

Changes in the viscoelasticity of maize starch pastes by adding sucrose at different stages

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Abstract

Effects of sucrose on the rheological properties of maize starch pastes were studied by steady and dynamic oscillatory viscoelasticity, DSC measurements, and microscopic observation. Sucrose concentrations varied between 0 and 55 wt%. When sucrose was added before heating of starch dispersions, the viscosity of the pastes increased with increasing sucrose concentration up to 20 wt% and decreased with increasing sucrose concentration above 20 wt%, although the viscosity of samples in the presence of sucrose was greater than that of the control at higher shear rate. A lower content of sucrose enhanced the swelling for starch granules, while a higher content of sucrose decreased the rate of swelling for starch granules and shifted the starch **gelatinization temperature** to higher temperatures. No decrease in the viscoelasticity of the pastes above 20 wt% sucrose concentration was observed by adding sucrose after

heating of starch dispersions. The viscoelasticity of maize starch paste to which sucrose was added after heating increased with increasing sucrose concentration.

Keywords: maize starch, sucrose, viscoelasticity, differential scanning calorimetry (DSC) measurement

1. Introduction

Starch products contain intricate mixtures of tastes. The tastes are mainly controlled by seasoning substances, some of which are known to have effects on the physical properties of starch pastes and gels. When we use starch as a thickener, a stabilizer, or a gelling ingredient, the properties of starch, such as dispersibility, solubility, stability, and the viscosity, are very important. It is necessary to investigate the effects of taste substances on the viscoelasticity of starch since it may lead to understanding how to control the viscosity of starch products.

Among the taste substances, sweet substances, e.g., sucrose, are the most widely added to starch products. Many researchers have reported concerning the effects of sweet substances on gelatinization and retrogradation of starch using various types of starch and many kinds of methods (Abu-Jdayil, Azzam, & Al-Malah, 2001; Ahmad, & Williams, 1999; Campbell, & Briant, 1957; Cheer, & Lelievre, 1983; Chungcharoen, & Lund, 1987; D'Appolonia, 1987; Eliasson, 1992; Evageliou, Richardson, & Morris, 2000; Evans, & Haisman, 1982; Katsuta, Miura, & Nishimura, 1992; Katsuta, Nishimura, & Miura 1992a,b; Kim, & Walker, 1992; Kohyama, & Nishinari, 1991; Lee, Kim, & Nishinari, 1998; Maauf, Che Man, Asbi, Junainah, & Kennedy, 2001; Paredes-

López, & Hernández-López, 1991; Perry, & Donald, 2002; Savage, & Osman, 1978; Spies, & Hosney, 1982). The viscoelasticity of starch pastes greatly depends on the origin of starch, the concentration of starch, and the kinds of sweet substances. Moreover, sweet substances have been added before heating of starch dispersions to investigate the effects of sweet substances on gelatinization and retrogradation of starch. By adding sucrose after heating of starch dispersions, it is expected that the effects of sucrose on the viscoelasticity of starch are distinct, in comparison with those by adding before heating.

In this study, maize starch and sucrose were selected. Maize starch is the most widely used starch in the food industry. Sucrose is widely used for an ingredient or a seasoning of sweetness in the food industry and in home cooking.

Our objective is to investigate how sucrose affects the physical properties of starch pastes and contributes to the effect of increasing the viscosity for starch pastes by adding sucrose at different stages.

2. Materials and methods

2.1. Materials

A maize starch sample (cornstarch Y) was kindly provided by Sanwa Starch Co. Ltd. (Nara, Japan). The concentration of maize starch was fixed at 3.0 wt%; this is the concentration generally used for thickeners in sauces or soups. The moisture (water) content of the sample was determined to be 13.4 % by drying it at 130°C in atmospheric

pressure. Sucrose was of commercial grade (granulated sugar, sucrose ≥ 99.92 %, water ≤ 0.04 %, glucose and fructose ≤ 0.015 %). The concentration of sucrose was varied between 0 and 55 wt%. Sorbic acid potassium salt (0.05 wt%) was added to prevent microbial growth.

2.2.Preparation of maize starch pastes

Powders of maize starch were dispersed in distilled water or in aqueous sucrose solutions prepared beforehand by adding various concentrations of sucrose to distilled water. A control sample was made of distilled water and maize starch. Maize starch dispersions were stirred at 200 rpm for 30 min at 25 °C, then the dispersions were heated up to 97 °C, stirring at the same rate and maintained at 97 °C for 60 min. Schematic diagram of the maize starch paste agitator is illustrated somewhere (Hirashima, Takahashi, & Nishinari, 2004). Then the hot dispersions were poured into glass vessels (140 mL volume) and cooled in a water bath (5 L volume) at 25 °C for 60 min equipped with a temperature regulator, stirring with magnetic stirrers. The samples to which sucrose was added after heating were also prepared. The final concentration of maize starch was 3.0 wt%.

2.3.Steady shear and dynamic oscillatory measurements

Steady shear and dynamic oscillatory measurements of starch pastes were carried out using a Fluids Spectrometer RFS II (Rheometrics Co. Ltd., New Jersey, U.S.A.) with a 50 mm diameter plate geometry. The gap was adjusted to 1.00 mm. All measurements were made at 25.0°C. From these measurements, shear viscosity ($\eta_{\dot{\gamma}}$),

storage modulus (G'), loss modulus (G''), and complex viscosity ($|\eta^*|_\omega = \sqrt{G'^2 + G''^2} / \omega$) were obtained. Strains for dynamic oscillatory measurements were chosen in the plateau range of each sample on the strain dependence test.

2.4. Microscopic observations

Microscopic observations were made using OPTIPHOTO (Nikon Co. Ltd., Tokyo, Japan) equipped with 2D-color CCD (1280 × 960 pixels). Pastes prepared by the method mentioned above were diluted with 2 times of distilled water. Granules of maize starch were dyed with a 10 mM iodine solution.

2.5. Differential Scanning Calorimetry (DSC) measurement

DSC measurements were conducted using a DSC6100S (Seiko Instruments Inc. Chiba, Japan) with 70 μ l silver (Ag) pans. Maize starch (3.0 wt%), sucrose (10, 20, 30, 40, and 50 wt%), and distilled water were mixed in the Ag pans. They were added up to total sample weights of 70 mg, then sealed. Each concentration of aqueous sucrose solution was used for a reference. Samples were heated from 25 °C to 130 °C at a heating rate of 1.0 °C / min. From DSC curves, onset temperature (T_o), peak temperature (T_p), **conclusion temperature of amylopectin gelatinization (T_c^1)**, **conclusion temperature of amylose–lipid complex disintegration (T_c^2)**, gelatinization temperature range ($\Delta T = T_c^2 - T_o$), and gelatinization enthalpy (ΔH) could be obtained.

3. Results and discussion

3.1. Microscopic observation

Fig. 1 shows microscopic photographs of starch granules for 3.0 wt% starch pastes with or without adding sucrose. The heated maize starch granules for pastes without adding sucrose (control) were swollen and partly ruptured (Fig. 1 top).

Microscopic photographs of the starch granules for samples with sucrose added before heating are lined along the left in Fig. 1. There were several larger granules in the samples with 10 or 20 wt% sucrose added as compared with those of the control, although all granules were not larger than that of the control because the original sizes of granules before heating were different (Fig. 1 top left). This result coincides with that of other researchers (Ahmad, & Williams, 1999; Cheer, & Lelievre, 1983). The size of starch granules for the sample with 30 wt% sucrose was smaller than those of 10 and 20 wt%. Then, as similar results have been reported by Savage and Osman (1978), there were no amylose and amylopectin chains (glucose chains) leaching out from starch granules of samples to which 40 or 50 wt% sucrose was added (Fig. 1 bottom left).

On the other hand, swollen granules and many glucose chains could be seen in the microscopic photographs for the samples with sucrose added after heating, compared with those added sucrose before heating (Fig. 1 right), especially for samples with 40 and 50 wt% sucrose.

To clarify the decrease in the degree of swelling for starch pastes with higher sucrose concentration, gelatinization temperatures and enthalpy for starch pastes were measured.

3.2.DSC measurements

Two peaks were observed in the DSC curve for a maize starch dispersion because maize starch contains lipid. A peak at lower temperature is induced mainly by amylopectin gelatinization and a peak at higher temperature is induced mainly by amylose–lipid complex disintegration (Biliaderis, 1990). We obtained these parameters from DSC curves: onset temperature (T_o), peak temperature (T_p), conclusion temperature of amylopectin gelatinization (T_c^1), conclusion temperature of amylose–lipid complex disintegration (T_c^2), gelatinization temperature range ($\Delta T = T_c^2 - T_o$), and gelatinization enthalpy (ΔH).

Fig. 2 shows the changes in T_o , T_p , T_c^1 , T_c^2 , ΔT , and ΔH . Transition temperatures, T_o , T_p , T_c^1 , and T_c^2 shifted to higher temperatures and ΔH increased with increasing sucrose concentration (C_s), while ΔT was not affected by sucrose. Namely peak heights became greater with increasing C_s , and the shape of DSC curves became sharper with increasing C_s . These are coincident with the results that have been reported previously (Ahmad, & Williams, 1999; Chiotelli, Rolée, & Le Meste, 2000; Eliasson, 1992; Evageliou, Richardson, & Morris, 2000; Kohyama, & Nishinari, 1991; Lee, Kim, & Nishinari, 1998; Maaurf, Che Man, Asbi, Junainah, & Kennedy, 2001; Paredes-López, & Hernández-López, 1991).

These results indicate that starch pastes cannot be gelatinized completely in the presence of higher C_s with the preparation methods used in this study. Therefore, the degree of swelling for starch granules with higher C_s was decreased. The cause of these phenomena is thought that sucrose binds starch chains in amorphous areas and the chains exhibit limited flexibility (Spies, & Hosney, 1982) and sucrose acts as an antiplasticizer, since its molecular weight is greater than that of the primary plasticizer of starch, water (Chungcharoen, & Lund, 1987; Schenz, 1995).

In this study, starch pastes were prepared by heating at 97 °C for 60 min. T_c^2 were higher than 97 °C for samples with $C_s \geq 20$ wt%, T_c^1 were higher than 97 °C for samples with $C_s \geq 40$ wt%, and T_p were higher than 97 °C for samples with $C_s > 45$ wt%. The decrease in the degree of swelling for samples with higher C_s is caused by the lack of enough water for starch to be gelatinized because affinity of sucrose for water is greater than that of starch for water (Chiotelli, Rolée, & Le Meste, 2000).

The viscoelasticity of starch pastes should be affected by the size of starch granules, the number of glucose chains leaching out from starch granules, and the degree of gelatinization.

3.3. Steady shear measurements for starch pastes with sucrose added before heating

Fig. 3 shows the shear viscosity ($\eta_{\dot{\gamma}}$) of 3.0 wt% maize starch pastes as a function of sucrose concentration (C_s). Sucrose was added before heating. $\eta_{\dot{\gamma}}$ of the control is illustrated in mesh. All samples showed typical shear-thinning behaviors. $\eta_{\dot{\gamma}}$ was affected by both $\dot{\gamma}$ and C_s . It was observed that $\eta_{\dot{\gamma}}$ with $10 \text{ wt}\% \leq C_s \leq 20 \text{ wt}\%$ increased with increasing C_s and shear-thinning behavior became remarkable in comparison with the control. On the contrary, $\eta_{\dot{\gamma}}$ with $20 \text{ wt}\% < C_s < 50 \text{ wt}\%$ decreased with increasing C_s and shear-thinning behavior became less pronounced.

C_s dependence of the shear viscosity observed at the same condition as in Fig. 3 is demonstrated in Fig. 4. $\eta_{\dot{\gamma}}$, where $\dot{\gamma}$ are 1, 10, and 100 s^{-1} , were chosen and represented by η_1 , η_{10} , and η_{100} , respectively. η_1 , which is $\eta_{\dot{\gamma}}$ at a lower $\dot{\gamma}$, increased

with increasing C_s up to 20 wt% and decreased inversely with increasing C_s above 20 wt%. The values of η_1 were greater than that of the control at $1 \text{ wt\%} \leq C_s < 40 \text{ wt\%}$ and almost the same at $C_s \geq 40 \text{ wt\%}$. η_{100} , which is $\eta_{\dot{\gamma}}$ at a higher $\dot{\gamma}$, had a similar tendency to η_1 , however, η_{100} had a maximum value for C_s between 20 and 35 wt% and the values of η_{100} were greater than that of the control at all C_s range examined. η_{10} had a similar tendency to η_1 .

$\eta_{\dot{\gamma}}$ decreased with higher C_s , because starch gelatinization did not completely occur by heating at 97 °C, and there were no entanglements of glucose chains and starch granules were smaller than those of the control and the samples with lower C_s . $\eta_{\dot{\gamma}}$, where $C_s \leq 20 \text{ wt\%}$, increased, nevertheless, the **gelatinization temperature** shifted to higher temperatures and the enthalpy increased. **The cause of this increase is thought to be one or both of the following structural changes: (1) the degree of swelling for starch granules, i.e., size of starch granules, and (2) the number of leached out glucose chains increases.**

From these results, it is thought that C_s ranges were divided into three: I) $0 \text{ wt\%} \leq C_s \leq 20 \text{ wt\%}$: a stage of increasing the degree of swelling and $\eta_{\dot{\gamma}}$ increased with increasing C_s . II) $20 \text{ wt\%} < C_s < 40 \text{ wt\%}$: a stage of decreasing the degree of swelling and $\eta_{\dot{\gamma}}$ decreased with increasing C_s , and III) $C_s \geq 40 \text{ wt\%}$: a stage of preventing the degree of swelling and $\eta_{\dot{\gamma}}$ did not change with increasing C_s .

3.4. Dynamic oscillatory measurements for starch pastes with sucrose added before heating

Fig. 5 illustrates the frequency (ω) dependence of storage modulus (G') and loss modulus (G'') of maize starch pastes with or without sucrose before heating. The paste of the control showed a behavior known for a concentrated flexible polymer solution, i.e., G' is less than G'' at lower ω and G' is greater than G'' at higher ω . G' , where C_s was below 35 wt%, did not depend on ω at lower ω and the long-time relaxation was observed. This plateau, similar in shape to the rubber plateau, appears only in the disperse system of spherical particles, where no entanglement couplings occur (Matsumoto, Hitomi, & Onogi, 1975). In the case of starch pastes, it is thought that the entanglements of glucose chains, which leached out from starch granules, increased and aggregations of starch granules occur. This behavior was obvious for the pastes with $1 \leq C_s \leq 25$ wt%. G' is greater than G'' at lower ω and G' is less than G'' at higher ω . The values of G' were less than those of G'' at all ω examined for pastes with C_s above 40 wt%. This behavior resembles that for liquid. The values of G'' were not different for the pastes with C_s above 40 wt%.

Therefore, this suggests that C_s range I is a stage of increasing the degree of swelling and the viscoelasticity increased. C_s range II and III are stages of decreasing the degree of swelling and the viscoelasticity decreased.

Complex viscosity ($|\eta^*|_\omega$) of 3.0 wt% maize starch pastes as a function of C_s is shown in Fig. 6. $|\eta^*|_\omega$ of the control is illustrated in mesh. Sucrose was added before heating. Comparison of $|\eta^*|_\omega$ in Fig. 6 with $\eta_{\dot{\gamma}}$ in Fig. 2 shows that $|\eta^*|_\omega$ and $\eta_{\dot{\gamma}}$ of all samples do not obey the Cox-Merz rule: $\eta_{\dot{\gamma}} = |\eta^*|_\omega$ because starch pastes contain swollen granules and glucose chains. The values of $|\eta^*|_\omega$ did not coincide with those of $\eta_{\dot{\gamma}}$, while $|\eta^*|_\omega$ had a similar tendency to $\eta_{\dot{\gamma}}$.

These results indicated that adding much sucrose led to a decrease in the viscosity for starch pastes because the swelling of starch granules was inhibited, although the viscosity was greater than that of the control at higher $\dot{\gamma}$. This increase of $\eta_{\dot{\gamma}}$ may be attributed not only to the viscosity of starch pastes but also to that of sucrose solution as shown in Fig. 7. Then, the relative viscosity increment ($\eta_i \dot{\gamma}$) was calculated to examine the contribution for the viscosity of maize starch.

$$\eta_i \dot{\gamma} = (\eta_{\dot{\gamma}} - \eta_0) / \eta_0$$

where η_0 is the viscosity of the solvent. In this case, the values of η_s were used as η_0 .

Fig. 8 indicates C_s dependence of $\eta_i \dot{\gamma}$, where $\dot{\gamma}$ are 1, 10, and 100 s⁻¹. $\eta_i \dot{\gamma}$ at all $\dot{\gamma}$, increased with increasing C_s up to 20 wt% (C_s range I) and decreased with C_s above 20 wt% (C_s range II and III). $\eta_i \dot{\gamma}$ with more than 28 wt% sucrose was less than that of the control. The value of $\eta_{\dot{\gamma}}$ was almost independent of $\dot{\gamma}$, where C_s was above 40 wt% (Fig. 3 and 4), while the value of η_s increased with increasing C_s (Fig. 7). Therefore, the effect of increasing viscosity for maize starch itself did not display so much at C_s range III.

When sucrose is added to the maize starch pastes after heating, the inhibition of swelling for starch granules was prevented (Fig. 1 right). Therefore, higher viscosity for starch pastes must be obtained.

3.5. The effects of adding sucrose after heating

Fig. 9 shows C_s dependence of $\eta_{\dot{\gamma}}$, where $\dot{\gamma}$ are 1, 10, and 100 s⁻¹, and they are represented by η_1 , η_{10} , and η_{100} , respectively. Sucrose was added after heating. No decrease in η_1 , η_{10} , and η_{100} was observed even at higher C_s . $\eta_{\dot{\gamma}}$ increased with increasing C_s and the values of $\eta_{\dot{\gamma}}$ for all samples were greater than that of the control.

The pastes with C_s above 40 wt% did not show the behaviors like liquid (Fig. 10). The plateau appeared at lower ω for the pastes with C_s below 50 wt%. The paste, with C_s 50 wt%, showed a solid like behavior, i.e., G' were greater than of G'' at all ω examined.

$|\eta^*|_\omega$ showed the same tendencies with $\eta_{\dot{\gamma}}$ (Fig. 11). Here, although $|\eta^*|_\omega$ was not coincidental with $\eta_{\dot{\gamma}}$, the values of $|\eta^*|_\omega$ for all samples were greater than that of the control.

The values of $\eta_{i\dot{\gamma}}$, where $\dot{\gamma}$ are 1, 10, and 100 s⁻¹, were also greater than those of the control, when sucrose was added after heating (Fig. 12). Adding sucrose after heating was effective for increasing the viscosity of maize starch pastes, compared with adding sucrose before heating. However, $\eta_{i\dot{\gamma}}$ at all $\dot{\gamma}$, increased with increasing C_s up to 20 wt% and decreased with C_s above 20 wt%. In this case, glucose chains of all samples leached out from starch granules (Fig. 1 right) and the gelatinization occurred. Therefore, it seems that maize starch itself has the effect of increasing viscosity. However, the values of $\eta_{i\dot{\gamma}}$ decreased at higher C_s range. It is thought that greater viscosity of sucrose solutions at higher C_s dominates the viscosity of maize starch pastes with sucrose further than that of maize starch pastes does.

4. Conclusions

When sucrose was added before heating of starch dispersions, smaller amounts of sucrose enhanced the swelling for starch granules, while larger amounts of sucrose decreased the degree of swelling for starch granules. Then, the starch **gelatinization temperature was** shifted to higher temperatures with increasing sucrose concentration and gelatinization was not completed in the presence of excessive sucrose.

From these results, the viscoelasticity of maize starch pastes increased with increasing sucrose concentration up to 20 wt% and decreased with increasing sucrose concentration above 20 wt%, although the viscosity of samples in the presence of sucrose was higher than that of the control. The higher viscosity of sucrose solution contributed to the higher values of the viscoelasticity of starch pastes with higher sucrose concentration. Therefore, the effect of increasing the viscoelasticity of maize starch was inhibited by adding much sucrose before heating.

No decrease in the viscoelasticity was noted for maize starch pastes by adding sucrose after heating of starch dispersions and the viscoelasticity of pastes increased with increasing sucrose concentration. The starch granules could be swollen even at a higher content of sucrose. The effect of increasing the viscoelasticity of maize starch was greater than that of the control, when sucrose was added after heating.

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Figure captions

Fig. 1. Microscopic photographs of starch granules for 3.0 wt% maize starch pastes. Pastes were diluted with distilled water and dyed with 10 mM iodine solution. Sucrose was added before and after heating. Image size is $390\mu\text{m} \times 725\mu\text{m}$. Observations were made at ambient temperature.

Fig. 2. Effects of sucrose on the gelatinization temperature of 3.0 wt% corn starch. T_o : onset temperature; T_p : peak temperature; T_c^1 : conclusion temperature for amylopectin gelatinization; T_c^2 : conclusion temperature for amylose and lipid complex disintegration; ΔT : gelatinization temperature range $T_c^2 - T_o$; ΔH : enthalpy for mg starch. Heating rate was $1.0^\circ\text{C}/\text{min}$.

Fig. 3. Shear viscosity ($\eta_{\dot{\gamma}}$) of 3.0 wt% maize starch pastes as a function of sucrose concentration (C_s). Sucrose was added before heating. $\eta_{\dot{\gamma}}$ of the control is illustrated in mesh. Measurements were made at 25.0°C

Fig. 4. Sucrose concentration (C_s) dependence of shear viscosity ($\eta_{\dot{\gamma}}$) of 3.0 wt% maize starch pastes at the shear rates of 1, 10, and 100 s^{-1} , respectively. Sucrose was added before heating. Measurements were made at 25.0°C .

Fig. 5. Frequency (ω) dependence of G' and G'' for 3.0 wt% maize starch paste. Sucrose was added before heating. Sucrose concentrations were 0 wt% (control), 5, 15, 20, 22.5, 25, 30, 35, 40, 50, and 55 wt%. Measurements were made at 25.0°C. The strain for the samples with 0 (control), 5, 15, 20, 22.5, 25, 30, 35, 40, 50, and 55 wt% sucrose concentrations, was 30, 20, 5, 3, 8, 10, 10, 15, 30, 20, and 30%, respectively.

Fig. 6. Complex viscosity ($|\eta^*|_\omega$) of 3.0 wt% maize starch pastes as function of sucrose concentration (C_s). Sucrose was added before heating. $|\eta^*|_\omega$ of the control is illustrated in mesh. The strain for the samples with 0 (control), 1, 5, 10, 15, 20, 25, 30, 35, 40, 50, and 55 wt% sucrose concentrations, was 30, 15, 20, 15, 5, 3, 10, 10, 15, 30, 20, and 20%, respectively. Measurements were made at 25.0°C.

Fig. 7. Sucrose concentration (C_s) dependence of shear viscosity for sucrose solution (η_s). Measurements were made at 25.0 °C.

Fig. 8. Relative viscosity increment for 3.0 wt% maize starch pastes with sucrose ($\eta_i \dot{\gamma}$) to the viscosity for sucrose solutions as a function of sucrose concentration (C_s). Shear rates ($\dot{\gamma}$) are 1, 10, and 100 s⁻¹. Sucrose was added before heating.

Fig. 9. Sucrose concentration (C_s) dependence of shear viscosity ($\eta \dot{\gamma}$) of 3.0 wt% maize starch pastes at the shear rates of 1, 10, and 100 s⁻¹, respectively. Sucrose was added after heating. Measurements were made at 25.0°C.

Fig. 10. Frequency (ω) dependence of G' and G'' for 3.0 wt% maize starch paste. Sucrose was added after heating. Sucrose concentrations were 0 wt% (control), 5, 20, 30, 35, and 50 wt%. Measurements were made at 25.0°C. The strain for the samples with 0 (control), 5, 20, 30, and 50 wt% sucrose concentrations, was 30, 10, 3, 3, and 3%, respectively.

Fig. 11. Complex viscosity ($|\eta^*|_\omega$) of 3.0 wt% maize starch pastes as function of sucrose concentration (C_s). Sucrose was added after heating. $\eta^*|_\omega$ of the control is illustrated in mesh. The strain for the samples with 0 (control), 5, 10, 19, 20, 25, 30, 35, 40, and 50 wt% sucrose concentrations, was 30, 8, 4, 3, 3, 3, 3, 3, 3, and 3%, respectively. Measurements were made at 25.0°C.

Fig. 12. Relative viscosity increment for 3.0 wt% maize starch pastes with sucrose ($\eta_i \dot{\gamma}$) to the viscosity for sucrose solutions as a function of sucrose concentration (C_s). Shear rates ($\dot{\gamma}$) are 1, 10, and 100 s⁻¹. Sucrose was added after heating.

Figures

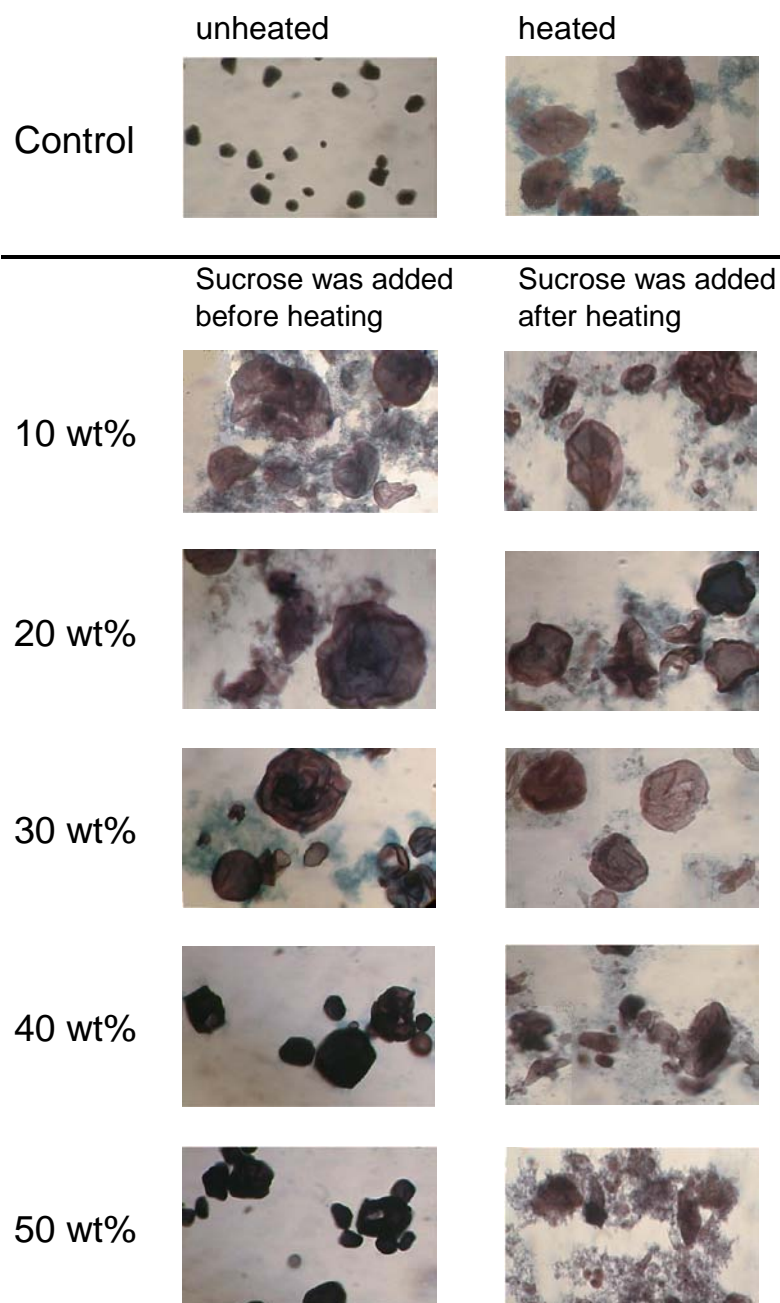


Fig. 1.

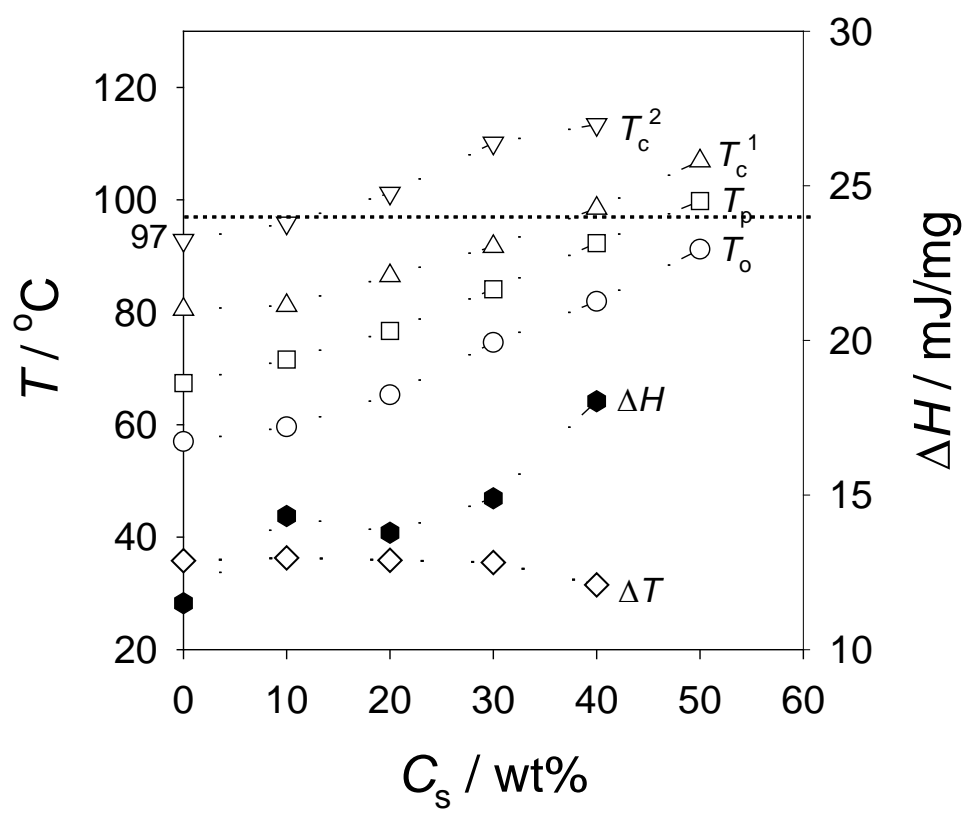


Fig. 2.

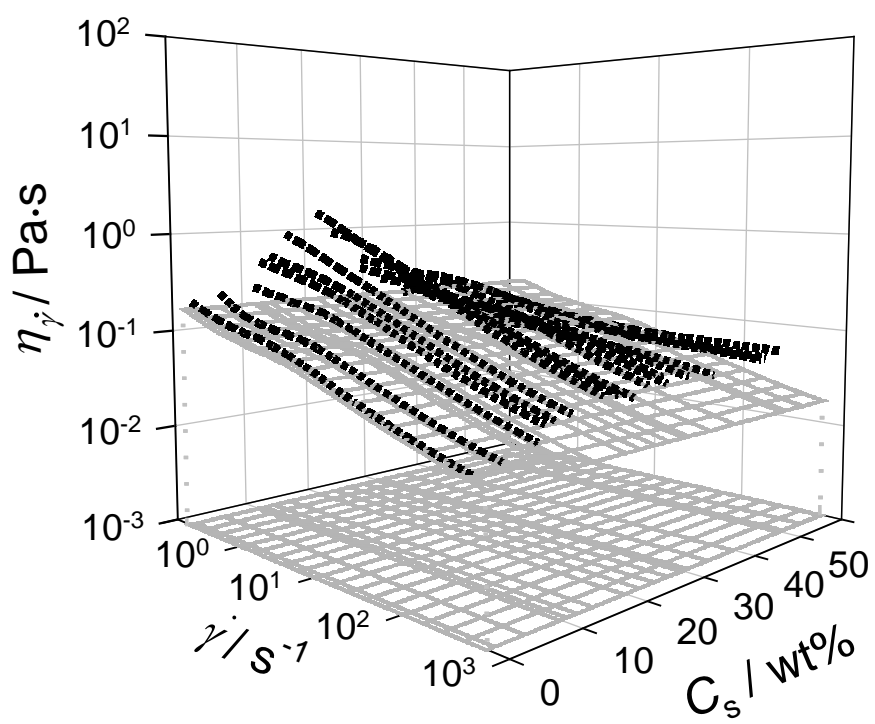


Fig. 3.

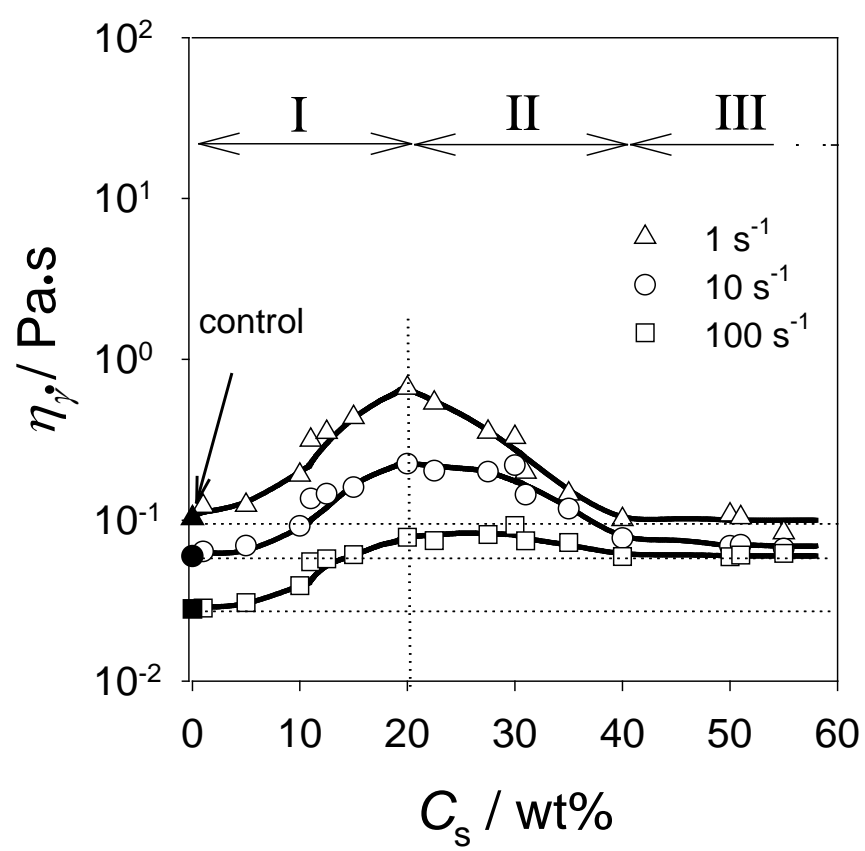


Fig. 4.

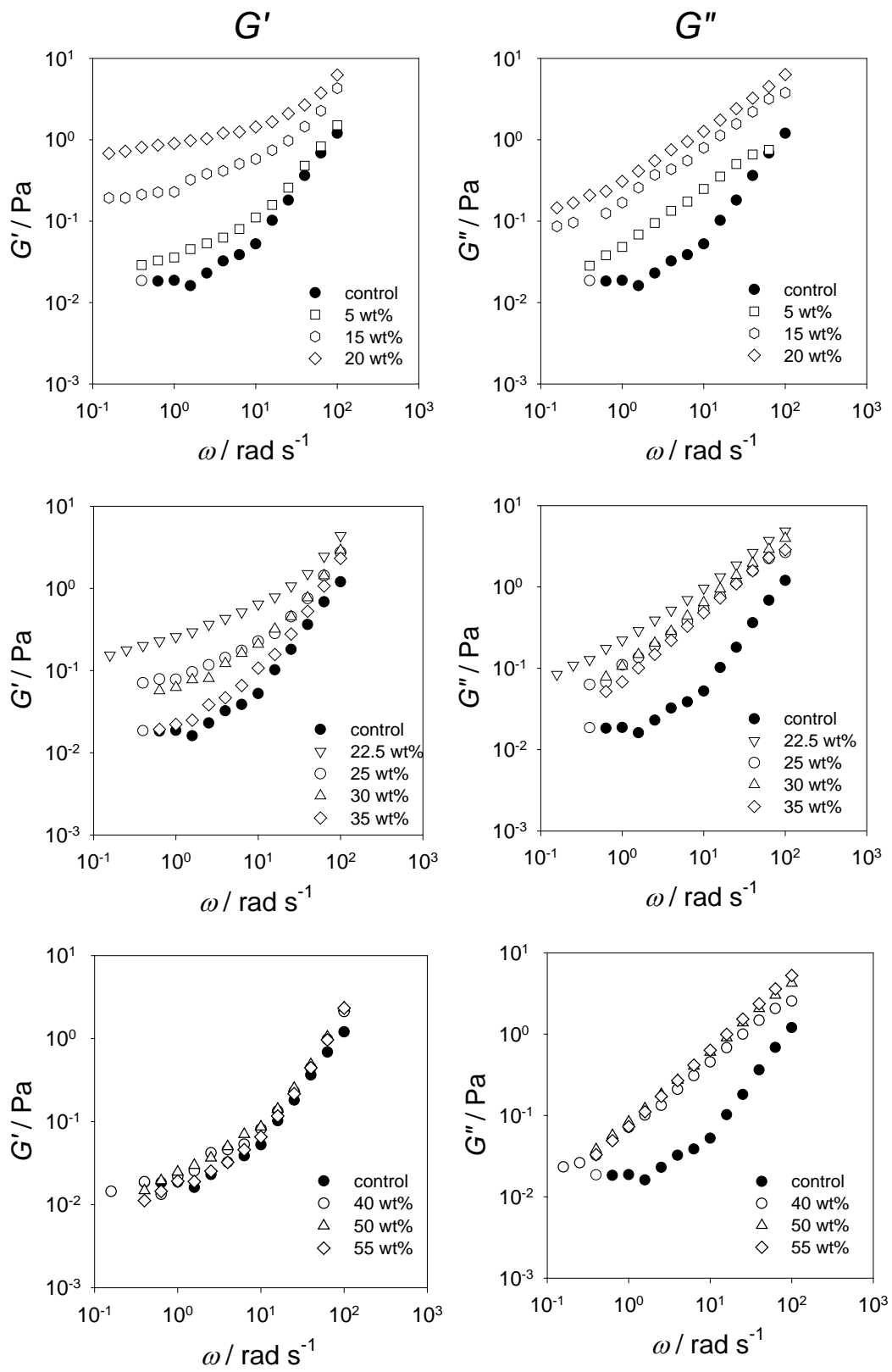


Fig. 5.

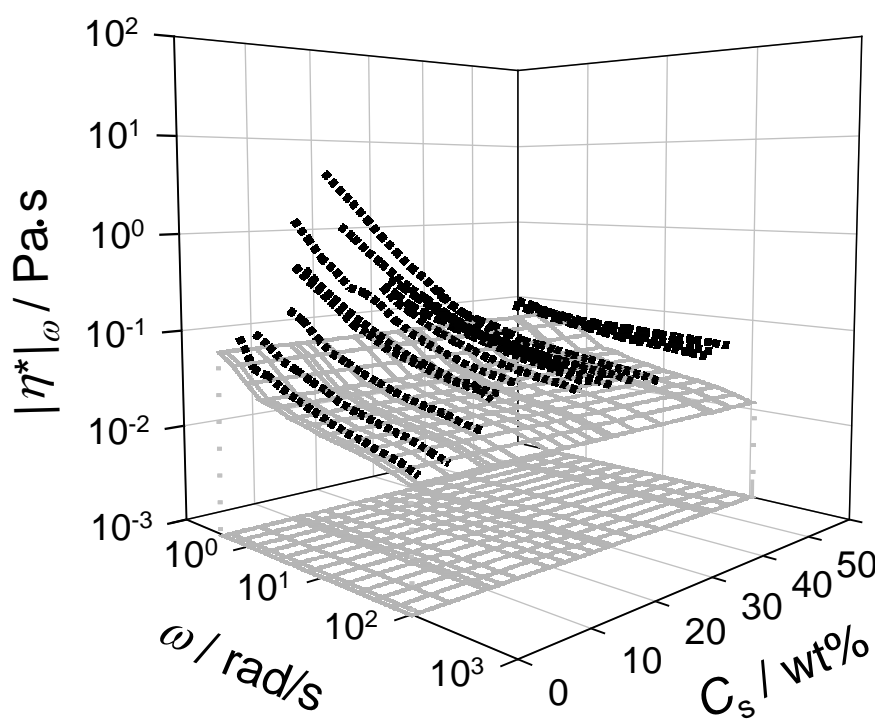


Fig. 6.

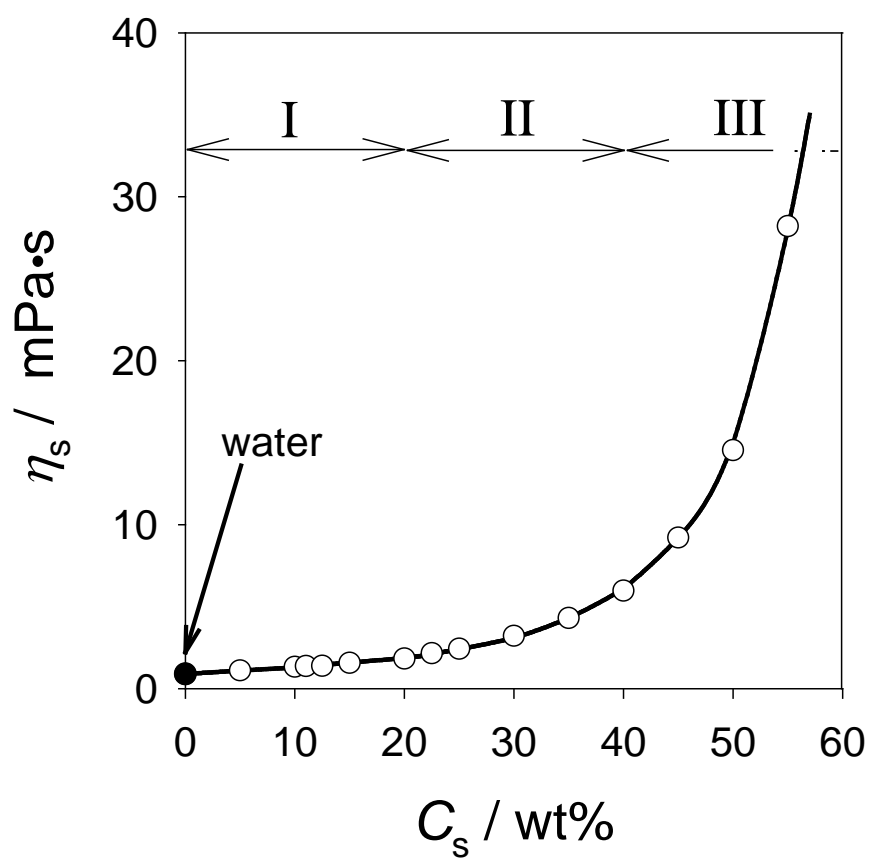


Fig. 7.

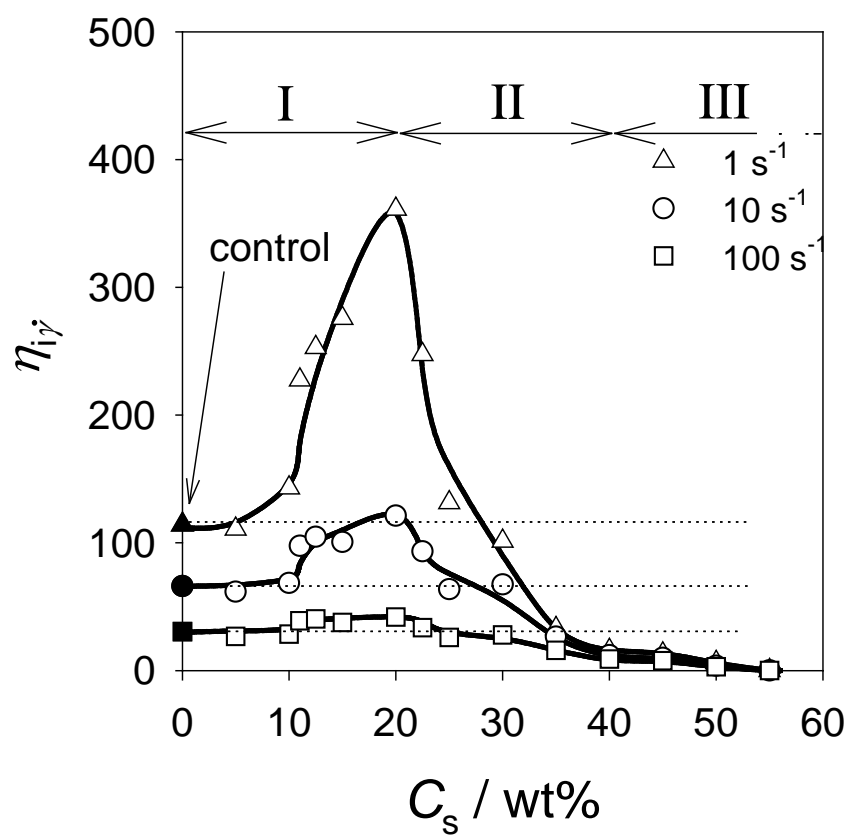


Fig. 8.

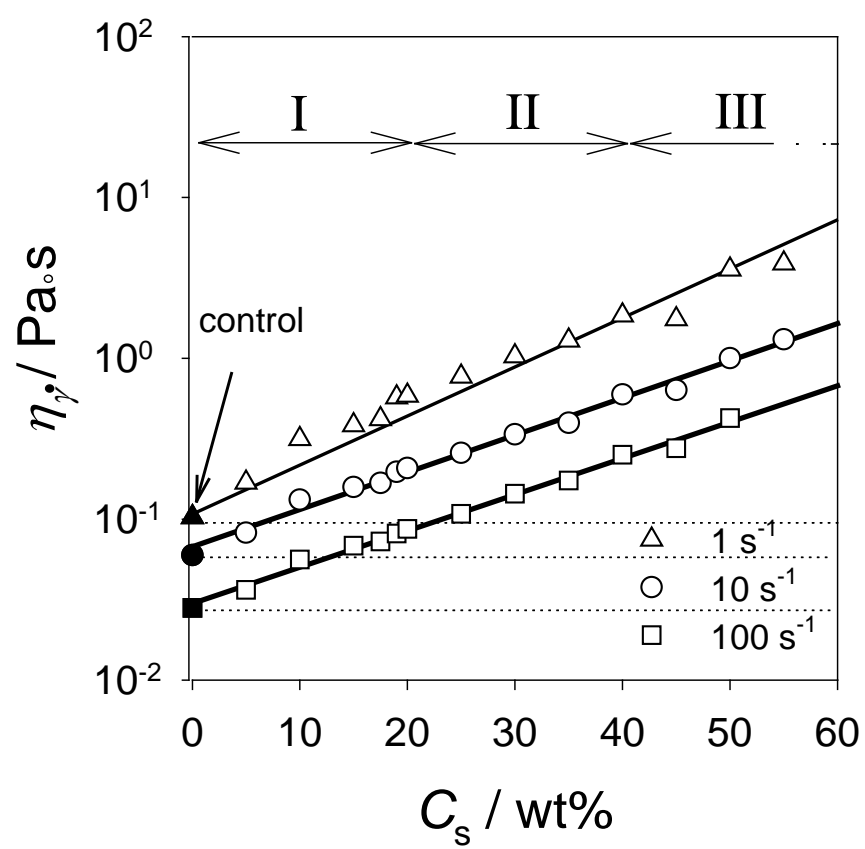


Fig. 9.

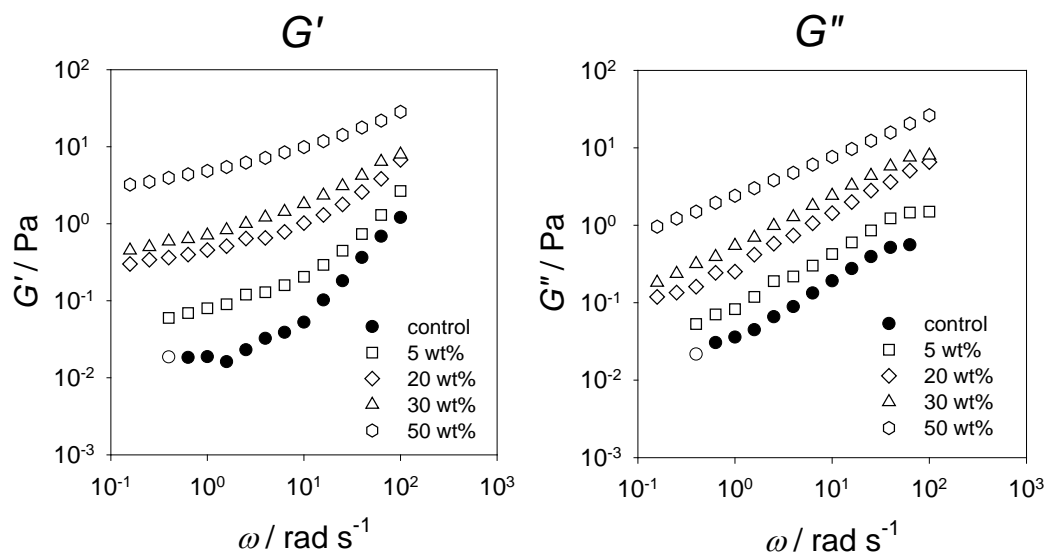


Fig.10.

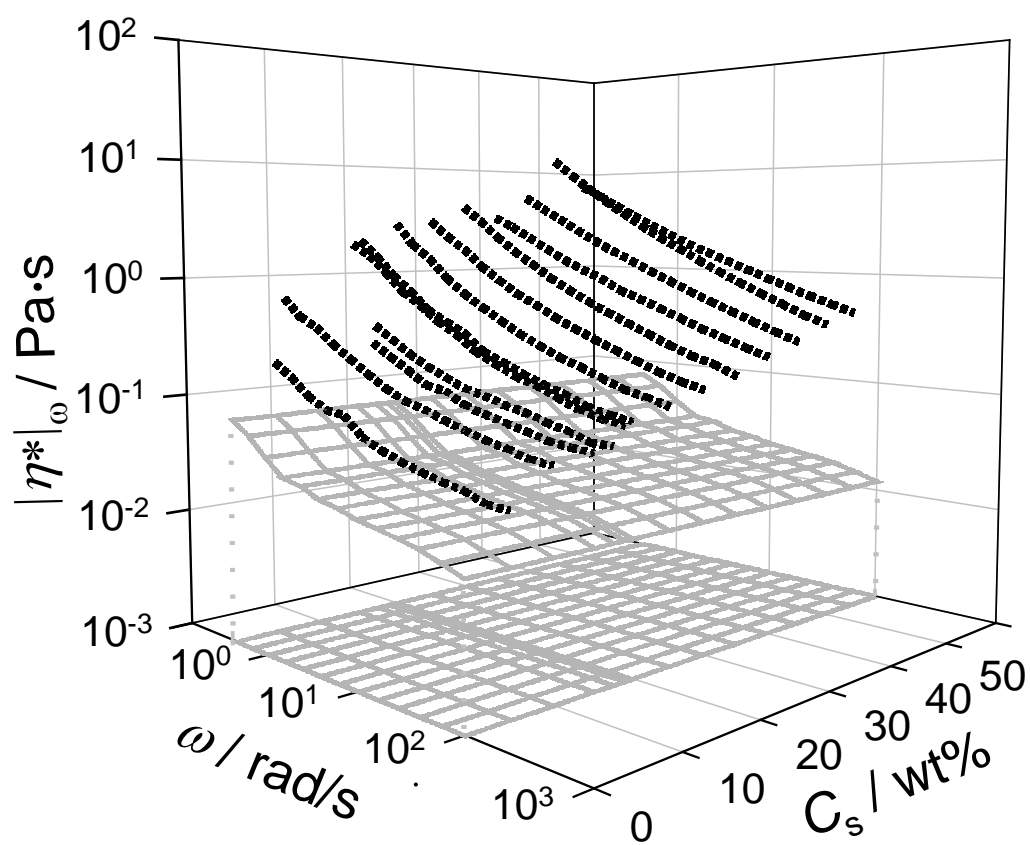


Fig. 11.

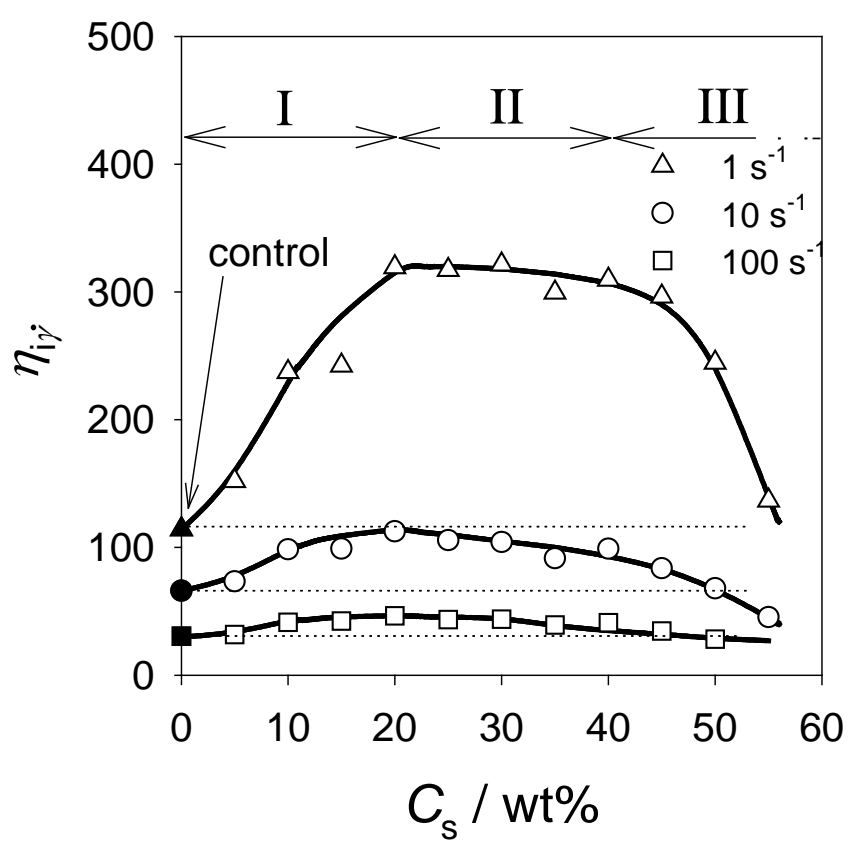


Fig. 12.