Carbohydrate Polymers 113 (2014) 189–193

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

The effect of thermal history on the elasticity of K-type gellan gels

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ARTICLE INFO

Article history:
Received 17 December 2013
Received in revised form 8 June 2014
Accepted 23 June 2014
Available online 10 July 2014

Keywords:
Gellan
Gelation
Thermal history
Storage Young's modulus

ABSTRACT

Elasticity of potassium type gellan gels prepared at different thermal histories was examined using dynamic viscoelastic measurements. The storage Young's modulus E' decreased with increasing cooling rate during gelation. Once gel formation occurred, thermal history at lower temperature did not influence the elastic modulus and thermal stability of the gellan gels. On the other hand, thermal history around gelation temperature influenced strongly the elastic modulus and thermal stability of resulting gels. When the gellan solution was kept for a certain time before cooling at a temperature near the gelation temperature, it was found that gels with higher elastic modulus and thermal stability were formed.

1. Introduction

Gellan gum is an extracellular polysaccharide produced by micro-organism *Sphingomonas elodea* (ATCC 31461) previously referred to as *Pseudomonas elodea*. The primary structure of gellan gum is composed of a linear tetrasaccharide repeat unit: →3)-β-D-Glcp-(1→4)-β-D-GlcpA-(1→4)-β-D-Glcp-(1→4)-α-L-Rhap-(1→ as reported by O'Neill and Jansson (Jansson, Lindberg, & Sandford, 1983; O'Neill, Selvendran, & Morris, 1983). The polymer is produced with two acyl substituents present on the 3-linked glucose, namely L-glyceryl, positioned at O(2) and acetyl at O(6). One of its main current areas of application is in beverages, where the acylated form ("high acyl" or "native" gellan) is commonly used. However, all early applications centred on gelation of the deacylated polymer ("low acyl" gellan, often known simply as "gellan" or "gellan gum"). Deacylated gellan gives firm, transparent gels which are brittle (i.e. fracture at low strain) and consequently give excellent "flavour release" (Morris, Nishinari, & Rinaudo, 2012; Sworn, 2000). An important area of current application is in dessert gels, as discussed by Kubo, Fujita, Nanbu, and Matsumura (2012), who examined the effects of calcium-ion concentration and cooling rate on the gelation of deacylated potassium gellan gum.

The mechanism whereby gellan gum forms gels in water was not fully understood. To understand gelation and gel properties of gellan gum further, the collaborative research group was organized in the research group of polymer gels affiliated to the Society of Polymer Science, Japan, in 1989 (Morris et al., 2012). The common deacylated gellan was used to study its properties with various techniques. The results of the collaborative studies were published in special issues of *Food Hydrocolloids* 7, 361–456 in 1993, *Carbohydrate Polymers* 20, 75–207 in 1996, and *Progress in Colloid and Polymer Science*, 114, 1–131 in 1999. Light scattering (Takahashi, Akutu, Kuboka, & Nakamura, 1999) and osmotic pressure measurements (Ogawa, 1999) showed that gellan changes from two single chains to a double helix on cooling and changes from a double helix to two single chains on heating, i.e. helix–coil transition occurs thermoreversibly. Rheological measurements (Miyoshi & Nishinari, 1999) showed that a gel formation followed the coil-to-helix transition on further cooling under appropriate conditions.

Thermal history is one of the factors to influence the gelation of gellan. The elastic modulus of gellan gels formed at a constant temperature reached a plateau value after a certain time and the plateau values decreased with decreasing temperature, for which the reason was not described (Nakamura, Harada, & Tanaka, 1993). The elastic modulus of gelatin gels, helix-forming thermoreversible gels like gellan gels, increased with time at lower temperatures not reaching an equilibrium value even after 100 h (Te Nijenhuis, 1997). The thermal stability of gelatin gels increased with increasing storage time at the temperature which is a little higher than gelation temperature (Michon, Cuvelier, Relkin, & Launay, 1997).

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http://dx.doi.org/10.1016/j.carbpol.2014.06.084
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For agarose gels, which are other helix-forming thermoreversible gels, the effect of the storage temperature near the gelation temperature on rheological and structural properties has been studied and it was found to differ from the behaviour of gelatin gels. Aymard et al. (2001) found that holding solutions of agarose for long times at high temperature decreased the strength of the gels formed on cooling. They interpreted this by structural observation that during the holding period the un-gelled solutions resolved progressively into regions of high and low polymer concentration, and that the resulting heterogeneity gave weaker networks when the solutions were gelled by cooling. On the other hand, Mohammed, Hember, Richardson, and Morris (1998) reported that agarose gels showed larger elastic modulus and were more thermally stabilized by cooling more slowly.

The effect of cooling rate, storage temperature and storage time on gel properties of gellan, gelatin and agarose remain ambiguous nevertheless it is expected that thermal history gives universal effects among these polymers since their gels are the same from viewpoint of cold-setting thermoreversible gels being composed of helices. It was already shown for gelatin that thermal history influenced the gel properties (Michon et al., 1997; Te Nihenhuis, 1997). It is widely accepted that polysaccharides such as gellan and agarose show a steeper structure ordering on cooling and less steep structure disordering on heating (Djabourov, Nishinari, & Ross-Murphy, 2013; Morris et al., 2012). On the other hand, it is reported that the triple helix of collagen shows a steep unfolding transition upon heating, whereas less steep and more gradual refolding is observed upon cooling (Mizuno, Boudko, Engel, & Bächinger, 2010). Therefore, we should be cautious in drawing direct comparisons between polysaccharide systems where nucleation is rate-limiting and gelatins where propagation is rate-limiting. In the present study, the effects of thermal history on the gelation behaviour of gellan were studied using longitudinal and shear oscillation measurements.

2. Experimental

2.1. Sample preparation

Gellan gum was supplied by San-Ei-Gen FFI Ltd., Osaka, Japan. It is the first common sample in the collaborative research published in *Food Hydrocolloids*, 7, 361–456 (1993). The metal contents in the sample are as follows: Na 1900 μg/g, K 20,800 μg/g, Ca 5120 μg/g and Mg 1460 μg/g. Since the major component of the metal is potassium, the sample is called K-type gellan. Another K-type gellan sample being used as the fourth common sample in the collaborative research in Japan was also used in the present study to check the effect of the metal content. The metal contents in the 4th sample are as follows: Na 3312 μg/g, K 21,755 μg/g, Ca 1230 μg/g and Mg 124.9 μg/g. The sample was not influenced by storage as for its molecular weight (Ogawa, 1996). The molecular weight was determined by converting it into the tetramethyl ammonium form and using light scattering (Okamoto, Kubota, & Kuwahara, 1993) and osmometry (Ogawa, 1993) as $M_w = 2.1 \times 10^5$ and $M_n = 0.5 \times 10^5$.

Gellan gum powder was swollen in distilled water and stirred at 25 ± 2 °C overnight. The 1.6% (w/w) gellan dispersion prepared in this way was heated at 80 °C for 2 h and at 90 °C for 10 min and then cooled at various cooling rates to obtain gels.

2.2. Sample preparation for longitudinal vibration and uniaxial compression

A stainless steel mould was used to obtain a gel cooled at a constant cooling rate. Fig. 1 shows the programmed temperature–time course of the circulator on cooling and the experimental temperature–time course of samples in the stainless steel mould which was detected by a thermo-couple. The sample could be cooled at a rate of 1 °C/min or 0.5 °C/min almost precisely. When a hot solution was poured into the stainless steel mould which was kept in circulating water at 25 °C, detected temperature of the sample was 70 °C at first and then decreased to 25 °C within 3 min. In this case, the cooling rate was regarded as ~15 °C/min. A cylindrical gel (10 mm diameter, 15 mm length) was formed. Gels were kept at 5 °C for 20 h until the measurement. As for the gels kept at 40 °C for 20 h, the teflon moulds (20 mm diameter, 30 mm length) were used for the preparation. Since the Young's modulus was shown to be not so much dependent on the shape of cylinders in the range used in the present experiment (Nishinari, Horiuchi, Ogino and Koide, 1974), the recalculation of the Young's modulus is not necessary.

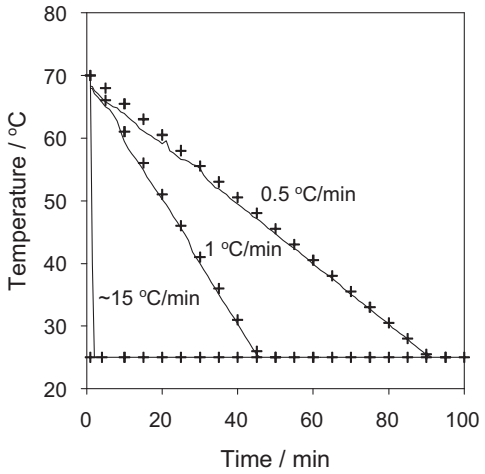

Fig. 1. Temperature of a circulator (+) and a sample (solid line) in cooling process from 70 to 25 °C at various cooling rates.

2.3. Longitudinal oscillation measurements

The storage and loss Young's moduli, E' and E'', were determined by the observation of longitudinal vibrations of cylindrically moulded gels. The apparatus used was a Rheolograph Gel (Toyo Seiki Seisakusyo Ltd., Japan) (Nishinari et al., 1980). Both the lower and upper ends of the cylindrical gel were fixed by an instantaneous adhesive alphacyano acrylate (Aron alpha, Toa Gosei, Ltd., Tokyo, Japan) to the Rheolograph Gel. Then, the temperature dependence of the elastic moduli observed by longitudinal vibration is free from slippage which sometimes causes an artefact in shear rheology.

Silicone oil was filled around the sample to control the temperature and to avoid the evaporation of water. Gels were kept at each measurement temperature for 15 min. Storage and loss Young's moduli, E' and E'', were measured at 5 °C interval. The frequency and the amplitude were 3 Hz and 100 μm, respectively.

2.4. Shear oscillation measurements

The storage and loss shear moduli, G' and G'', as a function of time were measured by a Rheostress (Haake, Germany). The 1.6% (w/w) gellan sample was poured into a cylinder geometry (diameter of the cylinder geometry = 38.02 mm; gap = 2.69 mm; length = 55 mm) at 70 °C and then immediately covered with silicone oil to prevent the evaporation of water. The serrated geometry was used to prevent slippage as mentioned in the study on the gelation of konjac glucomannan (Zhang et al., 2001). The temperature was controlled by the Haake circulator DC30-K10 (Haake, Germany). The applied

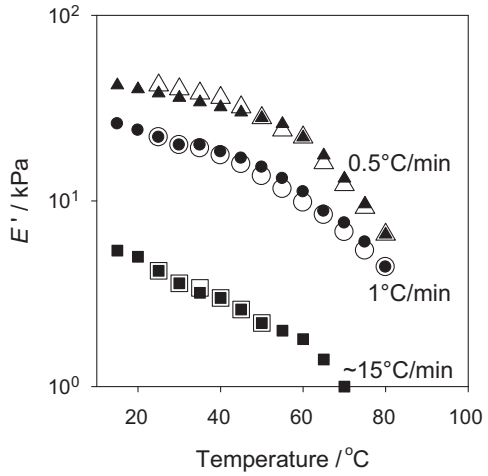

Fig. 2. Temperature dependence on heating of E' of 1.6 wt% K-gellan gels formed at various cooling rates and then kept for 20 h at 25 °C (open) or at 5 °C (closed). Frequency; 3 Hz.

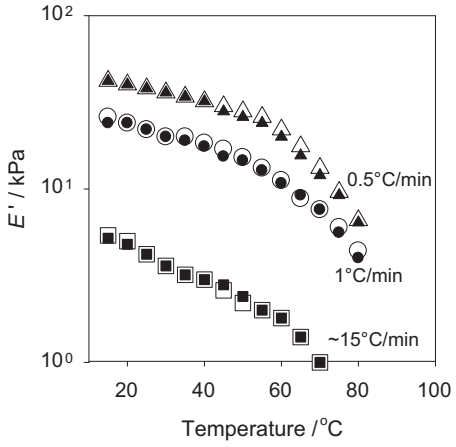

Fig. 3. Temperature dependence on heating of E' for 1.6 wt% gels formed at various cooling rates and then kept at 5 °C for 1day (open) or for 5 days (closed). Frequency; 3 Hz.

frequency and strain were 3 Hz and 0.5–1% which is within a linear viscoelastic regime, respectively.

2.5. Uniaxial compression

Cylindrical gels were compressed at 0.5 mm/s to examine the effects of cooling rate on the large deformation behaviour using a TA-plus Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK) at 22 ± 2 °C. In the compression tests, a flat (100 mm in diameter) plunger was used.

3. Results and discussion

After cooling, E' of 1.6 wt% K-gellan gels showed no further change on holding at 25 °C (over periods of up to 7 h), indicating rapid completion of network formation. E' decreased remarkably with increasing cooling rate. E' of gellan gels prepared at 0.5 °C/min and 1 °C/min was about 34 kPa and about 24 kPa, respectively. When the gellan sol was cooled rapidly (~15 °C/min) E' of the resulted gel was about 4 kPa at 25 °C (Fig. 2).

Fig. 2 shows temperature dependence on heating of E' of 1.6 wt% K-gellan gels prepared at various cooling rates and then stored at different temperatures, 25 or 5 °C. Storage Young's modulus E' of gels prepared at the slowest cooling rate 0.5 °C/min showed the

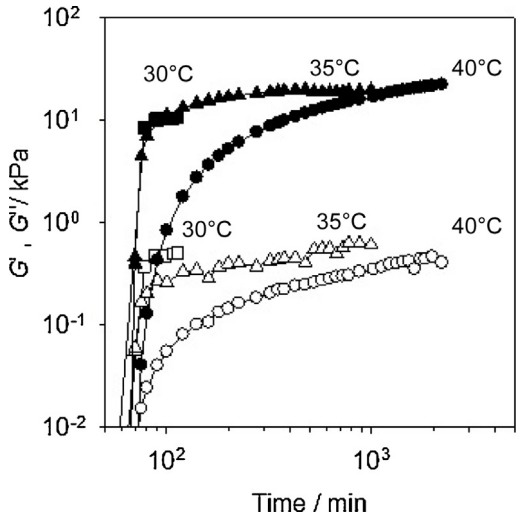

Fig. 4. Time dependence of G' (closed) and the loss shear modulus G'' (open) of 1.6 wt% K-gellan which was cooled from 70 to 30, 35, 40 °C at 0.5 °C/min and kept at each temperature.

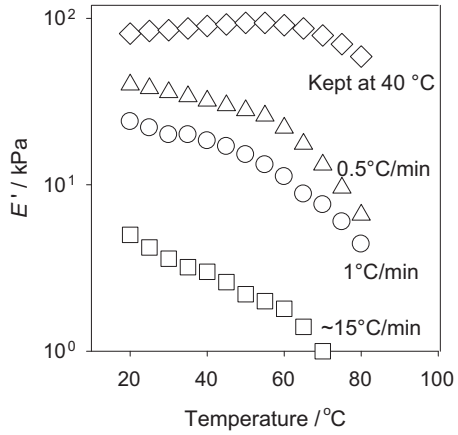

Fig. 5. Temperature dependence on heating of E' of 1.6 wt% K-gellan gels formed at various thermal histories. Diamond symbol represents a gel kept at 40 °C for 20 h and then kept at 5 °C for 20 h. Triangle, circle and square symbols stand for gels formed at various cooling rates shown beside each symbol and then kept at 5 °C for 20 h. Frequency; 3 Hz.

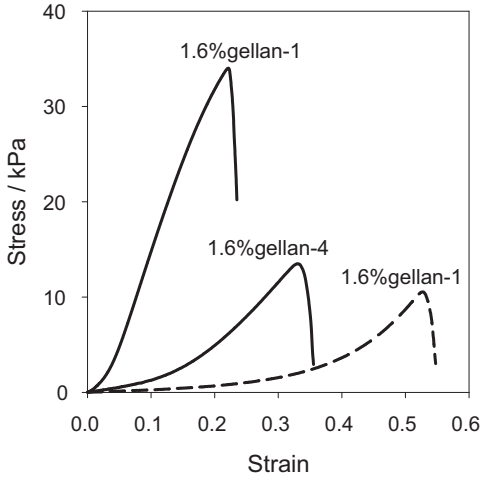

Fig. 6. Stress–strain curves for 1.6 wt% gellan gels (1st and 4th common samples, 1.6% gellan-1 and 1.6% gellan-4, respectively) prepared by slow (kept at 40 °C for 20 h and then kept at room temperature) (solid curve) and rapid (~15 C/min) cooling (broken curve). Compression speed, 0.5 mm/s. Measurement temperature 22 ± 2 °C.

largest values at any temperature indicating the largest number of elastically active network chains and the highest thermal stability. It is worthwhile to note that the melting process becomes progressively sharper (i.e. more co-operative) as the rate of cooling in the gel formation is decreased. When the temperature of gellan solution is lowered slowly, more chains could be incorporated into network chains which might not be incorporated by kinetic trapping at faster cooling. The E' of gels stored at 25 or 5 °C showed similar values and thermal stability, indicating that the elasticity of the gel did not change with the lapse of time below 25 °C. This is in contrast with gelatin gels of which elastic modulus increases by "maturation". As shown in Fig. 3, longer storage at 5 °C also did not change the elasticity and thermal stability of the gels.

Fig. 4 shows time dependence of G' and G'' at various temperatures for gelation of 1.6 wt% K-gellan. Gelation proceeded at different rate where slower gel formation led to higher elastic modulus if the storage time was sufficiently long. When the sample was cooled to 30 °C at 0.5 °C/min, gelation occurred around 35 °C and G' at 30 °C became almost constant in 5 min. The plateau value obtained at 30 °C was 10.5 kPa. When the same sample was cooled to 35 °C at 0.5 °C/min, gelation occurred at 35 °C and proceeded with time. The plateau value, about 20 kPa, was obtained after about 400 min. When the sample was kept at 40 °C by cooling at 0.5 °C/min both moduli were too small to be detected for ~10 min first. G' increased more slowly with time and became larger than 10.5 kPa after 325 min and continued to increase even after 2000 min. G' was 23 kPa at 2000 min.

As is well known, Young's modulus E is three times the shear modulus G for incompressible materials. In the present experiments, E' was more than three times G'. Gels used for the measurements of E' were kept at lower temperatures for a longer time to obtain good self-supporting gels therefore E' was greater than three times G'.

Fig. 5 shows temperature dependence on heating of E' of 1.6 wt% K-gellan gels formed by being kept at 40 °C for 20 h and then kept at 5 °C for 20 h. E' of gels kept at 40 °C showed highest values and thermal stability. The temperature dependence of E' was found similar to previous findings in which the gel was formed by cooling slowly; a hot gellan solution was poured into teflon mould and was kept at room temperature until the gellan sample reached room temperature (Watase & Nishinari, 1993). Gels prepared at slow cooling rates showed an entropic elasticity up to quite a high temperature ca. 60 °C. Such a temperature dependence of elastic modulus was explained on the basis of a reel chain model (Djabourov et al., 2013). In this model, molecular chains in the junction zone consisting of aggregated double helices are released out with increasing temperature and when they are released beyond a certain limit, they cease to contribute to the elastic modulus (Nishinari, Koide, & Ogino, 1985).

It is believed that gel formation of K-gellan accompanies helix formation and aggregation of helices. When the temperature of a gellan solution was lowered, the storage modulus began to increase at higher temperatures at a slower cooling rate than at a faster cooling rate (Supporting data 1). Although the helix formation and its aggregation did not take a long time, the process is not instantaneous and takes a finite time. At a faster cooling rate, molecular chains which might have formed helices and aggregates could not complete this process, and these chains might be incorporated into helices and aggregates at a slower cooling thus making helices and aggregates larger. The longer helix formation and/or larger aggregates should form more thermally stable network. These findings are in good agreement with previous reports on thermoreversible gels. Agarose gels also showed higher thermal stability when the gels were formed at lower cooling rates (Mohammed et al., 1998). Moritaka, Takahashi, and Kubota (2007) also reported that storage modulus of gels of K-type gellan, agar, and kappa-carrageenan increased when the cooling rate of the solution was decreased.

In addition to temperature effects, kinetic effects should be taken into consideration on the properties of K-gellan gels. Lower E' of gels formed at higher cooling rate suggests that kinetic trapping by network formation might occur in helix formation and/or aggregation between helices. The network with a certain elastic modulus will prevent further ordered structure formation by restricting movement of gellan chains. Rapid helix formation and their aggregation induce rapid network formation with a certain elastic modulus, which will prevent further ordered structure formation. Slow gelation induces a gradual network formation during which rearrangement of molecular chains can occur, and will allow gellan to form further helices and aggregates in process of the network formation. Consequently the elastic modulus of gels formed at slower gelation rate became higher since the number and size of helices and aggregates increased.

Fig. 6 shows stress–strain curves for 1.6 wt% gellan gels (1st and 4th common samples) prepared by slow and rapid cooling.

Since the content of divalent cations in the 4th common sample is much lower than in the 1st common sample, gels prepared by rapid cooling of the 4th sample solution were not self-standing, and the uniaxial compression could not be performed. A 1.6 wt% gellan gel prepared by rapid cooling showed a smaller elastic modulus which is proportional to the initial slope of the stress–strain curve and a fracture stress and a larger fracture strain than that prepared by slow cooling. Both the elastic modulus and the fracture stress

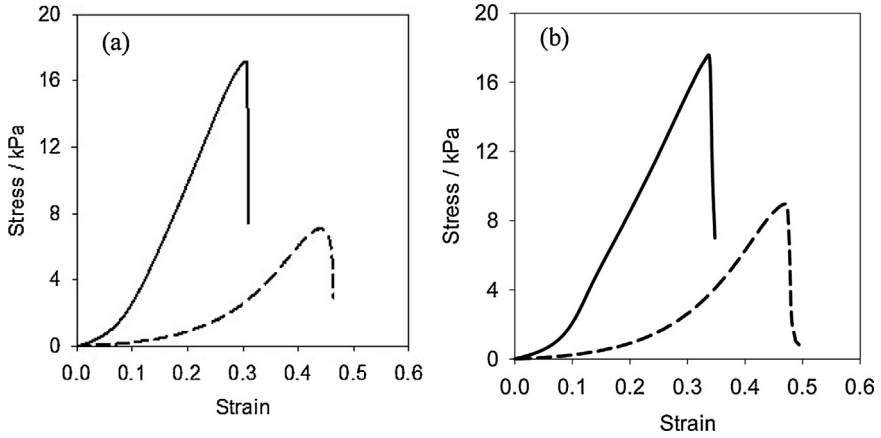

Fig. 7. Stress–strain curves (0.5 mm/s; 22 ± 2 °C) for 0.4 wt% gels incorporating 2 mM calcium chloride, prepared using (a) the 4th or (b) the 1 st gellan sample, by slow cooling (kept at 40 °C for 20 h, then at room temperature; solid curves) or rapid cooling (~15 °C/min; dashed curves.

were much smaller for the 4th-gellan gel than for the 1st gellan gel. These results are in good agreement with experimental results observed by small deformation measurements.

Fig. 7a shows the stress–strain curves for 0.4 wt% gellan gels of 4th common sample containing 2 mM calcium ions. These curves show again the general tendency that the gel prepared by slow cooling showed a larger fracture stress and a smaller fracture strain in comparison with the gel prepared by rapid cooling.

Stress–strain curves for 0.4 wt% gellan gels prepared from the 1st gellan sample showed similar behaviour (Fig. 7b). As shown in Fig. 6, the fracture stress is much larger for the 1.6 wt% gel of the 1st gellan sample than that of the 4th gellan sample because of higher content of divalent cations in the 1st gellan sample, while in the presence of 2 mM calcium ions the fracture stress of 0.4 wt% gels of the 1st and the 4th gellan samples show almost the same value (Fig. 7).

4. Conclusion

Effect of thermal history on the properties of K-gellan gels was investigated using dynamic viscoelastic measurements. K-gellan gel formation was controlled by kinetics of gelation as observed in gelation of agarose and gelatin. Higher values of the elastic modulus of gels induced by lower cooling rates were thought to be due to longer helix formation and less kinetic trapping of helix formation and/or aggregation between helices by network formation. The free chains or dangling chains could be incorporated into active network chains when the system is subjected to a slow cooling.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.06.084.

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