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An Enhanced Rheometer Inertia Correction procedure (*ERIC*) for the study of gelling systems using combined motor-transducer rheometers.

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Abstract

The rheological characterisation of viscoelastic materials undergoing a sol-gel transition at the Gel Point (GP) has important applications in a wide range of industrial, biological and clinical environments and can provide information regarding both kinetic and microstructural aspects of gelation. The most rigorous basis for identifying the GP involves exploiting the frequency dependence of the real and imaginary parts of the complex shear modulus of the critical gel (the system at the GP) measured under small amplitude oscillatory shear conditions. This approach to GP identification requires that rheological data be obtained over a range of oscillatory shear frequencies. Such measurements are limited by sample mutation considerations (at low frequencies) and, when experiments are conducted using combined motor-transducer (CMT) rheometers, by instrument inertia considerations (at high frequencies). Together, sample mutation and inertia induced artefacts can lead to significant errors in determination of the GP. Overcoming such artefacts is important however as extension of the range of frequencies available to the experimentalist promises both more accurate GP determination and the ability to study rapidly gelling samples. Herein, we exploit the frequency independent viscoelastic properties of the critical gel to develop and evaluate an Enhanced Rheometer Inertia Correction procedure (ERIC). The procedure allows acquisition of valid GP data at previously inaccessible frequencies (using CMT rheometers) and is applied in a study of the concentration dependence of bovine gelatin gelation GP parameters. A previously unreported concentration dependence of the stress relaxation exponent (α) for critical gelatin gels has been identified which approaches a limiting value (α = 0.7) at low gelatin concentrations, this being in agreement with previous studies and theoretical predictions for percolating systems at the GP.

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1.0 Introduction

Inertial effects can dominate rheological measurements performed by combined motor-transducer (CMT) rheometers (also known as controlled stress rheometers) on low viscosity systems or samples with weak gel network structures (Krieger, 1990), such effects being most severe at high frequencies (Klemuk & Titze, 2009; Krieger, 1990; Läuger & Stettin, 2016; Walters, 1975) . The design of CMT rheometers requires the torque developed by the instrument to both deform the sample under investigation and accelerate the moving components of the rheometer. Standard practice requires that the instrument is calibrated without the sample present in order to determine the inertial characteristics of the rheometer-geometry assembly, and to allow subsequent correction of the raw data. Separate motor-transducer rheometers (SMT) (also known as controlled strain) rheometers are not susceptible to instrument inertia artefacts since the torque sensing element remains static during data acquisition (Franck, 2003).

An inertia correction is routinely applied to raw storage modulus data (G'_{raw}) by the software controlling CMT rheometers such that:

$$G' = G'_{raw} - I_c \omega^2 k_g \qquad (1)$$

where G' denotes the apparent storage modulus of the material , I_c denotes a calibrated inertia constant, ω the angular frequency and k_g a geometry factor (Franck, 2005). However, the inertia constant can only be determined to finite precision and there will always be some uncertainty regarding the accuracy of G' extracted from the raw waveforms where the magnitude of the term $I_c \omega^2 k_g$ may represent a dominant part of G'_{raw} (Ewoldt, Johnston, & Caretta, 2015). Further, a momentum balance can be used to show that the inertial term has no imaginary component (Klemuk & Titze, 2009), and hence the loss modulus, G'', is unaffected by the presence of instrument inertia (Franck, 2005). The raw phase angle, δ_{raw}, measured by the instrument is often used as a measure of the extent to which the inertial artefacts pollute the raw data. Instrument manufacturers recommend caution where δ_{raw} exceeds a stated value which is dependent on the rheometer model, e.g. the TA Instruments AR-2000ex model has a limiting raw phase angle of 150° (TA Instruments, 2016).

Motivated by **a** significant discrepancy between gel point (GP) data obtained using CMT and SMT rheometers (δ_{GP} = 72.8 ± 6.9° and 62.7° ± 1.0°, respectively, for low concentration gelatin samples, see section 4.0) we exploit the characteristic rheological behaviour of the critical gel (i.e. frequency independent phase angle, δ) (Chambon & Winter, 1987) to develop an Enhanced Rheometer Inertia Correction procedure (ERIC). The ERIC procedure can be used to correct GP data in the post-acquisition phase. In the present work, the ERIC procedure was used in a study of the concentration dependence of the stress relaxation properties of bovine gelatin at the GP.

Characterisation of the sol-gel transition, which occurs at the GP of a material undergoing gelation, can provide information concerning the evolving microstructural properties of the material under investigation (Curtis et al., 2011; Lawrence et al., 2015). At the GP the storage and loss moduli scale as identical power laws in frequency ($G'{\sim}G''{\sim}\omega^{\alpha}$) (Chambon, Petrovic, Macknight, & Winter, 1986) with the parameter α ($0 < \alpha < 1$), termed the stress relaxation exponent, being sensitive to the microstructure of the sample spanning incipient gel network which forms at the GP. The measurement of α is often challenging and is limited by sample mutation (Mours & Winter, 1994) and, as demonstrated in the present work, instrument inertia artefacts (when using CMT rheometers). Examples of the utility of GP data are widespread in terms of physical and chemical gels (Djabourov, Leblond, & Papon, 1988; Hawkins, Lawrence, Williams, & Williams, 2008; Hsu & Jamieson, 1993; Djabourov, Jacques Leblond, 1988; Michon, Cuvelier, & Launay, 1993). Recently, GP characterisation of coagulating blood has been shown to provide a novel biomarker for healthy coagulation (Evans et al., 2010) and a predictor of clot microstructure (Curtis et al., 2013).

Gelatin is a common biopolymer derived from the hydrolysis of collagen. Whilst at temperatures above the maximum gelation temperature (~33.6°C) (Tosh & Marangoni, 2004) gelatin displays near Newtonian rheological properties, upon cooling a thermoreversible gelation process occurs to form a physically crosslinked biopolymer network (Boedtker & Doty, 1954; Tosh & Marangoni, 2004; Wolf & Keller, 1996). The thermoreversible nature of gelatin gelation makes the system an ideal test material for studies involving the validation of novel rheometric approaches (Curtis, Badiei, et al., 2015;

Curtis, Holder, et al., 2015; Hawkins et al., 2008).

2.0 Materials and methods

2.1 Gelatin preparation

Appropriate quantities of gelatin powder (Fisher G/0150/53) and type I deionised water were combined and shaken vigorously for five minutes before being placed in a 60°C water bath for 45 minutes to ensure complete dissolution. The solutions were agitated for one minute every 10 minutes and retained at 60°C for no longer than 45 minutes to prevent degradation. Aliquots of each concentration ($2.5wt\% \leq c \leq 30wt\%$) of gelatin were stored in a refrigerator (4°C) and melted at 60°C for 45 minutes prior to being loaded to the rheometer.

2.2 Rheometry

2.2.1 CMT Rheometers

Rheological measurements were conducted using a 60 mm acrylic plate geometry fitted to a TA Instruments AR-2000ex rheometer or a 60 mm aluminium plate geometry fitted to a TA Instruments AR-G2 rheometer; both systems used Peltier plate temperature control. Gelatin samples were loaded onto the rheometer at 34°C before the upper geometry was lowered into place and the test commenced at which point the temperature was quenched to the test temperature. In order to satisfy the gap loading condition (Schrag, 1977), **which ensures that the velocity gradient across the geometry gap is uniform and thus ensures negligible sample inertia effects**, a shearing gap of 150 μm was used. Inertia correction studies were completed using 2.5 wt% gelatin. A sequence of gel point experiments was performed with each experiment covering a decade of frequency, the highest frequency being systematically increased from 1 Hz to 12 Hz (with the corresponding lowest frequencies being 0.1 Hz to 1.2 Hz, respectively). All tests were performed at 19°C such that gelation occurred over approximately 900 s to minimise sample mutation effects at the GP (Mours & Winter, 1994) (typical sample mutation numbers, N_{mu}[†], under these conditions being $N_{mu} = 0.062$).

[†] Following Mours & Winter (1994), and where Δt denotes the data point acquisition time, the mutation number is calculated (at the lowest frequency) as follows:

$$N_{mu} = \frac{\Delta t}{G'_{GP}} \times \frac{dG'}{dt}\bigg|_{GP}$$

Concentration dependence of gelatin gelation was studied using the AR-2000ex rheometer fitted with a 60 mm acrylic plate geometry. A frequency range of 0.3—3.0 Hz was used for all experiments. Since the gel time of gelatin is strongly dependent on both concentration and gelation temperature, it was infeasible to maintain a constant temperature for this set of experiments. Hence, the temperature was varied from 19°C (for 2.5wt% Gelatin) to 32.5°C (for 30wt% Gelatin). No temperature dependence of the stress relaxation characteristics of the gels was observed in preliminary tests, in agreement with literature data (Hawkins et al., 2008). Sample mutation was assessed and data omitted where the mutation number was found to exceed 0.15 (Mours & Winter, 1994). Furthermore, for all experiments linear viscoelastic measurements were confirmed by ensuring that the magnitude of the third harmonic of the displacement signal remained insignificant at the gel point (Hawkins et al., 2010). A thin layer of low viscosity silicone oil (9.8 mPas, Brookfield) was applied to the free surface of the sample to prevent evaporation during the experiment.

2.2.2 SMT Rheometer

A TA Instruments ARES-G2 rheometer fitted with a 40 mm parallel plate geometry (and Peltier Plate temperature control accessory) was loaded with an appropriate volume of 2.5wt% gelatin. During loading the Peltier plate temperature was set to 30°C. As for the CMT rheometer studies, a gap of 150μm was employed to ensure the gap loading assumption was valid. A pre-shear of 100s^{-1} for 10s was applied to ensure symmetrical loading before multiple consecutive frequency sweeps were performed (0.9- 3.5Hz) at 20°C. The transient nature of the gelation process requires that the strain amplitude decreases throughout the experiment to i) achieve a resolvable torque signal at all stages of gelation and ii) maintain linear viscoelastic measurements. Hence, the strain amplitude was decreased by 25% (from an initial value of 100%) where the torque exceeded 3μNm, harmonic analysis of the signals at the GP revealed no higher harmonic contribution to the stress waveform confirming linear conditions (Hawkins et al., 2010) .

3.0 Enhanced Rheometer Inertia Calibration (ERIC)

Following Equation 1, the value of G' reported by the instrument is dependent on the both the true value of the storage modulus, G'_t, and the calibrated Inertia Constant, I_c. The value

of the latter is routinely determined during preparation of the instrument but the finite precision and accuracy of this calibration can cause the true Inertia Constant, which characterises the instrument-geometry assembly (I_t), to differ from the calibrated Inertia Constant (I_c) by a small deviation of ΔI. Hence it can be written from equation 1 that

$$G'_t(\omega) = G'(\omega) + (I_t - I_c)\omega^2 k_g = G'(\omega) + \Delta I \omega^2 k_g \qquad (2)$$

A MATLAB routine was used to incrementally change the value of ΔI in Equation 2 whilst monitoring the standard deviation of the roots of the gel point data (Evans et al., 2010). A root can be defined as the time and phase angle of the intersection between each pair of frequencies (see Figure 1a) with the deviation in the positions of the roots providing a measure of the accuracy of the GP data. The procedure was coded as a MATLAB (The MathWorks Inc., 2016.) GUI (ERIC) and is freely available from the corresponding author. Briefly, the procedure involved fitting a 5 parameter logistic equation to the $\delta(t)$ data for each frequency and determining the location of the intersection between each pair of fitted curves (i.e. each root), the apparent GP was then defined at the mean phase angle and mean time of all roots. The standard deviation (with respect to time) of the roots was taken as a measure of the accuracy of the GP. The procedure was repeated for $-0.2\ \mu Nms \leq \Delta I \leq 0.2\ \mu Nms$ with the true GP being identified where varying ΔI caused a minimum in the standard deviation of the GP roots.

The ω^2 dependence of the inertia correction (Equation 2) prevents the ERIC procedure from generating an erroneous pseudo-GP if the material does not display this phenomenon. This can be demonstrated by assuming hypothetical (and valid) non-GP data in which G' and G'' display separate power law dependencies on angular frequency such that:

$$G' = k_1\omega^\alpha, \quad G'' = k_2\omega^\beta, \quad (\alpha \neq \beta)$$

From Equation 2 a corrected value of G' would be expressed as:

$$G'_c = k_1\omega^\alpha + \Delta I.k_g.\omega^2$$

A pseudo-GP would require frequency independent tanδ, i.e.,

$$\frac{G''(\omega)}{G'_c(\omega)} = \frac{k_2\omega^\beta}{k_1\omega^\alpha + \Delta I.k_g.\omega^2} = c$$

where 'c' is a constant. Rearranging for $\Delta I.k_g$ gives

$$\Delta I.k_g = \frac{k_2}{c}\omega^{(\beta-2)} + k_1\omega^{(\alpha-2)}$$

and since ΔI and k_g are, by definition, independent of frequency, pseudo-GP data can only exist where $\alpha = \beta = 2$ or $k_1 = k_2 = 0$, neither of which are viable GP conditions (since at a GP $0 < (\alpha = \beta) < 1$, and both G' and G'' must be non-zero), hence the ERIC procedure cannot generate a GP if one does not exist.

4.0 Results and discussion

Figure 1a shows typical uncorrected GP data (acquired over a frequency range of 1.0 Hz – 10 Hz) as reported by the instrument with an apparent GP at 72.8 ± 6.9°. This value is significantly above the value of 62.7° ± 1.0° obtained using an SMT rheometer (ARES-G2 in the present study) and displays a high degree of uncertainty reflective of the large deviation of the positions of the GP roots. The deviation in the root positions (± 6.9°) should alert the experimentalist to inadequacies in the GP acquisition procedure which may be caused by several experimental issues, for example; sample mutation (Hawkins et al., 2010; Mours & Winter, 1994); sample inertia (Schrag, 1977); evaporation of the sample (Hellström, Samaha, Wang, Hultmark, & Smits, 2015); under/over/asymmetric loading (Ewoldt et al., 2015) or instrument inertia. In the present study, the effect of inaccurate instrument inertia calibration has been isolated by careful experimental design and data verification (see section 2.0). The corrected instrument inertia constant (I_t) was determined using ERIC, Figure 1b shows the deviation in the root positions for a range of I_t with the 'optimum' value being determined as 21.5422 μNm^2 (this representing a 0.46% change from the calibrated value). Figure 1c shows the 'corrected' GP data (after application of the ERIC routine) with an apparent GP at 62.4 ± 0.2° in excellent agreement with the SMT rheometer results.

Figure 2 shows both uncorrected (open) and corrected (filled) GP data for gelatin acquired over increasing frequency intervals (with the minimum frequency being a decade lower than the maximum frequency reported on the abscissa). It can clearly be seen that increasing the frequency window over which the data is acquired drives the uncorrected data to higher values of the δ at the GP with increasing deviation in the root positions (leading to less precise GP determination). However, appropriate correction of the data (using ERIC) allows both accurate and precise GP determination over the entire range of frequency windows studied herein. The result has important applications in the analysis of rapidly evolving strain sensitive gelling systems for which the use of techniques such as Fourier Transform Mechanical Spectroscopy has been shown to be inappropriate (Hawkins et al., 2008).

It should be noted that the dramatic improvement in both the precision and accuracy of the GP data has been achieved by an average 0.46% change in the calibrated inertia constant, whilst repeated calibration of the instrument inertia was found to generate a 0.15% deviation in this value. Hence, some systematic error in the calibrated inertia constant appears to exist for the AR-2000ex used in the present study which limits the validity of data where the raw phase angle is in excess of 150° (see Figure 3), thus preventing comparison of rheometric data obtained using other rheometers. This is in agreement with the manufacturers stated limitation of the AR-2000ex instrument (TA Instruments, 2016).

To test the hypothesis that the ERIC procedure could be applied to correct this systematic error, thus allowing valid data to be acquired at high raw phase angles, two TA Instruments AR-G2 rheometers were used to determine the apparent GP using a frequency range of 1 – 10 Hz, both rheometers had undergone manufacturer servicing and calibration within a period of 12 months prior to the present study. Using standard instrument inertia calibrations, a significant difference between the data obtained using the two instruments was apparent (AR-G2 I: 61.5 ± 2.0 ° / AR-G2 II: 69.0 ± 4.4° - see Figure 4). The ERIC procedure was then performed on all data obtained using these two instruments and a Student t-test ($n = 8$) was used to compare the distribution of δ_{GP} obtained using standard inertia calibration (SIC) procedures and ERIC. No significant difference was observed between the distributions of δ_{GP} using SIC and ERIC for AR-G2 I ($p = 0.39$) indicating that the standard procedure was sufficiently accurate for this rheometer. However, for AR-G2 II a significant

difference (*p = 0.003*) was found between the distributions indicating that the accuracy of the SIC protocols appears to be instrument specific. Following application of the ERIC procedures no significant difference was observed between the data acquired using the two AR-G2 instruments (*p = 0.16*).

In the present study, the concentration dependence of gelatin gelation has been studied (see Figure 5) using a CMT rheometer with data processing using both the SIC and ERIC procedures, the use of ERIC allowing the concentration range to be extended as low as 2.5 wt%. Figure 5a shows the data obtained in the present study (and corrected using the SIC procedure) with literature data from both Hawkins *et al.* (Hawkins et al., 2008) and Curtis *et al.* (Curtis, Holder, et al., 2015). Both studies show excellent agreement with the present data despite the studies using differing techniques. In the latter study, Curtis *et al.* used an SMT rheometer to study gelatin at 30wt% using multi-frequency rheometric techniques which allowed data to be accessed up to 10 Hz. Good agreement between the present data and the data of Curtis *et al.* should be expected given the high concentration of gelatin. This causes the torque generated by the (significantly more viscous) material to dominate the measurement thus reducing the raw phase angle (in the present CMT data) to within acceptable limits. Hawkins *et al.* employed FTMS (implemented using a CMT rheometer) to obtain data. Their data agrees with that presented herein at low concentrations and is likely to suffer from the same instrument inertia artefacts as described above, leading to the aforementioned discrepancy between data obtained using SMT and CMT rheometers.

Post-acquisition application of the ERIC procedure has allowed the data presented in Figure 5a to be corrected to account for inaccuracy in the inertia constant (as shown in Figure 5b). Agreement between the SMT and CMT rheometers is then recovered (CMT: 63.1 ± 0.6° / SMT: 62.7 ± 1.0°) confirming the validity of the ERIC procedure. Further, the corrected data appears to show that a maximum phase of angle of 63° is approached as gelatin concentration is decreased. This limiting value corresponds to a limiting stress relaxation exponent ($\alpha = \delta/90$) commensurate with theoretical predictions for percolating systems ($\alpha = 0.7$) (Adam et al. 1981; Martin, Adolf, & Wilcoxon, 1988; De Gennes, 1979) and experimental observations of other biopolymer systems (Audebrand, Garnier, Kolb, & Axelos, 2003; Axelos & Kolb, 1990; Werner, Bu, Kjøniksen, & Arne, 2006; Yu, Blacher,

Brouers, Homme, & Je, 1997).

5.0 Conclusions

The ERIC inertia correction procedure reported herein offers the experimentalist enhanced precision and accuracy of GP measurements and has facilitated the acquisition of valid GP data at previously inaccessible frequencies (where instrument inertia effects dominate the measurement). The typical modification to the inertia constant (of the order 0.1 μNms^2) is larger than the precision of the inertia constant as determined through repeated calibrations. This suggests that there may be an underlying inaccuracy associated with this form of instrument calibration procedure and that application of the ERIC procedure may provide a basis for identifying discrepancies between results obtained on different CMT instruments. A previously unreported concentration dependence of the stress relaxation exponent for critical gelation gels has been reported with a limiting value δ_{GP} of 63° (corresponding to $\alpha = 0.7$) being identified at low gelatin concentrations.

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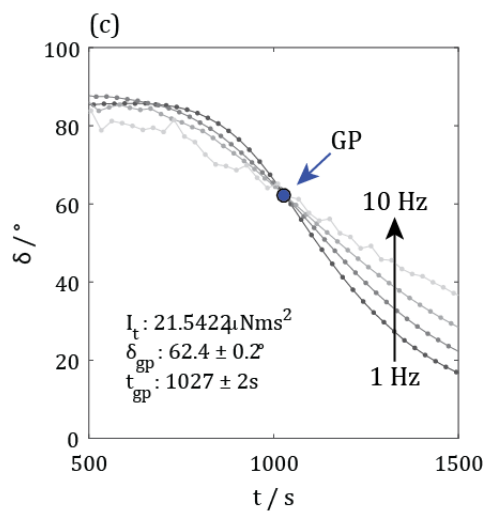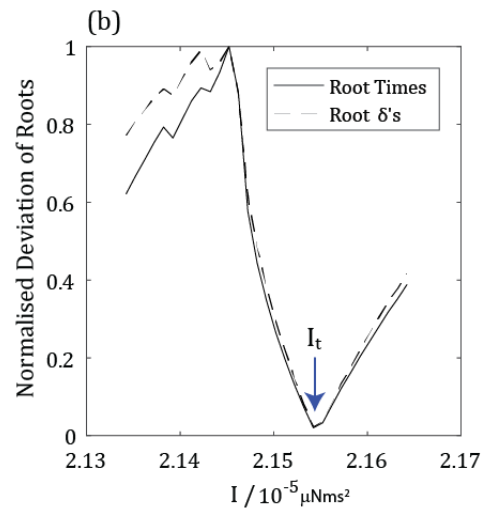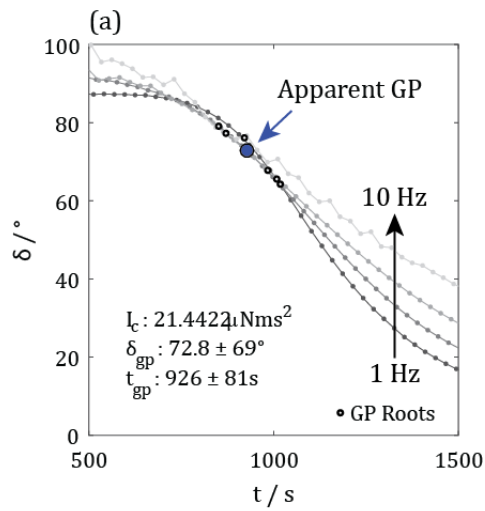
(a)

Apparent GP

10 Hz

1 Hz

I_c : 21.4422 μNms^2
δ_{gp} : 72.8 ± 69°
t_{gp} : 926 ± 81s

○ GP Roots

δ / °

100

80

60

40

20

0

500   1000   1500

t / s

(b)

Normalised Deviation of Roots

1

0.8

0.6

0.4

0.2

0

—— Root Times
- - - Root δ's

I_t

2.13   2.14   2.15   2.16   2.17

I / $10^{-5} \mu Nms^2$

(c)

GP

10 Hz

1 Hz

I_t : 21.5422 μNms^2
δ_{gp} : 62.4 ± 0.2°
t_{gp} : 1027 ± 2s

δ / °

100

80

60

40

20

0

500   1000   1500

t / s

Figure 1(a) an apparent gel point (1.0-10Hz) before correction showing the large deviation in the 'roots' position and resultant difficulty in defining the GP as a consequence of inertia induced artefacts **(lines refer to data recorded at different frequencies); (b)** optimisation data for determination of I_t and **(c)** a corrected gel point with converged roots.
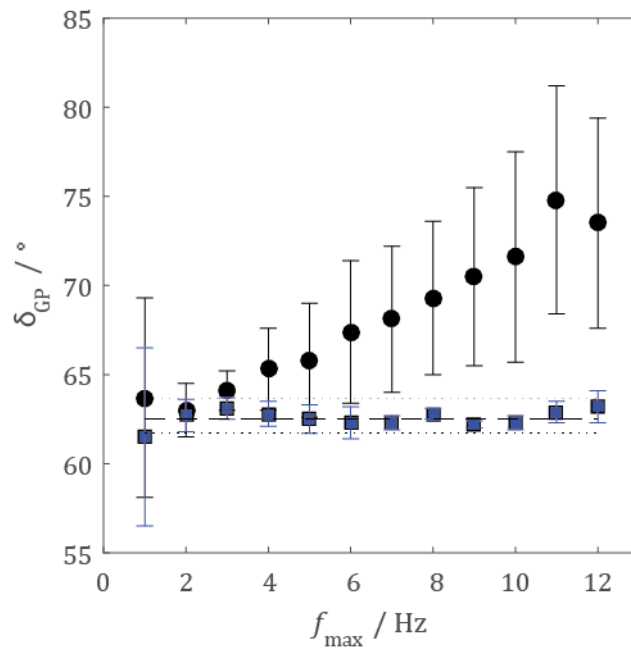
Figure 2 shows the phase angle of the raw GP data **(circles)** collected over a range of frequencies, along with the corrected GPs for the same data sets **(squares).** The corrected data is shown to be back within the acceptable limit for the concentration of gelatin. In order to reflect the uncertainties associated with the measurements, error bars reflect the larger of either (i) the standard deviation between repeats or (ii) the deviation of the root positions.
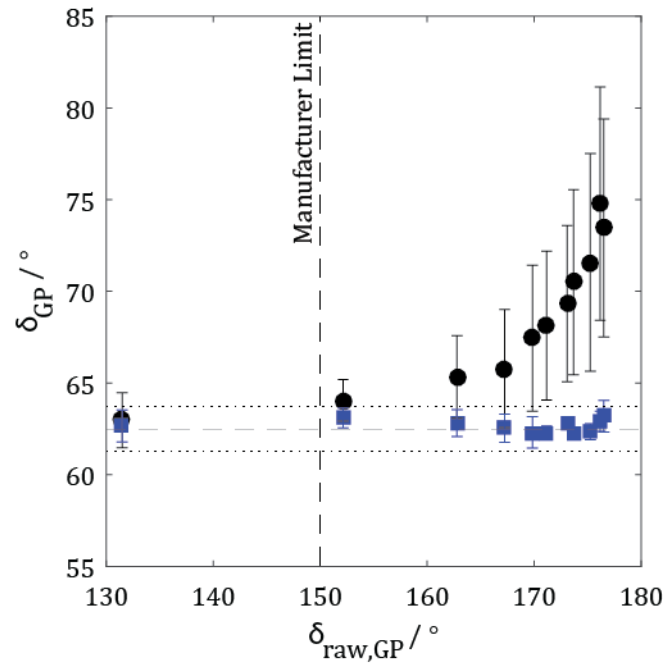
Figure 3 shows the apparent (black circles) and corrected (blue squares) GP data as a function of δ_{raw} at the apparent GP (highest frequency). Horizontal lines show the SMT rheometer mean (dashed) and standard deviation (dotted). The vertical line shows the manufacturers stated limiting δ_{raw} (TA Instruments, 2016). In order to reflect the uncertainties associated with the measurements, error bars reflect the larger of either (i) the standard deviation between repeats or (ii) the deviation of the root positions.
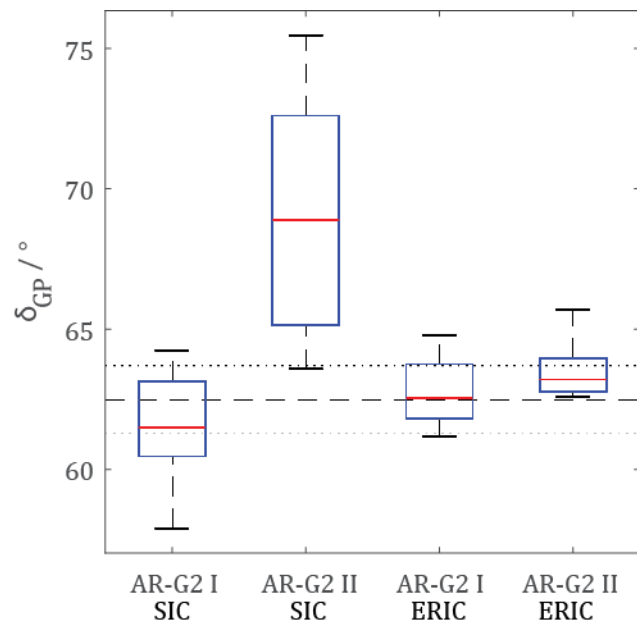
Figure 4 Comparison of gel point results from two AR-G2 rheometers using standard inertia calibration (SIC) and ERIC. A significant difference between the rheometers is apparent using the SIC (*p = 0.0004*), whist no significant difference is apparent after application of the ERIC routine (*p = 0.16*). Horizontal lines show the mean and standard deviation of data acquired using the SMT rheometer.
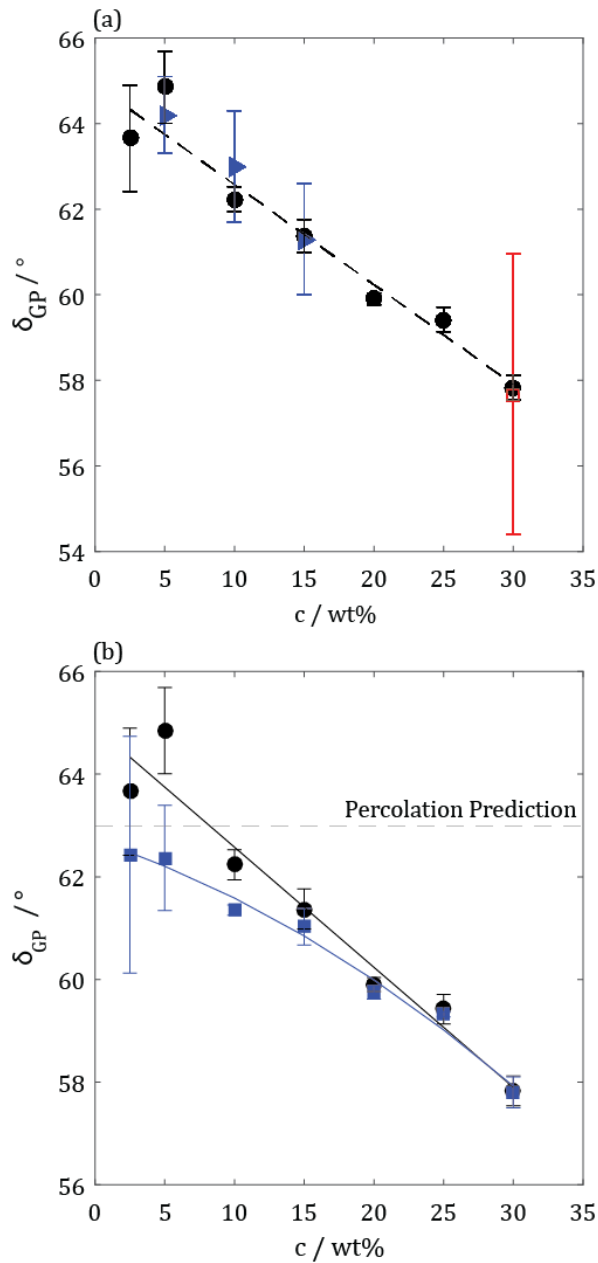
Figure 5 shows the concentration dependence **of** δ_{GP}. Figure 5a shows data from the present study (0.3 – 3 Hz, black circles) using standard instrument correction procedures, Hawkins *et al.* (0.2 – 3.2 Hz, SAOS and Fourier Transform Mechanical Spectroscopy, blue triangles) (Hawkins et al., 2008) and Curtis *et al.* (0.1 – 10 Hz, Optimal Fourier Rheometry and Fourier Transform Mechanical Spectroscopy, red squares) (Curtis et al., 2015). Excellent agreement between these CMT rheometer based studies is observed but a clear discrepancy is also evident at 2.5wt% with data obtained using a SMT rheometer (62.7 ± 1.0°), herein. Figure 5b shows the effect of applying the ERIC procedure to data from the present study (standard inertia correction – black circles, ERIC - blue squares) along with the percolation theory prediction of the value of δ_{GP}.