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Validation of Optimal Fourier Rheometry for rapidly gelling materials and its application in the study of collagen gelation ☆

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ABSTRACT

Rheological Gel Point measurements may incur errors in the case of rapid gelling systems due to the limitations of multiple frequency oscillatory shear techniques such as frequency sweeps and Fourier Transform Mechanical Spectroscopy, FTMS. These limitations are associated with sample mutation and data interpolation. In the present paper we consider how an alternative rapid characterisation technique known as Optimal Fourier Rheometry, OFR, can be used to study a rapidly gelling material, namely collagen at near physiological temperatures. The OFR technique is validated using a model reference gelling system whose GP characteristics have been widely reported. An analysis of the susceptibility of OFR measurements to rheometrical artefacts is made prior to its use in the study of rapid gelling collagen gels formed over a range of physiologically relevant collagen concentrations. The results of this OFR study are the first measurements of the stress relaxation characteristics of collagen gels performed in a single rheological experiment.

1. Introduction

The gelation characteristics/properties of biopolymer systems are of significant scientific interest and are a fundamentally important aspect of many biological processes. Salient examples include the formation of collagen gels, the microstructure of which depends on polymerisation conditions [1,2] and which are widely used as scaffolds in tissue engineering and 3D cell culture applications [3,4]. Other examples include the formation of fibrin gels which form the principal microstructural component of blood clots and provide the requisite mechanical properties for haemostatic functionality [5,6]. Such systems undergo a sol–gel transition which can be identified by rheological Gel Point, GP, measurements [7–11]. In many biopolymer systems such as fibrin–thrombin gels (or clots formed in whole blood) this can occur rapidly due, for example, to underlying prothrombotic conditions. The inherent rapidity of collagen gel formation at physiologically relevant conditions has restricted previous rheological studies of the GP in collagen based systems to sub physiological temperatures [12] or have necessitated the use of pepsin-solubilised collagen [13]. The ability to accurately measure the viscoelastic properties at the GP has particular relevance in characterising the fractal microstructure of biological systems such as blood clots [7,8,11,14].

A convenient and widely reported technique for detection of the GP involves measurements of the complex shear modulus, G^*, over a range of frequencies, ω, in oscillatory shear. At the GP the elastic and viscous components of the complex modulus, G' and G'', respectively scale in oscillatory frequency, ω, as $G'(\omega) \sim G''(\omega) \sim \omega^{\alpha}$ where α is termed the stress relaxation exponent [15]. Thus, the GP may be identified as the instant where the G' and G'' scale in frequency according to identical power laws [15], behaviour corresponding to attainment of a frequency *independent* phase angle, $\delta(= \text{atan}(G''/G'))$. GP measurements may involve 'frequency sweeps' with repeated consecutive application of a set of small amplitude oscillatory shear, SAOS, waveforms [15,16], or by Fourier Transform Mechanical Spectroscopy, FTMS, in which $G^*(\omega)$ is found by simultaneous application of several harmonic frequencies in a composite waveform and its subsequent Fourier analysis [17,18]. Frequency sweeps are limited to relatively slow gelation processes due to sample mutation and interpolation errors [9,19,20]. FTMS may overcome these limitations, but is unsuitable for markedly strain sensitive materials, such as fibrin gels, due to the strain amplitude of the composite waveform exceeding the linear viscoelastic range (LVR) [9].

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

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Due to the rapidity of gelation, GP measurements on acid-solubilised rat tail (type I) collagen are not viable at physiological (37 °C) temperatures and remain challenging at near-physiological temperatures. Yet the potential use of such biopolymer materials for *in vivo* tissue engineering applications for example [3], prompts the need to better understand their rheological properties. In the present paper we consider how recent developments in a rapid oscillatory shear characterisation technique, known as Optimal Fourier Rheometry, OFR [21] can be successfully applied to the study of such systems at near physiological temperatures.

2. Theoretical

2.1. Fourier Transform Mechanical Spectroscopy (FTMS)

In FTMS the input waveform, i.e. that applied to the test material, is generated by combining a sinusoidal waveform with several of its harmonics. The dynamic viscoelastic parameters at each of the discrete component frequencies are obtained by comparing the Fourier Transform, FT, of the input and response waveforms [17,18]. Whilst FTMS significantly reduces the time required to obtain G' and G'' over a range of frequencies, the amplitude of the applied waveform increases as more harmonics are included and may exceed the LVR for strain sensitive biopolymer systems even where a modest number of harmonics are employed [9]. Reducing the amplitude of the harmonics in an attempt to maintain linearity generally leads to a loss of resolution in the pre-GP data. This can cause inaccurate GP identification in a sample of low initial viscosity.

2.2. Optimal Fourier Rheometry (OFR)

Optimal Fourier Rheometry (OFR) is a 'multi-frequency' technique involving frequency modulated (chirp) waveforms of the following form [21].

$$\gamma(t) = \gamma_0 \sin(2\pi K(e^{t/L} - 1)) \tag{1}$$

where

$$K = \frac{Tf_1}{\ln(f_2/f_1)} \tag{2}$$

and

$$L = \frac{T}{\ln(f_2/f_1)} \tag{3}$$

where γ_0 denotes the strain amplitude, T denotes the waveform duration (herein set to $1/f_1$) and f_1 and f_2 denote the initial (lowest) and final (highest) frequency (Hz) of the waveform's frequency range, respectively. A typical OFR waveform is shown in Fig. 1. The dynamic rheological parameters $G'(f)$ and $G''(f)$ are calculated by comparing the FT of the strain input and stress response signals (see Section 3.3.2).

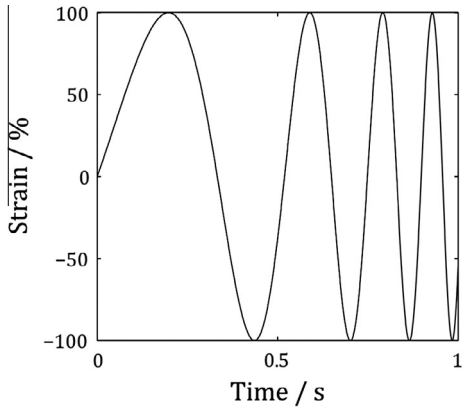

Fig. 1. OFR waveform characterised by the parameters $f_1 = 1$ Hz, $f_2 = 10$ Hz and $T = 1$ s with a peak strain amplitude, γ_0, of 100%.

In contrast to FTMS, in which the perturbation signal consists of discrete frequencies, the OFR waveform undergoes a continuous frequency modulation between two predefined limits. Hence, Fourier analysis of OFR waveforms identifies a number of frequency components limited only by the sampling rate of the original perturbation and response waveforms. As a result, OFR offers two significant advantages over FTMS, namely (i) the ability to obtain very high densities of data ($G'(f)$ and $G''(f)$) over a finite frequency window, and (ii) the strain amplitude is independent of the number of component frequencies sampled.

As the OFR technique has been relatively little reported, preliminary experiments were conducted to assess its validity and examine any potential restrictions on its application to biopolymer systems undergoing gelation. The test systems chosen for this aspect of the work were gels formed from aqueous solutions of gelatin. The GP characteristics of these systems have been widely reported and they have been used as model reference systems in previous rheometric studies invoking GP measurements [18].

3. Methods

3.1. Sample preparation

3.1.1. Gelatin gels

The required mass of general purpose gelatin powder (Fisher G015053) was added to deionised (type I) water (dH_2O) (heated to 60 °C) and agitated vigorously for 5 min to give a final gelatin concentration of 30 wt% (this high concentration allowing sufficient stress resolution in the pre-GP state). The gelatin solution was maintained at 60 °C for 45 min with further agitation every 10 min to ensure complete dissolution of the gelatin powder. The solution was then aliquoted and refrigerated until use. Aliquots were melted in a 60 °C water bath for 45 min before being immediately transferred to the temperature controlled stage of the rheometer which was maintained at 60 °C, see below.

3.1.2. Collagen gels

High concentration type I rat tail collagen (RTC) (10 mg/ml, BD Bioscience), dH_2O and 1M NaOH (Fluka) was placed on ice. The required amounts of dH_2O, 10x Phosphate Buffered Saline (Fluka) and RTC were then mixed well using a pipette tip before NaOH was added to initiate gelation (as per the manufacturer's instructions). The collagen gelation process is temperature dependent [1] with the rate of gelation being significantly reduced at low temperatures, hence the rheometer's Peltier plate temperature (see below) was lowered to 5 °C immediately prior to sample loading.

3.2. Rheometry

All experiments were performed using a TA-Instruments ARES-G2 controlled strain type rheometer fitted with a 50 mm Titanium parallel plate geometry. Temperature control was achieved through the use of a Peltier plate system, the gap zero setting being performed at the test temperature. A shearing gap, h, of 200 μm was employed throughout to minimise sample inertia effects. The free surface of the sample was coated with a thin layer of silicone oil (10 mPa s) to prevent evaporation. A preliminary study employing a range of gaps confirmed the absence of wall slip for gelatin samples while for collagen (in which sample inertia constraints require the use of small gaps) insignificant 3rd and 5th