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Assessment of the stress relaxation characteristics of critical gels formed under unidirectional shear flow by controlled stress parallel superposition rheometry [☆]



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ABSTRACT

Processes involving a unidirectional shear flow component are widespread in industrial manufacturing techniques such as printing and coating, or in physiological events such as blood coagulation. Standard rheometric techniques are usually employed under quiescent conditions and as such are inappropriate for the study of microstructural modification induced by the presence of a unidirectional shear flow. We demonstrate how controlled stress parallel superposition (CSPS) may be exploited to enable accurate detection of the Gel Point and analysis of Gel Point parameters for systems undergoing a viscoelastic liquid (VEL) to viscoelastic solid (VES) transition in the presence of a unidirectional flow field. Specifically, we note that certain features of the CSPS experiment, when performed near the Gel Point, may obviate previously reported concerns regarding the experiment. A biopolymer system (gelatin) which forms gels by thermoreversible gelation is employed as a model gelling material to confirm the ability of CSPS to characterise the stress relaxation characteristics of critical-gels in the presence of (a) progressively decreasing and (b) progressively increasing unidirectional strain rate and oscillatory strain amplitude. Additional validation of CSPS results is reported for a silicone dielectric gel used in the industrial production of printed electronic products. Finally, CSPS is used to investigate microstructural modification of fibrin–thrombin gels as a consequence of clot formation under a unidirectional shear stress. The results confirm the validity of the CSPS technique in gelation studies and the technique is used, for the first time, to directly record the thermally induced VES to VEL transition in aqueous gelatin systems.

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1. Introduction

A wide range of complex fluids are used as a basis for creating products by industrial processes involving flow, e.g. in manufacture by printing or coating. In biology the physiological process of blood coagulation involves a significant element of shear flow which may influence the formation and functionality of blood clots. There is a need to better understand and characterise the rheological properties of such systems and products, in particular the evolution of their viscoelastic properties and microstructure under imposed shear flows. One rheometric response to this requirement involves the superposition of a steady (or unidirectional) shear flow on a small amplitude oscillatory shear (SAOS) flow component. This may

involve parallel or orthogonal superposition of the unidirectional and oscillatory flow components, under controlled stress or controlled deformation rate conditions.

Superposition rheometry provides a basis for probing microstructural changes associated with a steady (or unidirectional) deformation. Interpretation of such experiments involves the assumption that the oscillatory (or ‘probe’) deformation has negligible effect on the material and hence any apparent microstructural changes may be attributed to the steady deformation alone [1–6]. Davies et al. [7] reported the implementation of superposition experiments in a controlled stress rheometer. The ability to perform controlled stress parallel superposition (CSPS) has since been available on many commercial rheometers but its use has not been widely reported in the context of systems undergoing gelation. In this paper we consider the application of CSPS to three material systems which are the basis of very different products but which share a rheological feature in terms of

[☆] Dedicated to Prof Ken Walters FRS on the occasion of his 80th Birthday.

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their gelation characteristics, all displaying critical-gel behaviour at the Gel Point, GP.

The first of these systems, a biopolymer gel, involves the thermoreversible gelation of aqueous gelatin solutions to form a physical gel, whereas the other systems considered herein involve the formation of chemical gels featuring permanent cross-linked branching networks. The second system is a commercial silicone dielectric gel (SDG) which is used in the production of electronic products created by industrial printing processes. The third experimental system is a fibrin gel formed by the thrombin-induced polymerisation of fibrinogen molecules. The gel network product in the latter case forms the principal microstructural component of a blood clot [8]. The latter case is particularly interesting as the critical-gel which is established at the GP serves as a 'template' for the ensuing development of microstructure and associated rheological properties in the post-GP phase of fibrin clot evolution [9].

In the present paper we report a further evaluation of the CSPS technique which focuses on its application to critical-gels. Dealy and Wissbrun [5] note that molecular rearrangement and disentanglement (as a consequence of the large deformation) *always* result in deviations from linear viscoelasticity and hence violation of the Boltzmann superposition principle. Whilst the material response to the steady deformation will be non-linear the response to the superposed oscillation should not affect the materials microstructure [10]. Previous workers have suggested that the Kramers–Kronig relations may be violated where the steady (or unidirectional) shear field causes significant enhancement of microstructure, with gelling systems near the GP being a possible example of such a situation [10]. We report herein how certain features of CSPS experiments on systems near the GP may obviate such concerns and how they may be exploited to enable accurate detection of the GP and analysis of GP parameters, and other rheological characteristics, in the presence of a unidirectional flow field.

2. Theoretical

2.1. Superposition rheometry for Gel Point detection

In parallel superposition an oscillatory deformation of amplitude γ_0 and frequency ω are superimposed upon a steady strain rate, with both deformations occurring in the same direction. The resulting equation for the total applied strain rate, is [5,11,12]:

$$\dot{\gamma}(t) = \dot{\gamma}_m + \gamma_0 \omega \cos(\omega t) \quad (2.1)$$

Booij [11] suggested that if the oscillatory deformation was carried out within the linear viscoelastic range (LVR) then the resulting stress waveform would take the form

$$\sigma(t) = \sigma_m + |G_{\parallel}^*| \gamma_0 \sin(\omega t + \delta_{\parallel}) \quad (2.2)$$

Thus G_{\parallel}^* (and hence G_{\parallel}' and G_{\parallel}'') can be defined for systems undergoing parallel steady and oscillatory shear, provided that the oscillatory deformation has negligible effect on the material (i.e. occurs within the LVR). The subscript \parallel is used to differentiate these parameters from their counterparts determined under quiescent conditions [13]. Alternatively, an oscillatory stress, σ_o , may be superimposed on a steady stress, σ_m , resulting in a similar pair of equations. In parallel superposition the effect of the oscillatory perturbation on $\dot{\gamma}_m$ can be assessed using:

$$\gamma_0 \ll \frac{\dot{\gamma}_m}{\omega} \quad (2.3)$$

Satisfying this inequality (in conjunction with the usual LVR considerations) confirms that the effect of the oscillatory deformation on the steady deformation will be minimal [3] (as shown in Fig. 1). And hence the molecular rearrangements and disentanglements can be assumed to be due solely to the steady deformation.

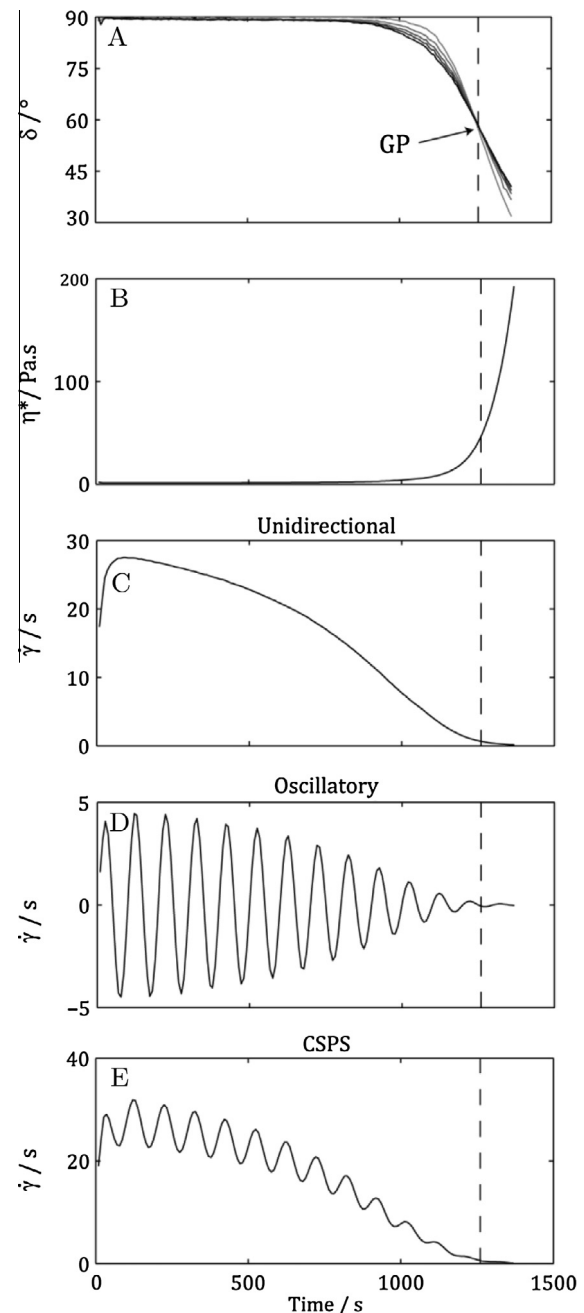


Fig. 1. Representative strain rate profiles during gelation of a sample of SDG for unidirectional shear (C), oscillatory (D) and CSPS (E) experiments. (A and B) Show data obtained using oscillatory and unidirectional stresses of 5 Pa and 30 Pa, respectively. Figures (C–E) are derived from η^* data (B) assuming a frequency of 0.01 Hz (for illustration). It should be noted that 0.01 Hz is not an appropriate frequency for GP studies unless gelation occurs extremely slowly.

It should be noted that in controlled deformation *rate* superposition experiments, the flow is usually described in terms of its oscillatory (SAOS) and *steady* components. This is not appropriate to the CSPS work reported herein which involves time-varying unidirectional shear flow developed under constant stress due to the rheological changes associated with gelation. Thus we refer herein to the *unidirectional* shear flow component, rather than the steady component of the superposition experiment.

Somma et al. [3] studied parallel superposition of oscillation on steady flows in polymer melts. They report significant differences between G_{\parallel}' , G_{\parallel}'' , η_{\parallel}^* or δ_{\parallel} and their corresponding quiescent parameters where $\omega > \omega_c$ (where ω_c can be defined as $\dot{\gamma}_m/\gamma_0$). In other

superposition experiments Boukany and Wang [14] report values of G_{\parallel} similar to under SAOS when G'_{\parallel} was probed at frequencies corresponding to timescales lower than that associated with the background steady shear.

Prompted by these findings we have sought to assess the application of CSPS to systems undergoing gelation. Specifically, we refer to systems which display a GP in terms of the 'Winter-Chambon' GP criterion [15]. For such systems the GP defines the transition between a viscoelastic liquid (VEL) and a viscoelastic solid (VES). At the GP, the elastic and viscous components of the complex shear modulus G^* (the dynamic rigidity, G' and loss modulus, G'' , respectively) scale as power-laws in frequency, ω , as $G' \sim G'' \sim \omega^{\alpha}$. This feature enables the 'critical-gel' which is established at the GP to be identified by the corresponding frequency independence of the loss tangent, $\tan \delta = G''/G'$ where δ represents the phase angle between stress and strain waveforms in SAOS measurements and is related to the stress relaxation exponent α as $\alpha = 2\delta/\pi$.

Alternatively the GP may be described in terms of the power law stress relaxation given by the Winter and Chambon gel equation

$$G(t) = St^{-\alpha} \quad (2.4)$$

where G denotes the stress relaxation modulus, S the gel strength parameter and α the stress relaxation exponent [15].

The following points are noteworthy in the context of the oscillatory and unidirectional shear flows arising in the application of CSPS in GP experiments. We refer to Fig. 1 which shows CSPS data obtained on a silicone dielectric gel, SDG. Fig. 1C–E shows shear rate profiles obtained from experiments involving unidirectional flow (C), small amplitude oscillatory flow (D) and parallel superposition flow (E), respectively. The shear rate profiles were derived from complex viscosity (η^*) measurements (Fig. 1B) using a 5 Pa oscillatory stress amplitude, σ_o , at a frequency of 0.01 Hz (D and E) and a unidirectional stress, σ_s , of 30 Pa (C and E). The profiles illustrate the main differences between unidirectional, oscillatory and CSPS experiments in terms of their pre-GP details. As expected, the strain rate profiles C and E show that the unidirectional shear rate tends to zero as the GP is approached (see Fig. 1A). It follows that the CSPS experiment approximates the SAOS experiment (D) under these conditions i.e. in the near vicinity of the GP. In this paper we report work designed to test the hypothesis that, under such conditions, CSPS measurements may be interpreted in terms of derived GP parameters (α , S) in the same way as their SAOS counterparts. The criterion used as a basis for assessing the validity of this assumption is discussed below (Section 2.2). Further work is then reported for a system in which a rheological VES to VEL transition is approached under conditions involving increasing amplitudes of oscillatory and unidirectional strain rates.

2.2. Assessing the validity of Gel Point parameters from CSPS measurements

A complete characterisation of the stress relaxation characteristics of the incipient gel network requires the gel strength parameter, S , in addition to the exponent α (Eq. (2.4)). S can be calculated using Eqs. (2.5) and (2.6) [15].

$$S = \frac{2\Gamma(\alpha) \sin(\alpha\pi/2)G'_c}{\pi} \quad (2.5)$$

$$G'_c = \frac{G'(\omega)}{\omega^{\alpha}} \quad (2.6)$$

Whilst α is obtained by identifying the frequency independent phase angle, δ , and is therefore sensitive to the power-law frequency scaling of G' and G'' , S is calculated based on the magnitude

of G' at a reference frequency (1 Hz in this study). Thus the value of α places no constraints on the value of S and the two parameters can be viewed as independent.

Scanlan and Winter [16] reported a linear relationship between $\ln(S)$ and α in several divinyl-terminated poly(dimethylsiloxane) prepolymers crosslinked with tetrakis(dimethylsiloxy) silane for which the critical-gel's microstructural characteristics were modified by controlling sample stoichiometry. In that work, S was considered to be composed of a short time constant λ_0 and a modulus G_0 , both being characteristic properties of the prepolymer, where

$$S = G_0\lambda_0^{\alpha} \quad (2.7)$$

It follows that for samples in which the critical gel network is formed from the same gelation precursor but which display different microstructures (and hence differing stress relaxation properties), valid rheological measurements yield a linear relationship between $\ln(S)$ and α , (as confirmed by measurements reported on polymer melts [16]). Conformity to this relationship (Eq. (2.7)) was used in the present study as a basis for the validation of estimates of α derived from CSPS measurements. For this purpose the GP criterion used was based on attainment of frequency independence of $\tan \delta_{\parallel}$.

3. Experimental

3.1. Materials

3.1.1. Gelatin gels

Aqueous gelatin solutions were prepared with general-purpose grade bovine gelatin powder, with a bloom strength of 100 and an average molecular weight of 80,000 (Fisher Scientific, UK). Each sample was freshly prepared for each experiment, gelatin being added to deionised water and dispersed vigorously for 2 min before being heated to 60 °C and held at that temperature for 45 min, the samples being removed every 10 min to be further agitated for 1 min.

3.1.2. Fibrin–thrombin gels

Human fibrinogen (43.0 mg/ml) and human- α -thrombin (500 NIH mL⁻¹) were obtained from Enzyme Research Laboratories Ltd., UK, and prepared as instructed by the manufacturer. Aliquots were stored at –80 °C until required. Samples of human albumin (Sigma Aldrich, 10 wt%), and CaCl₂ (Fluka, 1 M) were stored at 4 °C until required. Samples of fibrinogen and thrombin were allowed to thaw at room temperature before being placed on ice. Appropriate amounts of albumin diluted to 4.5 wt% in Tris Buffered Saline (Sigma Aldrich), fibrinogen and CaCl₂ were mixed (in that order) to give the required final concentrations of $c = 10$ mg/mL fibrinogen and 0.005 M CaCl₂. Thrombin was added to initiate gelation at the final concentration, ϕ , in the range $0.01 < \phi < 0.19$ NIH mL⁻¹ immediately prior to the sample's transfer to the rheometer.

3.1.3. Silicone dielectric gels

The silicone gel used herein was a 2 part addition cure system from ACC silicones Ltd. The gel comprised vinyl-ended silicone polymers, hydride cross-linker, bulking fillers, platinum catalyst, adhesion promoters and a pot life extender. During gelation vinyl polymers become cross-linked by the hydride cross-linker in the presence of the catalysts with no by-products. Samples were prepared by mixing equal masses of the components of the silicone gel at room temperature. The sample was then placed in a refrigerator at 4 °C for at least 30 min to allow degassing of the sample (gelation taking approximately 48 h at this temperature).

3.2. Rheometry

A TA Instruments AR-G2 rheometer fitted with a 60 mm 2° aluminium cone was used to perform all experiments on gelatin and silicone dielectric gels. The same system was used for CSPS studies of fibrin–thrombin gels to facilitate removal of the intact sample from the rheometer after gelation for further studies not reported herein. SAOS studies of fibrin–thrombin gels were performed using a double gap concentric cylinder geometry. Measurements on gelatin were performed using a frequency sweep experiment with 7 frequencies logarithmically spaced between 0.2 Hz and 2.0 Hz (limited by sample mutation and sample inertia considerations), experiments on fibrinogen–thrombin gels were also performed between 0.2 Hz and 2.0 Hz but with 4 logarithmically spaced frequencies to minimize interpolation errors [17]. A Fourier Transform Mechanical Spectroscopy, FTMS, approach to GP detection was employed for SDG experiments. FTMS combines several harmonic frequencies in a composite test waveform with the dynamic response of the material to each component frequency being studied separately via Fourier analysis [18,19]. The waveform employed in the study of SDG samples herein consisted of a fundamental frequency of 2 Hz with 3rd, 5th, 7th and 10th harmonic components.

An initial assessment of the LVR for the materials studied herein was made by performing amplitude sweeps in the pre and post Gel Point regions. A more rigorous assessment of linearity at the GP is difficult to achieve using the conventional stress or strain amplitude sweep due to the transient nature of the critical gel. Accordingly, Hawkins et al. [17] employed the relative amplitude of the 3rd harmonic and fundamental frequency components of the displacement signal (i.e. H_3/H_1) at the GP as a measure of deviation from linearity. In the present study H_3/H_1 was used to assess linearity over a range of unidirectional stress levels, a ratio (H_3/H_1) greater than 0.001 being taken to indicate the onset of non-linearity. This threshold value was established by monitoring H_3/H_1 during a stress amplitude sweep on a suitably slowly gelling gelatin sample. The absence of mutation artefacts was ensured by excluding data associated with rapid gelation where the mutation number (N_{mu}) exceeded 0.15 as proposed by Winter et al. [20].

4. Results

4.1. Gelatin

The progressive decrease in the unidirectional shear field in the approach to the GP under constant stress suggests that interpretation of CSPS experiments might allow accurate GP determination given sufficiently small $\dot{\gamma}$ (see Fig. 1). In the present work this assumption was tested by comparing GP data obtained under FS and CSPS for gelatin systems. In addition, experiments were devised in order to test whether CSPS could yield valid data for systems undergoing rheological change involving a progressive increase in the amplitudes of the unidirectional shear field and oscillatory strain under constant stress. In the present work that rheological change was represented by a transition from a previously gelled viscoelastic solid (VES) state back to its precursor viscoelastic liquid (VEL) state.

These experiments involved exploiting the thermoreversible gelation of aqueous gelatin solutions by inducing a temperature increase in the post-GP regime. The result is to produce a VES to VEL transition (i.e. the opposite of that exhibited in the initial GP experiment). In the present experiments the temperature was maintained constant throughout the gelation process at $T = 27^\circ\text{C}$ until the post GP phase was attained, following which (after 4000 s) the temperature was increased by $0.05^\circ\text{C}/\text{min}$ from 27°C to 33°C , this latter value exceeding the maximum gelation temperature T_{GPmax} for this system (the temperature above which t_g is infinitely large) [21].

Fig. 2A shows the results of FS (SAOS) experiments. The initial values of δ (approximately 90° at all test frequencies) reflect the near-Newtonian fluid response of the material, this being followed at later times by the development of a frequency dependence of δ characteristic of a viscoelastic liquid (VEL), with the recorded values of δ declining with increasing oscillatory frequency. The frequency dependence of δ declines progressively as the GP is approached, the GP being marked by frequency independence of the loss tangent, as previously reported for this system [17,19]. The post-GP phase is marked by development of a viscoelastic

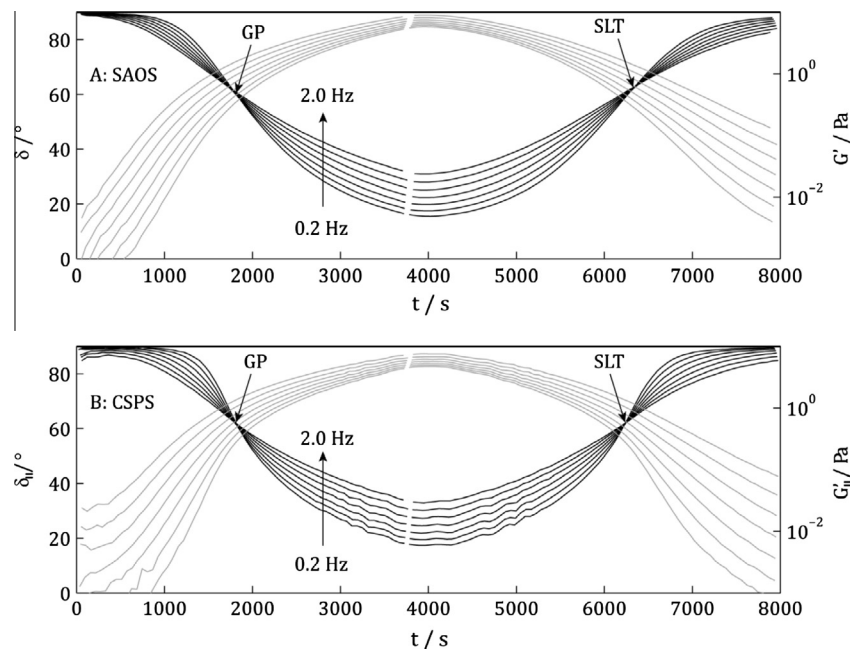


Fig. 2. Rheological transitions (VEL to VES and VES to VEL) for gelatin samples studied under SAOS (Fig. 2A) and CSPS (Fig. 2B). GP marks the Gel Point which in CSPS is measured under conditions of progressively decreasing unidirectional strain rate and oscillatory strain amplitude whilst SLT marks the solid–liquid (VES to VEL) transition. Note that under CSPS the latter is approached under conditions of increasing unidirectional strain rate and oscillatory strain amplitude.

solid-like response with the recorded values of δ increasing with increasing oscillatory frequency.

It is noteworthy that the corresponding results obtained by CSPS reveal the same rheological features in terms of the frequency dependence of $\delta_{||}$ (see Fig. 2B). The value of δ recorded at the GP by FS (SAOS) is approximately $\delta = 62^\circ$, the corresponding frequency independent value of recorded by CSPS being also 62° , in excellent agreement with published data [17,19]. Moreover the values of G' (FS SAOS) and $G'_{||}$ (CSPS) corresponding to frequency independence of δ and $\delta_{||}$, respectively were also indistinguishable ($G'_{||} \sim G' \sim 1.4$ Pa).

We now consider the results obtained on these systems following an increase of the test temperature to a constant value above T_{CPmax} . These results are obtained during the resulting transition from a previously gelled viscoelastic solid state, back to the precursor viscoelastic fluid state (SLT in Fig. 2). This increase in temperature to a value above the maximum gelation temperature causes a marked and progressive decrease in G' and $G'_{||}$, with a corresponding increase in δ and $\delta_{||}$, the significant point being that their VES-like frequency dependence is initially preserved. A striking feature of the ensuing rheological change is that both δ and $\delta_{||}$ subsequently pass through a second frequency independent point as the system loses its solid-like characteristics, this being accompanied by a rapid decline in the value of both G' (FS) and $G'_{||}$ (CSPS). It is noteworthy that the frequency independent values of δ and $\delta_{||}$ at the SLT are also indistinguishable at this 'de-gelling' (VES to VEL) transition; and are identical to those recorded during the initial gel formation (GP) experiments. Thereafter, the frequency dependence of δ and $\delta_{||}$ change, becoming characteristic of a VEL, the values of δ and $\delta_{||}$ both increasing progressively towards 90° as the temperature is maintained above T_{CPmax} .

A significant feature of Fig. 2 is the increase in the frequency dependence of $\delta_{||}$ (CSPS) in the pre-gel region ($t < t_g$) compared with that of δ (FS) in the same region. This phenomena is consistent with previously reported findings that the presence of a unidirectional shear component has greatest effect on measurements performed at frequencies corresponding to shear rates lower than those associated with the background unidirectional shear flow [3,14]. Hence, decreasing frequency is expected to generate larger differences between $\delta_{||}$ and δ resulting in a more pronounced frequency dependence of $\delta_{||}$ than δ where the unidirectional shear rate is significant (i.e. only in the pre-gel region).

The experiments reported above established that when conducted under appropriate conditions (in terms of linearity and mutation number criteria) the results of FS and CSPS experiments on a system undergoing rheological transition (from VEL to VES, or vice versa) may be taken as indistinguishable. This conclusion is drawn on the basis that, during gelation, attainment of frequency independence of $\delta_{||}$ indicates the same rheological significance (in terms of the GP) as frequency independence of δ . A further (and hitherto unreported) feature of the present experiments is revealed during the transition from a previously gelled VES state back to the precursor VEL state. The significant feature of the latter CSPS results is that they are obtained from experiments in which both the oscillatory strain amplitude and the unidirectional shear field increase progressively during the approach to the rheological transition (SLT).

The findings reported above for thermoreversible gelation, in which no permanent cross-links are formed in the gel network, suggest that CSPS could be used as a basis for investigating critical-gel formation under the influence of unidirectional flow. Accordingly, a further series of experiments was devised to further assess the validity of the application of the CSPS technique using a criterion which has previously been employed in studies of critical-gels formed in systems involving permanently cross-linked gel networks (Section 2.2 and Scanlan and Winter [16]). The first system

chosen in the present study for this assessment was a silicone dielectric gel.

4.2. SDG systems

The results shown in Fig 3 below were obtained on samples of SDG by separate FTMS (SAOS) and CSPS tests. In order to generate a range of α in the FTMS tests, the test temperature, T , of the system was varied over the range $20^\circ\text{C} < T < 50^\circ\text{C}$. Higher temperatures resulted in values of t_g which incurred mutation effects due to the rapidity of gelation, while values of T lower than 20°C resulted in extremely slow gelation, the large values of t_g ($>10^4$ s) being deemed *too long to be of any process relevance for this material*.

The corresponding range of α achieved in the CSPS tests results from the application of the unidirectional stress, σ_s , in the range $0\text{ Pa} < \sigma_s \leq 20\text{ Pa}$ with tests being conducted at constant temperature ($T = 50^\circ\text{C}$). Note that under the superposed unidirectional stress the values of t_g are markedly reduced relative to those resulting from temperature variation in the FTMS tests, with CSPS test values being in the range $800\text{ s} \leq t_g \leq 1100\text{ s}$.

The data shown in Fig. 3 lie on a single exponential curve of the form $S = G_0 \alpha_0^2$. ANCOVA tests [22] showed no significant difference between exponential fits to the CSPS and FTMS data sets. Thus the results of determining GP parameters by CSPS are valid in terms of satisfying the assessment criterion (i.e. conformity to Eq. (2.7)) – a finding further confirmed by the fact that the estimated GP parameters based on CSPS measurements of $G'_{||}(\omega)$ are essentially indistinguishable from the corresponding GP parameters estimated from FTMS (SAOS) measurements of $G^*(\omega)$. For comparison, examples of pre-GP, GP and post-GP data sets for $G'_{||}(\omega)$ and $G^*(\omega)$ are shown in Fig. 4.

4.3. Fibrin–thrombin gels

The results shown in Fig 5 below were obtained on fibrin–thrombin systems undergoing gelation in FS and CSPS tests. In order to generate a range of α in the FS tests, the thrombin concentration, ϕ , was varied in the range $0.01 < \phi < 0.19$ NIH/ml, at constant temperature ($T = 10^\circ\text{C}$). The range of α achieved in the CSPS tests results from the application of the unidirectional stress σ_s in the range $0\text{ Pa} < \sigma_s \leq 0.4\text{ Pa}$. The results obtained are shown in Fig. 5.

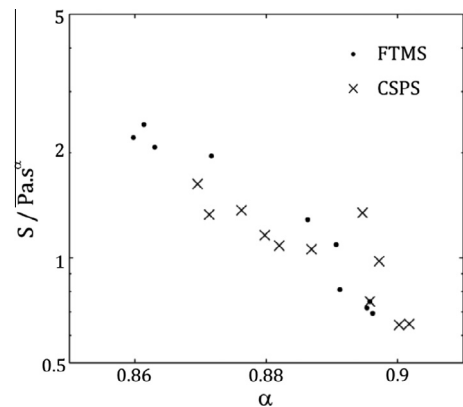


Fig. 3. Values of S and α derived from the results of separate FTMS and CSPS experiments on SDGs. The data lie on a single exponential curve of the form $S = G_0 \alpha_0^2$. The insignificant difference (p -value* = 0.097) between the CSPS and FTMS data sets confirms that the results of determining Gel Point parameters by CSPS are valid in terms of satisfying the assessment criterion. * p -Value reported is that for an ANCOVA test for separate linear regressions with p -value < 0.05 being the standard acceptance of significant differences between the regression slopes.

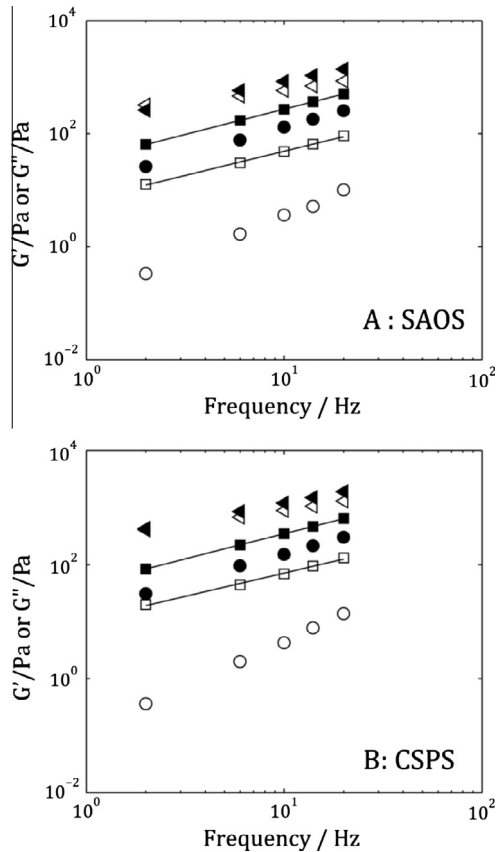


Fig. 4. Examples of pre-GP (o), GP (\square) and post-GP (\triangleleft) data sets for (A) $G'(\omega)$ and $G''(\omega)$ (obtained under SAOS conditions) and (B) $G'_i(\omega)$ and $G''_i(\omega)$ (obtained under CSPS conditions) for the SDG system. Both data sets were obtained at 50 °C and $\sigma_s = 10$ Pa for the CSPS data. Solid lines show power law fits to the GP data with equivalent scaling in (A) $G'(\omega)$ and $G''(\omega)$ and (B) $G'_i(\omega)$ and $G''_i(\omega)$.

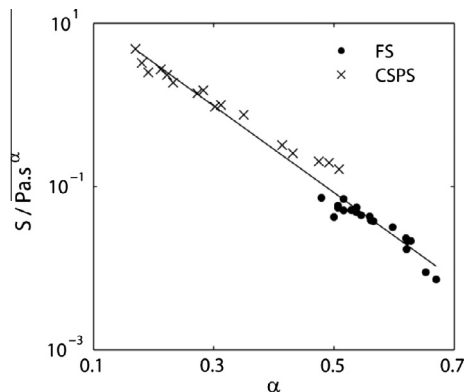


Fig. 5. Values of S and α derived from the results of separate FTMS and CSPS experiments on fibrin–thrombin gels. As in the case of the SDG results the data lie on a single exponential curve of the form $S = G_0 \lambda_0^\alpha$. ANCOVA tests [22] showed no significant difference (p -value* = 0.300) between exponential fits to the CSPS and FS data sets. Thus, for this system also, the results of determining Gel Point parameters from CSPS measurements may be deemed valid in terms of satisfying the assessment criterion based on conformity to Eq. (2.7) [16]. * p -Value reported is that for an ANCOVA test for separate linear regressions with p -value < 0.05 being the standard acceptance of significant differences between the regression slopes.

5. Discussion

In the work reported herein the biopolymer gel system formed by the thermoreversible gelation of aqueous gelatin systems was

employed as a model gelling material to confirm the ability of CSPS to characterise the stress relaxation characteristics of critical gels in the presence of (a) progressively decreasing and (b) progressively increasing unidirectional shear field and oscillatory strain amplitude. In addition to confirming the validity of the CSPS technique in gelation studies, the latter feature involved CSPS being used to record the thermally induced VES to VEL transition. In previous work Michon et al. [23] conducted frequency sweeps on gelatin gels which had previously gelled at a fixed/aging temperature (for an aging period) then subsequently melted by incrementally increasing the temperature and performing measurements on the material following a 1 h period of equilibration at each temperature. These authors plotted values of $\tan \delta(\omega)$ versus temperature and, by a process of extrapolation between data points obtained at each temperature, estimated the location of an apparent gel-sol transition from which estimates of α and “critical melting temperature” were derived. They recognised that the material was not at a thermal equilibrium following an hour at each test temperature as indicated by the increasing values of G' with time, and that this could lead to an overestimation of critical melting temperature. However, no comment was made on the validity of the values of α which were found to range from 0.47 to 0.81, and were dependent on concentration, aging time and aging temperature. In the present work the gel–sol transition was measured directly, for the first time using both SAOS and CSPS.

The results obtained for the SDG system are particularly interesting in terms of illustrating the potential applications of CSPS in industrial process settings. The ability to manipulate the characteristics (S and α) of the SDG critical gels by temperature change was restricted to a relatively narrow of α (ca. 0.86–0.9, corresponding to a δ range of 77–81°). This was due to the fact that temperatures greater than 50 °C resulted in values of t_g which incurred mutation effects, while values of T lower than 20 °C resulted in extremely slow gelation, the large values of t_g ($>10^4$ s) being deemed too great to be of process relevance. However, when gelation proceeded under CSPS (i.e. in the presence of a superposed unidirectional shear flow) the range of α which could be generated was significantly enhanced to $0.75 < \alpha < 0.9$ ($67^\circ < \delta < 81^\circ$), at constant temperature. Significantly, the corresponding range of t_g was also markedly reduced. The present results indicate that manipulation of the CSPS unidirectional flow shear stress component can be used to both alter the microstructural characteristics of the formed gel, and the time required to achieve gelation. This may prove valuable in process settings such as printing and coating which involve exposure of process materials to flow induced shear stresses.

Turning to the results obtained for fibrin–thrombin gels, it is useful to recall that the blood clotting process involves fibrinogen–fibrin transformation due to the catalytic action of thrombin, and the subsequent polymerisation and network formation of fibrin fibres [24,25]. In vivo the network of fibrin fibres forms the primary microstructural basis of a clot whereas in vitro they form a fibrin gel [26]. Viscoelastic properties are among the most sensitive measures of fibrin polymerisation and clot microstructure [25]. In haemorrhological measurements the clotting time may be identified with t_g , the time required to form the incipient clot’s sample-spanning network of fibrin fibres at the Gel Point [24,25].

In discussing the results for the fibrin gels it is useful to convert the values of α to their corresponding values of the fractal dimension, D_f [27]. The exponent α obtained from GP measurements is a measure of gel network branching and is related to D_f as $D_f = (D + 2)(2\alpha - D)/2(\alpha - D)$, where D is the Euclidian dimension ($D = 3$). Gel Point measurements have been used to accurately detect incipient clot formation in samples of whole blood [8,28,29] and estimates of D_f derived therefrom provide a microstructural biomarker for altered clotting and a Healthy Index for normal clotting (based on $D_f = 1.74 \pm 0.04$ [29]). For fibrin gels under

SAOS the increase in D_f ($1.8 < D_f < 2.0$) is solely due to gel formation under progressively higher levels of ab initio thrombin concentration, with $D_f = 2.0$ being the upper limiting value. For fibrin gels in CSPS the higher values of D_f – up to a maximum of 2.3 – reflect the effects of clot formation under unidirectional flow and may be attributed to enhanced monomer activation resulting from mixing in shear flow [30]. The potential clinical significance of the increase in D_f under CSPS is that fractal networks such as the incipient clot require large amounts of additional mass to produce small increments in D_f and given the incipient clot's role as a microstructural template for ensuing clot development [9] elevated values of D_f might lead to the establishment of denser, less permeable clots with enhanced resistance to fibrinolysis [31–33] and deformation [34].

The unidirectional shear stresses employed in this CSPS study are lower than those previously reported to influence the elasticity of fully formed clots in SAOS tests following exposure of coagulating plasma to flow [35]. A significant aspect of the CSPS results is that they reveal the marked sensitivity of incipient fibrin clot microstructure (D_f) to unidirectional flow shear stresses < 0.3 Pa. Such low stresses have physiological relevance insofar as they are commensurate with estimates of the mean wall shear stress for flow in the aorta, inferior vena cava and other venous structures [36,37]. The results obtained on fibrin–thrombin clots suggest that CSPS may be a valuable haemorheological tool for studying the effects of physiological and pathological levels of shear on clot properties.

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