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INFLUENCE OF SHEAR DIRECTION ON GELATION ABILITY OF COLLOIDAL CHITOSAN SOLUTIONS

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This paper discusses the influence of the direction of applied deformation on the ability to gelation of thermosensitive chitosan hydrogels. The application of the shear rate equal in value to the classically performed oscillatory measurements leads to significantly different shapes of experimental curves. It was found that the type of mechanically applied deformation has a significant impact on the gelation ability of colloidal chitosan solutions and conditions of sol-gel phase transition. Simple shear leads to a phase transition at a lower temperature or in a shorter time compared to oscillatory tests. Moreover, based on the final values of dynamic viscosity in rotational measurements, it was found that stronger crosslinking of the polymer structure was observed.

Keywords: sol-gel phase transition, chitosan hydrogels, rheometry measurements, oscillatory shear, rotational shear

1. INTRODUCTION

Recently, thermosensitive chitosan hydrogels are the object of interest of many researchers. It results from their natural ability to undergo a sol-gel phase transition (Owczarz et al., 2018; Rwei et al., 2005), which makes it possible to prepare minimally invasive injection scaffolds for tissue engineering applications (Chenite et al., 2000). The relatively low viscosity and the formation of the three-dimensional matrix *in vivo* speaks for their use (Liu et al., 2017; Solouk et al., 2014). In order to precisely determine gelation conditions and mechanical properties of the obtained hydrogels, oscillatory rheometry measurements are commonly carried out (Chenite et al., 2001; Cho et al., 2006; Jiang et al., 2016; Owczarz et al., 2017). These measurements are dedicated for viscoelastic media, which include colloidal chitosan solutions. Furthermore, the oscillatory tests are low invasive. They almost do not damage the internal structure of the medium and are relatively easy to interpret and unambiguous. Nevertheless, the use of this type of deformation leads to bidirectional oscillatory shear.

The high unidirectional, simple shear stress generated in the syringe during injection can affect the sol-gel phase transition conditions. It seems reasonable to determine the effect of these stress conditions on the ability to gelation of colloidal chitosan solutions. No previous studies have been carried out to evaluate the effect of unidirectional shear on the sol-gel phase transition of colloidal chitosan solutions. However, Owczarz et al. (2018) claimed that simple shear leads to a change in the spatial configuration of chitosan chains in solutions with disodium β-glycerophosphate. Thus, it seems reasonable to determine the effect

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of unidirectional shear as the only type of applied deformation on the conditions of the sol-gel phase transition of chitosan solutions.

The purpose of this paper is to investigate the influence of rotational deformations on the sol-gel phase transition condition of low-concentrated chitosan solutions. The obtained data will be compared with the results of classical oscillatory tests under the same deformation.

2. MATERIALS AND METHODS

2.1. Materials

Colloidal solutions of chitosan (Sigma Aldrich) were prepared by dissolving 0.4 g of biopolymer (DD = 81.8%, Mw = 680 kDa) in 16 ml of 0.1 M hydrochloric acid (Fluca). A suspension of sodium β-glycerophosphate (Sigma Aldrich), obtained by dissolving 2.0 g of salt into 2.0 ml of distilled water, was added to the chitosan solutions. During the preparation of the research material, the procedures described in the literature were followed (Chenite et al., 2001; Kamińska et al., 2017; Owczarz et al., 2017).

2.2. Methods

Measurements were carried out in a cone-plate measurement system (50 mm diameter, 1° slope angle, 48 µm truncation) of Anton Paar Physica MCR 301 rotational rheometer. In order to determine the influence of direction of the applied mechanical deformation, oscillatory and rotational tests were performed with constant deformation under non-isothermal (5–60 °C, heating rate of 1 deg/min) and isothermal (37 °C) conditions. Measurements at 37 °C were preceded by rapid heating (1 deg/s) from the storage temperature (5 °C).

The shear rate in rotational measurements has been determined from the calculation of deformation in oscillatory measurements (strain amplitude $\gamma = 1\%$, angular frequency $\omega = 5\ \mathrm{s}^{-1}$). The geometry of the measurement system (diameter and angle of cone) was also taken into account. During the conversion of the oscillatory shear deformation on the rotational, unidirectional shear deformation, the main determinant was cone displacement during measurements. The calculations were carried out in accordance with the following formula:

$$\dot{\gamma} = 2 \cdot A \cdot \frac{\omega}{\pi} \cdot \frac{1}{\tan(\theta) \cdot D} \tag{1}$$

Based on the proposed equation, a shear rate ($\dot{\gamma}$) value of 0.03 s^{-1} was determined and it was applied to all measurements. In Equation (1), the value of parameter A was determined as cone displacement at a known strain amplitude, and was equal to 0.008 [mm].

3. RESULTS AND DISCUSSIONS

The results of measurements carried out under non-isothermal conditions are shown in Fig. 1. It is visible that the unidirectional, simple shear leads to a significantly different shape of the dynamic viscosity η curve compared to the experimental curve of the real part of the complex viscosity η' obtained in oscillatory tests. Particularly noteworthy is the initial gelation stage, the so-called viscoelastic flow region. In the case of oscillatory measurements, the real part of the complex viscosity decreases with temperature increase. This course of the experimental curve is widely known and consistent with Stokes' law. However, for rotation measurements, an increase in the dynamic viscosity η was noted. Thus, the increase of viscosity during simple shear from the beginning of the process may indicate a process of building the internal structure of the medium. Further heating of the experimental medium led to a continuous slow increase in the dynamic viscosity and the real part of the complex viscosity. Slow polymerization occurred, limited by the diffusion of polymer molecules. At 35 °C, regardless of the type of deformation applied, a rapid increase in the value of both quantities, which is associated with the fastest changes occurring in the sample, was observed. Temperature increase and thus decrease of medium viscosity caused an increase of the diffusion rate of polysaccharide molecules.

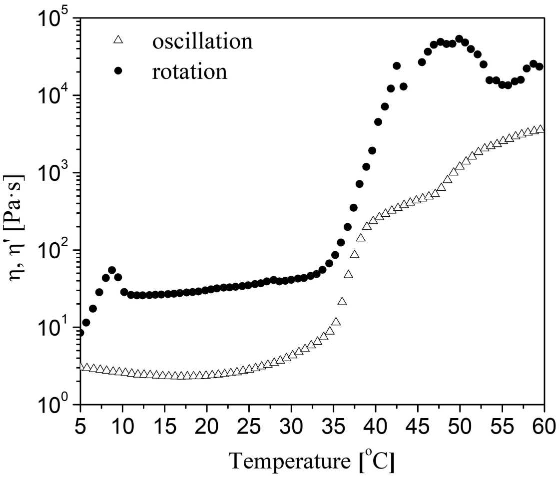

Fig. 1. The experimental curves of dynamic viscosity η and the real part of the complex viscosity η' obtained under non-isothermal conditions

Above 43 °C, the dynamic viscosity η curve reaches the second plateau. In the case of oscillatory measurements, this area is identified with a range of slow gelation, in which the existing polymer structure is being expanded (Cho et al., 2006). However, further heating, above 52 °C, caused partial destruction of the structure and dynamic viscosity η decreased. That result was not obtained during oscillatory measurements, the observed value of real part of the complex viscosity η' continuous increased. This means that oscillatory measurements being less invasive did not damage the internal structure. Simultaneously, the second plateau was not observed, which indicates that the final, highly cross-linked polymer structure was not formed during the test. The values of dynamic viscosity η were higher compared to the real part of complex viscosity η' in the whole range of temperatures. Simultaneously, the values obtained at the beginning of the measurements reached nearly the same values regardless of the applied deformation. This shows a significant influence of the applied deformation on the behaviour of chitosan chains in the shear field and on the formation of the spatial structure.

The changes of dynamic viscosity values in non-isothermal rotational measurements depending on the applied shear deformation are shown in Fig. 2. The analysis of the obtained experimental data indicates that the value of shear rate significantly influences the shape of experimental curves. In the initial heating stage (up to 10 °C), an increase in the shear rate caused a decrease in the dynamic viscosity value. This confirms the shear-thinning behaviour of colloidal chitosan solutions (Owczarz et al., 2017). The shapes of the experimental curves almost do not change to about 30 °C, the viscosity values are relatively independent of temperature change. In this range only the curve determined for the shear rate of 0.06 s^{-1} differs slightly from the others. It was also found that as the shear rate increased, the rapid change in the shape of the experimental curve and the increase in dynamic viscosity shifted towards higher temperatures. Above 55 °C, the occurrence of the second plateau is observed for all shear rates tested. However, with the increase of applied deformation, the values of dynamic viscosity decreased. It is worth noting that these changes are more significant than those observed at low temperatures. Hence, they cannot be equated only with shear-thinning behaviour. Changes of the final values of dynamic viscosity may indicate a lower degree of crosslinking of the polymer structure.

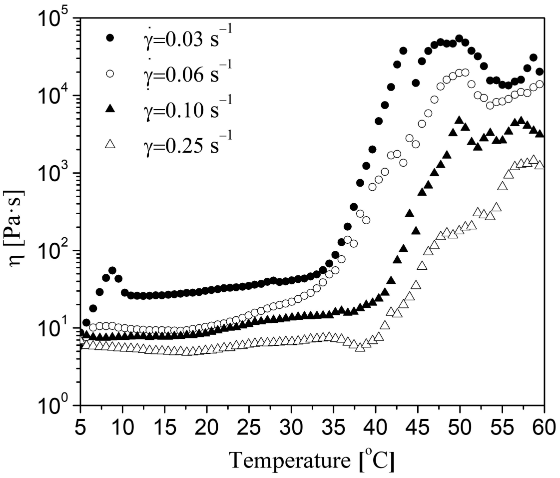

Fig. 2. Effect of shear rate on change of dynamic viscosity η in non-isothermal measurements

The results of isothermal measurements (at 37 °C) at constant deformation are shown in Fig. 3. It is clearly visible that the impact of the applied deformation on the conditions of the sol-gel phase transition is even greater compared to non-isothermal tests. The experimental curve of dynamic viscosity η is consistent with the experimental curve of the real part of complex viscosity η' obtained in oscillatory measurements. The initial decrease of viscosity is related to the rapid heating of the test medium to the set measurement temperature. After reaching the minimum value, a rapid increase in the dynamic viscosity by four orders of magnitudes is observed. The second plateau is reached after approximately 940 seconds. In the case of oscillatory measurements, more than three times longer storage at 37 °C did not lead to the occurrence of the second plateau area. This means that time of measurements was not long enough to form an infinite three-dimensional polymer structure. The biggest differences between oscillatory and rotational measurements were observed from 100 s to 1000 s. In this range, in the oscillatory measurements, small changes of the real part of complex viscosity η' are visible. Simultaneously in the same range, the most rapid changes of dynamic viscosity were observed. This means that the applied direction of deformation significantly affects the observed course and time necessary to form a three-dimensional polymer structure.

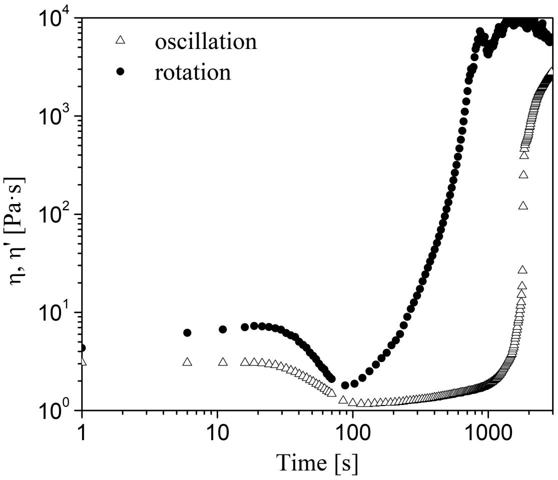

Fig. 3. Changes of dynamic viscosity η and the real part of the complex viscosity η' during sol-gel phase transitions in rotational and oscillatory measurements

Moreover, as in the case of non-isothermal measurements, it was also found that at each stage of structure forming, dynamic viscosity η was significantly higher than the real part of complex viscosity η' from oscillatory tests.

4. CONCLUSIONS

The obtained results indicate a significant influence of the direction of applied deformations on changes in rheological properties of low-concentrated, colloidal chitosan solutions. The experimental curves of rotational measurements are consistent with the characteristic regions of curves obtained in oscillatory measurements for thermosensitive chitosan hydrogels. The application of very low shear rate values led to the formation of a three-dimensional structure from the beginning of the heating process and also in significantly shorter time for isothermal measurements. Probably it resulted from increased mobility of molecules and changed their spatial configuration (disentangling of polymer chains along the shear field). The acceleration of the sol-gel phase transition during unidirectional shear can also result from the ordering of the entangled long polymer chains along the shear field. The investigations are consistent with the literature (Owczarz et al., 2018). Simultaneously it was found that simple shearing of the formed, unlimited polymeric structure caused its destruction. This phenomenon was not observed during oscillatory measurements. Further development of the problem undertaken in the article seems justified due to the potential application of colloidal solutions of chitosan as injectable scaffolds in tissue engineering and high shear forces occurring in the syringe during their application.

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SYMBOLS

A	displacement of the cone at known strain amplitude [mm]
D	cone diameter [mm]

Greek symbols

η	dynamic viscosity [Pa·s]
η'	real part of complex viscosity [Pa·s]
$\dot{\gamma}$	shear rate [1/s]
ω	angular frequency [rad/s]
θ	cone angle [°]

Abbreviation

DD	degree of deacetylation

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