

Effect of high hydrostatic pressure on structure of gelatin gels

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Abstract. The present contribution reports on the rheological investigations concerning influence of high hydrostatic pressure on the molecular structure of gelatin gels. For the purpose of the study, a torsional shear wave rheometer for in-situ investigations of viscoelastic substances under high pressure was developed. Small amplitude vibrations generated by piezoelectric elements are used to determine the storage modulus of the investigated medium. The system is able to stand pressures up to 300 MPa. The experiments have been carried out with household gelatin (0.1 w/w aqueous solution). The gelification curves revealed similar time course. However, the values of G' obtained for the gels curing 300 minutes under 100 MPa and 200 MPa were observed to be respectively 2.1 and 4 times higher than at ambient conditions. The increased number of triple helix junction zones is hypothesised to be the cause of this phenomenon as a result of reinforcement of the hydrogen bonds due to pressure. An attempt to cognize the characteristic dimensions of the molecular structure based on the theory of rubber elasticity is made.

Key words: high hydrostatic pressure, in-situ measurement techniques, rheology, gelification, gelatin, molecular structure.

1. Introduction

High pressure technologies (pressures up to 1 GPa) have proved a great potential in modern bioengineering as a promising alternative to conventional methods of modification of biotechnological materials. Due to extreme mechanical loads, achieving the order of magnitude of molecular forces, the pressure can induce conformational changes of macromolecules. As a result high pressure treatment enables denaturation of proteins [1], inactivation of enzymes [2] and microorganisms [3] while the amount of aromas, vitamins and nutrients is not substantially decreased [4]. Additionally, application of high pressure allows producing unique food textures unobtainable with classical technologies, as it was observed at example of protein gels [5,6]. Furthermore, elevated pressure can serve as valuable research tool for investigations of phase behaviour [7], reaction energetics [8] and structure of biomolecules [1,5,6,9]. Not least, use of high pressure contributes to saving the total amount of energy required for industrial food processing [10]. Already numerous commercial applications for high pressure food treatment have been found, ranging from meat and seafood products, fruit and vegetable juices, jams to pre-cooked rice and complete ready-to-eat meals, which shows the practicability of this technique [4].

Obviously, due to mechanical stresses and resulting molecular alterations, the macroscopic physical properties of the treated biomatter can undergo significant variations [11]. The knowledge of physical properties of treated substance is, in turn, essential for understanding, design and control of the process technology. From special interest are

rheological data as they provide information on flow behaviour of the matter, which is necessary for thermofluid-dynamic design and scaling of vessels, piping, pumps etc. Furthermore, rheological characterisation allows insight into the molecular structure of the investigated substance and into the phenomena governing the microstructural modifications. Certainly, only the throughout knowledge of the molecular and cellular mechanism underlying the observed effects can contribute to adequate exploitation of the tremendous potentials connected with high pressure [12]. Thus, suitable measurement techniques for in-situ investigations under high pressure become a matter of particular importance.

The present contribution reports on the design of an in-situ rheometer for non-destructive investigations of viscoelastic substances under pressure up to 300 MPa. The idea of the system bases on works by Carozza [13], where food gels are studied under atmospheric conditions. Although, numerous measurement techniques have been established for equilibrium shear rheological investigations under high pressure conditions, mostly for Newtonian liquids (e.g., [14,15]), a suitable small amplitude dynamic shear rheometer for pressures up to 300 MPa is still missing. Due to the small deformations within the measurement, the sample structure is not influenced. That is particularly important when investigating shear sensitive samples or shear sensitive processes, e.g., sol-gel transition. The setup is specifically designed for investigations of food-related biomatter, however, the potential field of application is much broader (e.g., polymer science, lubricants technology, geophysics).

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Numerous food systems, for which high pressure technology might potentially bring significant advantages [4], feature viscoelastic behaviour (e.g., jams, fruit jellies, pastes, creams, yoghurt). A prevalent component of those foodstuffs is gelatin. This animal origin protein is used as a gelling agent, stabiliser, thickener and texturizer [16]. Gelatin plays an important role in production of foods with reduced fat content to simulate the mouth feel of fat without extensive calories. Therefore, due to high practical importance, household gelatin was chosen for current study.

2. Materials and methods

2.1. Theory. Considering viscoelastic sample between two plates within the linear regime [17], the storage and loss modulus are calculated by

$$G' = \frac{\rho\omega^2 \left(\frac{4\pi^2}{\lambda^2} - \frac{1}{z_0^2} \right)}{\left(\frac{4\pi^2}{\lambda^2} + \frac{1}{z_0^2} \right)^2}, \quad (1)$$

$$G'' = \frac{\rho\omega^2 \frac{4\pi}{\lambda z_0}}{\left(\frac{4\pi^2}{\lambda^2} + \frac{1}{z_0^2} \right)^2}, \quad (2)$$

where ρ represents the sample density, ω the angular frequency of plate vibration, λ the wave length and z_0 the exponential damping length. By introduction of the dimensionless wave length

$$s = \frac{\lambda}{2\pi z_0} \quad (3)$$

and the wave velocity

$$c = \frac{\omega\lambda}{2\pi} \quad (4)$$

equations (1) and (2) can be transformed to

$$G' = \rho c^2 \frac{1 - s^2}{(1 + s^2)^2}, \quad (5)$$

$$G'' = \rho c^2 \frac{2s}{(1 + s^2)^2}. \quad (6)$$

In this case the loss tangent, being the ratio G''/G' , equals

$$\tan \delta = \frac{2s}{1 - s^2}. \quad (7)$$

The dimensionless wave length s can be calculated from equation (7) for known values of the loss tangent. Consequently, storage and loss modulus may be determined by measurement of the wave velocity c and by the loss tangent $\tan \delta$ for known sample density ρ .

In linear viscoelastic medium between two plates, where one undergoes forced harmonic displacement, resonances can occur as the frequency ω is varied. It can be shown [17] that

$$\tan \delta = \frac{\omega_2 - \omega_1}{\omega_0}, \quad (8)$$

where ω_0 is the resonant frequency. The values ω_1 and ω_2 name the frequencies at which the energy absorbed from the oscillator reaches half of its maximum value. This is equivalent to points at which the amplitude of the oscillation decreases by the factor $1/\sqrt{2}$. Thus, the loss tangent can be determined by application of harmonic oscillations to one of the sensor plates and measurement of the amplitude at the second plate as the frequency of oscillation is varied. The result only applies if the loss tangent is treated as being independent of frequency. In practice it is considered as valid if $(\omega_2 - \omega_1)$ is small in comparison with ω_0 . The wave velocity c can be determined from the time of flight that the disturbance travels from one sensor plate to the other. Thus, storage and loss moduli can be calculated according to Eqs. (5) and (6). It is worth considering, that for the case of an ideally elastic solid, where the wave is not attenuated z_0 is infinite, and the Eq. (1) changes to

$$G' = \rho c^2. \quad (9)$$

Similarly, for a predominantly elastic material, as the gels investigated in the present work, where λ is much smaller than z_0 calculation of the storage modulus with Eq. (9) leads to only a marginal error [17].

2.2. Measurement setup. The sample fills the gap between two parallel plates of 23 mm diameter (see 2 in Fig. 1). Each plate is connected with a piezoelectric element – one working as a driver, the other as a sensor. Piezoelectric elements are manufactured as composites of plumbum-zirconate-titanate and carbon fibres in a polymeric coating. Hence, under driving voltage they undergo torsional oscillations. In this way, small amplitude torsional shear waves are applied to the sample.

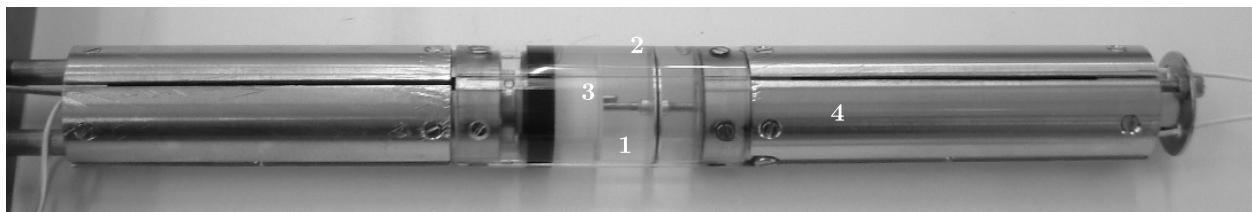


Fig. 1. High pressure torsional shear wave rheometer. 1-contracting sample chamber, 2-sensor/sender plates, 3-thermocouple, 4-piezo element shielding

The measurement system is placed in a high pressure cell capable to hold pressures up to 300 MPa. Water is used as a pressure transmitting fluid. The temperature of the pressure chamber is set by means of a controlled thermal bath. The temperature inside the sample chamber can be gauged by a thermocouple.

In order to determine the storage modulus from Eq. (9), the measurement of the time of flight τ , a disturbance travels between driver and sensor plates is used to assess the wave velocity. The distance between the plates h is set in the range of 0.3 to 1 mm. Thence, the wave velocity equals

$$c = \frac{h}{\tau - \tau_0}, \quad (10)$$

where τ_0 is the effective response time of the instrument. The value of τ_0 is determined by calibration. For this purpose, a comparative measurement is carried out under atmospheric pressure by means of a dynamic stress rheometer (Rheometric Scientific SR-5000).

However, due to compression-induced change of the mechanical properties of materials used for the construction of the experimental setup, particularly of the polymeric materials applied in the piezoelectric composites, the dynamic characteristics of the system changes with pressure. Therefore, the effective response time of the instrument is a function of pressure. In order to determine the change of response time, both piezoelectric composites were connected with a brass stalk and the response time was measured at different pressure levels. The determined pressure correction for the response time is negative, i.e., the response time decreases with pressure due to compression-induced stiffening of the mechanical elements.

2.3. Sample preparation and experimental procedure. Gelatin solution is used as a model system for the current investigations. The sample is prepared according to the method given in [18] as a 0.1 w/w household gelatin aqueous solution.

Prior to the measurement, the calibration procedure is carried out in order to determine τ_0 (see Eq. 9). The course of storage modulus G' over time during gelification by cooling of the solution from 40°C to 20°C is tracked in the high pressure rheometer and dynamic stress rheometer with cone-and-plate geometry at 1 Hz. After 300 minutes of measurement the results are compared and the effective response time is determined.

However, at this stage, special attention must be paid to the frequency spectrum of the storage modulus. Namely, it must be proven that the storage modulus is independent of frequency in the frequency range used. Otherwise, the measurement at 1 Hz by dynamic stress rheometer and the measurement at high pressure rheometer, which typically takes place at the frequency of several hundred Hz could not be compared. Thus, the frequency spectrum for the storage modulus of the investigated sample is measured firstly in order to prove the applicability of

the measurement system for the aimed investigation. This preparatory experiment carried out for 0.1 w/w aqueous gelatin solution showed that the storage modulus can be treated as frequency independent in the interesting frequency range.

After the calibration procedure the measurement setup is heated up to 40°C and kept at that temperature for several hours in order to ensure the complete reversion of the gelification process. Next, the rheometer is placed inside the pressure cell and the pressure is increased up to 100 MPa. Having the pressure increased, the sample is held at 40°C for 30 minutes in order to equilibrate the temperature inhomogeneities developed due to polytropic compression. Afterwards, the temperature of the thermal bath is set to 20°C. Due to large thermal inertia of the pressure chamber, it takes around 25 minutes until the quench temperature of 20°C is reached inside the chamber. The measurement is being carried out for 300 minutes and the temperature is increased again up to 40°C. The chamber is held at that temperature for several hours and afterwards the pressure is increased up to 200 MPa. After the equilibration time the temperature is decreased again to 20°C and the measurement is started. The course of temperature and pressure is schematically depicted in Fig. 2.

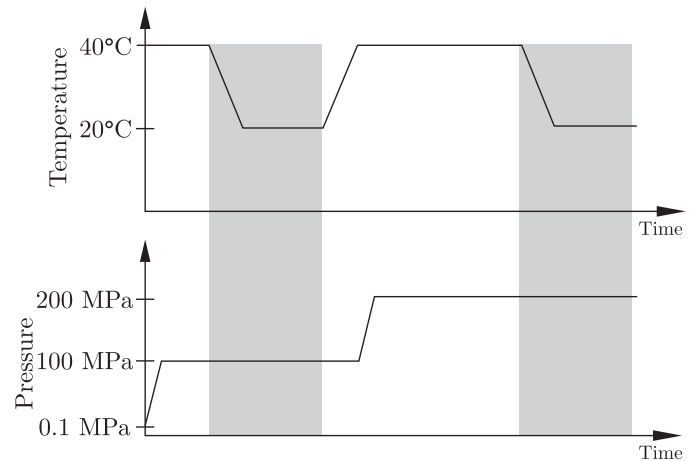


Fig. 2. The course of temperature and pressure during experiment. The periods of data registration during gel building are marked grey

The density of the sample necessary for the calculation of the storage modulus G' (see Eq. 9) is determined using the following approach. Firstly, the difference between the measured density of 0.1 w/w aqueous gelatin solution and that of pure water is calculated at ambient pressure. An assumption is made that this offset remains constant in the investigated pressure range. Thus, the density of the sample under high pressure is calculated as the density of water under high pressure (according to [20]) increased by the determined offset.

3. Results and discussion

The measurements carried out at 100 and 200 MPa reveal that gelatin has kept its jellifying properties under high pressure. The development of the storage modulus G' over time is depicted in Fig. 3. The time is referred to the total measurement time t_{end} and the storage modulus to the value at $t = t_{\text{end}}$. The measurement at atmospheric pressure and comparative measurement with dynamic stress rheometer Rheometric Scientific SR-5000 used for means of calibration are shown as well.

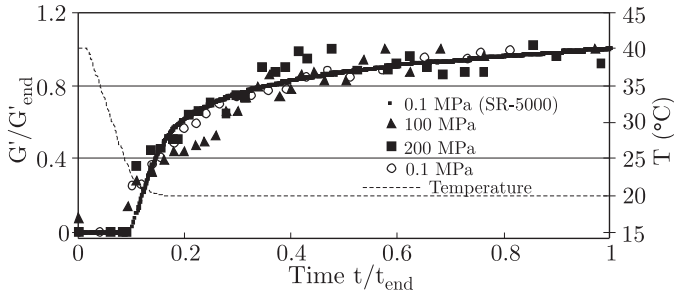


Fig. 3. Development of the storage modulus G' over time during gelification of gelatine (after Ref. 19)

The storage modulus at the end of experiment at ambient pressure equals to 3.8 kPa, at 100 MPa reaches 8 kPa and 15 kPa at 200 MPa. This correlates with the ex-situ investigations by Montero et al. [21], who have observed the gelatin of cod and megrim skin cured under pressures up to 400 MPa to reveal considerably higher values of the storage modulus G' upon melting in comparison to non-pressurized reference probe. However, the authors point out strong variation of the gelatin gel properties in respect of the animal species, the extraction method and the pressure-temperature trajectory during gelification.

Gelatin is a biopolymer made from collagen through hydrolysis process. The native collagen units are rods of 300 nm length, each one built of 3 amino acid strands of molecular weight of 100 000. The three strands have a form of a triple helix, where single chains are connected by hydrogen bonds perpendicular to the chain axes. Gelatin, being denaturated collagen, above melting temperature exhibits flexible random coil conformation. In the literature there is an agreement [9,16,18] that upon cooling the gelatin undergoes conformational change, where parts of the molecular chain transform into helix. At the helix sites the molecular strands connect with other strands being in helix conformation. As a result, junction zones develop, which resemble the ones of the native collagen. With time, the built gel network spans through the whole sample. However, the junction building process is understood to be completed in two steps: nucleation and wrapping. The nucleation process is in this case the rate limiting one, since it requires positive activation energy. The following process of wrapping the strands is thermodynamically favoured to be fast. The critical nucleus can further transform into helix junction zone if it exceeds a certain critical length. This critical helix length increases

with temperature and tends to infinity at gelation point as shown by Guo [16]. Let us now consider the influence of high pressure on that limiting parameter. Since the helix junctions are stabilised by hydrogen bonding, it seems valid that the stability of the hydrogen bonds is the crucial factor influencing the critical helix length. Consequently, it can be expected that the stronger the hydrogen bonds are, the shorter the critical helix nuclei must be. The hydrogen bonds are known to be promoted under high pressure due to their negative activation volume [1]. Thus, it is postulated, that the stable length of the helix junction zone is shifted towards lower values at elevated pressures and hence the gelification temperature is shifted to higher values.

Due to inapplicability of the measurement of G' for determination of gel point it is difficult to recognize the changes in the gelification temperature within the present experiment. However, the earlier investigations of Shimada et al. [22] by means of hot-wire method as well as the study of Gekko and Fukamizu [9] with the falling ball method show indeed a remarkable increase of gelification temperature upon pressurization by 2÷3 K per 100 MPa.

The rheological experiment carried out within the present contribution indicates higher values of the storage modulus for the gels cured at elevated pressures. This allows a statement that the average length of the parts of the molecular chain remaining in random coil conformation is shorter. Thus, either the triple helix junction zones in the gelatin gel cured under high pressure are longer than at ambient pressure, or they are shorter but more numerous. Taking into account the considerations presented above, it follows that the latter is true. This means that the gels cured under elevated pressures exhibit more triple helix junction zones per molecular chain compared to the gels cured at normal conditions.

By use of the thermodynamic theory of rubber elasticity (see for example [23,24]) it is possible to correlate the storage modulus G' and the average molecular weight of the chain parts between the junctions \bar{M}_c . Including the correction for free ends (see [23]) the following relation applies

$$\bar{M}_c = \left(\frac{G'}{uRT} + \frac{2}{\bar{M}} \right)^{-1}, \quad (11)$$

where u is the concentration of the dissolved polymer, R the universal gas constant, T the absolute temperature, and \bar{M} the average molecular weight of the whole molecular chain. The average number of junctions per molecular chain n can be, hence, calculated

$$n = \frac{\bar{M}}{\bar{M}_c} + 1. \quad (12)$$

Application of the theory of rubber elasticity to the experimentally acquired storage modulus after 100 minutes of gelling delivers the average number of junctions per molecular chain equal to 3.9 at ambient pressure, 4.8 at 100 MPa and 6.4 at 200 MPa. The junctions are treated

here as point-wise, which is an approximation. Specifically after longer curing the cross linking zones can reach considerable length due to rewrapping processes. However, investigations presented in [9] and [16] reveal that the cross linking junction zones of gelatin consist of rather a small size of triple helix of only few nm length, whilst the overall length of a gelatin molecule reaches ca. 300 nm.

4. Summary

A torsional shear wave rheometer for in-situ high pressure investigations (up to 300 MPa) of viscoelastic substances has been developed. This measurement system uses transmission of small amplitude torsional shear waves generated by piezoelectric elements to determine the storage and loss moduli.

The measurements upon the gelification of 0.1 w/w gelatin aqueous solution under elevated pressure by quenching from 40°C to 20°C show that the gels achieved at pressures of 100 and 200 MPa reveal values of the storage modulus higher by factor 2.1 and 4 respectively in comparison to gels cured at ambient pressure. Montero et al. [21] have observed similar phenomenon before with use of ex-situ methods. A hypothesis is presented that the higher values of the storage modulus originate from the increased number of triple helix junction zones per molecular chain. It is postulated that the governing effect is the decrease of the critical length of helix nucleus preceding development of a triple helix junction zone originating from the increased stability of the hydrogen bonds due to pressure. The presented hypothesis seems to be consistent with the earlier works by Gekko and Fukamizu [9] and Shimada et al. [22] as well as investigations of gelatin gels in deuterium oxide by Oakenfull and Scott [24]. The characteristic parameter of the gel network, i.e. number of junctions per molecular chain is calculated by use of the theory of rubber elasticity [23]. After 100 minutes of gel curing, the number of cross links per molecular chain amounts 3.9 at ambient pressure, 4.8 at 100 MPa and 6.4 at 200 MPa.

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