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Rheological study of reinforcement of agarose hydrogels by cellulose nanowhiskers

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ABSTRACT

The influence of the addition of tunicate cellulose nanowhiskers on the structural and rheological properties of an agarose hydrogel matrix has been studied, with the objective to design innovative green material, with good mechanical properties. The cellulose nanowhiskers were characterized using transmission electron microscopy, and their charge surface density was determined by a titration method. Oscillatory shear and stress relaxation tests were performed in order to characterize the rheological properties of the agarose matrix, and of the agarose hydrogels filled by nanowhiskers at volume fractions below 0.2%. The results show a significant reinforcement effect due to the addition of nanowhiskers, and suggest changes in the matrix network structure induced by the cellulose nanoparticles.

1. Introduction

Hydrogels are tridimensional, natural or synthetic, polymeric networks able to retain large quantities of aqueous solutions. The junctions are either permanent (chemical hydrogel) or transient (physical hydrogel). Their applications are numerous, as for instance in the biomedical field, where they are sometimes used because of biocompatibility and interesting rheological properties (Peppas, Bures, Leobandung, & Ichikawa, 2000). They are also widely used in food industry when composed of natural polymers. In this study, we have chosen to work on a composite system, agarose being the hydrogel matrix, and cellulose nanowhiskers acting as insoluble fillers.

Agarose is a neutral linear polysaccharide extracted from red algae (*Rhodophyceae*), and is the major component of agar, the other component being agaropectin. It is composed of alternating β-D-galactopyranose and anhydro-α-L-galactopyranose. Agarose is used, for example, in separation techniques and characterization of biomolecules such as electrophoresis or affinity chromatography (Cuatrecasas, 1970; Sparks & Phillips, 1992).

The gelation of agarose is dependent on two parameters: temperature and concentration. The agarose chain conformation changes with temperature, from a random coil conformation, at high temperatures, to a helical conformation, with decreasing temperature, the transition occurring between 40 °C and 60 °C (Tako & Nakamura, 1988). This thermally induced conformational change is essential for the formation of an agarose gel, if the concentration is high enough. At very low agarose concentrations, the polymer chains in helical conformation aggregate and form clusters (Sugiyama, Rochas, Turquois, Taravel, & Chanzy, 1994); when the polymer concentration increases, a sol–gel transition appears through the clusters connection, *via* fibrillar junctions of variable composition (Pines & Prins, 1973), forming a network spanning the whole sample, that is a gel. The agarose network mesh has a characteristic size which varies as a function of concentration: from 200 nm at 1 wt% to 80 nm at 3 wt% (Rochas, Hech, & Geissler, 1999); it is about 450 nm at 0.2 wt% (Bica, Borsali, Geissler, & Rochas, 2001). However it should be noted that these mesh size values are only averages: indeed, there is a mesh size distribution within the hydrogel (Rochas et al., 1999).

In this study, agarose hydrogels have been studied in the presence of cellulose nanowhiskers. These rod-like nanocrystalline cellulose particles, have been extensively studied because of very attractive properties (biodegradability, renewability, non-toxicity, high elastic modulus, light weight, etc) (De Souza Lima, Wong, Paillet, Borsali, & Pecora, 2003; Eichhorn, Dufresne, Aranguren, Capadona, & Rowan, 2010; Habibi, Lucia, & Rojas, 2010). Cellulose nanowhiskers can be extracted from various plant and animal sources. An acid hydrolysis of cellulose fibers is usually performed in order to degrade the amorphous regions of the cellulose

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microfibrils, and to get the nanowhiskers. The hydrolysis conditions strongly influence the cellulose nanowhiskers properties, mainly the surface charge and nanowhiskers size, whose dimensions also vary according to the cellulose source, from hundred nanometers to several microns (Araki, Wada, Kuga, & Okano, 1998; Dong, Revol, & Gray, 1998). For example, the aspect ratio, *i.e.* the length/diameter ratio, is of the order of 15 for cotton nanowhiskers and 70 for tunicate ones. Besides, nanowhiskers exhibit very high potential reinforcement capability due to their high surface area (of the order of several hundred m^2/g), and to their very high rigidity: their Young's modulus lies between 100 GPa and 160 GPa (Sturcova, Davies, & Eichhorn, 2005). Cellulose nanowhiskers have been mainly studied as reinforcement agents in synthetic thermoplastic matrices (poly (vinyl chloride), polypropylene, etc) (De Souza Lima & Borsali, 2004; Samir, Alloin, & Dufresne, 2005). More recently, they have also been investigated in biopolymer matrices (poly (lactic acid), poly (hydroxyalkanoate)) (Abdul Khalil, Bhat, & Ireana Yusra, 2012) and more particularly in polysaccharide matrices (Abdollani, Alboofetileh, Rasaei, & Behrooz, 2013; Khan, Khan, Salmieri, Tien, & Riedl, 2012). The study of nanocomposites composed of hydrogel matrices filled with cellulose nanowhiskers is much more recent (Dash, Foston, & Ragauskas, 2013; Gómez Martínez, Stading, & Hermansson, 2013; Spagnol, Rodrigues, Neto, Pereira, & Fajardo, 2012a; Yang, Han, Duan, Ma, & Zhan, 2013).

The objective of this study is to contribute to a better understanding of the effect of the addition of cellulose nanowhiskers on the structural and rheological properties of agarose hydrogels, focusing on the strengthening effect brought by cellulosic nanofillers. From a more applied point of view, the present work aims at designing new green hydrogels, with good mechanical properties.

2. Materials and methods

2.1. Materials

2.1.1. Agarose

The agarose sample has been provided by EUROGENTEC (Belgium). The main characteristics of the agarose used in this study have been given by the supplier. The intrinsic viscosity is 280 cm^3/g, corresponding to an average molecular weight of about 101,000 Da. The sulfate content is inferior to 0.1% and the melting temperature lies between 88 °C and 90 °C.

2.1.2. Preparation of agarose solutions

To prepare the aqueous solutions of agarose studied in this work, the desired amount of agarose powder was dispersed in deionized water under mechanical stirring at a temperature close to 90 °C. The solutions, from 0.1 wt% to 0.35 wt%, form gels on cooling at room temperatures in Petri dishes, and were characterized the day after preparation.

2.1.3. Elaboration of cellulose nanowhiskers

The cellulose source used in this work is the tunic of marine animals (*Phallusia mammillata*), provided by the Station Biologique de Roscoff (France). The proteins were extracted from washed pieces of the tunics by three successive bleaching treatments, alternating the washing with potassium hydroxide 5% at ambient temperature during 3 h and the washing with chlorite at 70 °C during 4 h. The tunicate nanowhiskers were prepared by acid hydrolysis of the cellulosic residue dispersed in water at a concentration of about 10%, using 96 wt% sulfuric acid, following a two-step procedure: in a first step, sulfuric acid was added drop by drop under continuous vigorous stirring of the mixture, and the temperature of the mixture was maintained at 32 °C, then, in a second step, the reaction mixture was kept at 70 °C during 45 min.

2.1.4. Preparation of nanowhiskers suspensions

The cellulose nanowhiskers were dispersed in deionized water, and the suspension was dialyzed until the pH of the suspension reaches pH = 7, then it was sonicated during 10 min in order to disperse the cellulose nanoparticles. The suspension was then treated with a mixed-bed ion-exchange resin (*Mixed bed resin TDM-8* from Sigma Aldrich), and 0.02 wt% sodium azide, which acts as a bacteriostatic agent, was added to the suspension. The resulting 0.2 vol% (or 0.31 wt%) nanowhiskers suspension was stored at 4 °C.

2.1.5. Preparation of agarose gels filled with cellulose nanowhiskers

The nanowhiskers suspension, previously sonicated during 10 min in an ice bath, was agitated under mechanical stirring at about 800 rpm, and heated. When the temperature was close to 90 °C, the desired amount of agarose was added to the suspension. In the present study, the concentration of agarose mass fixed at 0.2 wt%, whereas the cellulose nanowhiskers volume fraction, Φ, varied from 0.013% up to 0.2%.

2.2. Methods

2.2.1. Transmission electron microscopy (TEM)

Transmission electron microscopy was used in order to determine the geometrical characteristics of cellulose nanowhiskers. A 0.2 vol% nanowhiskers aqueous suspension was placed on a carbon coated TEM copper grid. Samples, negatively stained with uranyl acetate (1%), were let to air dry before observation, using a JEOL JEM-1230 microscope (Nikon, Tokyo, Japan), equipped with a LaB6 gun filament (lanthanum hexaboride), operating at a voltage acceleration of 80 kV. The images were analyzed using SigmaScan Pro 5.0.0 sofware.

2.2.2. Atomic force microscopy (AFM)

AFM observation was performed in order to investigate the structure of the agarose gels filled with the cellulose nanowhiskers. The samples were deposited on a freshly cleaved mica plane, then dried under an Argon flux. The images were acquired in the air using a microscope AutoProbe CP Park Scientific Instrument (USA). The tips were made of silicon doped with phosphorus (Veeco Probes, USA). The resulting images were processed with the WSxM 4.0 Sofware (Nanotec Electronica).

2.2.3. Titration

10^{-4} mol/l sodium hydroxide was added to a 0.17 vol% nanowhiskers aqueous suspension in order to titrate the charged sulfate groups resulting from the reaction of the sulfuric acid with the hydroxyl groups of cellulose. The number of sulfate groups at the surface of nanowhiskers per glucose unit was inferred from the overall number of sulfate groups per glucose unit (derived from the titration measurements), divided by the ratio of surface chains to total chains in a nanowhisker, which can be calculated from the average dimensions of a nanowhisker and from the crystallographic characteristics of the cellulose crystal (Goussé, Chanzy, Excoffier, Soubeyrand, & Fleury, 2002).

2.2.4. Rheometry

All rheological measurements were carried out using a controlled stress rheometer Gemini (Bohlin Instruments). Oscillatory shear and stress relaxation tests were performed at 20 °C, in parallel-plate geometry (diameter: 25 mm, gap: 1.5 mm), in order to characterize the rheological properties of the agarose matrix and of the filled hydrogels. In both cases, preformed gel samples, with a

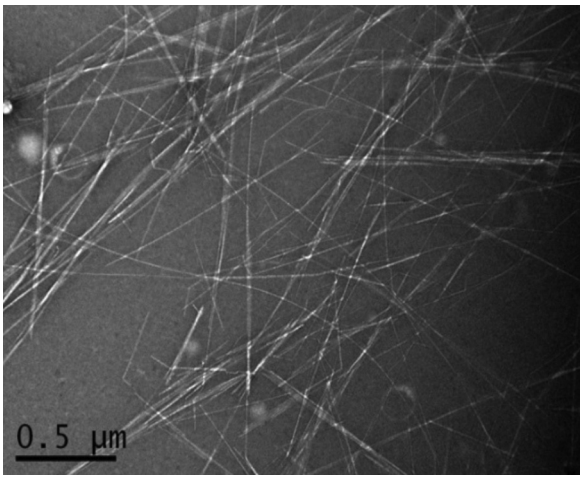

Fig. 1. TEM micrograph of a 0.2 vol% aqueous suspension of nanowhiskers.

diameter of 25 mm, were cut in Petri dishes using a punch and were very carefully transferred with a large spatula from the Petri dishes, with a diameter of 60 mm, to the plate geometry of the rheometer. Both plates were covered with waterproof abrasive paper of roughness of about 80 μm in order to prevent slippage. Low viscosity silicone oil was used to prevent water evaporation during rheometrical tests.

3. Results and discussion

3.1. Transmission electron microscopy (TEM)

Fig. 1 shows, as an example, a TEM image of a 0.2 vol% nanowhiskers suspension, and allows to visualize long and entangled rigid rods. From different pictures taken from the same sample, the diameter and length of about 400 nanowhiskers were measured. The average rod length $L = 960 \pm 475$ nm, and the mean rod diameter $D = 16 \pm 4$ nm, leading to a mean aspect ratio $L/D = 60$. It should be noted that a large polydispersity (mostly for the length) is observed for the nanowhiskers here studied.

The average aspect ratio value is in accordance with the values found in the literature for nanowhiskers of the same origin (Favier, Chanzy, & Cavaille, 1995). It should be noted that the aspect ratio of tunicate nanowhiskers is high, at least compared with that reported for cotton nanowhiskers ($p = 12$) (Roohani, Habibi, Belgacem, Ebrahim, & Karimi, 2008), or ramie nanowhiskers ($p = 29$) (Habibi, Goffin, Schiltz, Duquesne, & Dubois, 2008). It has also to be mentioned here that the average length of the tunicate nanowhiskers used in this study is about two times higher than the average mesh size of a 0.2 wt% agarose hydrogel, used as a matrix in the present work.

3.2. Surface charge

The number of SO_3^- groups per glucose unit, determined by titration, was found to be about 0.04. When the extent of acid hydrolysis is low, substitution occurs essentially at the surface of the nanowhiskers, so that the number of charged sulfate groups per glucose unit at the surface, DS_s is higher, and can be deduced from the ratio of surface chains to total chains in a nanowhisker (Goussé et al., 2002), which is equal to 0.23 in our case, leading to $DS_s = 0.17$. This result means that, due to acid hydrolysis of cellulose, 17% of accessible OH groups have been substituted by sulfate groups bearing a negative elementary charge on glucose units at the nanowhiskers surface.

3.3. Rheology

3.3.1. Strain sweep oscillatory shear

Strain sweep tests were used to determine the critical strain, γ_c (%), characterizing the extent of the linear viscoelastic response regime. The critical strain was determined from two different classical representations, which gave the same results: the first one from the storage modulus G' *versus* imposed strain amplitude curves, and the second one from the stress–strain curve. The values of the critical strain for different agarose concentrations and for 0.2 wt% agarose hydrogels, filled at nanowhiskers volume fractions ranging from 0.013 to 0.2%, are reported in Table 1.

Table 1 shows that the critical strain of the filled hydrogels is slightly lower (1%) than that of the pure agarose hydrogel (4%), suggesting that the filled hydrogel network is slightly more fragile than that of the agarose one. Moreover, contrary to what is observed for pure agarose hydrogels, for which the critical deformation is a power law decreasing function of the concentration, $\gamma_c - C^{-1.4}$, the results show that the critical strain is hardly dependent on nanowhiskers concentration, at least over the concentration range studied. This result suggests that non-linearity in the rheological behavior of filled hydrogels is primarily due to the non-linear response of the agarose network.

3.3.2. Frequency sweep oscillatory shear

A frequency sweep from 0.01 Hz to 1 Hz has been carried out in the linear regime with pure agarose hydrogels and agarose hydrogels filled with nanowhiskers. Fig. 2(A) shows a typical frequency sweep curve for a 0.2 wt% agarose hydrogel, and Fig. 2(B) for a 0.2 wt% agarose hydrogel filled with 0.13 vol% nanowhiskers.

Fig. 2(A) shows that, for a pure 0.2 wt% agarose gel, the storage modulus G' is much higher than the loss modulus G'', and that both viscoelastic moduli are slightly dependent on the frequency, which is characteristic of a solid-like viscoelastic behavior.

The viscoelastic behavior shown in Fig. 2(B) exhibits similarities with that shown in Fig. 2(A), proving that filled hydrogels also exhibit solid-like viscoelastic behavior. Nevertheless, the values of the viscoelastic moduli, G' and G'', are about 1 decade higher in the case of the filled hydrogel. Moreover, in Fig. 2(A), there is a slightly marked minimum in the frequency dependence of the loss modulus G'' of the agarose gel, which is reminiscent of weak gel behavior (Gabriele, De Cindio, & D'Antona, 2001). Such a minimum is not present in Fig. 2(B) for the agarose gel filled with 0.13 vol% nanowhiskers. This qualitative difference is a rheological signature of differences in gel structure.

The frequency dependence of viscoelastic moduli being weak, in order to have a better understanding of the influence of the presence of cellulose nanowhiskers in the agarose hydrogels, values of G' and G'', and of the loss angle, $\tan\delta = G''/G'$, for different agarose and nanowhiskers concentrations, at a fixed frequency of

Table 1
Critical strain for different agarose concentrations and at different nanowhiskers volume fractions, for a 0.2 wt% agarose matrix.

	Agarose concentration C (%)						Nanowhiskers volume fraction Φ (%)					
	0.1	0.15	0.2	0.25	0.3	0.35	0	0.013	0.032	0.065	0.13	0.2
γ_c (%)	9.5	6	4	3	2.5	1.5	4	2	2	1.5	1	1

Table 2
Storage modulus G', loss modulus G'' and loss angle ($\tan\delta$) at different agarose concentrations and different nanowhiskers volume fractions, for a 0.2 wt% agarose matrix.

	Agarose concentration C (%)						Nanowhiskers volume fraction Φ (%)					
	0.1	0.15	0.2	0.25	0.3	0.35	0	0.013	0.032	0.065	0.13	0.2
G' (Pa)	7	30	80	140	180	350	80	200	320	590	940	830
G''(Pa)	1	3	7	15	25	50	7	15	40	60	125	150
$\tan\delta$	0.14	0.1	0.1	0.11	0.14	0.14	0.1	0.08	0.12	0.1	0.13	0.18

0.1 Hz, were reported in Table 2. These viscoelastic characteristics were also reported in Table 2 for filled 0.2 wt% agarose hydrogels, at different nanowhiskers volume fractions.

It can firstly be seen that increasing the agarose concentration leads to an increase of the storage and loss moduli, showing a strengthening of the hydrogels, in agreement with numerous previous studies on biopolymer gels. At 0.1 Hz, the storage modulus of the agarose gels follows a power law as a function of agarose concentration, with an exponent close to 3 as shown in Fig. 3(A). The data plotted in Fig. 3(A) comply with those plotted in the paper by Tokita and Hikichi (1987): $G' \sim C^3$. From these data, these authors have shown that, in the vicinity of the percolation threshold, the exponent of the concentration dependence of G' rescaled with the critical concentration for percolation was very close to 2, as predicted by scalar percolation theory. However, in our work, we are far from the percolation threshold (~one decade higher), and the rescaling with the critical concentration has nearly no effect, so that the exponent of the concentration dependence of G', either rescaled or not with the critical concentration for percolation, is close to 3. As suggested by Tokita & Hikichi, this could be due to the change from scalar elasticity percolation (exponent about 2) to vector elasticity percolation (exponent about 3) at concentrations far from the percolation threshold.

Furthermore, Table 2 shows that the loss angle stays roughly constant, which suggests that the global structure of the agarose hydrogel is the same at all concentrations investigated.

Finally, we would like to point out that the relatively low G' values, in comparison to those found in the literature, are due to the elaboration technique used in this work, for which cooling, which occurs at ambient temperature, is more rapid in

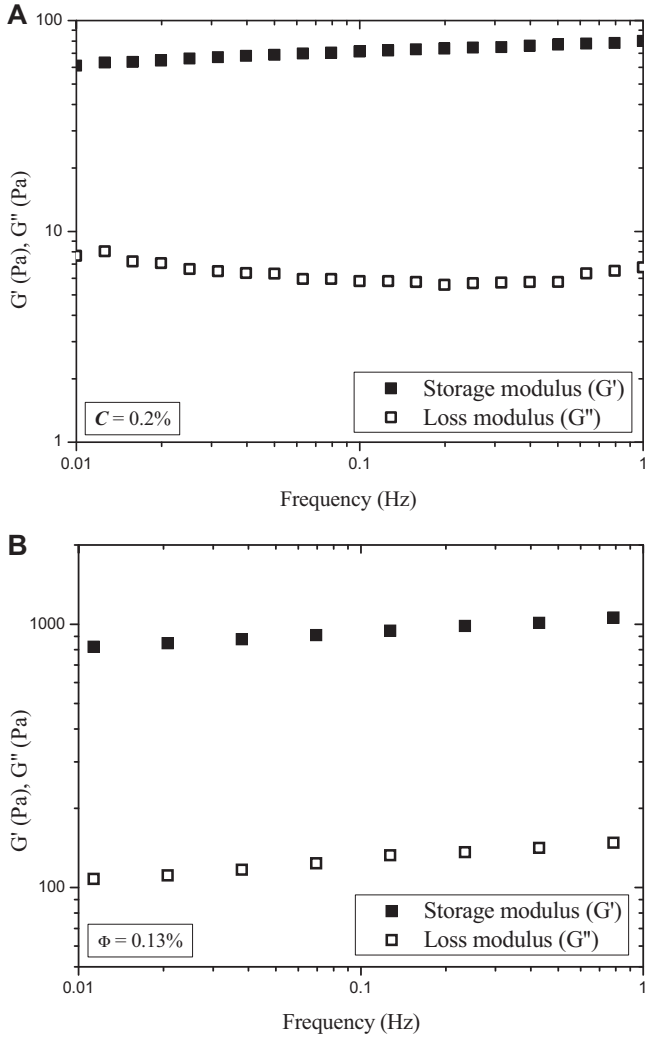

Fig. 2. Storage modulus G' and loss modulus G'' as a function of frequency for (A) a 0.2 wt% agarose hydrogel (B) a 0.2 wt% agarose hydrogel filled with 0.13 vol% nanowhiskers (strain = 1%).

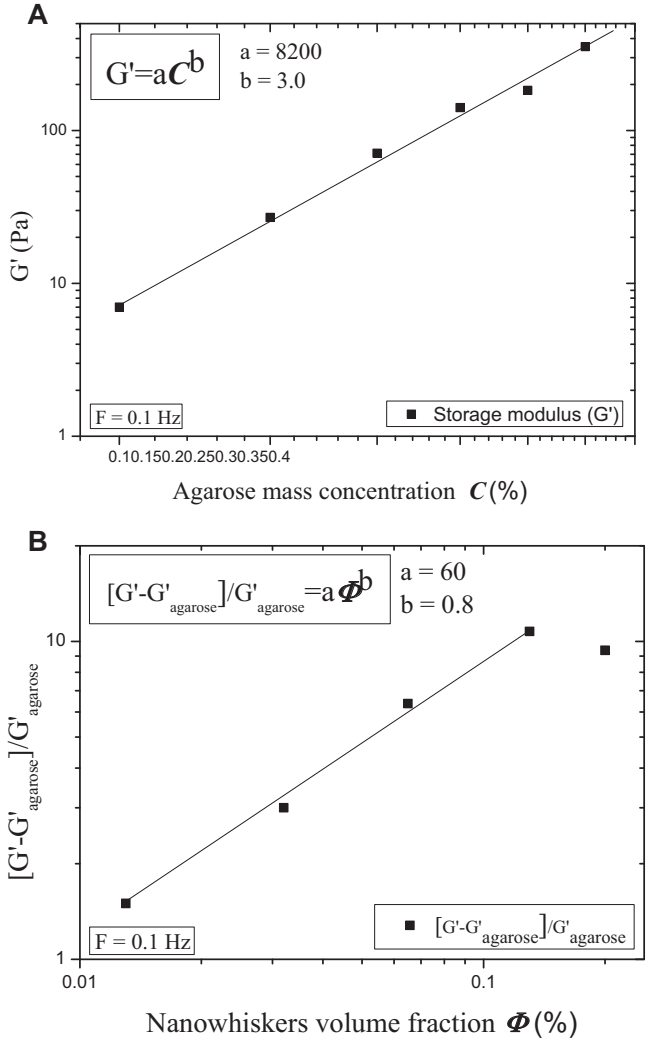

Fig. 3. (A) Storage modulus G' as a function of the agarose mass concentration C (B) $(G'\text{-}G'_{agarose})/G'_{agarose}$ as a function of the nanowhiskers volume fraction Φ for a 0.2 wt% agarose hydrogel, fitting by a power law (frequency = 0.1 Hz).

Table 3
Gel strength S and relaxation exponent n for different mass concentrations of agarose and at different volume fractions of nanowhiskers, for a 0.2 wt% agarose matrix.

	Agarose concentration C (%)						Nanowhiskers volume fraction Φ (%)					
	0.1	0.15	0.2	0.25	0.3	0.35	0	0.013	0.032	0.065	0.13	0.2
S (Pa)	3	30	65	125	175	375	65	140	270	480	1015	1150
n	0.08	0.07	0.1	0.09	0.2	0.1	0.1	0.1	0.06	0.07	0.06	0.1

comparison to what is performed in other works, where controlled cooling is performed over longer times (Mohammed, Hember, Richardson, & Morris, 1998).

As far as filled hydrogels are concerned, results in Table 2 show that the reinforcement effect due to the addition of cellulose nanowhiskers is quite significant. Indeed, the addition of 0.13 vol% nanowhiskers increases about 12 times the elastic modulus of a 0.2 wt% agarose hydrogel.

In order to study the nanowhiskers contribution to elasticity enhancement, we plotted a scaled storage modulus with respect to agarose, that is $(G' - G'_{agarose})/G'_{agarose}$ as a function of nanowhiskers volume fraction, in Fig. 3(B). The volume fraction dependence of the scaled storage modulus follows a power law with an exponent equal to 0.8, at least for volume fractions up to 0.13%. This result shows that the reinforcement effect is not due to the existence of a tridimensional network formed by the nanowhiskers within this volume fraction domain, but suggests that nanowhiskers strengthen the agarose network by affecting its organization, through topology modifications and/or network connectivity changes, resulting in an increase of the number density of network junctions, which leads to enhanced elastic properties, as predicted by transient network models (Green & Tobolsky, 1946). Besides, it should be noticed that the increase in G' (reinforcement effect) is accompanied by an increase in the loss angle with nanowhiskers concentration, reflecting an even higher increase of the dissipative phenomena with the addition of nanowhiskers. This could be due to enhanced viscous dissipation induced by water mediated interactions between agarose molecules and cellulose nanowhiskers and/or between nanowhiskers.

The fact that nanowhiskers have a characteristic size larger than the average mesh size of the pure agarose network, but also the method used to prepare filled hydrogels, where agarose molecules are added to a nanowhiskers suspension, might explain why nanowhiskers are able to modify the agarose network structure.

Finally, in the absence of nanowhiskers percolation network, the stress can be assumed to be transferred mostly through individual nanowhiskers/hydrogel matrix interactions. The AFM picture in Fig. 4, which shows individual cellulose nanowhiskers very well dispersed within the agarose matrix, supports this assumption.

However, we would like to point out that the storage modulus of the filled hydrogel with 0.2 vol% nanowhiskers is lower than that obtained with 0.13 vol% nanoparticles. This result, perfectly reproducible, suggests that, from a volume fraction of 0.2%, the nanowhiskers affect in a more extensive way the agarose network organization, leading to a lower connectivity between agarose helices, and therefore to a loss of elasticity.

If we compare with literature, the reinforcement effect induced by addition of cellulose nanowhiskers has been observed with other hydrogels, either made from synthetic polymers (Zhou, Wu, Yue, & Zhang, 2011) or natural ones (Dash et al., 2013). These observations were, however, made in ranges of volume fractions higher than those used in our study (between 1 wt% and 10 wt%). Moreover, it has been shown that the reinforcement effect was even more pronounced as the nanoparticle aspect ratio was higher. Indeed, the study of an epoxy matrix reinforced by nanowhiskers showed that the stress transfer between the matrix and the fillers was more efficient for tunicate nanowhiskers than for cotton ones (Rusli, Shanmuganathan, Rowan, Weber, & Eichhorn, 2011). Besides, it should be highlighted that the reinforcement effect sometimes occurs at high volume fractions (20 wt%) (Spagnol, Rodrigues, Pereira, Fajardo, & Rubira, 2012b). Finally, it was also shown that the reinforcement effect due to the presence of nanowhiskers is lower than that obtained with synthetic clay nanoparticles, such as Laponites; whose contact area with the matrix is much larger (Kvien, Sugiyama, Votrubec, & Oksman, 2007).

3.3.3. Stress relaxation

The stress relaxation results show that for both pure agarose and filled agarose, the time dependence of the relaxation modulus $G(t)$ can be quite correctly fitted by a decreasing power law (1):

$$G(t) = St^{-n} \quad (1)$$

where S is the gel strength and n the relaxation exponent. This type of law is characteristic of the relaxation of a critical gel (Winter & Chambon, 1986). The model originally proposed by Winter and Chambon can be applied each time the gel structure presents self-similarity (or scale invariance), that is the reason why it can describe the viscoelastic behavior of a great number of covalently and physically cross-linked polymer gels (Matsumoto, Kawai, & Masuda, 1992; Michon, Cuvelier, & Launay, 1993; Scalan & Winter, 1991), but also of various rod-like or disk-like particle gels (Cocard, Tassin, & Nicolai, 2000; Reddy, Zhang, Lettinga, Dhont, & Vermant, 2012).

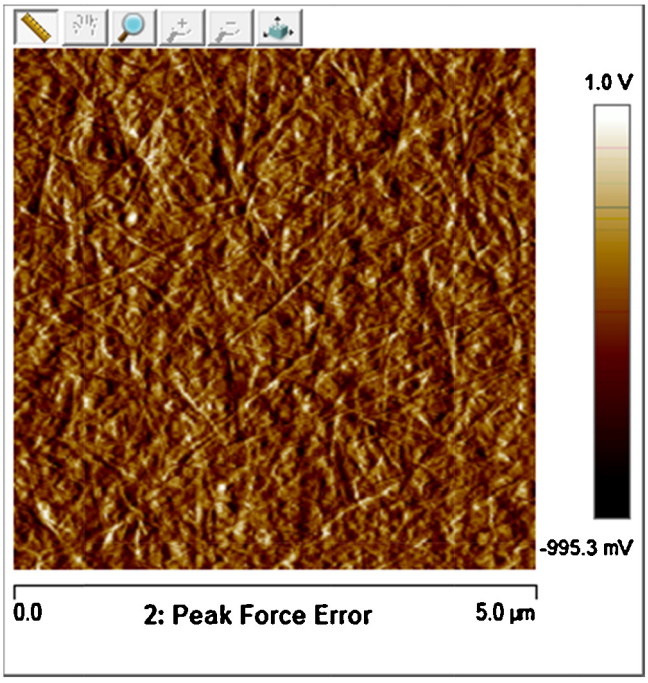

Fig. 4. AFM micrograph of a 0.2 wt% agarose hydrogels filled with 0.13 vol% nanowhiskers.

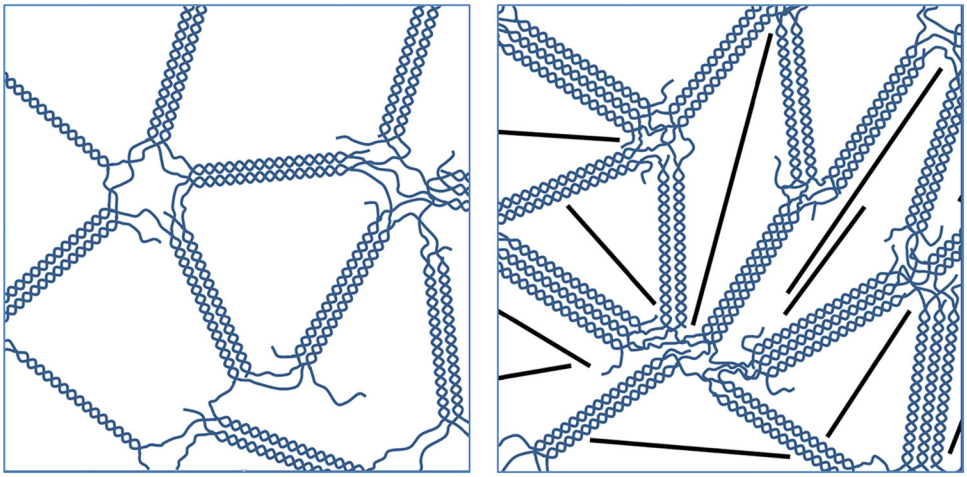

Fig. 5. Schematic illustration of an agarose hydrogel structure (left) and the modification of an agarose hydrogel structure induced by the addition of nanowhiskers at volume fraction less than 0.2% (right).

The values of S and the relaxation exponent n for pure agarose and filled agarose hydrogels are shown in Table 3.

Table 3 shows that the relaxation exponent of agarose hydrogels, at the different concentrations studied, is rather low: $n \sim 0.1$. In addition, Table 3 shows that the values of the relaxation exponent of the filled hydrogels, at least in the range of volume fractions studied, are very close to those obtained for pure agarose hydrogels.

This result confirms that the agarose gel seems to impose the viscoelastic behavior to the filled systems, as outlined above.

4. Conclusion

The small amplitude oscillatory shear properties of either pure agarose hydrogels or agarose hydrogels filled by tunicate cellulose nanowhiskers have been studied. The results tend to show that the rheological properties of filled hydrogels are governed by the agarose matrix structure, at least at low nanowhiskers volume fractions ($\Phi < 0.2\%$). However, even at these low volume fractions, where nanowhiskers do not percolate, the fillers have a significant reinforcement effect, characterized by an increase of more than one decade of the storage modulus when adding 0.13 vol% nanowhiskers to a 0.2 wt% agarose hydrogel.

Finally, the results of this study suggest that nanowhiskers can modify the agarose network topology and/or connectivity, resulting in a significant reinforcement effect, the stress being transferred mostly through nanowhiskers/hydrogel matrix interactions. Fig. 5 shows a schematic illustration of the modifications of the agarose network structure that could be induced by addition of nanowhiskers at volume fraction less than 0.2%.

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