Structural and Mechanical Properties of Agar/Gelatin Co-gels. Small-Deformation Studies

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ABSTRACT: Composite aqueous gels based on the thermoreversible cold-setting gelling components agar and gelatin have been studied by electron and light microscopy and mechanical spectroscopy. The microscope evidence showed phase separation of the two polymer networks, with phase inversion occurring at specific mixture compositions. Mechanical characterization of the gels was achieved by measurement of the shear modulus at small deformation, and a theoretical model, constructed by using ideas of solvent partition between components and of upper and lower bound limits for G' vs. composition behavior, provided a useful rationalization of these data.

Introduction

Over the years much work[1-14] has been devoted to the study of gel formation by biopolymers such as polysaccharides and proteins, and of the methods of inducing this phenomenon in aqueous solution, thermal setting has probably been the most widely investigated. Consequently, for these individual polymer types, the structures and mechanisms of formation of the gel networks involved, and their mechanical properties, are now well understood, and fundamental distinctions between the cold-setting behavior of polysaccharides (and gelatin) and the heat-setting behavior of many globular proteins can now be made.

Recently, work has been carried out in this laboratory to examine thermally induced gel formation in aqueous solutions containing two such polymer types, and as part of this program mixed gels formed by combining the water-soluble polysaccharide agar (see Figure 1) and protein gelatin have received particular attention. In the present paper structural and mechanical properties of these gels are described, these properties being determined by electron and light microscopy and mechanical spectroscopy. Although structural and mechanical data for composite gels involving gelatin have already been reported[15] by some of the present authors, the second component on these occasions was glass in the form of discrete particles, i.e., an insoluble material. As will become clear from the work described here, the structural and rheological properties that emerge when a soluble, and independently gelling, second component is added to gelatin are somewhat different, though certain basic similarities between the two types of system remain.

In the present work the mechanical data discussed are restricted to measurements of the shear modulus at small deformation. Results of large-deformation, destructive, testing will be described later.

In terms of their molecular structure and mechanism of formation, gelatin gels have more in common with polysaccharide gels than those formed by heat-setting globular proteins. In particular, the initial gelation of gelatin is believed to proceed by formation of ordered quasi-crystalline triple-helical junction zones separated along a single polymer chain contour by flexible regions (see, for example, ref 1 and 16), and these zones (analogous to the collagen triple helix) form on cooling solutions to ~28 °C. The gelation of agarose proceeds in a similar way, although at a somewhat higher temperature and with double helices constituting the junction zones.[17] From these facts, it is to be expected that sequential (or, under circumstances of very rapid cooling, simultaneous) gelling of these two types of polymer will occur when a solution containing them both is cooled. For such a mixed gel there is a need to investigate structural aspects of the process such as the relationship between the agar and gelatin in the network that forms and mechanical properties such as the relationship between the composite gel shear modulus and the amounts of the constituents present. Although such studies have been made in the past on mixed interpenetrating networks based on synthetic polymers, for example, by Sperling and co-workers[18] and by Frisch et al.,[19] the present system differs from these and from most polymer–polymer blends or "alloys" in that a large volume fraction of solvent (water) is present in both the sol and gel states. It is this extensive presence of water (volume fraction ~0.8), and the competition between the polymers for it, that clearly distinguishes biopolymer gels from most synthetic cross-linked networks and makes any theoretical description of their properties additionally complicated. In the present work such a description is attempted, however, and a theoretical model is constructed to relate the small-deformation shear moduli of the agar/gelatin gels studied to their compositions and microstructures. In the future we intend to apply this model to results for other mixed biopolymer gel systems and so investigate its general applicability.

Experimental Section

Mixed gels of agar and gelatin were prepared by adding suitable amounts of agar to water, autoclaving the mixtures to dissolve the agar, cooling the resultant solutions to 45 °C, and adding and dissolving appropriate amounts of gelatin. The weights of agar used were adjusted to provide two series of agar/gelatin gels: one in which the agar content was constant at 1.0% w/w and another in which this constant amount was 2.0% w/w. In both series, the gelatin content was allowed to vary between 0 and 25.0% w/w.

Mechanical measurements were made by introducing the warm solutions between the plates of a Rheometrics mechanical spectrometer (RMS-605) and allowing gelation to occur in situ.

For the microscopy studies blocks of gel were obtained by cooling solutions in glass containers, and the procedures of fixing, staining, and embedding samples were as described previously[14] for similar investigations of agar/bovine serum albumin gels; i.e., fixing procedures involved use of tannic acid, glutaraldehyde, and osmium tetraoxide, dehydration involved ethanol–water mixtures, and embedding was carried out by using Araldite epoxy resin. Optical microscope images were photographed with a Leitz Ortholux II microscope. For this purpose, specimens were sectioned to a thickness of 1 μm (10^{-6}m) and were stained with toluidine blue. Electron micrographs were recorded with a JEOL 100 CX electron microscope. In this case staining procedures involved treatment with uranyl acetate and lead citrate, and the section thickness was 800 Å (8×10^{-8} m).

In these various experimental studies the agar and gelatin samples used were crude commercial products (the gelatin was acid-treated 250 Bloom material supplied by Croda, and the agar was Portuguese agar from Lysander Foods) since one object of

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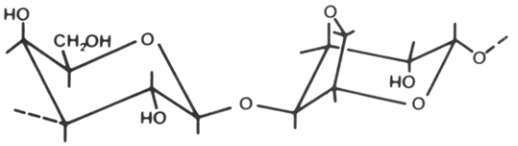

Figure 1. Basic repeating dissacharide unit of the polysaccharide component agarose (agar).

the present investigation was to compare rheological trends at small deformation with large-deformation tensile testing of the same systems. In the latter type of experiment large volumes of sample are needed and both the agar and gelatin must be available in kilogram quantities.

Results

Microscopy. Figure 2a (in which the top row of micrographs shows light microscope results and the bottom row electron microscope details) provides clear evidence concerning the microstructure of agar/gelatin co-gels. The micrographs presented are for samples selected from the series of 1% agar systems and, interestingly, they appear quite similar to those published for a number of synthetic

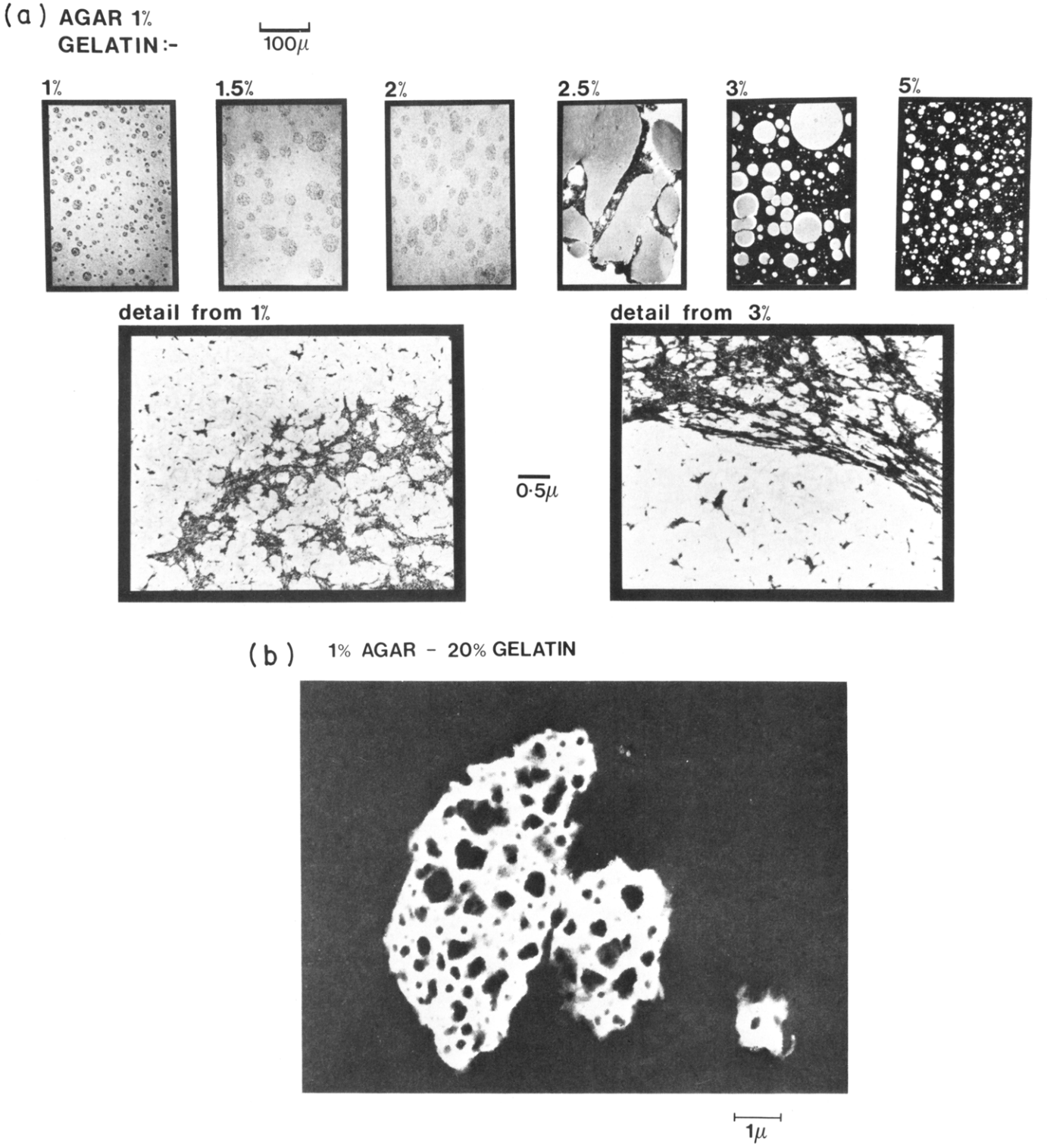

Figure 2. (a) Light microscope (top row) and electron microscope (bottom row) results for the 1% w/w agar series of agar/gelatin gels. Gelatin appears as the darkly staining material, the maximum concentration shown being 5% w/w. (b) Electron microscope view of the microstructure of a 1% agar/20% gelatin mixed gel. Again dark areas indicate gelatin.

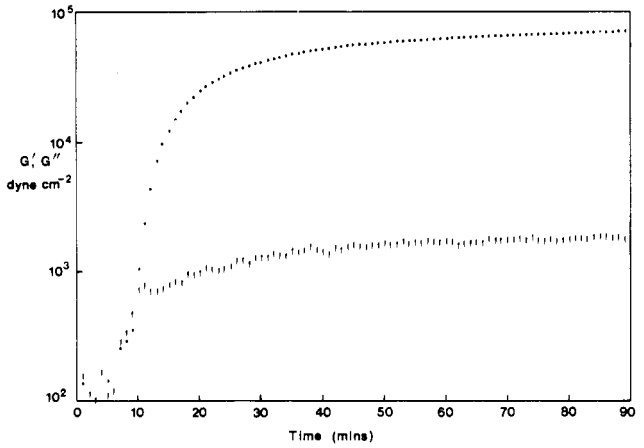

Figure 3. Plots of G' (···) and G'' (' ' ') at 10 rad s^{-1} against time as a 1% agar/10% gelatin gel forms on cooling from 80 to 25 °C.

interpenetrating networks including polybutadiene/styrene[20,21] and poly(ethyl acrylate)/polystyrene.[22] The regions of agar gel network appear as light areas, with dark areas indicating gelatin. When only 1% w/w gelatin is present, the agar forms a continuous supporting phase and contains roughly spherical inclusions of gelatin. As the gelatin content increases, however, the volume fraction of gelatin network also increases, until phase inversion occurs at a gelatin concentration of approximately 2.5%. Beyond this point, as the gelatin content increases further, the gelatin network clearly becomes the supporting phase, and agar inclusions present become smaller. Figure 2b shows an electron micrograph of the 1% agar/20% gelatin system and makes clear the predominance of the gelatin phase at high gelatin content.

Another feature of the microstructures of agar/gelatin co-gels that is evident in both Figure 2a and Figure 2b, and that is apparently also common in the microstructures of synthetic polymer composites,[20] is the lack of homogeneity displayed by both the supporting and the supported gel phases. This is true whichever polymer network has the supporting role, for it may be seen from Figure 2b that the agar inclusions contain small pieces of gelatin network randomly distributed through them, and Figure 2a indicates that the reverse is true for gelatin inclusions at low gelatin content. Indeed, in some cases (see Figure 2a, detail from the 3% gelatin gel) both polymer networks simultaneously demonstrate this property.

Finally, it should be added that results very similar to those shown in Figure 2a,b were obtained for the 2% agar gel series, but this time the phase inversion point was estimated to lie between 5 and 6% gelatin content.

Mechanical Measurements. Figure 3 shows a typical trace of shear modulus (at 10 rad s^{-1}) against time as a warm agar/gelatin solution (1% agar/10% gelatin) is cooled. Both G' and G'', the storage and loss components of the shear modulus, increase (rapidly at first) and then level off to reach a constant value on overnight storage. After this time G' and G'' can be measured as a function of frequency, the 1% agar/10% gelatin system giving the results shown in Figure 4. Clearly, G' is almost independent of frequency, while there is a slight dip in G'' centered on 0.4 rad s^{-1}. This last feature is characteristic of the gelatin component since it is observed for pure gelatin[15] but not for agar.[14] As in earlier work, because of the flatness of the frequency response, the datum at 10 rad s^{-1} was chosen when plotting G' against composition for the two series of gels studied. Before examining these composition results, however, it is of interest to consider the consequences of heating and remelting the 1% agar/10% gelatin system. Figure 5 shows the variation of modulus with temperature that accompanies reheating and recooling the sample. Even though the agar gel, in pure form, is stable to ~80 °C, the composite gel structure may be seen to have melted out by ~37 °C, while on recooling it forms again by 28 °C. Although some hysteresis is involved when melting and re-forming gelatin gels, at least part of the above effect is due to the comparatively rapid heating (1 deg min^{-1}) and cooling rates applied.

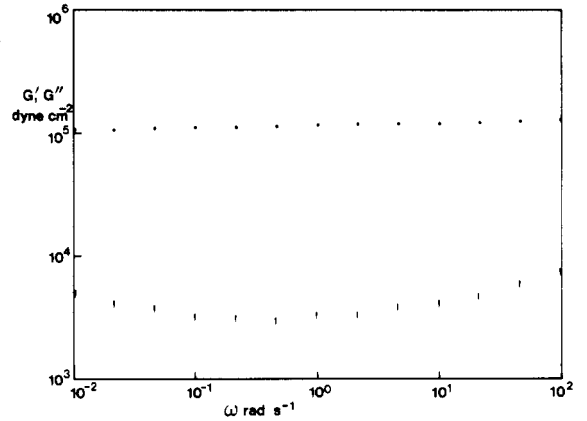

Figure 4. Frequency dependence of G' (···) and G'' (' ' ') for a 1% agar/10% gelatin gel measured at 25 °C.

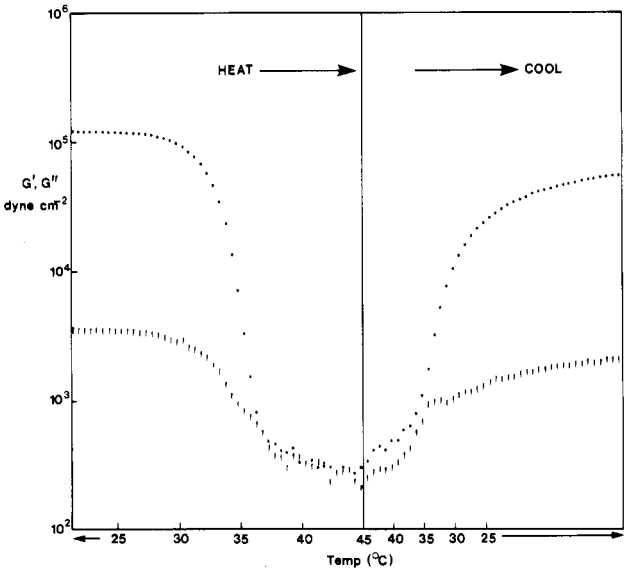

Figure 5. Changes in G' (···) and G'' (' ' ') at 10 rad s^{-1} with temperature as a 1% agar/10% gelatin gel is melted by heating and allowed to re-form on cooling.

Finally, Figure 6 shows the composition dependences of G' for the 1% and 2% agar gel series. G' is plotted against the weight percent gelatin present, and results for the corresponding pure gelatin gels are included for comparison. G' values for pure 1% and 2% agar gels are also included as horizontal broken lines. Figure 6 makes it clear that for both agar/gelatin gel series, the shear modulus first falls as gelatin is added and then, after reaching a minimum near, but somewhat beyond the phase inversion point, increases again finally to follow a gelatin concentration dependence close to that for pure gelatin itself. Although this last behavior is understandable in terms of the high gelatin content, it is by no means as easy to interpret the low-gelatin composition dependence of the modulus, particularly on the agar-rich side of the phase inversion point. Here the fall in gel modulus in relation to the value appropriate to the parent agar system is

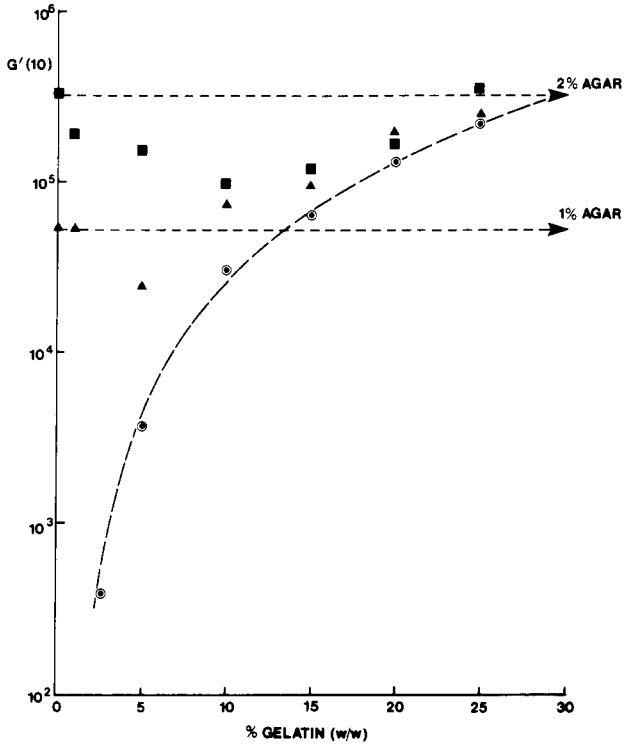

Figure 6. G'(10 rad s^{-1}) plotted against gelatin content for the 1% (▲) and 2% (■) agar gel series. G' values for pure 1% and 2% agar gels are represented by horizontal broken lines, and the concentration dependence of G' for pure gelatin gels is also indicated (⊙).

surprising. Its significance in terms of the properties of the two pure polymer constituents and the gel microstructures is considered at length in the next section.

Theory

The microscope data make it clear that there is an antagonistic effect operating between agar and gelatin during gel formation. This is clearly indicated by the heterogeneous microstructures demonstrated in Figure 2a,b. It turns out, however, that this property of polymer segregation provides a simplifying feature when a quantitative correlation is sought between gel composition and shear modulus. The simplifying feature is that a body of theory already exists for treating heterogeneous composites containing well-defined phase domains, the upper and lower bound approach introduced by Takayanagi et al.[23] and described in some detail by Manson and Sperling[24] being the simplest starting point. The only difficulty expected when applying this theory to aqueous gels is that these are three-component systems, while the original treatment applied to binary composites containing pure, mutually insoluble, components whose individual rheological properties were independent of the macroscopic amounts present. The existence of a variable amount of solvent able to partition itself between two polymer constituents is a complicating factor but, as the present section will show, the difficulties it presents can be overcome by suitable elaboration of the original approach.

The Takayanagi Approach. To understand this extended version of the simple theory it is necessary to begin with formulation of the Takayanagi approach as it is normally applied to two-component composites. If X and Y are these components and they have individual shear moduli (real parts only considered here) G_X and G_Y, then the Takayanagi equations provide upper (isostrain) and lower (isostress) bound limits for the value of the shear modulus G_C of a composite formed from X and Y. If ϕ_X and $\phi_Y = 1 - \phi_X$ are the volume fractions of X and Y making up the composite, these limits are given by the equations

$$G_C = \phi_X G_X + \phi_Y G_Y \quad \text{(upper bound)} \tag{1}$$

and

$$1/G_C = \phi_X/G_X + \phi_Y/G_Y \quad \text{(lower bound)} \tag{2}$$

and it is clear that this theory requires the experimental G_C always to lie between G_X and G_Y. Although eq 1 and 2 do not allow calculation of the exact dependence of G_C on ϕ_Y in a given situation, it is to be expected[24] that for a simple phase-separated structure, G_C will follow lower bound behavior prior to phase inversion when the supporting phase X is the weaker one (isostress situation) and then show a transition to upper bound behavior after this. For aqueous three-component gels, however, which are the subject of the present work, the situation is different as these contain water in large amounts, and the way in which this solvent partitions itself between components X and Y during gelation determines (for any arbitrary system composition) the correct values of G_X, G_Y, ϕ_X, and ϕ_Y to be inserted into eq 1 and 2. This complicating factor can be introduced into the theory, however, provided that some reasonable assumptions are made.

Relative Affinity Parameter p. Let x and y be weight percentages of X and Y in the solution and let $w = 100 - x - y$ be the mass of associated solvent (water). The nominal concentrations of X and Y (C_X^{nom} and C_Y^{nom}) are, of course, just x and y. When the system gels, however, it is assumed that phase separation of X and Y occurs, there being formation of a composite gel system containing two distinct aqueous gel phases based on X and Y. It is further assumed that during this process αw of the solvent is associated with X, and $(1 - \alpha)w$ with Y. In this situation the real, i.e., effective, concentrations of X and Y in the two phases are

$$C_X^{eff} = 100x/(x + \alpha w) \tag{3}$$

and

$$C_Y^{eff} = 100y/(y + (1 - \alpha)w) \tag{4}$$

If the densities of the two phases are now assumed equal (since they are both dilute aqueous gels), then the volume fractions of regions X and Y are

$$\phi_X = (x + \alpha w)/100 \tag{5}$$

and

$$\phi_Y = (y + (1 - \alpha)w)/100 \tag{6}$$

In eq 3–6, α appears as a variable fraction which depends both on the composition of the system (i.e., x and y) and on the relative powers of the two polymers to attract solvent. An attempt may be made to separate these factors by introducing a parameter p which is assumed to be independent of x and y and which is intended to measure the relative affinities of networks X and Y for solvent. In terms of p, α may be written as

$$\alpha = px/(px + y) \tag{7}$$

and clearly $p < 1.0$ implies that Y is more solvent attracting than X, the opposite being true for $p > 1.0$. Possibilities for the experimental determination of p for given X and Y and a particular solvent will be mentioned later, but if for the moment eq 7 is assumed, eq 3–6 can be rewritten in terms of p as

$$C_X^{eff} = 100(px + y)/(100p + (1 - p)y) \tag{8}$$

$$C_Y^{eff} = 100(px + y)/(100 - (1 - p)x) \quad (9)$$

$$\phi_X = x(100p + (1 - p)y)/100(px + y) \quad (10)$$

and

$$\phi_Y = y(100 - (1 - p)x)/100(px + y) \quad (11)$$

It follows that for all x and y, provided p is known for the polymer pair and solvent in question and is constant over the concentration range studied, upper and lower limits for G_C can be calculated via eq 1 and 2 and the phase volume and effective concentration information supplied by eq 8–11. To do this the volume fractions ϕ_X and ϕ_Y from eq 10 and 11 are used directly in eq 1 and 2, while the effective concentrations C_X^{eff} and C_Y^{eff} are used to determine moduli G_X and G_Y appropriate to the gel phases X and Y. This step of converting these real polymer concentrations to shear moduli can be performed either by interpolating extensive experimental modulus vs. concentration data for the individual polymer systems or, if only limited data of this kind are available, by using this to evaluate constants in an analytical expression proposed by Hermans[25] to relate gel modulus to polymer concentration.

Calculated Bounds for Agar/Gelatin. In the present work this analytical approach was adopted to obviate the necessity of making detailed experimental studies of the concentration dependences of the shear moduli of agar and gelatin gels. The analytical expressions were adjusted to pass through the data points shown for gelatin in Figure 6 and through similar but more limited concentration data for agar gels. Further comments on this procedure and on the Hermans formula and its underlying assumptions are given in the Appendix. Using these methods the simple Takayanagi approach was modified and used to calculate upper and lower bounds for G_C for the agar/gelatin systems studied, X being identified with the weaker gelatin component and Y with agar. To calculate these bounds it was necessary to decide upon a suitable value for p, and though this quantity could conceivably be determined experimentally via Flory–Huggins polymer–solvent interaction parameters, χ,[26,27] appropriate to the gel state, an absence in the literature of suitable values for these precluded this objective approach. Instead, it was accepted, temporarily at least, that p was a parameter of the theory to be optimized in relation to the experimental data, and pairs of upper and lower bounds for G_C were calculated as a function of the gelatin concentration x for a range of p values surrounding $p = 1.0$. Selected results are presented for the $y = 1.0\%$ agar systems in Figure 7 and for the $y = 2.0\%$ systems in Figure 8. In these figures the bounds shown are for $p = 0.5$, 1.0, and 2.0 only, since comparison of these limits with the experimental data suggests that p is not less than 0.5, and consideration of the relative hydrophilic character of the two polymers suggests that it is unlikely to be greater than 1.0, let alone 2.0. The $p = 2.0$ result is included for completeness, however.

From Figures 7 and 8 it may be seen that in the 1% agar case the experimental behavior of G_C at high gelatin content coincides closely with the lower bound calculated for $p = 0.5$, while in the 2% case the lower bound for $p = 1.0$ (or slightly less) seems more appropriate. In what follows the remainder of the discussion will refer to the bounds calculated for $p = 1.0$, it being recognized that the uncertainty surrounding this quantity is potentially resolvable by experimental methods such as one or more of the following: (a) measurement of the appropriate χ values for agar and gelatin gels as mentioned previously, (b) measurement of the volume fractions ϕ_X and ϕ_Y by microscopy, and (c) direct determination of α (and hence p) by studies of water NMR relaxation.

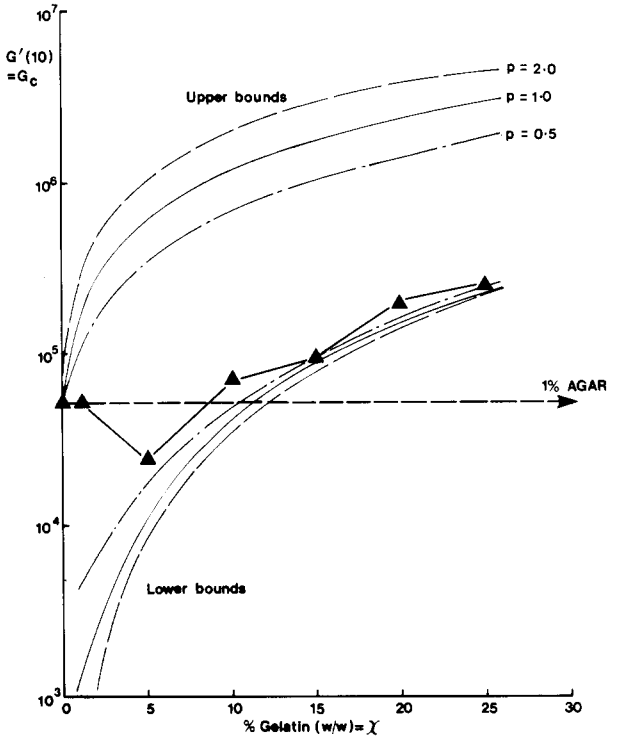

Figure 7. Upper and lower bounds ($p = 0.5$, 1.0, and 2.0) for the composition dependence of G' for the 1% agar gel series as calculated using a modification of the Takayanagi approach. For details, see text.

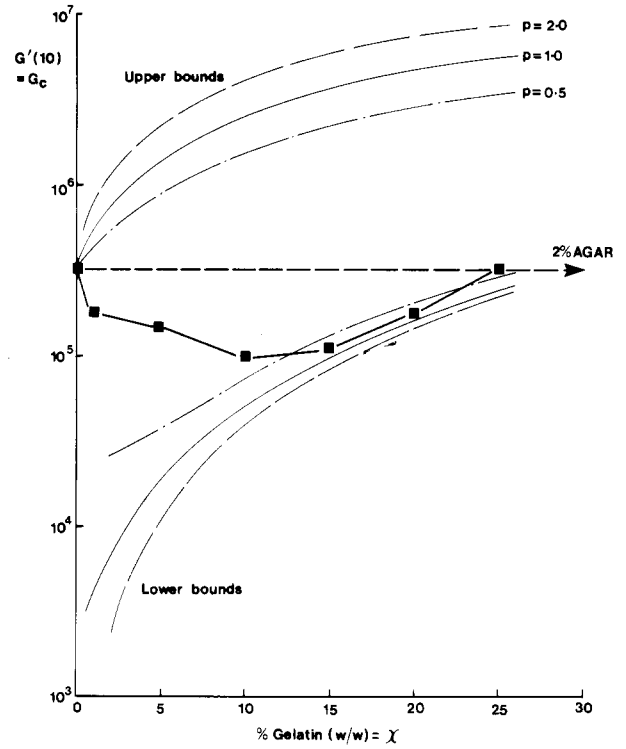

Figure 8. Upper and lower bounds ($p = 0.5$, 1.0, and 2.0) for the composition dependence of G' for the 2% agar gel series as calculated using a modification of the Takayanagi approach. For details, see text.

Assuming the $p = 1.0$ bounds, it may now be seen how successful these limits are in explaining the experimental composition dependence of G_C for the two gel series to be studied. From Figures 7 and 8 it is clear that only at high

gelatin content do the two systems show behavior consistent with the theory, for, in both cases, as the gelatin content is increased beyond that necessary for phase inversion the modulus G_C approximates predicted lower bound behavior. This is reasonable since the simplest expectation of the G_C vs. x behavior for both series of gels must be that G_C follows upper bound behavior up to the phase inversion point (since the supporting agar phase is the more rigid one) and then collapses to the lower bound curve as x increases beyond the inversion point, and the softer gelatin phase becomes the supporting gel. While the lower bound expectation is fulfilled, it is clear from Figures 7 and 8 that the initial upper bound behavior does not occur, for, in both cases, G_C falls as gelatin is added and reaches a minimum in the vicinity of the inversion point. Clearly, in the agar/gelatin case the simple prediction of upper and lower bound behavior with a transition between these limits is only partly verified experimentally. It is worth noting, however, that such conclusions as can be drawn from Figures 7 and 8 are not crucially affected by the choice made for p and that the real behavior of the gels is always to be found on or between the upper and lower limits specified.

Modified Bounds for Agar/Gelatin. Following these preliminary attempts to rationalize the mechanical properties of agar/gelatin gels, a modification to the theoretical treatment described above was considered with a view to making it provide a more satisfactory explanation of the experimental data. This reexamination focused on the assumption made initially that the modulus contributions for the component gel phases X and Y (i.e., G_X and G_Y) could be estimated from effective concentrations of the molecular species present and from experimental modulus vs. concentration data for these species studied as individual gelling systems. Questioning of these assumptions was prompted by the realization that the anomalously low values for G_c found experimentally (particularly at low x) could only be explained if the agar contribution to the gel strength in such systems was considerably less than would be expected on the basis on the effective concentration (C_Y^{eff}) calculated from eq 9 and the known experimental concentration dependence of the modulus for pure agar gels. Since suitable modifications of calculated C_Y^{eff}'s would require a drastic change in p and a substantial relaxation of the condition that p was independent of x and y, it was decided to explore the alternative possibility that agar gels formed in a gelatin environment are softer than pure agar gels formed alone at the same effective concentration. This was done by assuming that the agar phases present at all x behaved mechanically as if their concentrations were closer to C_Y^{nom} than C_Y^{eff}, and accordingly earlier calculations of upper and lower bounds were repeated with the modification that everywhere C_Y^{eff} was replaced by C_Y^{nom} (equal to 1.0 or 2.0% w/w). The new upper and lower bounds determined in this way are compared with earlier estimates in Figures 9 and 10, and from these figures it is clear that while the new lower bounds are only slightly altered in relation to the previous ones, the new upper bounds are substantially changed. These now agree well with the experimental data points from both the 1.0% and 2.0% agar gel series, this agreement being particularly important at small values of x, where upper bound behavior would be expected to occur prior to phase inversion. Since the new upper and lower bounds tend to come together as x increases beyond the inversion point, the experimental data agree well with theory in this composition range as well. In summary, what has been achieved is that the experimental dependence of G_c on x has now been modeled on the low, as well as the high, x side of the inversion point, albeit at the cost of sacrificing some of the simple logic of the original argument. For the agar/gelatin system at least, it seems that the activity of agarose molecules as they gel in a mixed agar/gelatin environment is not significantly different from

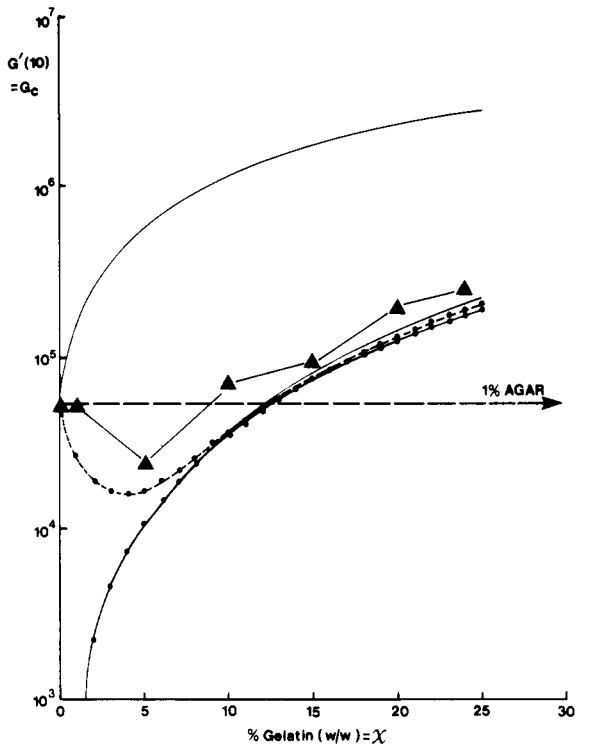

Figure 9. Modified (p = 1.0) upper and lower bounds (-·-) calculated using C_{agar}^{nom} instead of C_{agar}^{eff} are compared with the original estimates of Figure 9 (—) for the 1% agar gel series. Only the upper bound is substantially changed.

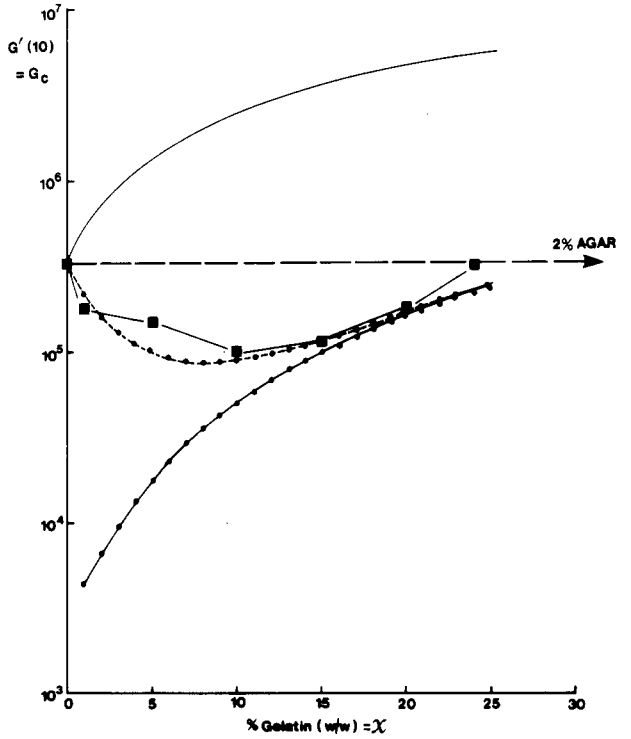

Figure 10. Modified (p = 1.0) upper and lower bounds (-·-) calculated using C_{agar}^{nom} instead of C_{agar}^{eff} are compared with the original estimates of Figure 10 (—) for the 2% agar gel series. Only the upper bound is substantially changed.

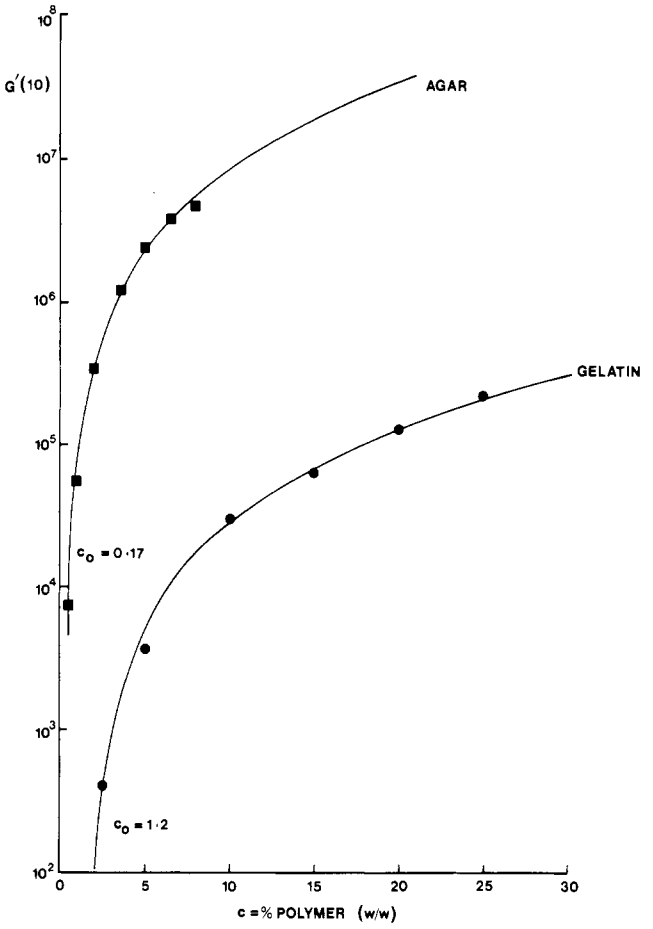

Figure 11. Fits of experimental G' (at 10 rad s^{-1}) vs. concentration data achieved for agar and gelatin gels using Hermans' theoretical expression.

that which would apply if no gelatin molecules were present at all. Also, as the gelatin network inclusions form at a later stage in the gelling process, the agar network does not respond in the fashion required by the Hermans treatment (see Appendix); i.e., it does not increase its cross-link density in the way prescribed by Hermans' equations for a network suffering concentration. This could be a particular kinetic property of agar gel formation, for the hysteresis behavior of agar as a gelling polymer is well-known,[17] but further study is needed to prove this. Indeed, the whole question of whether or not cross-link formation in biopolymer gels can generally be described as a mobile equilibrium readily shifted by changes in temperature, concentration, etc. remains to be established even though there is no doubt that in mathematical terms Hermans' equation fits experimental G' vs. concentration data for individual gelling systems and conditions very well indeed.

Discussion

The mechanical properties of agar/gelatin gels have received some limited attention in the literature, but no previous study has been set up to investigate the microscopy of such gels nor to relate observed microstructure to rheological data. In the past, however, Watase et al. have studied[28,29] the effect of composition on gel melting temperature and on the real part of the extension modulus E' ($\approx 3G'$). From the dependence of the gel melting temperature on the proportion of gelatin present, they conclude that the two polymers form individual networks, and judging by the point at which this temperature changes suddenly with composition, phase inversion occurs in their systems at 0.5% agar/5% gelatin. The present work confirms their conclusions about the phase-separated nature of the microstructures of these gels, and no doubt the slight contradiction between present estimates of the composition required for phase inversion and theirs can be reconciled with differences in the polymer samples used (e.g., "primary chain" molecular weight and molecular weight distribution) and differences in heating and cooling regimes.

In terms of the gel moduli, Watase et al. also report minima in plots of E' against composition, and in one article[28] they attempt, with what they admit is little success, to fit extended equations of the Takayanagi type to the data. Eventually, they attribute the minima observed to a specific interference effect that causes the agar network to be softer than normal when formed in the presence of ungelled gelatin. Their theoretical approach is always likely to fail, however, because of the incorrect way they assess the gel component phase volumes in terms of the volumes of the two gelling polymer solutions originally mixed together. In the present work the problem of finding a theoretical model for such systems is attacked in a more realistic way, that is, one in which the likely reorganization of water between the two components is taken into account. This theory can be used to provide a quantitative description of the experimental data including the minima just mentioned, provided that it is accepted that the agar gel strength contribution to the modulus is less than would be expected on the basis of the amount of agar present and the volume finally occupied by the agar network in the composite. In the present work this effect is ascribed to the fact that the agar network forms first and that the subsequent concentrating action of the aggregating gelatin is insufficient to increase the strength of the agar phase to a value equivalent to a pure agar gel at the new effective concentration. This property could indeed be identified with the interference effect discussed by the Japanese, and to this extent there is no contradiction between the findings presented here and their results. The present paper offers a more quantitative description of the small-strain rheology of mixed aqueous gels, however, and in the case of the agar/gelatin systems also provides experimental evidence regarding their microstructures.

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Appendix

In his original paper[25] Hermans derived a theoretical expression to relate the equilibrium shear modulus of a polymer gel to the polymer concentration. This was based on the Flory–Stockmayer theory of gelation[30–32] and assumed that an equilibrium between cross-link association and dissociation was established at any temperature. This equilibrium was controlled by an equilibrium constant, which, in turn, depended on the intrinsic stability of the cross-links formed. Using this concept, the degree of reaction of the available polymer cross-linking sites could be formulated at any concentration and hence the appropriate cross-link density established. Using ideal rubber theory,[33] this last quantity allowed the shear modulus G to be expressed as a function of c, the concentration, or, more generally, as a function of c/c_0, where c_0 was the critical concentration for gelation appropriate to the system concerned. Hermans' final result can be written as

$$G = (RT/2fMK)(w_g(2 - w_g)c/c_0 - 2w_g)c/c_0 \quad \text{(A1)}$$

and in this equation f is the functionality of the gelling polymer, i.e., the available number of cross-linking sites per molecule, M the molecular weight, K the equilibrium constant for cross-link formation, and w_g the gel fraction, assumed by Hermans to be given by the Flory–Stockmayer relation

$$w_g \sim 1 - \exp(-w_g c/c_0) \quad \text{(A2)}$$

This is valid for large f only, and indeed throughout his analysis Hermans made the assumption that f was very much greater than 2. Fortunately, for the agar and gelatin systems studied here, this assumption should not be too limiting since for gels that arise via formation of ordered junction zones and that have x zones per chain and n chains per zone, the functionality f is equivalent to (see ref 16) $1 + (n - 1)(x - 1)$. For gelatin, experimental data[16] suggest that $n = 3$ and $x \sim 5$–8 and this makes $f \sim 10$. Though similar experimental results are not yet available for agar gels, an analogous situation is likely to obtain with the zones involving double rather than triple helices, and again f is likely to be moderately large.

From the nature of eq A1 it is clear that it can be used to fit any set of G vs. concentration results, if two variable scale factors are introduced corresponding to c_0 and $RT/2fMK$. In the present case this was done using experimental data (G' at 10 rad s^{-1}) for pure gelatin and agar gels and a simple least-squares fitting procedure. The fits achieved are shown in Figure 11 and are generally very good. This shows that, within the concentration range readily accessible to experiment (it is difficult to study agar gels at concentrations much greater than 8% w/w for reasons of polymer insolubility and gel syneresis), the Hermans treatment provides an adequate means of interpolating experimental modulus vs. concentration data. However, for the agar in particular, it was necessary in many of the calculations described earlier to use the Hermans function as a means of extrapolating G vs. c results to values of c/c_0 greater than could be studied with accuracy experimentally. This meant that a considerable reliance was placed on the correctness of eq A1 in all the calculations described earlier in this paper, and because of this an alternative treatment of the modulus vs. concentration problem was subsequently investigated to establish what effects if any uncertainties in the assumed G vs. c relationship might have on general conclusions drawn from this work. This alternative analysis was also based on the concept of a cross-linking equilibrium but allowed f to take on any value greater than 2. Another difference was that the methods of the cascade approach[34-36] to the theory of gelation were used to calculate G in terms of the degree of reaction achieved at any concentration. Details of this investigation will be published elsewhere but the main outcome was that the cascade treatment provided an alternative G vs. c/c_0 relationship to that given by (A1) and one that depended on f. It was found that for $f < 100$ and $c/c_0 > 10$ this function differed significantly from the Hermans expression. Since this finding cast some doubt on the reliability of the agar contribution to the composite modulus calculated by the Hermans approach (i.e., implied the possibility of error in extrapolations beyond $c = 10\%$ w/w), these calculations were repeated using the $f = 10$ cascade version for the G vs. c relationship. Though the upper bound curves in Figures 7 and 8 were substantially lowered on doing this, the lower bounds were largely unaffected. The change in upper bound did not, however, explain the unexpected behavior of the agar discussed previously, and it was still necessary to replace C_Y^{eff} by C_Y^{nom} to achieve a good fit to the experimental data. It was concluded that, significant as errors in the chosen G vs. c/c_0 theoretical relationship might prove in certain situations, they were not likely to affect conclusions reached here regarding the properties of agar/gelatin gels. Other uncertainties in the adopted model such as the exact value of p to use or whether or not in principle G vs. c relationships are suitable for application in co-gelling situations seem to be of greater importance.

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References and Notes

(1) J. D. Ferry, *Adv. Protein Chem.*, **4**, 2 (1948).
(2) M. Joly, "A Physico-chemical Approach to the Denaturation of Proteins", Academic Press, London, 1965.
(3) M. P. Tombs, in "Proteins as Human Foods", R. A. Lawrie, Ed., Butterworths, London, 1970, p 126.
(4) M. P. Tombs, *Faraday Discuss. Chem. Soc.*, **57**, 158 (1974).
(5) M. G. Bezrukov, *Angew. Chem., Int. Ed. Engl.*, **18**, 599 (1979).
(6) A. H. Clark and C. D. Tuffnell, *Int. J. Pept. Protein Res.*, **16**, 339 (1980).
(7) A. H. Clark, D. H. P. Saunderson, and A. Suggett, *Int. J. Pept. Protein Res.*, **17**, 353 (1981).
(8) A. H. Clark, F. J. Judge, J. B. Richards, J. M. Stubbs, and A. Suggett, *Int. J. Pept. Protein Res.*, **17**, 380 (1981).
(9) D. A. Rees and E. J. Welsh, *Angew. Chem., Int. Ed. Engl.*, **16**, 214 (1977).
(10) E. R. Morris, D. A. Rees, D. Thom, and E. J. Welsh, *J. Supramol. Struct.*, **6**, 259 (1977).
(11) D. A. Rees, *Pure Appl. Chem.*, **53**, 1 (1981).
(12) R. K. Richardson and S. B. Ross-Murphy, *Int. J. Biol. Macromol.*, **3**, 315 (1981).
(13) R. K. Richardson and S. B. Ross-Murphy, *Br. Polym. J.*, **13**, 11 (1981).
(14) S. B. Ross-Murphy, A. H. Clark, R. K. Richardson, G. Robinson, and A. C. Weaver, *Prog. Food Nutr. Sci.*, **6**, 149 (1982).
(15) R. K. Richardson, G. Robinson, S. B. Ross-Murphy, and S. Todd, *Polym. Bull.*, **4**, 541 (1981).
(16) C. A. L. Peniche-Covas, S. B. Dev, M. Gordon, M. Judd, and K. Kajiwara, *Faraday Discuss. Chem. Soc.*, **57**, 165 (1974).
(17) I. C. M. Dea, A. A. McKinnon, and D. A. Rees, *J. Mol. Biol.*, **68**, 153 (1972).
(18) D. L. Siegfried, J. A. Manson, and L. H. Sperling, *J. Polym. Sci., Polym. Phys. Ed.*, **16**, 583 (1978).
(19) S. C. Kim, D. Klempner, K. C. Frisch, and H. L. Frisch, *Macromolecules*, **10**, 1187 (1977).
(20) L. H. Sperling, *J. Polym. Sci., Polym. Symp.*, **No. 60**, 175 (1977).
(21) D. A. Thomas, *J. Polym. Sci., Polym. Symp.*, **No. 60**, 189 (1977).
(22) D. Klempner, *Angew. Chem., Int. Ed. Engl.*, **17**, 97 (1978).
(23) M. Takayanagi, H. Harima, and Y. Iwata, *Mem. Fac. Eng. Kyushu Univ.*, **23**, 1 (1963).
(24) J. A. Manson and L. H. Sperling, in "Polymer Blends and Composites", Heyden Press, London, 1976.
(25) J. R. Hermans, *J. Polym. Sci., Part A*, **3**, 1859 (1965).
(26) P. J. Flory "Principles of Polymer Chemistry", Cornell University Press, Ithaca, NY, 1953.
(27) R. Koningsveld, *Adv. Polym. Sci.*, **7**, 1 (1970).
(28) M. Watase and K. Nishinari, *Rheol. Acta*, **19**, 220 (1980).
(29) H. Moritaka, K. Nishinari, H. Horiuchi, and M. Watase, *J. Texture Stud.*, **11**, 257 (1980).
(30) P. J. Flory, *J. Am. Chem. Soc.*, **63**, 3083, 3091 (1941).
(31) W. H. Stockmayer, *J. Chem. Phys.*, **11**, 45 (1943).
(32) W. H. Stockmayer, *J. Chem. Phys.*, **12**, 125 (1944).
(33) L. R. G. Treloar, "The Physics of Rubber Elasticity", Clarendon Press, Oxford, 1958.
(34) M. Gordon, *Proc. R. Soc. London, Ser. A*, **272**, 54 (1962).
(35) M. Gordon and S. B. Ross-Murphy, *Pure Appl. Chem.*, **43**, 1 (1975).
(36) K. Dušek, *Makromol. Chem., Suppl.*, **2**, 35 (1979).