Potential of Thromboelastometry and Multispeckle Diffusing Wave Sprectroscopy for Monitoring Acid-Induced Casein Gelation

Norbert Raak, Rebecca Leidner, Harald Rohm, and Doris Jaros

Chair of Food Engineering, Technische Universität Dresden, 01062 Dresden, Germany

ABSTRACT

In this study we evaluated two techniques which may be able to give complementary information to, or even replace small amplitude oscillatory shear rheology in investigations of milk protein gelation. Thromboelastometry, a method established since long for the clinical characterisation of blood clotting, is comparable to strain-controlled oscillation in a Couette rheometer but requires only 360 µL of sample. Rheolaser™ applies Multispeckle Diffusing Wave Spectroscopy and therefore monitors gelation processes non-invasively by particle tracking. Both techniques were found to correlate well with conventional rheological measurements. The gelation onset times were comparable, and it was possible to establish non-linear regression fits for the maximum values of the respective gelation curves. Both are important parameters for the characterisation of gelation processes.

INTRODUCTION

Small amplitude oscillatory shear (SAOS) rheology is a well established tool for monitoring gelation processes since it allows to determine the time course of gel stiffness under non-destructive conditions. However, some disadvantages of this technique (for instance large sample volumes in the case of liquid samples that require the use of concentric cylinder geometries, and time-inefficiency) encourage the search for alternatives.

Thromboelastometry (TEM) originates from clinical laboratories where it is used for the routine analysis of blood clotting characteristics. Its advantage arises from the possibility to use several autonomous measuring channels simultaneously, and the small required sample volumes (360 µL). Two different types are available[1], for which analogies to Searle- and Couette-type rheometers can be drawn. In a previous work, a "Searle-type" thromboelastometer was evaluated and deemed to be suitable for monitoring acid-induced casein gelation[2]. In addition, a "Couette-type" thromboelastometer is applied in the present study.

Diffusing Wave Spectroscopy (DWS) is a technique similar to Dynamic Light Scattering (DLS), but it requires more concentrated and highly opaque samples to achieve a pronounced multiple scattering[3,4]. Instead measuring the scattered light at a single position as in conventional DWS, Multispeckle Diffusing Wave Spectroscopy (MS-DWS) uses charge-coupled device (CCD) array detectors to collect scattering intensities over a broader area[4]. Such a measurement system is, for example, integrated in the Rheolaser™ series offered by Formulaction SA (L'Union, France). These instruments monitor interfering backscattering intensities over time to measure particle mobilities which are related to the viscoelastic properties of the sample[5,6]. In a recent study, a good correlation between SAOS and Rheolaser™

was found for acid-induced gelation of heat-treated milk[7].

In this study, we aim to assess the suitability of the two techniques to monitor and characterise the acid-induced gelation of enzymatically cross-linked casein. Cross-linking with microbial transglutaminase (mTGase) has a huge potential for improving the texture properties of yoghurt[8,9], but little is known about the mechanisms that affect acid milk gels. Innovative techniques like MS-DWS and time- and sample-efficient instruments like TEM could contribute to meaningful information.

MATERIALS AND METHODS

Sample Preparation

Acid casein powder (Sigma-Aldrich GmbH, Steinheim, Germany) was dispersed at 27 g/kg protein in 0.1 mol/L phosphate buffers (pH 6.8) and stirred overnight to ensure complete dissolving. Sodium azide was added in a concentration of 0.3 g/kg to avoid microbial growth.

The solutions were incubated with 3 U mTGase (Activa MP, Ajinomoto Foods Europe SAS, Paris, France) per g casein at 40 °C for 0-48 h to cross-link casein to different extents. The incubation was stopped by heat treatment at 85 °C for 15 min, followed by cooling in ice water.

To induce gelation, the temperature equilibrated casein solutions were blended with glucono-δ-lactone (GDL) in concentrations of 40, 60, or 80 g/kg immediately prior to measurements. The mixtures were then transferred into the vessels of the corresponding instruments, and the tests started after a defined period of time which was respected in the evaluation. Gelation measurements were carried out at 20, 30, or 40 °C.

Small Amplitude Oscillatory Shear

Time-based SAOS experiments were conducted using a strain-controlled ARES RFS3 rheometer (TA Instruments, Eschborn, Germany) with a cup-and-bob-geometry (d_i=32 mm; d_o=34 mm, h=33.5 mm). Frequency and strain were kept constant at ω=1.0 rad/s and γ=0.003, respectively, and the temperature was adjusted by a computer-controlled circulator. The gelation onset was determined at G'=1 Pa, and G'_{max} was extracted as an indicator for maximum gel stiffness. All results are shown as mean values of duplicate experiments.

Thromboelastometry

TEM was carried out using the automated thromboelastometer MultiTEM:a from Framar Hemologix SRL (Roma, Italy) with disposable cup-and-pin-units (d_i=6 mm; d_o=8 mm, h=7 mm). The temperature was kept constant by a heated steel block, and the angular displacement of the cup was φ=4.75°. The increasing displacement of the pin over time as a consequence of increasing sample stiffness during gelation was converted into an arbitrary amplitude parameter (A) by the software TEMAwin (Version 1.7.1; Framar Hemologix SRL, Rome, Italy). Maximum amplitude, and gelation onset time, read at an amplitude A of 2 mm, were obtained directly from the software. All results are shown as mean values of quadruplicate experiments.

Multispeckle Diffusing Wave Spectroscopy

A Rheolaser™ Master (Formulaction SA, L'Union, France) was used for MS-DWS experiments. TiO_2 particles were added to the casein solutions in a concentration of 0.75 g/kg and served as tracer particles. After GDL addition, the samples were transferred into glass vials (d_i=2.4 mm, h=5.3 mm) and placed within the measurement cell. Time-based backscattering of the samples was observed using laser light with a wave length of λ=650 nm in combination with a multi-pixel detector, and internally converted into curves of Mean Square Displacements (MSD) over decorrelation time by the software Rheosoft Master (V1.4,

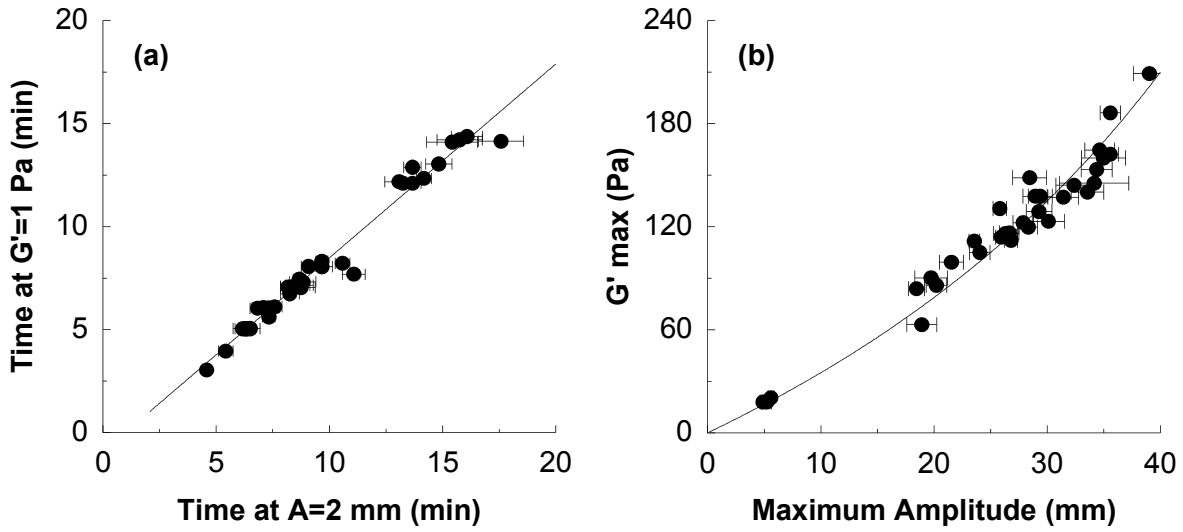

Figure 1. Relationship between gelation parameters obtained from thromboelastometry (x-axis) and small amplitude oscillatory shear (y-axis): gelation onset time (a), and maximum value (b).

Formulaction SA, L'Union, France). The temperature was kept constant by a peltier element. The elasticity index (EI) was determined from the inverse mean MSD at short decorrelation times (<0.1 s). The maximum EI was extracted from the curves, and the gelation onset was determined at $EI=10^{-3}$ nm^{-2}. All results shown are mean values of triplicate experiments.

RESULTS AND DISCUSSION

Thromboelastometry

Output data from TEM show the displacement of the inner pin with time, normalised to an arbitrary amplitude parameter (A) ranging from 0 to 100 mm. This means that the amplitude equals 0 mm if the sample is liquid and the pin remains unmoved, and 100 mm if the cup displacement (φ=4.75°) is completely transmitted to the pin. Gelation curves obtained from SAOS and TEM were qualitatively comparable (not shown), as was already shown[2].

The gelation onset criteria (G'=1 Pa for SAOS and A=2 mm for TEM) were in good agreement, showing a linear correlation (R^2=0.98; Fig. 1a). The slope (=0.94) suggests a similar sensitivity of both methods concerning the detection of the gelation onset. However, the shift in the x-axis of approx. 0.9 min indicates a consistent delay in the detection of the gelation onset via TEM, probably because the required sample stiffness for a pin displacement of A=2 mm is greater than 1 Pa.

A good correlation was also found between the maximum amplitude from TEM and the maximum G' from SAOS (Fig. 1b). It has been previously reported that this relationship is a non-linear one because the amplitude ranges between 0 and 100 mm. while G' could theroretically rise into the infinite[2]. A model for the relationship between pin displacement as expressed through the amplitude parameter A and actual elasticity of the sample has already been described[10]:

$$\text{Elasticity} = 100 \cdot A / (100 - A) \qquad (1)$$

Eq. 1 was multiplied by a regression coefficient and applied to fit the data points from Fig. 1b. The coefficient was calculated to be 3.15 (R^2=0.95) and was slightly lower compared to a previous study (3.59)[2].

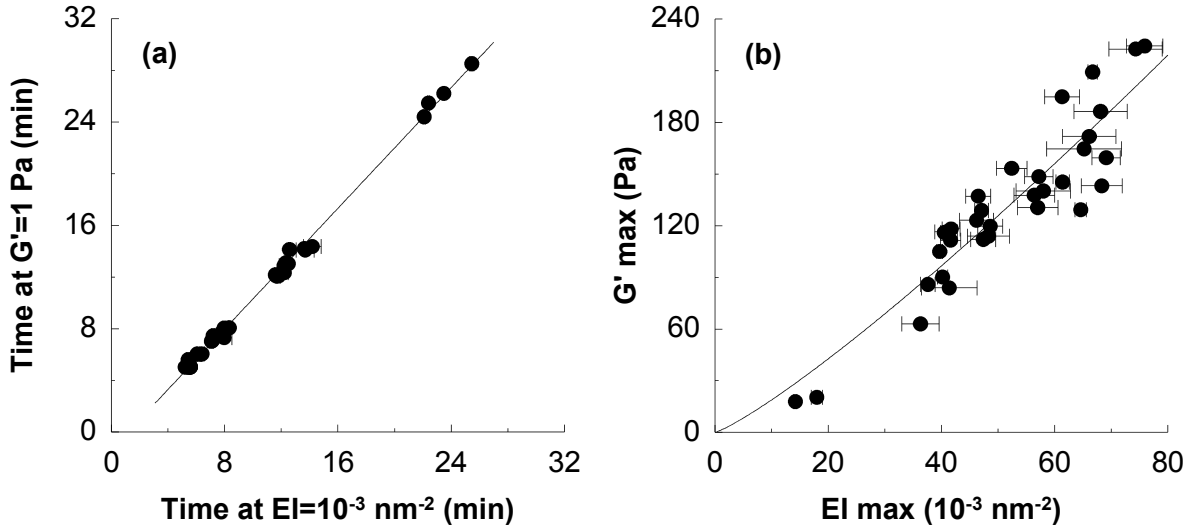

Figure 2. Relationship between gelation parameters obtained from Rheolaser™ (x-axis) and small amplitude oscillatory shear (y-axis): gelation onset time (a), and maximum value (b).

Multispeckle Diffusing Wave Spectroscopy

Output data from Rheolaser™ is expressed as the development of the elasticity index (EI) over time, reflecting the decrease of particle movements in the sample. The gelation onset criterion of $EI=10^{-3}$ nm^{-2} is therefore the first measurable hindrance of particle motion, comparable to the first measurable elasticity of G'=1 Pa in our SAOS experiments. These criteria showed a good linear relationship (R^2=0.99; Fig. 2a). However, gelation onset was detected slightly earlier by Rheolaser™. This is not surprising since the onset is directly determined from observations of particle movements and no reliable mechanical signal is required.

As has also been done by Rohart et al.[7], we applied a power-law function ($y=a \cdot x^b$) to correlate EI_{max} and G'_{max} (Fig. 2b). The regression parameters of a~4300 and b=1.18 (R^2=0.85) were much lower compared to the findings of Rohart et al.[7] for acid-induced skim milk gels (a=220000; b=2.4; R^2=0.98). This is probably connected to a lower oscillation frequency in our SAOS experiments (ω=1 rad/s compared to ω=6.28 rad/s) because G' of acid milk gels increases with frequency[11].

CONCLUSION

TEM requires only small sample volumes (360 µL) and is hence of particular interest for gelation experiments with material of limited quantities. MS-DWS, which is based on particle tracking, could probably provide additional information on the microstructure of the gels. However, more research has to be conducted to fully exploit the possibilities which by Rheolaser™.

TEM and MS-DWS show a huge potential to complement or replace SAOS experiments in investigating acid-induced casein gelation because most important gelation parameters (gelation onset and maximum curve value) correlate well with conventional rheological measurements.

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