Large deformation and ultimate properties of biopolymer gels: 1. Single biopolymer component systems

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The large deformation and ultimate properties of gelatin and agarose gels were investigated, in simple tension, as a necessary preliminary to an examination of mixed biopolymer gel systems. The underlying structure of these systems is sufficiently similar in certain respects to that of a 'rubber-like' network, to suggest that an analysis along these lines might be fruitful. It is found that under tensile test conditions, the factorization of strain and time dependence appears to be valid over all experimental conditions encountered. Modulus values from the initial slope of stress/strain curves confirm previously derived modulus/concentration relationships. Doubly logarithmic plots of stress at break against strain at break ('failure envelopes') provide a means of comparing ultimate properties as a function of polymer concentration. Since both break stress and break strain exhibit strong dependence on strain rate, minimum observed values of these quantities have been chosen to characterize the failure process.

(Keywords: gelatin; agarose; composite; biopolymer gel; tensile testing; stress relaxation; rubber elasticity; failure envelope; ultimate properties; stress function; network)

INTRODUCTION

A recent publication reported the results of an investigation into the small deformation mechanical properties of mixed (phase-separated) agarose/gelatin gels[1]. The shear moduli of these composite biopolymer gels were derived from the moduli of their components by a novel method. A subsequent publication will report the continuation of this work to include the large deformation regime. As a preliminary, however, the behaviour of the single biopolymer component systems under larger (tensile) strain (already summarized elsewhere[2]) has been examined in greater detail. Attention was focused on two particular aspects, viz. the dependence of stress/strain profiles on polymer concentration, and the dependence of failure properties on both concentration and tensile strain rate.

A diverse range of structural and molecular organizations can give rise to gel systems[3]. It is now widely accepted, however, that the behavioural similarities of these systems are of as much interest as their structural differences, and a common feature of gel systems is the existence of some kind of three-dimensional network structure.

It might be argued that the best understood such system, in the theoretical sense, is that envisaged in theories of 'rubber' elasticity. This is formally constructed by the covalent crosslinking of linear polymer molecules into a network, the chain vector distribution between junction points being described by a Gaussian probability function. The detailed behaviour of this type of network is currently receiving considerable attention in the literature[4]. Such theories might be expected to provide at least a starting point for the understanding of biopolymer gels, which are, however, more complex in a number of respects.

It is now generally accepted that the 'cross-links' in many biopolymer systems are due to cooperative sequences of ordered chains ('junction zones') separated along the chain contour by disordered regions. In particular, it is believed that in some biopolymer systems these junction zones are multiple helices, triple (collagen type) helices for gelatin gels[5] and double helices for agarose gels[6]. There is the additional possibility of further, quasi-crystalline, inter-helix association. (Gelatin, e.g. exhibits very marked photoelastic properties usually associated with orientable ordered regions.) Both types of interaction provide effective physical crosslinks which are permanent over the time-scale of our experiments, if the deformation is not too large. Nonetheless, a degree of dissociation/reassociation is likely to occur due to thermal fluctuations[3], if the properties are monitored over a sufficiently long time. The ease with which this can occur will be altered by the application of a finite strain.

The lengths of disordered chains between junction zones are generally shorter than the large sections of random coil described by rubber theories. Thus the assumption of a Gaussian distribution of chain lengths is likely to be of limited validity. Further, these chains are certainly less flexible than in a synthetic random coil polymer because of the restricted rotation about peptide or glycosidic bonds. Therefore, a discussion based on the consideration of purely entropic contributions to elastic phenomena is almost certain to be inappropriate.

The gels as normally prepared contain a very large volume fraction of water, and can be considered to be swollen networks. Additional complications may arise due to the polar nature of water as a solvent, and the polyelectrolyte substituents on the gelation chain. Also present in these systems at low concentration is a 'sol fraction', i.e. polymeric material not physically connected to the 'infinite' network.

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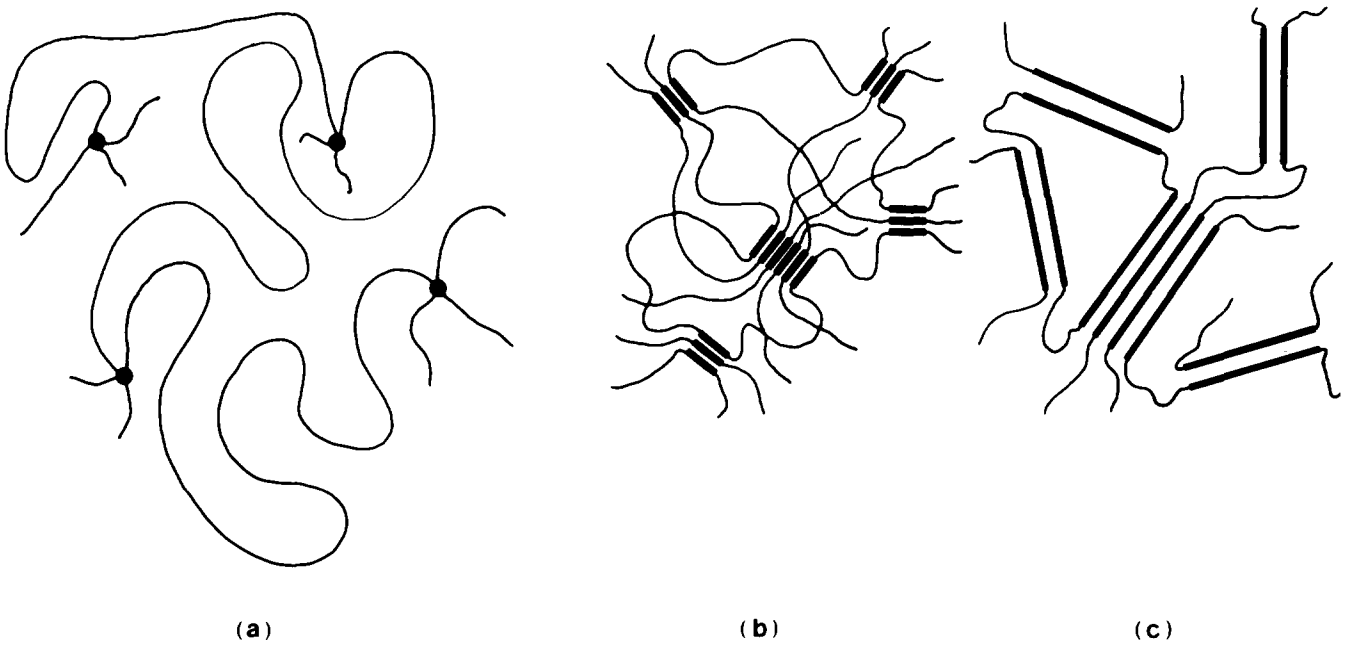

Figure 1 Schematic representation of network types: (a) 'Ideal rubber'. Circles are covalent crosslinks. (b) Gelatin and (c) Agarose. Heavy lines indicate helical junction zones

The similarities and differences between these different types of network are illustrated schematically in *Figure 1*. In view of the above comments, it was felt that no satisfactory theoretical treatment of these more complex networks has yet been developed, and, at least initially, the characterization should be largely phenomenological in nature.

When mixed gels are prepared, according to our recent model[1] the two components partition the available water between them. If one component gels before the other, the gelation of the second component may result in further redistribution of water between the two components. A full analysis requires a knowledge of how a system which has already undergone gelation behaves when the water content is further altered.

THEORY

Throughout the following discussion, the strain, extension ratio, stress (force per unit original cross-sectional area) and true stress (force per unit actual area) will be denoted by ε, λ, σ and $\bar{\sigma}$ respectively.

The assumption of incompressibility implies that Poisson's ratio is 0.5, or equivalently, that the Young's modulus E, at equilibrium, is three times the shear modulus, G.[7] Although values of Poisson's ratio significantly different from 0.5 have been reported for other systems, comparison of the Young's modulus from our tensile experiments with previously reported values of G,[1] allowing for the different temperatures at which the experiments were performed, indicates that the assumption of incompressibility is reasonable for our systems. In this case, $\bar{\sigma}=\lambda\sigma$.

Strain and time dependence

The time dependence of stress, for synthetic polymer systems experiencing small strains, is well understood[7]. In a stress relaxation experiment, the stress (at constant strain), and hence the modulus (which is the ratio of stress to strain) decreases with time. This time dependence is frequently, although simply, expressed as a sum of exponentials, $E(t)=\sum_{i=1}^{n} E_i e^{-t/\tau_i}$, where $E(t)$ is the time-dependent Young's modulus, the E_i are scaling constants, the τ_i are relaxation times, and n is large (usually of the order of the number of sub-units per molecular chain). For larger deformations, the dependence of stress on strain is non-linear. Further analysis is considerably simplified, if, in a stress relaxation experiment, the effects of strain and time are separable at all deformations. In this case we can write, formally

$$\sigma(t)=E(t).\varepsilon=E(t)(\lambda-1) \tag{1a}$$

for small deformation, and for larger strains,

$$\sigma(t)=E(t).f(\lambda) \tag{1b}$$

where f denotes some functional relationship, as yet unspecified.

This separability is established experimentally by ensuring that doubly logarithmic plots of stress against time are parallel for different initial step strains.

Experiments frequently employ more complex loading patterns, the application of strain at a constant rate being particularly popular. When only small strains are considered, the Boltzmann superposition principle is invoked[7], to produce a constitutive equation of the form

$$\sigma(t)=\int_0^t E(t-u)\dot{\varepsilon}\,du \tag{2}$$

where $$\dot{\varepsilon}=\frac{d\varepsilon}{du}$$

We now consider two approaches which extend this treatment to larger strains at constant strain rate. Smith[8] has attempted to include the separability of equation (1b) at all strain rates by writing

$$\sigma(t)=F(t)\Gamma(\lambda) \tag{3a}$$

where the function Γ represents the strain dependence, and the 'constant strain rate modulus', F, is defined via equation (2) as

$$F(t)=\sigma(t)/\varepsilon(t)=\sigma(t)/\dot{\varepsilon}t$$

$$=\frac{1}{t}\int_0^t E(t-u)\mathrm{d}u \qquad (3b)$$

(Since the strain rate is constant, $\dot{\varepsilon}$ in equation (2) can be taken outside the integral as a factor.)

Alternatively, Bloch *et al.*[9] have developed a more formal analogue of the small deformation constitutive equation, which for constant strain rate gives

$$\bar{\sigma}(t)=\dot{\varepsilon}\int_0^t E(t-u)\frac{\mathrm{d}f}{\mathrm{d}\lambda(u)}\mathrm{d}u \qquad (4)$$

The integral formulation is only valid if it can be shown that strain and time effects uncouple in a stress relaxation experiment, and would imply that no such separability should be observed for more complicated loading patterns, including the constant strain rate test.

However, if either of the functions E or f' $(=\mathrm{d}f/\mathrm{d}\lambda)$ vary slowly enough, they can, to a good approximation, be taken outside the integral as a factor. The latter case has been discussed by Bloch *et al.*[9], but in the present study, it has been found more fruitful to set $E(t)$ approximately constant. It can then be seen from equation (3b) that $F(t)\sim E(t)$ ($\sim$constant), and since $\mathrm{d}\lambda=\dot{\varepsilon}\mathrm{d}u$, evaluation of equation (4) gives $\sigma(t)=\bar{\sigma}(t)/\lambda=E(t).f(\lambda)/\lambda$. Thus, in this special case, if $\Gamma(\lambda)$ is identified with $f(\lambda)/\lambda$, and $E(t)$ with $F(t)$, equations (3a) and (4) become equivalent.

When the experimental results are discussed, it will be seen that, from stress relaxation experiments, the time-dependent modulus does indeed vary only slowly with time, while the dependence of stress as a function of strain is by no means linear. However, this effect of strain could be well fitted by the so-called BST equation (to be discussed later). The equivalence of equations (3a) and (4) could then be checked by direct numerical evaluation of the stress.

It should be noted that this procedure requires a knowledge of $E(t)$ at times much shorter than those accessed experimentally. In this particular case, values for $E(t)$ of these short times were estimated by the addition of further exponential terms to the stress relaxation data, with relaxation times chosen to fit two constraints: (i) the calculated values of stress did not noticeably depend on strain rate, for those rates achieved experimentally (this restriction enforces agreement with experimental observation), (ii) the additional terms should decay just enough not to contribute significantly to the values obtained from stress relaxation experiments, within the time scale of the measurements.

While somewhat artificial, this procedure demonstrates two effects:

(a) The stress/strain curves do depend on $\dot{\varepsilon}$ at higher $\dot{\varepsilon}$ (the magnitude of this effect depends on the exact form chosen to model $E(t)$).

(b) Even for the most extreme dependence on strain rate consistent with the constraints discussed above, values of stress calculated from equations (3a) and (4) respectively do not diverge significantly for calculated strain rates >100 times higher than were imposed experimentally.

This 'mathematical modelling' indicates that the more tractable Smith formulation, equation (3a), adequately represents the dependence of stress on both strain and time up to fairly high strain rates. The relevance of this should be apparent from the following discussion of failure properties.

Ultimate properties

Discussions of failure (fracture, rupture) are usually couched in terms of concentration of stress at some microscopic crack or heterogeneity. Crack growth initiates when some local failure criterion (such as a critical strain, stress, surface energy, etc.) is achieved. Where there are mechanisms which allow the dissipation of stored elastic energy, it is usually necessary to postulate a period of slow crack growth, before catastrophic failure occurs[10-12]. The relevance of the preceding discussion is now clear. A material which shows very little dependence of stress on strain rate may behave very differently locally, where the stress, strain and hence strain rate are considerably higher (frequently by a factor 10^3 to 10^4)[10]. Further, most attempts to analyse this behaviour theoretically start from an assumed strain–time separability of the form of equation (2) (e.g. ref. 12).

The failure of biopolymer gels in a tensile test is of the 'tearing' type commonly exhibited by elastomeric materials, and usually associated with the breaking of bonds within the network structure, rather than the 'ductile' fracture related to the slippage of macromolecules past each other. One of several[12] theoretical treatments of this type of behaviour is that due to Bueche and Halpin[10]. This treatment would give the stress and strain at break in a tensile test as

$$\sigma_B=K.E(t/q) \qquad (5)$$

$$f(\lambda_c)=K.E(t/q)/E(t) \qquad (6)$$

where t is the time to break, and $K=f(\lambda_c)/s$. The value of λ_c, the critical (local) strain at which failure initiates, is given physical interpretation as the maximum extensibility of the network chains. Stress is concentrated by a factor s, which depends only on the local geometry of the microcrack, or equivalent inhomogeneity, and a distribution of such flaws accounts for the large degree of scatter normally observed for failure properties in supposedly replicate experiments. The constant q can, essentially, be related to the distance over which slow crack growth proceeds.

Although other theories have been postulated[12], since they are intended to rationalize the same type of data, the predicted curves exhibit the same type of shape. Further, the need to invoke stress concentration, slow crack growth, and strain–time separability is generally accepted. The main intent of the discussion is to demonstrate that the rate-dependence of ultimate behaviour is controlled by the dissipation of energy at the crack tip. There are obvious attractions in using a theory which attributes this dissipation solely to those viscoelastic mechanisms which determine the bulk stress response, with the additional hope of relating the derived parameters of the theory to network structure.

The failure envelope

The physical extent of slow growth must be small for our gel systems, since no appreciable drop in stress due to a reduction in load-bearing area occurs prior to rapid failure. Thus, plots of (log) break strain against (log) break stress, the failure envelope, lie on the stress–strain curve defined by bulk behaviour. The factor which does depend on strain rate is how far along this curve we can proceed before failure occurs. Due to the rapid initiation and propagation of cracks under local regions of high stress (and, presumably, high strain rates), the specimen as a whole fails at a relatively low extension. The failure envelope is a convenient way to compare the ultimate properties of, say, gels of different concentrations.

It is also of note that we cannot extend the experimentally accessible range of strain rates by attempting to apply time–temperature superposition (see, e.g. ref. 7) since for the present gel systems, this is likely to alter the macromolecular structure (number of crosslinks, 'junction zone' size, etc.) of the network.

Since quantities referring to local behaviour have been eliminated, scatter due to a distribution of inhomogeneities is not reflected in the data presentation. However, the failure envelope does not yield any information which is not implicit in the full stress–strain profile. Any additional insight must be derived from the behaviour of stress and strain at break with rate or time.

Elastic properties and network structure

We have already commented on a number of differences between a biopolymer gel and the idealized network assumed in rubber elasticity theories. Some insight is gained, nonetheless, from a consideration of properties which depend only on the network-type structure. If a network in a state of tensile strain is allowed to attain equilibrium then the nominal stress, which is the derivative of the elastic free energy with respect to length, is a function of the extension ratio. The elastic energy is stored in the network chains, and a problem which must be addressed is how to determine the number of such chains per unit volume. This comment can be clarified by a brief, qualitative review of the gelation process.

Prior to gelation, there is a concentration of polymer molecules in solution, each bearing a number of sites (or for biopolymers, zones) which are potentially able to react with similar sites on other molecules, or on a different part of the same molecule. As the course of the reaction proceeds, a distribution of species develops (dimers, trimers and so on). There will be a critical degree of reaction, the gel point (which, for a given temperature, occurs at concentration c_0) when one such species becomes effectively infinite. Many authors have examined the details of this process (e.g. ref. 13), but two points are of particular relevance. Even beyond the gel point, there will still exist a distribution of species, the sol fraction, not physically attached to the infinite network and therefore not contributing to elastic phenomena. Secondly, even within the network there are a number of chains which, at equilibrium, are not capable of supporting stress. Moreover, these can be more complex than single molecular 'dangling ends'. Indeed, close to the gel point, whole aggregates loosely attached to the network do not contribute to its load-bearing properties. Thus the problem becomes, for any given degree of reaction, one of counting the number of network chains which contribute to these load-bearing properties, i.e. those which are elastically active (the EANCs).

Modulus and concentration

Gordon and Ross-Murphy[13] give the equilibrium Young's modulus, E_e, as

$$E_e = 3G = 3g\frac{kTN_A}{V_{mol}}.N_e \qquad (7)$$

G = shear modulus

The parameter g, the so-called front factor, is a number of the order of unity which appears in theories of rubber elasticity. The temperature is T, k is Boltzmann's constant and N_A is Avogadro's number. The quantity V_{mol} is the volume per mole of polymer chains in solution prior to gelation (primary chains). If each EANC contributes an amount gkT to the shear modulus, it is easily seen from equation (7) that the quantity N_e is effectively the number of EANCs per primary chain.

In essence, different statistical approaches to gelation derive N_e as a function of α, the degree of reaction of available crosslinking sites. Hermans[14] derived α as a function of polymer concentration, on the assumption that an equilibrium between crosslink association/dissociation is established at any temperature. Using α values based on this assumption of an equilibrium, Clark *et al.*[1] have calculated the shear modulus as a function of concentration, using both Flory–Stockmayer gelation theory[15–17] and cascade theory[13,18,19] to evaluate N_e. Results based[20] on the cascade treatment confirm a value of g close to unity for gelatin, but indicate a considerably larger value for agarose (a contribution to the modulus of approximately $20\,kT$ per elastically active chain). This confirms the contention that the elastic phenomena, for agarose at least, cannot be accounted for solely in terms of entropic arguments.

In network terms, we can express the Young's modulus as a product of two parameters. The first parameter is the contribution per EANC ($3\,kT$ for the classical ideal rubber, where $g = 1$). The second is the number of EANCs per unit volume, ν_e, defined via equation (7) as

$$\nu_e = \frac{N_A}{V_{mol}}.N_e \qquad (8)$$

This treatment relates the modulus to α, the fraction of available crosslinking sites which have reacted. Strictly speaking, it is implicit in this type of argument that such a crosslink is permanent, in the sense that, once formed, it will not dissociate (all other factors, such as temperature, remaining unchanged). The assumption of an association/dissociation equilibrium, discussed above, is a way of relating α to concentration (Hermans[16] in fact, assumed a dimerization reaction, but other kinetic schemes could be considered). However this notion implies that a dynamic equilibrium is attained. Indeed, more rapid relaxation of stress at very long times has been attributed to such processes (ref. 7). However, the insensitivity of the storage modulus to frequency which is observed for these systems[1] is typical of a system where the crosslinks form irreversibly, i.e. are 'permanent'. In what follows, it is assumed that any change in α due to changes in such parameters as temperature, water content, etc., has been allowed to proceed long enough for

equilibrium to have been attained. In practice, of course, for both systems, the modulus will continue to increase as a function of log(time), perhaps for ever, as secondary 'crystallization' occurs. However, in the timescale of the present experiments, such changes are assumed to be, and may be seen to be, very slight and are neglected for the purposes of this study.

These ideas are of importance when considering the modulus of air-dried gels. Removal of water alters the polymer concentration. If the number of crosslinks can redistribute themselves according to the equilibrium assumed in the gelation process, the modulus as a function of concentration should lie on the curve defined by gels as normally prepared. If the crosslinks, once formed are permanent, a number of further possibilities occur. It is convenient to express the ratio of the modulus at a given concentration to that at some other concentration as some power, p, of the ratio of the respective volume fractions of the two gels. In the simplest case, the number of EANCs stays the same, but the density alters because of the volume change, giving $p=1$. If we regard the gel as originally prepared as a swollen network, Flory–Rehner theory[21] would give $p=1/3$. For networks crosslinked in solution values of $p=2/3$ have been suggested[22]. Essentially, these theories consider, in addition to the volume change, changes in the free energy due to mixing of polymer and solvent and elastic contributions due to the stretching of chains by the swelling process. It should be noted that if electrolytic or Donnan type effects contribute[21] there will be additional elastic contributions, which will further reduce the value of p.

Stress–strain response and stored energy

Current theories of rubber-like elasticity, when applied to a tensile test, predict contributions to the stress in addition to the simple form derived from the classical statistical theory. These predictions are based on such considerations as topological constraints on junction motion[23] and constraints on the motion of the chains themselves ('entanglements'[4]). An examination of our experimental data shows that the deviations from ideal rubber elasticity are much greater than would be expected on the grounds of these theories, and a more empirical approach has been adopted. Anticipating the discussion of results, we mention here that our stress/strain data could be well fitted by the phenomenological equation of Blatz, Sharda and Tschoegl (BST equation)[24], viz.

$$\sigma=\frac{3E}{2n}(\lambda^{n-1}-\lambda^{-(n+2)/2}) \qquad (9)$$

When $n=2$, this is the equation of ideal rubber elasticity, and thus, this parameter can be regarded as a measure of the deviation from ideal behaviour.

Nonetheless, we can still argue qualitatively as follows. If all the strain energy is stored in the EANCs, then, since the density of EANCs is proportional to the modulus, normalization of the stress by the modulus should give the same functional form for a given polymer, regardless of concentration. If other contributions to the strain energy are important they might be expected to manifest themselves as a concentration dependence of the strain function, and to be particularly important in the case of gels concentrated by the removal of water.

As the deformation becomes larger for elastomers the distribution of chain vectors becomes increasingly non-Gaussian. In addition, for example for natural rubber, there is the possibility of strain-induced crystallization. Both these effects lead to a much more rapid increase of stress with strain than discussed above. Since biopolymer gels break at relatively low extensions, we cannot obtain any information about their behaviour in the 'upswing' region of the stress–strain curve. For the purposes of this presentation, therefore, consideration of this region of the stress response will be neglected.

EXPERIMENTAL

Gelatin used in the investigation was supplied by Croda as Croda bone 250 gelatin (acid form), i.e. the nominal Bloom strength was 250. Gels were prepared by dispersion in cold water, followed by heating at 60°C for roughly 1 h. Solutions were held at this temperature for a further period, without stirring, to allow deaeration.

Agarose was supplied by Lysander foods. Solutions were prepared by cold water dispersion, then autoclaved. The same degassing procedure was followed. In all cases gel slabs were prepared by pouring the solutions into a mould and cooling. The mould consisted of two glass plates separated by precision metal spacers (1 cm), which also served as mould boundaries. Gels thus formed were stored for 24 h at 0°C. The gels were then allowed to equilibrate (1 h) at room temperature, 22°C, and measurements were carried out at this temperature. This storage procedure was adopted in order to minimize any further time evolution of material properties.

Deswelling was achieved by placing the gel slabs on wire trays in open air at ambient temperature, for periods ranging from 24 h to 72 h. If this process was allowed to proceed too far, gradients across the slab thickness produced a brittle surface layer, which could not be reversibly reswollen. For somewhat shorter drying periods, the process was halted by the application of a light surface coating of paraffin oil. It is assumed that the water left in the gel then redistributes itself homogeneously, and that the only likely effect is an increase in the number and/or the effectiveness of microflaws.

As mentioned earlier, it is known that for gelatin, at least, the modulus will continue to increase slowly over a period of weeks. Storage at 0°C was intended to bring the gels to 'equilibrium' more quickly. All gels, with the exception of the air-dried gels, were compared under the same storage conditions. While equilibration to room temperature required roughly one hour, the mechanical testing of a given gel required, usually, approximately 7 h. Repeat experiments, at the same strain rate, at the beginning and end of an experimental session did not give significantly different stress–strain profiles. This suggests that, as asserted earlier, no significant degree of further crosslinking occurred in this time.

The method of deswelling gels by drying in air, for periods of up to three days, could well have allowed the formation of additional crosslinks. This question will be addressed in the Results section.

Tensile testing is difficult to perform on gel strips or 'dumbbells', particularly at lower concentrations. Attempts to clamp the specimen introduce irrecoverable damage. This problem was circumvented by using ring-shaped specimens, which are hung over two dowel pins, one mounted on the transducer stage and the other on the

driven stage of the testing machine. At the concentrations studied here specimens tested in this manner displayed no tendency to fail preferentially at the points of support.

Specimens were cut from the gel slab with two concentric circular knife-edged cutters (inner diameter 37.0 mm, outer diameter 48.5 mm), and a hand press. The experiments were carried out on an Instron model 1122 Universal Tester, utilizing 12.5 mm dowel pins and four decades of crosshead speeds (1–1000 mm/min). The instrument resided in a temperature controlled room, with an ambient temperature of 22 ± 0.5°C. Force–time data were collected using a DEC MINC 11/03 laboratory computer.

Stress was calculated on the basis that the tension in each leg was half the measured force. Strains were derived from the increase in ring circumference equalling twice the increase in pin separation. The original length was based on a mean circumference passing through the centroid of the cross-section. Since the position of the centroid shifts slightly as the area contracts, it is necessary to apply a correction, derived by Smith[25].

Myers and Wenrick[26] have compared the behaviour of strips of material, ring samples and 'racetracks' (i.e. specimens cut to be tested in a manner similar to the rings, but with semicircular ends and initially straight 'legs'). Over the range of extensions and strain rates which we can access, these different geometries gave the same stress–strain curves. Therefore, this arrangement can justifiably be considered to be an experiment in simple tension.

When the ring geometry is employed, there is a 'tail' in the force–time profile associated with the straightening of the ring. Subsequent deformation is assumed to involve simple tension. The strain when the ring just becomes straight, as calculated from the initial dimensions, lies in the range 1 to 2% (extension ratio = 1.01 to 1.02). The vast majority of replicates attain strains in the range 30 to 100% before failure. Thus, extrapolation of the initial (near-linear) portion of the curve to zero strain does not lead to significant error, and this procedure was adopted. However, some replicates broke at strains lower than 10%, i.e. this 'tail' region accounts for a significant portion of the force curve. In these cases, only the failure values are quoted, and no attempt has been made to estimate other quantities (e.g. modulus).

RESULTS

Strain and time separability

As a check on separability of strain and time effects (cf. equation (2)) a series of stress relaxation experiments were conducted on a 10% (w/w) gelatin gel, accessing a range of time scale similar to that encountered in a constant rate experiment (about 1000 s). Parallel curves were obtained (*Figure 2*). Since real step loading cannot be physically achieved, these curves must show some effects due to transients, and only represent the relaxation function at times longer than about ten times the loading interval. Nonetheless, certain features are obvious.

The initial relaxation (glassy and transition zone behaviour) is very fast, and cannot be accurately observed in these experiments. Over most of the time scale, the stress relaxes very little, remaining almost constant. This is in good agreement with the flat frequency-dependence of shear storage modulus reported for all the gel systems studied (ref. 1).

The almost constant 'pseudoequilibrium' value of the relaxation modulus, and hence the constant strain rate modulus, indicates that stress–strain behaviour should exhibit little dependence on strain rate, except perhaps under very fast or very slow test regimes. For comparative purposes, values of stress for each extension ratio, calculated from the BST equation (equation (9)) with $n = 3.0$, are shown as solid lines in *Figure 2*.

In fact, when stress–extension curves obtained at different strain rates are superposed, they show no greater degree of spread than is encountered for several replicates tested under the same conditions. This spread can easily be accounted for as error in the estimate of the initial cross-sectional area. When the stress profiles are normalized by the initial slope, this small envelope of curves becomes one single curve, within the limits of transducer noise. By virtue of the arguments expounded previously, the experiments access a range of strain rates where the time dependence does not contribute significantly to the stress response. Thus, the initial slope can be taken as a value of the 'equilibrium' Young's modulus, and the normalized curves then represent the 'equilibrium' strain function.

Modulus estimates

Shear modulus values for gelatin gels (that is, Young's modulus divided by three, using the assumption of incompressibility) are plotted in *Figure 3* as a function of concentration. On the same plot are shown values previously obtained in small deformation oscillatory shear, giving generally good agreement between the two experiments. Also shown are values for the air-dried gels, the concentration being recalculated simply on the basis of weight loss due to water removal.

As already discussed, the ratio of the modulus to that in the reference state (in this case, the 10% gel before drying) might be expected to be some power p of the ratio of the volume fractions of the gel network in the two systems. In *Figure 3*, curves are shown for $p = 1/3$ (curve I) and $p = 1$ (curve II). Since the value $p = 1$, allowing for a change in volume but nothing else, is extremely unrealistic, and our modulus values sit higher than this curve, it seems very probable that some change in the number of junction zones has occurred. However, the extent of this change is not as much as would be accounted for by the equilibrium assumed in the Hermans treatments.

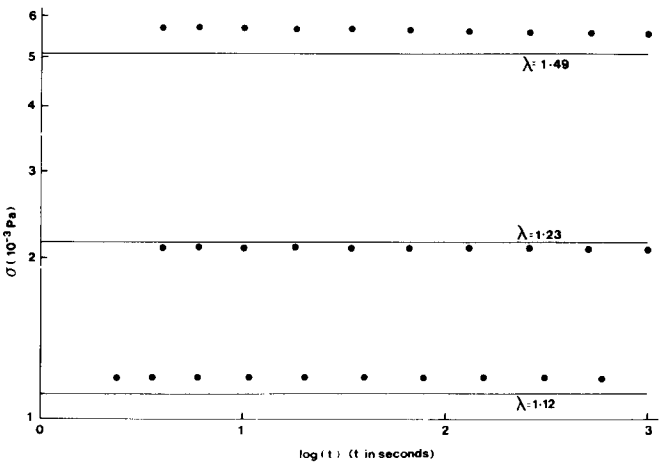

Figure 2 Stress relaxation of gelatin (10% w/w). Solid lines indicate the value of stress calculated from the BST equation, with $n = 3$

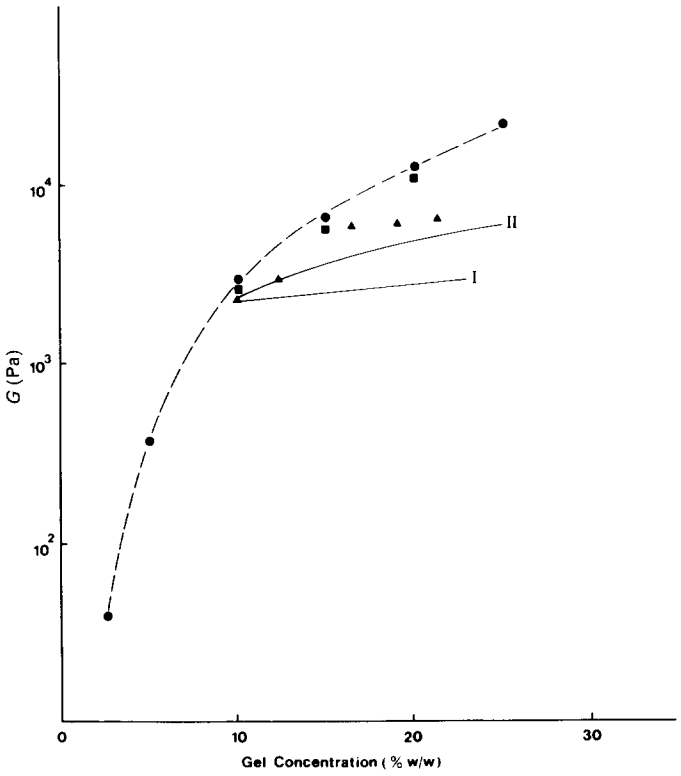

Figure 3 Modulus against concentration (% w/w): from oscillatory shear (●), from tensile tests (■) and for air-dried gels (▲). Curve I, $p=1/3$, curve II, $p=1$

The problem of the slow attainment of equilibrium has already been mentioned. Strictly, the air-dried gels should be compared with gels of the same polymer concentration, as prepared normally, and aged for a similar length of time without water loss. The effect of this would be to give a curve sitting somewhat higher than the upper curve in *Figure 3*. However, this does not qualitatively alter the reasoning given above.

THE STRAIN FUNCTION

For gelatin, stress profiles for different gels, whether formed by crosslinking in solution or by air-drying, define a single curve when normalized by the modulus. Further, there is no evidence of an upturn in any of the curves. This is interpreted as indicating that the replicates fail before this part of the stress profile can be attained. Values of the exponent n obtained from a least squares regression to the BST equation (9) are given in *Table 1*, giving an approximate value $n=3$. Also given are values of the modulus from this fit, and from the initial slope.

The modulus–concentration relationships already discussed, and verified by experiment[20], set modulus proportional to the density of EANCs. This implies that for a given temperature, once chemical equilibrium has been attained, the gels of the same biopolymer at different concentrations differ only in the number of EANCs per unit volume. Theoretically, at least, we have to assume that the entropic and enthalpic contributions per EANC to the strain energy will be the same for these two gels, independent of concentration. Thus, any differences in the strain functions will be due to differences in the interactions between chains (entanglements, etc.). Moreover, the change in volume when a gel is air-dried will bring the network chains closer together, but the modulus data would indicate that any redistribution of junction zones is not sufficient to give a network exactly equivalent to that of a gel of the same polymer concentration, as prepared normally. If interaction between chains is important, it would seem likely that the relative contribution in these air-dried systems would be greater. The coincidence of the strain function for all gelatin gels, regardless of how they are prepared, would suggest that such interactions do not contribute to the stress–strain isotherm to any noticeable degree.

A similar analysis is difficult to perform for agarose gels, which fail much more readily. Significant force–deformation data, from that part of the experiment when the ring is straight and in simple tension, can only be obtained from the fastest strain rate experiments. Nonetheless, the results were sufficiently good to define a failure envelope for each concentration which, as already discussed, is a portion of the equilibrium stress–strain curve. When these data are subjected to a non-linear least squares fit of the BST equation, a value of 4.2 is obtained for the exponent, n. Thus, the two biopolymers give different strain functions. However, the same exponent is obtained for both concentrations investigated for agarose, i.e. as for gelatin, the strain function is not concentration dependent.

Regardless of any possible molecular interpretation, it is of importance to establish experimentally that the shape of the stress response does not depend on concentration, and to be able to give this functional form (e.g. via the BST equation), since this will greatly facilitate any discussion of the properties of composite gel systems.

Ultimate properties

Plots of failure envelopes are shown in *Figure 4* for gelatin gels. (Note that the abcissa is log strain, rather than log extension ratio, for plotting convenience.) These curves define part of the full stress–strain profile, and superpose well within the limits of scatter when stress is divided by modulus.

Plots of break stress and break strain against strain rate for gelatin gels are shown in *Figures 5* and *6* respectively. Each point is the mean value of at least six replicates. This averaging procedure was adopted, because these curves markedly illustrate the statistical nature of failure—a distribution of values indicates a distribution of microflaws of different 'strength'. The extent of this scatter is indicated by error bars for the 15% gel. These bars correspond to the standard deviation about the mean.

Table 1 Modulus and exponent n from BST equation and modulus from initial slope

Gelatin concentration (% w/w)	G (N/m^2) (from initial slope)	G (N/m^2) (from 9)	n (from 9)
10	2317	2699	2.94
15	4739	5705	2.94
20	8329	9309	2.64
Air-dried gels			
12.5	2959	3435	3.11
16.5	5908	6976	2.86
19.1	6112	7283	2.99
21.5	6487	7641	3.94

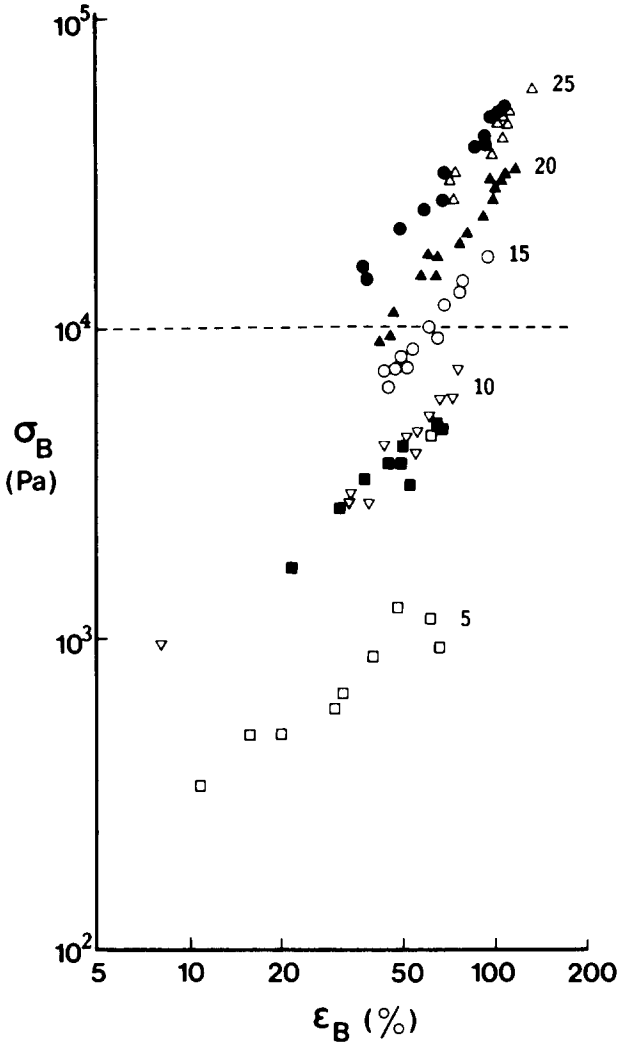

Figure 4 Failure envelopes for gelatin gels (% w/w concentration indicated against each set of points)

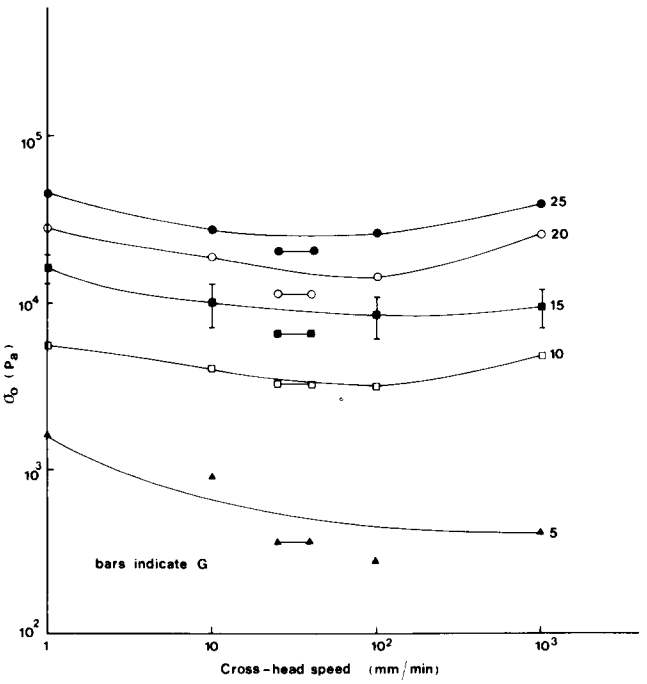

Figure 5 Dependence of break stress on strain rate (as cross-head speed) for gelatin gels (% w/w concentration indicated for each curve)

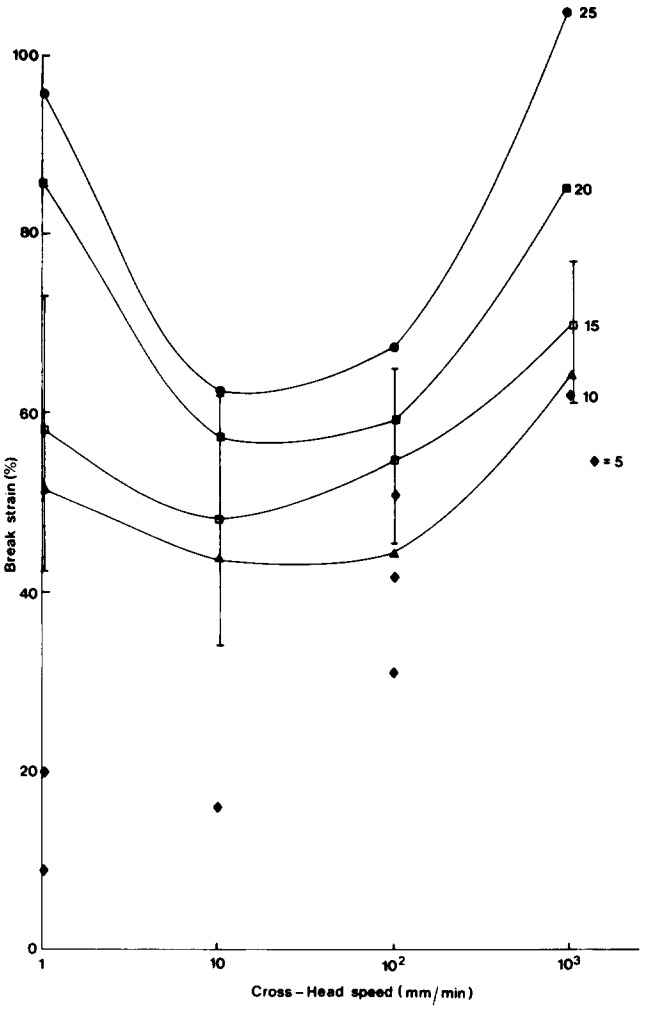

Figure 6 Dependence of break strain on strain rate (as cross-head speed) for gelatin gels (% w/w concentration indicated for each curve)

Since failure values lie on the bulk stress–strain curve, it is sufficient to examine, say, the dependence of break stress on strain rate for a given gel, since the break strain–strain rate curve can be derived from it. These curves go through a shallow minimum, falling with decreasing strain rate and rising again at the slowest rates. The high degree of data scatter indicates a broad distribution of microscopic heterogeneities capable of acting as stress raisers. Because of this, it was felt necessary to perform a statistical analysis of the results, to verify that the apparent trends were real.

For each log σ_B vs. log $\dot{\varepsilon}$ curve, a test based on the *F*-distribution[27] indicated that a quadratic fit was better than a linear fit was better than the mean stress. The quadratic fit could be suitably characterized by the coefficient of the quadratic term, and the co-ordinates of the minimum. The statistical comparison of these parameters can be summarized as follows: each gelatin concentration gives a curve of the same shape, but these curves are displaced relative to each other along the ordinate axis. The minimum break stress could be related linearly to the modulus, at a significance level of 95%, i.e.

$$\sigma_{B_{min}} = A.E + B \qquad (10)$$

This relationship is illustrated in *Figure 5*, where values of G ($= E/3$) are indicated by short horizontal bars.

These findings are at odds with the predictions of equation (5). In particular the higher break stress at lower rates is not expected. It cannot be attributed to creep,

since we do not see any drop at long times in the stress relaxation data, and also because any such effect would manifest itself in a less steep stress–strain profile at these slow rates. Various attempts were made to modify the theory, e.g. by allowing the stress concentration factor to depend on applied stress, but no great improvement was achieved. However, the difficulty is resolved, at least qualitatively, if some mechanism is proposed whereby the critical strain can increase with decreasing rate.

Consider, for the moment, a covalent, crosslinked network, and focus attention on the section of random coil chain between two crosslinks. If a further crosslink forms somewhere along the chain contour, this chain effectively becomes two shorter chain segments. Now, if both these 'new' segments are nearly fully extended, and the 'new' crosslink breaks, we have our original, longer chain segment, which will not be fully extended (except, of course, in the limiting case that both 'short' segments were parallel to the original tensile force).

The existence of helix–helix or 'quasi-crystalline' association in biopolymer networks has already been considered. If these associations are relatively weak, dissociation will require less energy than further chain extension. This situation is more complex than that described above, but this 'unzipping' should lead to an increased extensibility of the network in a similar fashion. If this process itself takes time, it will occur most markedly at the slowest rates, and the bulk material will be able to deform further before failure occurs. Since a shorter chain becomes fully extended at a lower extension ratio, networks composed of these shorter chains, i.e. gels of higher concentration, will undergo this type of dissociation more readily.

It should be emphasized that the mechanism proposed above, although plausible, is speculative and by no means proven. However, it is worth noting that data for the 5% gel (longer EANCs) although limited because of handling difficulties, are tending towards what might be expected using arguments based on viscoelasticity only. We further note that this idea of a dissociation which occurs more slowly than the initial association at the time of gelation is in keeping with the observed behaviour of the modulus of air-dried gels.

Agarose gels were only studied at two concentrations, but exhibit trends similar to those displayed by gelatin. Failure envelopes for an agarose and a gelatin gel at similar values of c/c_0 (and hence similar densities of EANCs) are shown in *Figure 7*. The curves have not been normalized by the modulus, to emphasize that the agar curve is displayed further vertically, i.e. for agarose it appears that each EANC contributes more to the modulus (cf. ref. 20). Note also that the break strain is generally lower. This evidence would support our contention of shorter network chains for agar. However, the inadequacy of the Bueche–Halpin theory precludes any quantitative estimate of chain length. Thus, any prediction of failure properties (e.g. in composite gel systems) must be based on the empirical observations from our statistical analysis (via equation (10)).

SUMMARY

For the gel systems studied, strain and time effects factor in stress relaxation. However, the 'pseudo-equilibrium' modulus is attained so rapidly that the stress profile of the bulk material is insensitive to strain rate over at least four decades.

The form of the strain function (2) could be adequately described by a BST-type equation. The modulus parameter is dependent on polymer concentration in a way which is consistent with previous data on shear modulus concentration. The exponent parameter, n, is insensitive to concentration, but agarose and gelatin give different values. That for agarose deviates further from ideal rubber elasticity than for gelatin, consistent with the hypothesis of more extended junction zones, with shorter 'flexible' chain segments between zones.

Removal of water from pre-formed gels does not affect the strain function, expressed as stress divided by equilibrium modulus. However, although the resulting modulus is somewhat less than that of the equivalent polymer concentration crosslinked in solution, it is higher than would be expected on the basis of the simple Flory–Rehner type swelling theory, suggesting that neither the Hermans equilibrium assumption nor completely labile crosslinks are involved.

Both tensile strength and break strain depend markedly on strain rate, both falling as the test rate is reduced, and rising again at the slowest rates. The minimum values of these quantities increase with polymer concentration. In general, agarose gels exhibit the same behaviour, but break at smaller strains.

The data have been presented as a set of experimental observations related in an empirical fashion. Nonetheless, the behaviour is consistent with that of a polymer network made up of non-Gaussian chain segments. There is little doubt from previous work that the network chains are shorter than normally encountered in rubber-like systems, or that the 'crosslinks' are multiple-helix junction zones. It also seems likely that there are additional, but weaker (possibly multiple helix–helix) associations of some kind (cf. carrageenan gels where such ion mediated behaviour dominates the gelation mechanism). The kinetics of this type of association/dissociation are currently unclear, but would assert some influence on the rate dependence of ultimate properties.

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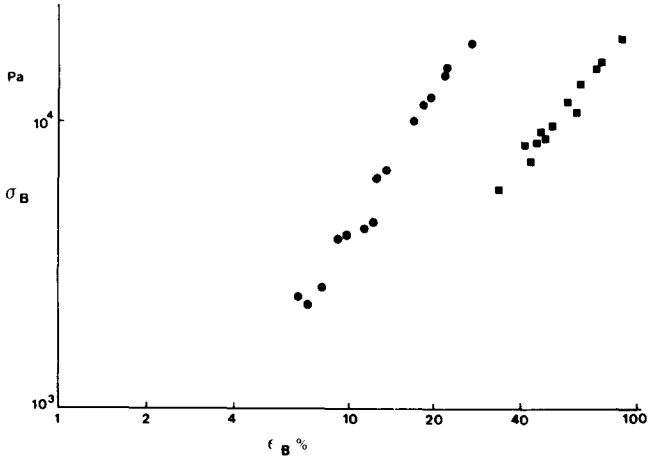

Figure 7 Comparison of failure envelopes for agar (●) and gelatin (■) gels of similar values (c/c_0)

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