) versus M relationship, and decides whether the data of Rochas *et al.* (1990) can be fitted adequately by the present cascade model.

That the observed behaviour can be described, at least qualitatively, by the current model, can be seen by examining the mathematical properties of eqn (2). First, there is always a trivial solution $v = 1$ for all α and f. More important, however, it can be shown by algebraic manipulation, or by numerical computation, that a second solution exists which, for constant α and variable f, has limiting value zero as f becomes very large, but increases to unity as f decreases to a critical value f_c satisfying the relation $\alpha = 1/(f_c - 1)$. This last relation is of course the well-known critical value for α ($\alpha = \alpha_c$), below which a gel will not form (Flory, 1941). As M, and hence f, decrease still further, the only solution to eqn (2) is a constant $v = 1$. Since $v = 1$ ensures $G = 0$, and $v = 0$ produces a constant limiting modulus value independent of M, it is evident that for constant α, G (or E) will remain zero until a limiting critical molecular weight is reached ($M_c = f_c/k$). The modulus will then increase, and eventually reach a constant limit. Thus, in principle, the data of Rochas *et al.* (1990) can be reproduced by the current cascade model, though whether the sharpness of the transition, and the very flat plateau behaviour beyond, can be accurately fitted, will depend on the detailed behaviour of v as a function of f, a point which is explored by least-squares fitting of the data in the next section.

Before passing to this detailed analysis, however, it is interesting to note that since, in the situation where

$M = M_c$ and $f = f_c$, the polymer concentration C is equal to the critical gel concentration C_0, and since from previous work (Clark & Ross-Murphy, 1985; Clark, 1987)

$$C_0 = M(f-1)/Kf(f-2)^2 \quad (4)$$

an equation for M_c in terms of C, K and k is the following:

$$C = (kM_c - 1)/Kk(kM_c - 2)^2. \quad (5)$$

This shows that for any system, M_c can vary with molecular factors and system conditions. Also, if kM_c is large, $M_c = 1/k^2KC$ to a good approximation. Evidently, the greater is K or C, the smaller is M_c, and this is a result that already appears to be indicated by the experimental data for kappa-carrageenan (Rochas *et al.*, 1990).

Finally, in this section on theory, it should be added that the above results and conclusions are not totally dependent on the assumption of cross-linking equilibrium, and hence of gel thermoreversibility. Recent work (Clark, 1993) has shown that almost exactly the same mathematics and logic will apply to gels formed by irreversible cross-linking, provided such cross-linking occurs against the background of a competing wastage process (cyclisation, for example). In this case K is no longer an equilibrium constant, but equals the ratio of rate constants for the cross-linking and wastage events.

RESULTS

A computer program was written to obtain a least-squares fit to experimental E versus M_w data, assuming that the relation $E = 3G$ could be applied, and eqns (1)–(3) above hold. The variable parameters were k, K and a. Data were taken as accurately as possible from the article by Rochas *et al.* (1990) and calculations performed: (a) on their three sets of data corresponding to increasing kappa-carrageenan concentrations (5, 10 and 20 g/litre) but constant KCl level (0·1 M); (b) on their three sets of data for constant (10 g/litre) kappa-carrageenan concentration but increasing salt level (0·05, 0·10 and 0·50 M KCl).

In all least-squares calculations, as in previous applications of the cascade approach to biopolymer gelation (for example, to modulus concentration data), it was not possible to vary all three parameters independently. The functionality-determining parameter k turns out to be highly correlated with K (in effect the data really determine the product k^2K); hence, it was necessary to conduct a series of refinements for increasing fixed values of k, varying in each case only K and a. Not surprisingly, in view of the correlation property, the fits obtained for the various k-values assumed, were comparable in quality except for values of k lower than 0·0005, where the agreement between theory and experiment fell off dramatically. Given, however, that a value of $k = 0{\cdot}001$ corresponds to one bonding segment per three disaccharide units, which is about the minimum length expected for a site of practical importance, it appears that values of k much higher than 0·001 are unrealistic. (Note, however, that a somewhat higher value could be justified if it is accepted, as seems likely from the junction zone model of kappa-carrageenan networks, that each functional segment may bond to more than one other similar site, rather than form only isolated double helical cross-link.) The practical range of realistic k-values is thus limited, but an exact value cannot be specified without introducing additional assumptions. Accordingly, in discussion of the least-squares fits which follows, this matter will be left open, with results for the $k = 0{\cdot}001$ analysis being presented as typical, and realistic, but not necessarily indicating that this value of k is the best answer.

Results for parameters a and K (and corresponding values for M_c from eqn (5) are given in Tables 1 and 2. Table 1 shows results obtained for the situation where the KCl level is constant. Table 2 is for data where the polymer concentration is constant. The nature of the fits achieved in these two cases using the $k = 0{\cdot}001$ model is made clear in Figs 1 and 2. The correlation problem already described means that had results from other k models been considered, Figs 1 and 2 would change little, but the K-values in the tables would decrease as k increases. On the other hand, values for a and M_c would change only slightly.

Although very good fits have been achieved for all data sets, the parameter values in Tables 1 and 2 are

Table 1. Parameters a and K obtained by least-squares fitting ($k = 0{\cdot}001$) of modulus–molecular weight data (0·1 M KCl) of Rochas *et al.* (1990)

C (g/litre)	a	K (litre/mol)	$M_c \times 10^{-5}$
5	14·4 (0·7)	6·2 (0·2)	0·3546
10	23·7 (0·3)	4·36 (0·02)	0·2589
20	42·7 (0·3)	2·43 (0·01)	0·2351

Estimated standard deviations are in parentheses.

Table 2. Parameters a and K obtained by least-squares fitting ($k = 0{\cdot}001$) of modulus–molecular weight data ($C = 10$ g/litre) of Rochas *et al.* (1990)

M KCl	a	K (litre/mol)	$M_c \times 10^{-5}$
0·05	18·8 (0·2)	2·50 (0·01)	0·4296
0·10	23·7 (0·3)	4·36 (0·02)	0·2589
0·50	34·7 (0·3)	5·57 (0·02)	0·2089

Estimated standard deviations are in parentheses.

surprising, and require comment. From past experience it would have been expected that both a and K in Table 1 would be independent of concentration but, as can be seen, a increases by a factor of three, and K falls by almost the same amount over the concentration range $C = 5$ to 20 g/litre. The error limits show that this trend lies outside the limits of parameter uncertainty calculated during the fits, and both this fact, and the trends observed, are unchanged on selecting other k models. To explain such variability of a and K one might attribute the effect to model limitations, such that it demands rigorous applicability of the relationship $f = kM$ at all concentrations and molecular weights, which may not be true for the real systems, or that it ignores molecular weight polydispersity, which is undoubtedly present. There may be another explanation, however. Recent unpublished analyses (by the present author), of extensive amounts of modulus–concentration data for various biopolymer systems using the cascade approach, suggest that other aspects of the theory may be at fault. These treatments have involved data fitting to subsets of modulus points along segments of the full concentration range, and they suggest that, in reality, a increases substantially with concentration, and K falls, over this range. Also, the predicted size of the effect is comparable to that shown in Table 1. In fact, such an increase in a is not entirely unexpected. The possibility that a is in reality a function of gel concentration has already been discussed (Clark & Ross-Murphy, 1987) in view of the fact that EANCs at the gel point will necessarily be very different entities from those of more concentrated versions of the same gels; that is, they are much more likely, at low C/C_0 values, to approximate ideal Gaussian chains. The fall-off in K is more difficult to justify, however, but one can realistically consider explanations based on non-ideal solution behaviour, and the use of a concentration variable in weight per cent, rather than an activity.

Turning to Table 2, and the fits at constant concentration, we can see that again excellent fits are achieved, but once more the parameters give cause for thought. In this situation the expectation would be that K would increase with added KCl, as cross-linking becomes more favoured through reduced repulsion. The factor a might be expected to change in response to salt addition, perhaps becoming lower as the charge on the EANCs becomes screened. In practice K does increase, but not dramatically, and a, rather than fall, increases by a factor of roughly two. This is not inconsistent with the results of Table 1, however. Gels made at the same concentration C, but at increasing salt levels, will correspond to increasing values of the ratio C/C_0, so that in the absence of other effects, there will be a natural tendency for a to increase, and K to fall, if the previous discussion of the implications of Table 1 is accepted. Overall, these effects counter the expectations for salt effects originally held and could well be consistent with the moderate increase in K, and rise in a, actually observed.

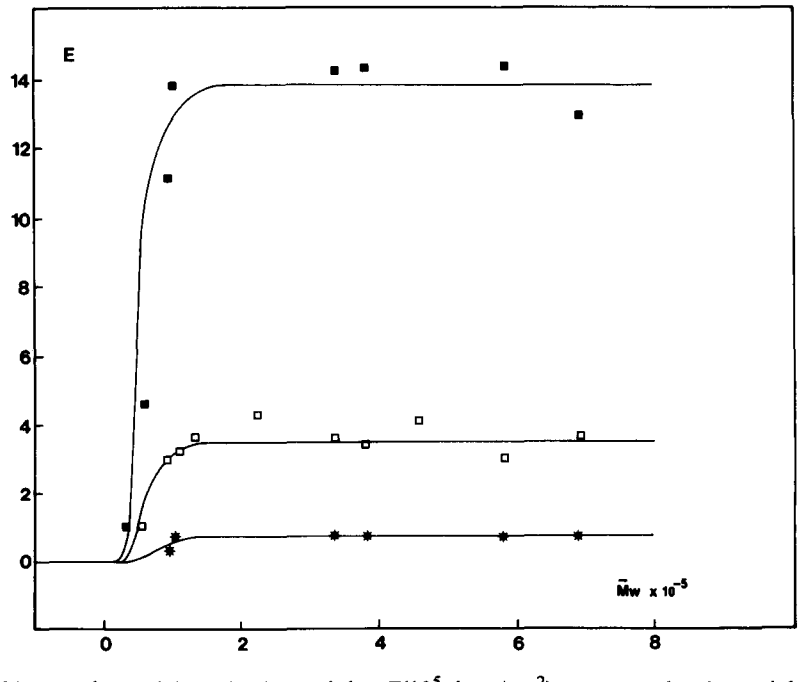

Fig. 1. Best fits of $k = 0{\cdot}001$ cascade model to elastic modulus $E(10^5$ dyne/cm$^2)$ versus molecular weight data (Rochas *et al.* 1990) for kappa-carrageenan in 0·1 M KCl. ✱, □ and ■ are polymer concentrations of 5, 10 and 20 g/litre, respectively.

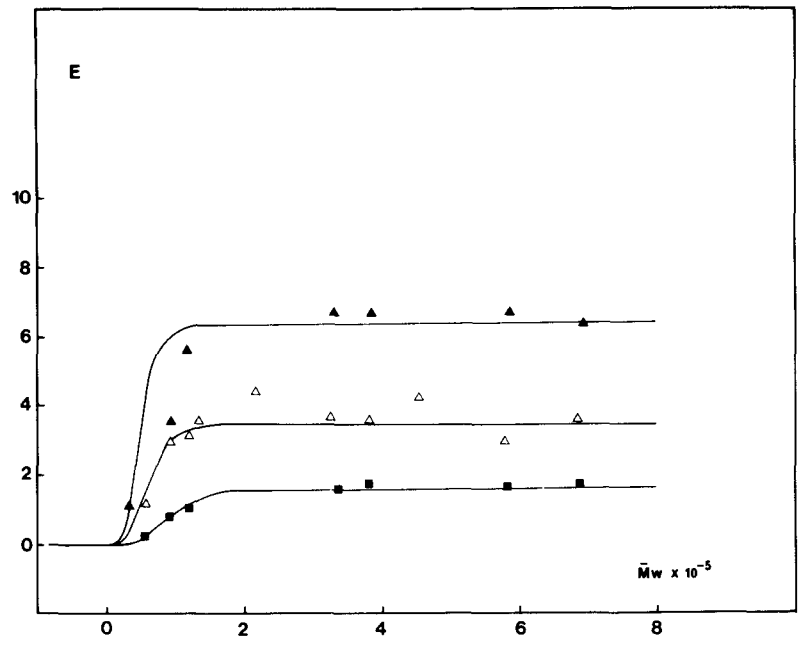

Fig. 2. Best fits of $k = 0{\cdot}001$ cascade model to elastic modulus E (10^5 dyne/cm^2) versus molecular weight data (Rochas *et al.* 1990) for kappa-carrageenan at a concentration 10 g/litre. ■, △ and ▲ are KCl concentrations of 0·05 M, 0·1 M and 0·5 M, respectively.

CONCLUSIONS

The fits in Figs 1 and 2 demonstrate the effectiveness of the cascade approach in describing in detail the forms of the data generated by Rochas *et al.* (1990). Qualitatively, this behaviour is explained in terms of the critical nature of gelation imposing a lower molecular weight threshold below which gelation does not occur. Subsequent increase in the modulus, followed by a levelling off, are then explained in terms of an increased efficiency of cross-linking up to a limiting threshold, as the molecular weight increases. The quantitative relationship between G (or E) and M depends crucially on the detailed variation of the extinction probability v, as f increases in proportion to molecular weight, and α remains constant. It should be noted, particularly, that behaviour of the type observed for kappa-carrageenan (and for some other polysaccharides) can only occur if the proportionality relationship between f and M is closely followed. For polysaccharides gelling *via* junction zone formation this seems to be a reasonable expectation, and it may also apply to gelatins, but for globular proteins, f is almost certainly unrelated to molecular weight except in very special situations. The behaviour found for kappa-carrageenan is not therefore expected to be universal.

Finally, as has been discussed at some length, the parameters obtained by least-squares fitting are not as initially expected. However, the trends observed for a and K are consistent with information emerging very recently from detailed analyses of modulus–concentration data for various gelling systems. These analyses will be published in due course.

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