



Tunable viscoelastic features of aqueous mixtures of thermosensitive ethyl (hydroxyethyl)cellulose and cellulose nanowhiskers

Erfan Dashtimoghdam^{a,b,c}, Hamed Salimi-Kenari^{b,d}, Rasool Nasseri^{a,e}, Kenneth D. Knudsen^f, Hamid Mirzadeh^a, Bo Nyström^{b,*}

^a Department of Polymer Engineering and Color Technology, Amirkabir University of Technology, Tehran, Iran

^b Department of Chemistry, University of Oslo, Oslo, Norway

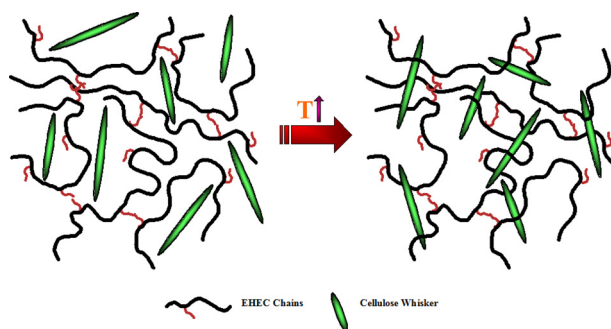
^c Department of Chemistry, University of North Carolina, Chapel Hill, NC, USA

^d Faculty of Engineering and Technology, University of Mazandaran, Babolsar, Iran

^e Department of Chemical Engineering, University of Waterloo, Waterloo, Ontario, Canada

^f Department of Physics, Institute for Energy Technology, P.O. Box 40, N-2027, Kjeller, Norway

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:

Cellulose derivative
Cellulose nanowhiskers
Thermosensitive hydrogels
Rheology
Gel temperature

ABSTRACT

Ethyl(hydroxyethyl) cellulose (EHEC) is known to form reversible hydrogels in water at elevated temperatures in the presence of an ionic surfactant. However, the toxicity of common ionic surfactants (like SDS and CTAB) hampers pharmaceutical and biomedical applications of EHEC-based thermogels. Addition of a nature-based material to EHEC solutions - in the form of negatively charged cellulose nanowhiskers (CNWs) - will introduce an internal electrostatic repulsion that could also produce the balanced swelling necessary for forming a stable gel at elevated temperatures (ca. 37 °C). This may therefore be an alternative way of designing low toxicity thermoresponsive hydrogels of high mechanical strength for pharmaceutical and biomedical applications without the use of ionic surfactants. The properties of the temperature-induced gelling systems (EHEC/CNW and EHEC/SDS/CNW) were characterized by rheological methods and rheo-small angle light scattering (rheo-SALS), whereas the structure and morphology of CNWs were examined by transmission electron microscope (TEM) and small angle neutron scattering (SANS). Oscillatory shear results for the EHEC/CNW system showed that the gel temperature (ca. 37 °C) was virtually unaffected by the amount of added CNWs, while the fractal dimension values (2.2–2.3) suggested the evolution of a tighter incipient gel network with increasing level of added CNWs. Furthermore, a threefold increase of the gel strength parameter was observed with increasing concentration of CNWs. For the EHEC/CNW/SDS system, a more open network evolved with increasing amount of CNWs, and for this system, higher values of the gel strength parameter were found. Pronounced shear-thinning, even at very

* Corresponding author.

E-mail address: b.o.g.nystrom@kjemi.uio.no (B. Nyström).

<https://doi.org/10.1016/j.colsurfa.2020.124489>

Received 5 December 2019; Received in revised form 18 January 2020; Accepted 19 January 2020

Available online 20 January 2020

0927-7757/ © 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

low shear rates, was observed for the EHEC/CNW system at all levels of CNW addition, whereas for the EHEC/CNW/SDS system, Newtonian-like behavior was detected at low shear rates.

1. Introduction

In recent years, stimuli-responsive hydrogels have attracted a great deal of attention in the biomedical field due to their swelling and shrinking behavior that can be controlled in a reversible way by using stimuli such as pH, temperature, light, magnetic fields, and chemical entities [1–8]. Many amphiphilic water-soluble polymers exhibit a sol-gel transition upon heating a semidilute solution [1,2,7,8]. A classic example of a polymer belonging to this family of systems is ethyl(hydroxyethyl) cellulose (EHEC), which is a temperature-sensitive non-ionic amphiphilic polysaccharide that has been widely studied in aqueous media in the presence of an ionic surfactant [1,2,9–14]. In the past, typical used ionic surfactants are SDS and CTAB [1,2]. A special feature of EHEC in aqueous solution is that it exhibits macroscopic phase separation when the temperature is raised above the lower critical solution temperature (LCST) (ca. 34 °C) as a consequence of the intermolecular aggregation of hydrophobic domains [15]. However, in the presence of an ionic surfactant the large domains produced by the temperature-induced hydrophobic association of polymer chains are fragmented into smaller hydrophobic microdomains. These “lumps” [10] bring connectivity to the gel-network in the semidilute concentration regime, while the charged surfactant provides electrostatic repulsion and chain swelling due to the retention of water molecules. A gel-network is evolved, and the gelation is governed by a delicate balance between connectivity and swelling [10]. Physically crosslinked hydrogels frequently possess poor mechanical properties, which reduce their potential for practical applications, such as tissue engineering.

It has recently been observed that reinforcement and enhanced mechanical stability of the gel-structure in hydrogels of various origins can be accomplished by incorporating cellulose nanowhiskers (CNWs) into the gel matrix [16–21]. CNWs are rod-like objects that have gained considerable interest as a promising biomaterial because of their outstanding properties, such as high surface area, high mechanical strength, hydrophilicity, biocompatibility, and biodegradability [22,23]. CNWs are frequently obtained by controlled acid hydrolysis of native cellulose fibers, where the hydrolysis conditions and cellulose source influence the surface chemistry and particle size of the individual whiskers. The typical dimension of CNWs is 5–10 nm in width and 100–300 nm in length [19,24,25]. When the CNWs are dispersed in water, they form a colloidal suspension and in dilute suspension the particles are stabilized by the negative charges introduced during the isolation procedure [23]. The zeta potential of the CNWs is usually around –30 mV [26], so they will be electrostatically stabilized in suspension. Rheological experiments [26] revealed that suspensions of CNWs were isotropic up to ca. 3 wt% (in the present work only lower concentrations are considered); at higher concentrations the system is biphasic and there are ordered domains in the system. At low concentrations (< 3 wt%), it is found that increasing shear rate induces shear thinning of the system and lower viscosity values are observed as the temperature rises.

In the semidilute concentration regime, aqueous solutions of EHEC form gels in the presence of an ionic surfactant at elevated temperatures. However, in the absence of ionic surfactant a macroscopic phase separation occurs. The principle aim of this study is to find out whether well-defined hydrogels can be designed by heating semidilute solutions of EHEC in the presence of CNWs. Cellulose nanoparticles have been studied before in connection with hydrogels, but this has been either with chemical crosslinking (Dai and Kadla [16] using divinyl sulfone crosslinked hydroxyethyl cellulose; Dash et al. [19] with gelatin and dialdehyde cross-links to cellulose crystals); Chen et al. [21] using free

radical polymerized NIPA including initiator and catalyst additives), or on different systems (Abitbol et al. [17] considered poly(vinyl alcohol) gels made via repeated freezing and thawing; Martinez et al. [18] investigated κ -carrageenan gels; Le Goff et al. [20] studied agarose gels). Given the potential for pharmaceutical and biomedical use of EHEC-containing materials, there should be a particular interest for exploring the properties of EHEC-based gels that are stabilized only with CNWs, without the addition of ionic surfactants that may have higher toxicity, such as SDS and CTAB. To the best of our knowledge, there are no studies on the temperature-induced gelation of EHEC in the presence of CNWs. In the light of the importance of EHEC for various uses, the EHEC/CNW hydrogels can offer new applications in different fields.

Another objective of this work is to survey the influence of added whiskers on the temperature-induced gelation process of EHEC and to observe the impact of vital parameters such as gel-point, gel strength, viscoelastic exponent, and structure of the incipient gel. This investigation is focused on the change of the rheological properties in aqueous semidilute solutions of EHEC and also in EHEC solutions with SDS when CNWs are added.

Both oscillatory shear and rheo-small angle light scattering experiments are carried out. Together with the improvement of the mechanical properties, the addition of CNWs should advance the biocompatibility of EHEC gels, in comparison to the addition of commercial ionic surfactants to the polymer. This makes these hydrogels potential candidates for tissue engineering and other biomedical applications.

2. Experimental

2.1. Materials

The polysaccharide EHEC labelled as DVT 89017 was manufactured by Akzo Nobel Surface Chemistry AB, Stenungsund, Sweden. EHEC had the following specifications, provided by the manufacturer: number average molecular weight (M_n) = 80,000, degree of substitution of ethyl groups $DS_{\text{ethyl}} = 1.9/\text{anhydroglucose unit}$, molar substitution of ethylene oxide groups $MS_{\text{EO}} = 1.3/\text{anhydroglucose unit}$, and the sample had a dispersity index (M_w/M_n) of about 2. The anionic sodium dodecylsulfate (SDS) was obtained from Fluka.

Dilute EHEC solutions were dialyzed against pure water for at least one week (until the conductivity of the expelled water showed no further decrease) in order to remove salt (impurity from manufacturing) and other low molecular weight components. EHEC was thereafter recovered by freeze drying. As the dialyzing membrane, regenerated cellulose with a molecular weight cut-off of 8000 (Spectrum Medical Industries) was employed. CNWs were obtained from commercial cotton fibers. The cellulosic whiskers were characterized via CHNS elemental analysis (Perkin Elmer 2400 analyzer). The total contents of nitrogen, carbon, hydrogen, and sulfur were found to be 0.0754, 34.75, 5.60, and 2.84 %, respectively.

2.2. Preparation of nanocomposite thermogels

EHEC solutions were prepared by dissolving the polymer in water and the solutions were homogenized by slow stirring at room temperature for more than 12 h. All measurements were carried out in a semidilute (2 wt%) solution of EHEC.

The CNWs were prepared by sulfuric acid hydrolysis, under strictly controlled conditions of temperature, agitation, and time, of commercial cotton fibers as described elsewhere [27,28]. In brief, the cotton

fibers were first finely chopped and passed through a 10-mesh sieve. Afterwards, 2.5 g of fibers were dispersed in 50 ml of 6.5 M sulfuric acid at 45 °C and stirred vigorously for 45 min. Subsequently, 250 ml of cold distilled water was added to stop the reaction and dilute the suspension. CNWs were collected after successive centrifugation and washing with distilled water to partly remove excess sulfuric acid. The fibers were then re-suspended and the non-reactive sulfate groups were removed by dialysis against distilled water. Finally, the suspension was ultra-sonicated to achieve a stable colloidal suspension. The negative charges on CNWs are due to the ionization of sulfate ester groups ($-\text{OSO}_3^-$), which are formed after reaction of sulfuric acid with hydroxyl groups. The average zeta potential of the prepared CNWs at 25 °C, pH 6.8, and zero salt concentration was measured to be -35 ± 3 mV, which should secure the stability of the CNWs suspension. It has been shown that the natures of acid and acid-to-cellulosic source ratio are important factors that affect the extraction of CNWs [24]. Sulfuric acid as a hydrolyzing agent reacts with the surface hydroxyl groups of cellulose to yield charged surface sulfate esters that promote dispersion of the CNWs in aqueous media. The effect of the amount of negative charges on the surface of CNWs is estimated through conductometric titration measurements; this method can be utilized to approximate the number of sulfate ester groups on the surface of CNWs. The results are presented in Supporting Material (Fig. S1).

To prepare EHEC/CNW suspension, we added CNW suspension to the EHEC solution in water to reach the predetermined final concentration of both components. The total concentration of EHEC was fixed (2 wt%) and various concentrations of CNW were prepared. In the presence of SDS, EHEC/SDS solutions were prepared and the CNW suspension was added and adjusted to the desired concentration.

2.3. Small angle neutron scattering

The SANS experiments were conducted at two temperatures, 25 °C and 50 °C, using the SANS instrument at the JEEP-II reactor at Kjeller, Norway. A RISØ-type (^3He) neutron detector mounted in an evacuated chamber was employed in this work. Two different neutron wavelengths (5.1 and 10.2 Å) and two sample-detector distances were used (1.0 and 3.4 m) in order to give a q-range of 0.01 to 0.25 Å $^{-1}$. Here q is defined by $q = (4\pi/\lambda)\sin(\theta/2)$, with θ being the scattering angle and λ the neutron wavelength. The CNW suspension was held in 2 mm Starna quartz cuvettes. Accurate temperature control was achieved by placing the sample cell onto a copper base with internal water circulation. The normalized scattering intensity, i.e. the absolute scattering cross section (cm^{-1}), was calculated by incorporating the contribution from the blocked-beam background and the empty cell, including independent measurements of the transmissions. In order to reduce incoherent background and enhance the contrast, the samples were prepared in heavy water.

2.4. Transmission electron microscopy

The suspensions of CNWs were adsorbed on hexagonal formvar grids, and subsequently washed three times with MQ-water stained with 3 % uranyl acetate and then dried at room temperature. A Phillips transmission electron microscope CM 100 (Phillips, Eindhoven, The Netherlands) was employed for the imaging, and images were recorded digitally with a Quemsa TEM CCD camera (Olympus Soft Imaging Solutions, Germany) and iTEM software (Olympus Soft Imaging Solutions, Germany).

2.5. Zeta potential measurements

Zeta potential data were collected with a Malvern Zetasizer Nano ZS (Malvern instruments Ltd, U.K). Here Laser Doppler Velocimetry is used to extract the zeta potential via the electrophoretic mobility of the samples at 25 °C. The measurements were carried out in a glass cell

PCS1115 cuvette (12 mm) with a cap, using a dip-cell with palladium electrodes having 2 mm spacing. The electrophoretic mobility values for the CNWs in the suspension were subsequently converted to zeta potential values. The measurements were carried out at 25 °C, pH 6.8, and zero salt concentration.

2.6. Rheo-small-angle light scattering (rheo-SALS)

Rheology and small-angle light scattering (rheo-SALS) data were collected during oscillatory shear or shear flow using a Paar-Physica MCR 300 rheometer, configured with a specially designed parallel plate-plate setup made of glass, where the plates have a diameter of 43 mm and the gap between the plates is 0.25 mm. A sketch of the setup as well as the experimental details has been given elsewhere [29–31]. The temperature was changed from 10 to 44 °C for all samples, following a step-wise protocol.

The two-dimensional scattering images were collected on a semi-transparent screen and then captured with a CCD camera (driver LuCam V. 3.8), positioned with its plane parallel to the screen. An exposure time of 200 ms were used to acquire the CCD images.

2.7. Analysis of rheological data

The rheological behavior of an incipient gel can be described by a simple power law model, where the storage modulus G' and the loss modulus G'' are related via [32]:

$$G' = S\omega^n\Gamma(1-n)\cos\delta \quad (1)$$

where n is the relaxation exponent, $\Gamma(1-n)$ is the gamma function, and S is the gel strength parameter, which depends on the crosslinking density and the molecular chain flexibility and mobility [32]. This relationship has been used in this study to calculate values of S . The phase angle (δ) between stress and strain is independent of angular frequency (ω) but proportional to the relaxation exponent [33]:

$$\tan\delta = \tan\left(\frac{n\pi}{2}\right) \quad (2)$$

The following scaling relation can then describe the incipient gel:

$$G'(\omega) \propto G'' \propto \omega^n \quad (3)$$

The gelation temperature of a thermoreversible gel can be determined by observation of a frequency-independent value (Method 1) of $\tan\delta$ ($=G''/G'$) acquired from a multi-frequency plot of $\tan\delta$ versus temperature [2,34]. An alternative method [35] to find the gel point is to plot against temperature the “apparent” viscoelastic exponents n' and n'' ($G' \sim \omega^{n'}$, $G'' \sim \omega^{n''}$) obtained from the frequency dependence of G' and G'' at each temperature and finding a crossover where $n' = n'' = n$ (Method 2). These methods will be illustrated and discussed below.

To acquire information about the structure of incipient gels, Muthukumar elaborated a theoretical model [36] in which variations of the strand length between crosslinks of the incipient gel network produce changes in the excluded volume interactions. It is assumed that increasing strand length will enhance the excluded volume effect. Muthukumar suggested that if the excluded volume interaction is fully screened, the parameter n for a polydisperse system is given by

$$n = \frac{d(d+2-2d_f)}{2(d+2-d_f)} \quad (4)$$

where d_f is the fractal dimension and d ($d = 3$) is the spatial dimension. The change of the structure of the incipient gels when CNWs are added will be discussed in terms of the fractal dimension.

3. Results and discussion

3.1. Characteristic properties of the cellulose nanowhiskers

Before we discuss structural and rheological characteristics of the EHEC/CNW composites at the gel point, we will give some basic information about the CNWs utilized in this study. By employing transmission electron microscopy (TEM) and small angle neutron scattering (SANS) on the suspensions of CNWs, we gain some information about the CNWs. However, our focus is not on the characterization of the CNWs but the impact of these whiskers on the gelation features of EHEC.

Fig. 1 (a) shows a transmission electron micrograph of CNWs. This illustration displays bundles of rodlike microcrystalline fibers. The fibers have a rather broad size distribution as is frequently observed for CNWs [18–21]. It is usually found that most of the variation is in the length of the whiskers [20]. Based on image analysis of the TEM micrograph, the average diameter and length of CNWs were found to be 10 ± 1 and 121 ± 17 nm, respectively (see Fig. S2).

SANS results for 1 wt% suspension of CNWs are depicted in Fig. 1(b) at temperatures of 25 °C and 50 °C. A strong upturn of the scattered intensity at low q -values is registered at both temperatures. This pronounced upturn in the intensity and no visible flattening of the profile at low q , show that the rod-like objects have an average length that are outside the q -window accessible in the SANS instrument (< 100 nm), in line with what was observed in the TEM micrographs. In view of this, an “infinitely” long cylinder is effectively pictured, which would give a scattering slope of -1 if the cylinder was very thin. It is evident from the results at 25 °C that we have a power-law behavior with a slope of approximately -2 in the intermediate region of the scattering profile. This is characteristic of particles with a finite cross section. The lack of any plateau in the intermediate q -range indicates that there is substantial variation in the cross-sectional dimensions for these CNWs, possibly due to some particles adhering laterally, thus effectively increasing the dimensions. At 50 °C, the overall scattering features are similar to those measured at 25 °C, but with a certain drop in intensity compared to the data at 25 °C. This may indicate scattering from species of smaller size. In this context it is interesting to note a previous rheology study [26] of suspensions of CNWs, where the viscosity for a dilute suspension (3 wt%) was found to monotonously decrease with increasing temperature. This was attributed to changes in the microstructure of the CNWs and disappearance of ordered structure.

Some years ago, Terech et al. [37] carried out small angle scattering experiments (both SANS and SAXS) on dilute aqueous suspensions of CNWs and in their scattering profile ($\log(I)$ versus q) a plateau located around $q = 0.02 \text{ \AA}^{-1}$ was found. From this a well-defined width of the cylindrical object (radius of ca. 8 nm) could be determined. As is obvious from Fig. 1(b), no sign of a plateau region is detected and this suggests that the CNWs in this study have a broader size distribution, and that a population of significantly larger species dominates over the smaller entities in the system. It is well-known that the CNWs are quite polydisperse and this feature and the size of the species depend on the source of CNWs, the protocol of acid hydrolysis [22], and how effective the sonication is [26]. The difference in SANS-behavior between the present system and that of Terech et al. [37] can probably be traced to differences in the preparation of the cellulose nanowhiskers as described above.

3.2. Rheological results of the composite systems

In this section, we will discuss temperature-induced rheological and structural properties of EHEC samples with added cellulose whiskers, both in absence and presence of SDS. It should be noted that in the absence of an ionic surfactant and/or CNWs, a semidilute solution of EHEC does not form a hydrogel at higher temperatures, but a macroscopic phase separation takes place.

3.2.1. EHEC/CNW suspensions

To illustrate the effect of incorporated whiskers in aqueous semidilute EHEC solutions on the temperature-dependent gel characteristics, the rheological behavior of the systems in the course of gelation was analyzed (see Fig. 2). It is evident that the two methods (Method 1 – $\tan\delta$ versus temperature at various frequencies and Method 2 – n' and n'' versus temperature) yield the same gel-temperature for the considered systems (the values are given in Table 1). The general feature observed from Method 1 is that the loss tangent, $\tan \delta$, decreases during the gel formation, indicating that the samples become more and more elastic. At the gel point temperature, the values of the relaxation exponent n are determined from a log-log plot of G' and G'' versus ω (parallel lines).

Characteristic data for the incipient EHEC gels with different amounts of incorporated CNWs are displayed in Table 1. Interestingly, the gel point is virtually unaffected by increasing the concentration of CNW, although the cloud point of the samples decreases. The kinship between gelation and macroscopic phase separation has been pointed out in a number of theoretical investigations [38–41] on thermoreversible gels. In these studies, a close correlation between thermoreversible gelation and phase separation was established. A previous study [2] on thermoreversible gelation of EHEC in the presence of SDS disclosed a close correlation between cloud point and gelation. In this study [2] it was also shown that at a constant EHEC concentration of 2 wt%, increasing SDS concentrations lead to both lower cloud and gel

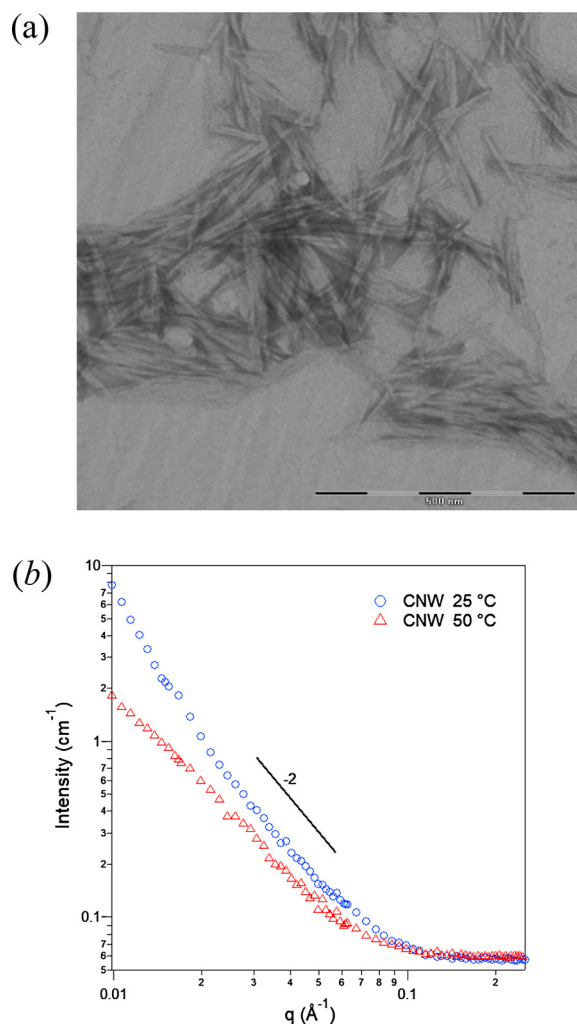


Fig. 1. (a) Transmission electron micrograph of cellulose nanowhiskers (scale bar is 500 nm). (b) SANS scattering profiles for 1 wt% suspension of CNWs at the temperatures indicated.

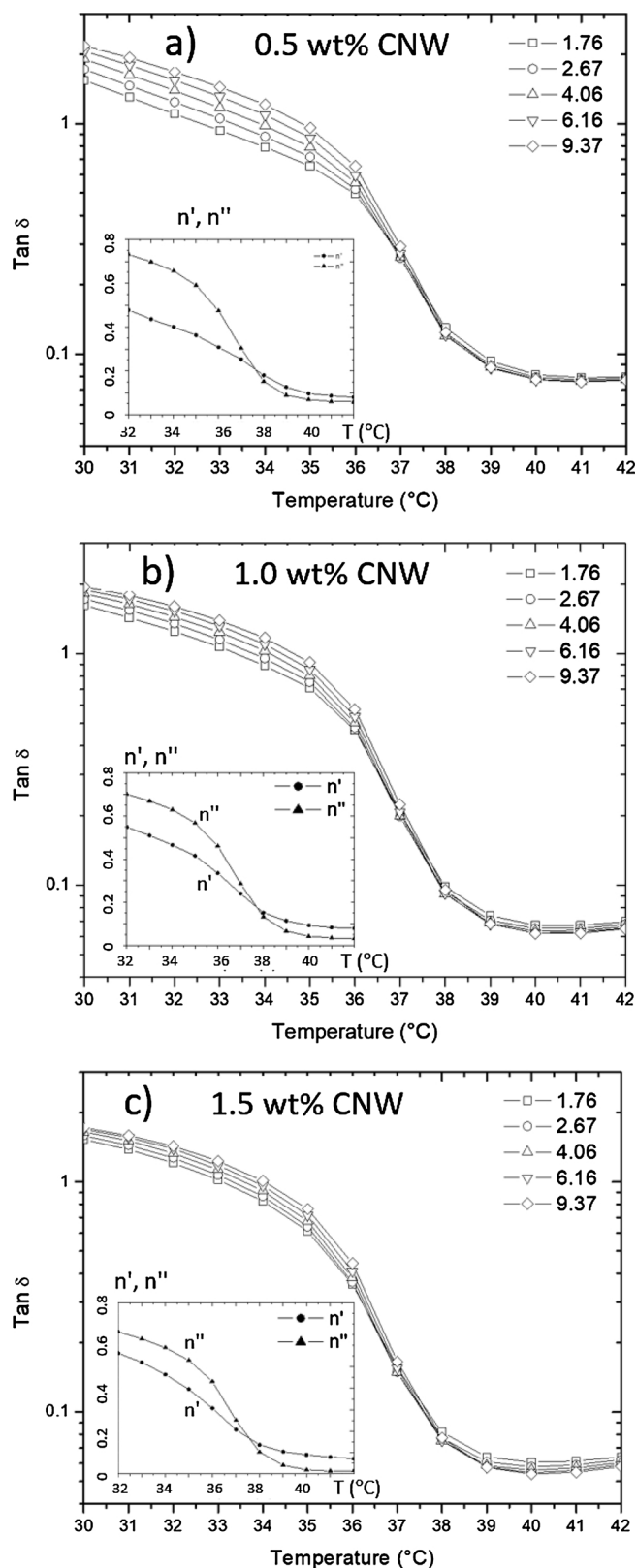


Fig. 2. (a-c) Typical illustration of the two methods (Method 1 and Method 2, see Section 2.7) for the determination of the gel point (see the Experimental Section). Viscoelastic loss tangent as a function of temperature at the indicated frequencies for EHEC (2 wt%)/CNW(0.5,1.0,1.5 wt%) nanocomposite systems. The inset plots (Method 2) show changes of the apparent relaxation exponents, n' for the storage and n'' for the loss modulus at various temperatures and the intersection determining the gel point. The right hand numbers in the panels are the angular frequencies (rad/s).

points. However, as pointed out above, we find that a higher level of CNW addition had practically no influence on the gel temperature. This shows that at the considered level of CNW incorporation, the presence of CNWs is not a determining factor during the initial phase of gel formation. The conjecture is that when SDS is added to the EHEC sample more hydrophobic microdomains are solubilized and higher temperatures are needed to establish the gel point, whereas for the EHEC/CNW system addition of more CNWs will not affect the size and number of hydrophobic microdomains.

The value of the viscoelastic relaxation exponent n falls off with increasing amount of CNW (see Table 1); in the framework of the Muthukumar approach [36] this trend suggests that the structure of the incipient gel changes upon incorporation of CNWs. The fractal dimension increases with increasing level of CNW addition (Table 1); this behavior indicates the formation of a more homogeneous and tight gel network [32,33,42] as the CNW concentration increases.

The most conspicuous finding is the threefold increase of the gel strength parameter as the incorporation of CNW increases (Table 1). The result clearly shows that by increasing the number of dispersed CNWs in the system, the strength of the gel network is substantially improved. In the absence of CNWs in the EHEC solution, a temperature rise leads to gradually larger hydrophobic microdomains and eventually macroscopic phase separation takes place. However, the presence of CNWs in the EHEC sample seems to prevent the evolution of too dominating hydrophobic junction zones and phase separation at elevated temperatures, and stabilizes the gel network. It is known [43,44] that cellulose displays both a hydrophilic and hydrophobic nature and our hypothesis is that as more hydrophobic microdomains are evolved in the EHEC network matrix at elevated temperatures, the CNWs are interacting with the EHEC network and strengthening of the network occurs as illustrated in Fig. 3a. In the absence of CNWs, a macroscopic phase separation occurs with only EHEC because of the dominating hydrophobic zones. A schematic illustration of the temperature-induced gelation is depicted in Fig. 3(a), where the CNWs act as physical crosslinkers in the network, thereby strengthening the gel-network. In addition, the CNWs carry charges that contribute to the swelling characteristics of the gel-network. The conjecture is that the CNWs improve the connectivity of the network and also impose some structural changes of the network as indicated above. This matches the observation [45] for chemically crosslinked hydrogels, where the gel strength of incipient gels was found to increase with increasing cross-linker agent. Rheological studies on hydrogels consisting of agarose [20], gelatin [19], κ -carrageenan [18], poly(vinyl alcohol) [17], and carboxymethyl cellulose/hydroxyethyl cellulose [16] all exhibited a significant reinforcement effect upon incorporation of CNWs.

To gain insight into the rheological behavior of semidilute EHEC solutions (2 wt%; 25 $^{\circ}\text{C}$) at different levels of CNW addition, the effect of shear rate on the viscosity is illustrated in Fig. 3(b). The idea is to obtain information about the stability of the transient network under shear flow and how the viscosity of the EHEC solution is changed upon addition of CNWs. It is evident that the viscosity at low shear rate gradually increases as the amount of CNWs rises. This indicates that even at ambient temperature there is considerable interaction between EHEC chains and CNWs. All samples are very sensitive to shear rate and the suspensions exhibit shear-thinning already at very low shear rates; at high shear rates the viscosity curves fuse and the reinforcement effect is lost. The strong shear-thinning effect is attributed to shear-induced disruption of the network. Although suspensions of CNWs also show shear thinning [26], we believe that network disruption is observed in Fig. 3(b), since the EHEC solution without CNWs shows a similar feature. These results clearly show that even in the presence CNWs, the network in the pre-gel region is easily breakable.

Rheo-SALS methods can be utilized to obtain information about macroscopic structural changes in EHEC solutions in the presence of CNWs under the influence of shear flow. Fig. 4 shows typical 2D SALS scattered intensity patterns in EHEC solutions (fixed polymer

Table 1
Characteristic gel properties of EHEC (2 wt%)/CNW (0.5, 1.0, 1.5 wt%) formulations.

| EHEC (wt%) | CNW (wt%) | Gel Point (°C) | Viscoelastic Exponent (n) | Gel strength (Pa.s ⁿ) | Fractal Dimension | Cloud Point (°C) |
|------------|-----------|----------------|-------------------------------|-----------------------------------|-------------------|------------------|
| 2.0 | 0.5 | 37.6 | 0.21 | 9.4 | 2.31 | 35.2 |
| 2.0 | 1.0 | 37.7 | 0.18 | 17.7 | 2.34 | 33.9 |
| 2.0 | 1.5 | 37.6 | 0.16 | 27.9 | 2.36 | 33.2 |

concentration of 2 wt%) with different amounts of added CNWs at different shear rates. In this wave vector region, all the SALS patterns are practically isotropic, which suggests that on this dimensional scale no major structural changes take place. However, there are some alterations that should be noted. It appears that enhanced scattered intensity is promoted by both increasing CNW concentration and augmented shear rate. It seems that at the highest shear rate (100 s⁻¹) the 2D-images are little affected by the amount of CNWs. These results suggest that a shear-induced rearrangement of the CNWs occurs at high shear rates and this tendency is favored by higher concentration of CNW.

3.2.2. EHEC/CNW suspensions in the presence of SDS

In the classical rheological studies on EHEC [1,2,9] hydrogels, usually SDS was added to achieve a balance between connectivity and swelling in the gel-matrix. In light of this it is interesting to observe the effect CNWs on the gelation features in the presence of SDS. In a similar

way as for the EHEC/CNW systems (see the discussion above), the gel points for the EHEC(2wt%)/SDS(4mm)/CNW (0, 0.05, 0.1, 0.2wt%) samples could easily be determined by employing Method 1 (Fig. S3) or Method 2 (Fig. S4). Both methods give the same gelling temperatures and they are accurately determined by these approaches.

The values of the characteristic gelation parameters for the EHEC(2wt%)/SDS(4mm)/CNW system measured at the same CNW concentrations as without SDS are collected in Table 2. The values of the gel points for the systems are near to 37 °C, which is close to the value observed for the systems without SDS. It is interesting to note that the values of the gel strength parameter S are much higher for these systems with SDS than for the corresponding ones without surfactant. The results clearly show that as the amount of CNWs increases, the value of S rises but the impact of CNWs on S is less pronounced than in the absence of surfactant. In general the values of the fractal dimension are somewhat smaller than for the systems without SDS. This trend suggests the evolution of a more “open” and less homogeneous gel-

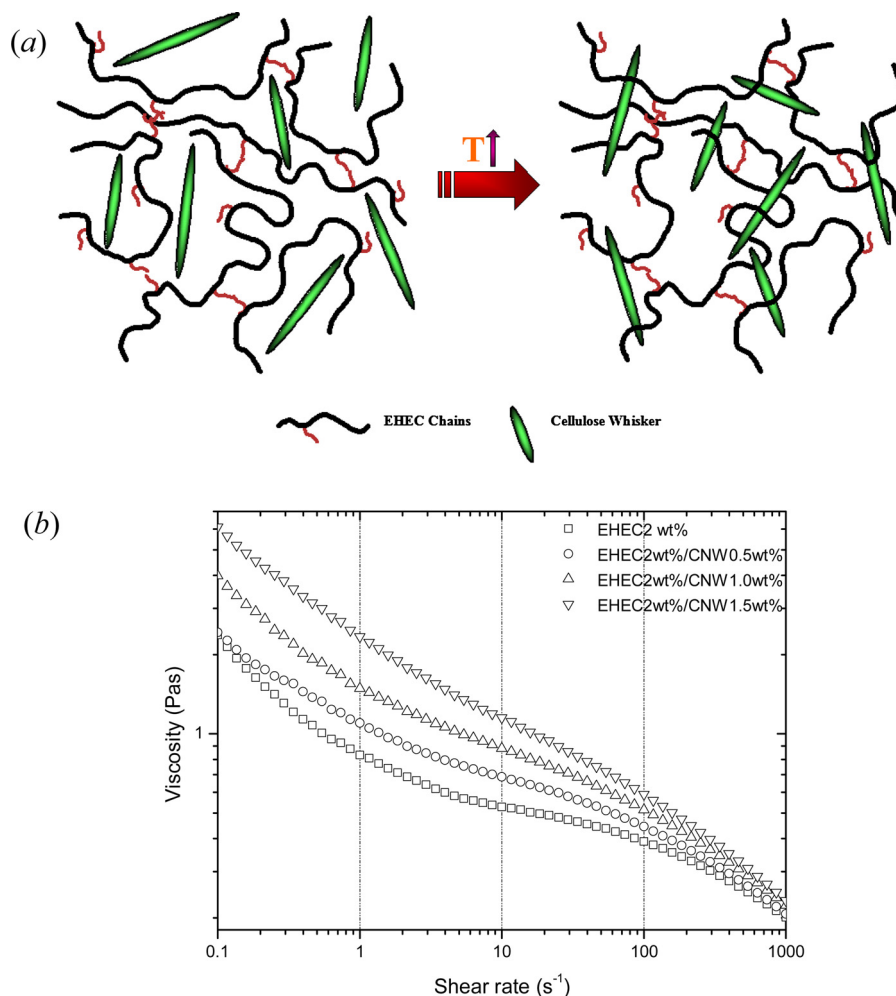


Fig. 3. (a) Schematic illustration of temperature-induced gelation of aqueous EHEC in the presence of CNWs. The small red chains represent hydrophobic stickers. (b) Effect of shear rate on the viscosity of EHEC (2wt%) aqueous solutions at the indicated levels of CNWs addition at 25 °C (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

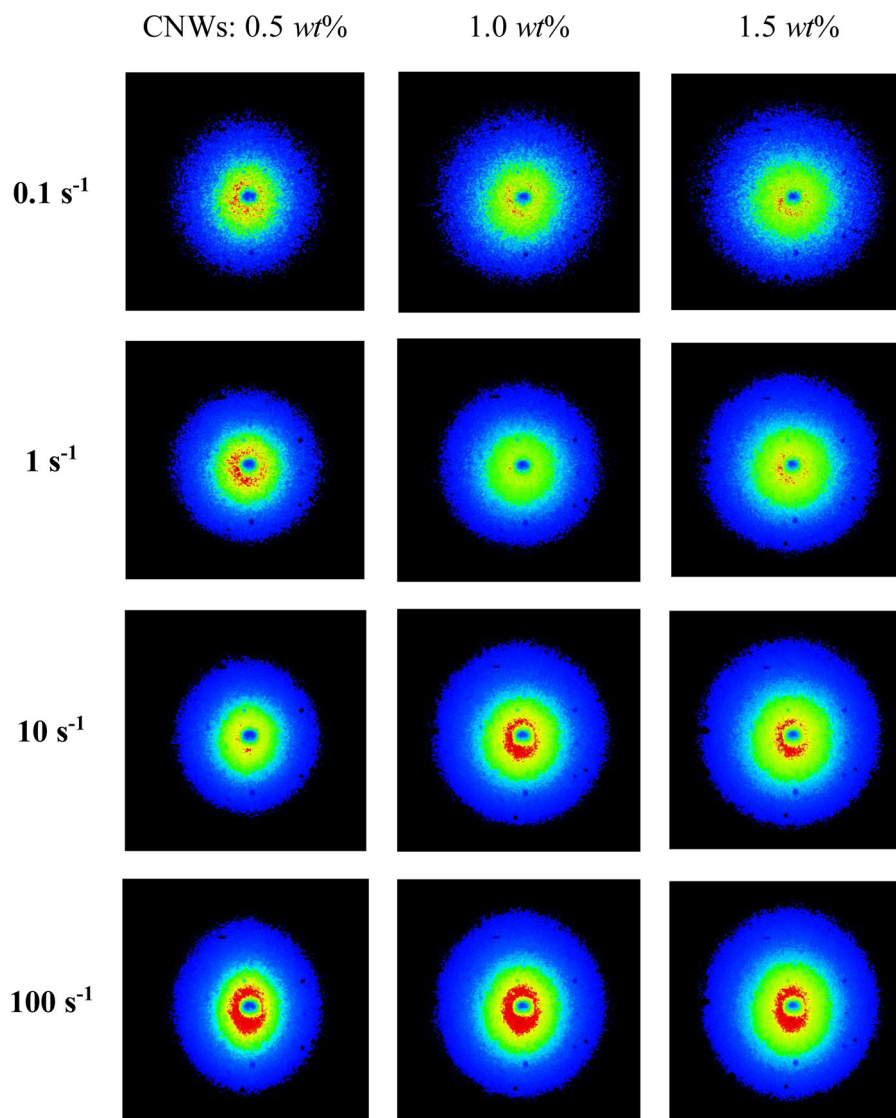


Fig. 4. 2D-SALS patterns of EHEC (2 wt%)/CNWs (0.5, 1.0, 1.5 wt%) aqueous suspensions at 25 °C and the indicated shear rates.

Table 2

Characteristic gel properties of EHEC (2 wt%)/SDS (4 mm)/CNW (0.5, 1.0, 1.5 wt%) formulations.

| EHEC(wt %)/SDS (mm) | CNW (wt%) | Gel Point (°C) | Viscoelastic Exponent (n) | Gel Strength (Pa.s ⁿ) | Fractal Dimension |
|---------------------|-----------|----------------|-------------------------------|-----------------------------------|-------------------|
| 2 : 4 | 0 | 36.7 | 0.32 | 35.1 | 2.20 |
| 2 : 4 | 0.05 | 36.9 | 0.30 | 38.2 | 2.22 |
| 2 : 4 | 0.1 | 36.8 | 0.29 | 42.5 | 2.23 |
| 2 : 4 | 0.2 | 37.2 | 0.27 | 54.3 | 2.25 |

network when SDS is added to the samples.

These results show that the gel strength is substantially enhanced in the presence of SDS. The picture that emerges is that both polymer-surfactant interactions and polymer-CNW connections contribute to the connectivity and strength of the gel-network. A schematic illustration of the interactions in connection with gel formation is displayed in Fig. 5(a).

Shear-rate viscosity curves of semidilute EHEC/SDS solutions (2 wt %; 25 °C) at different levels of CNW addition are shown in Fig. 5(b). The values of the viscosity at low shear rate are virtually the same as without SDS (between 2 and 6 Pa.s depending on the CNW

concentration) and the effect of SDS is marginal at all levels of CNW addition. The effect of increasing the amount of CNW on the viscosity is the same as before, but the impact of shear thinning is less pronounced and with SDS present almost Newtonian behavior is observed at low shear rate. This is in contrast to the strong shear thinning features, even at low shear rates, observed for the systems without SDS (cf. Fig. 3(b)). Since a similar weak shear-thinning behavior is also observed for the EHEC/SDS system, it seems that the EHEC-SDS interactions are crucial for the plateau-like region at low shear rates and the stabilization of the network. Addition of CNWs to the system increases the zero-shear viscosity and this supports our conjecture that the whiskers contribute to the strengthening of the network.

Rheo-SALS results at different shear rates for EHEC (2 wt%)/SDS (4 mm) aqueous solutions with various levels of added CNWs are displayed in Fig. 6. We discern that the 2D scattered intensity profiles become more pronounced with increasing amount of whiskers at all shear rates. This can be partly ascribed to the increased number of scattering entities and correlated with the strengthening of the network as the level of CNWs rises, in accordance also with the increase of the shear viscosity as the concentration of CNWs is increased. At low amounts of CNWs, the SALS patterns are virtually isotropic, which suggests that on this dimensional scale no major structural changes occur. However, at high shear rates and high levels of whiskers

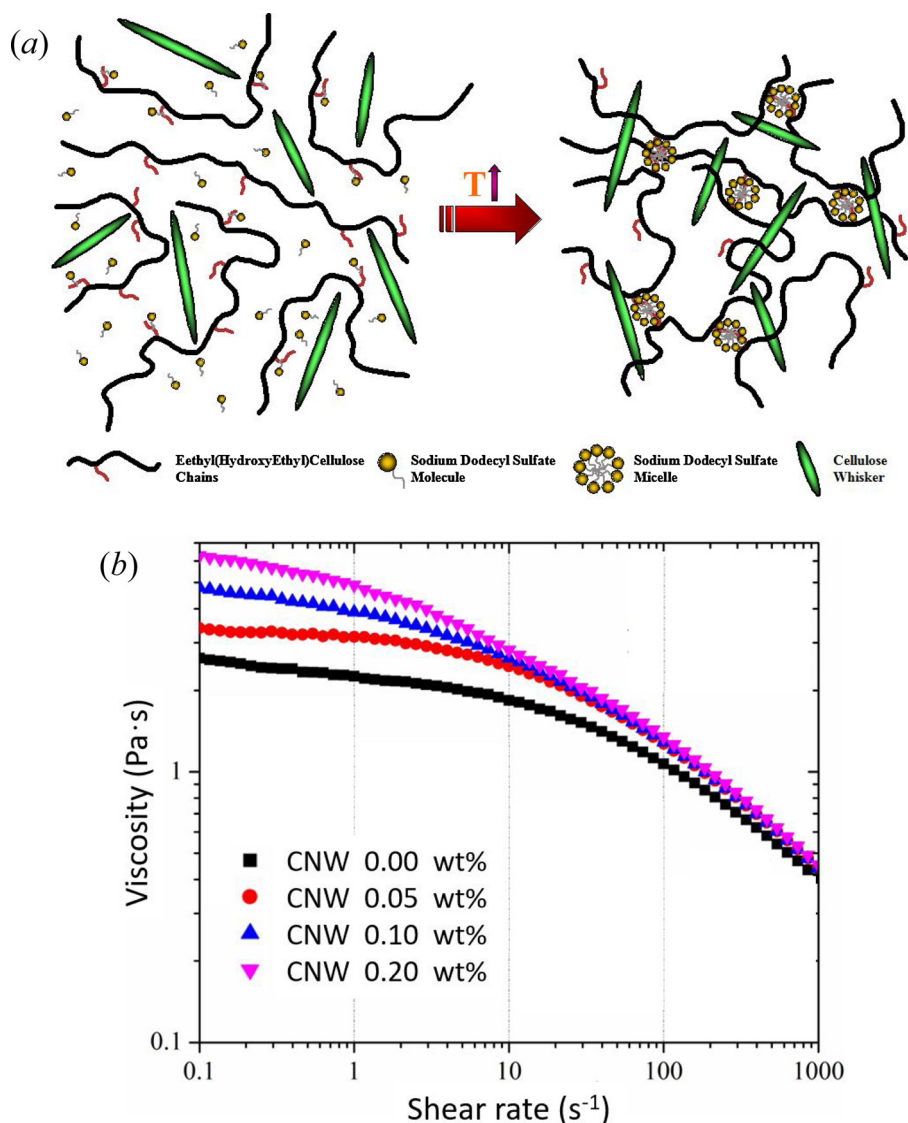


Fig. 5. (a) Schematic illustration of presumptive interactions involved in the temperature induced sol-gel transition of the EHEC/CNW/SDS aqueous system. The small red chains represent hydrophobic stickers. (b) Effect of shear rate on the viscosity of EHEC(2wt%)/SDS(4mm) aqueous solutions consisting of 0, 0.05, 0.1, and 0.2wt% cellulose nanowhiskers at 25 °C (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

addition, the scattered intensity profile exhibits an anisotropic pattern (elliptical image) with the major axis perpendicular to the flow direction. This type of behavior is frequently a signal of strong concentration fluctuations that are coupled to the applied mechanical stresses [46,47]. Since the scattered intensity profile for the EHEC/SDS system is practically unaffected by the shear rate and the intensity rises with increasing amount of added CNW, it is likely that shear-induced structural changes can be attributed to orientation and alignment of CNWs.

4. Conclusions

In previous studies [1,2,9–14] it has been established that semi-dilute aqueous solutions of EHEC in the presence of an ionic surfactant form hydrogels at elevated temperatures. However, potential toxicity issues of common ionic surfactants hamper the pharmaceutical use of EHEC-based thermogels. In this study, we confirmed our hypothesis that addition of negatively charged cellulose nanowhiskers to semi-dilute EHEC solutions can lead to formation of a stable hydrogel at elevated temperatures (ca. 37 °C), without the use of an ionic surfactant. Hydrogels combined with cellulose nanoparticles have been

studied previously, but this has been either with chemical crosslinking [16,19,21], or on different systems ([17,18,20]). To our knowledge this is therefore the first description of the properties of EHEC/CNW gels, formed without the use of an ionic surfactant or any other additives. We find that the gel temperature is practically independent of the amount of added CNWs, whereas the trend of the fractal dimension indicates the development of a “tighter” incipient gel-network with increasing amount of added CNW to the EHEC sample. It is shown that the whiskers contribute to the strength of the incipient gel-network, with the gel strength increasing as the level of CNWs rises. In the presence of an ionic surfactant, the system (EHEC/CNW/SDS) exhibits similar gelling features as without SDS, but in this case the incipient gel-network is more open and the gel strength parameter is significantly higher, suggesting that both polymer surfactant interactions and connectivity provided by CNWs contribute to the strength of the incipient gel network. The shear viscosity measurements on EHEC/CNW suspensions revealed marked shear thinning effects even at low shear rates, indicating that the network prior to gelation is quite fragile and easy to disrupt. In the case of the EHEC/CNW/SDS system, pronounced shear thinning was found at high shear rates, but a virtually Newtonian behavior was detected at low shear rates, suggesting that polymer-

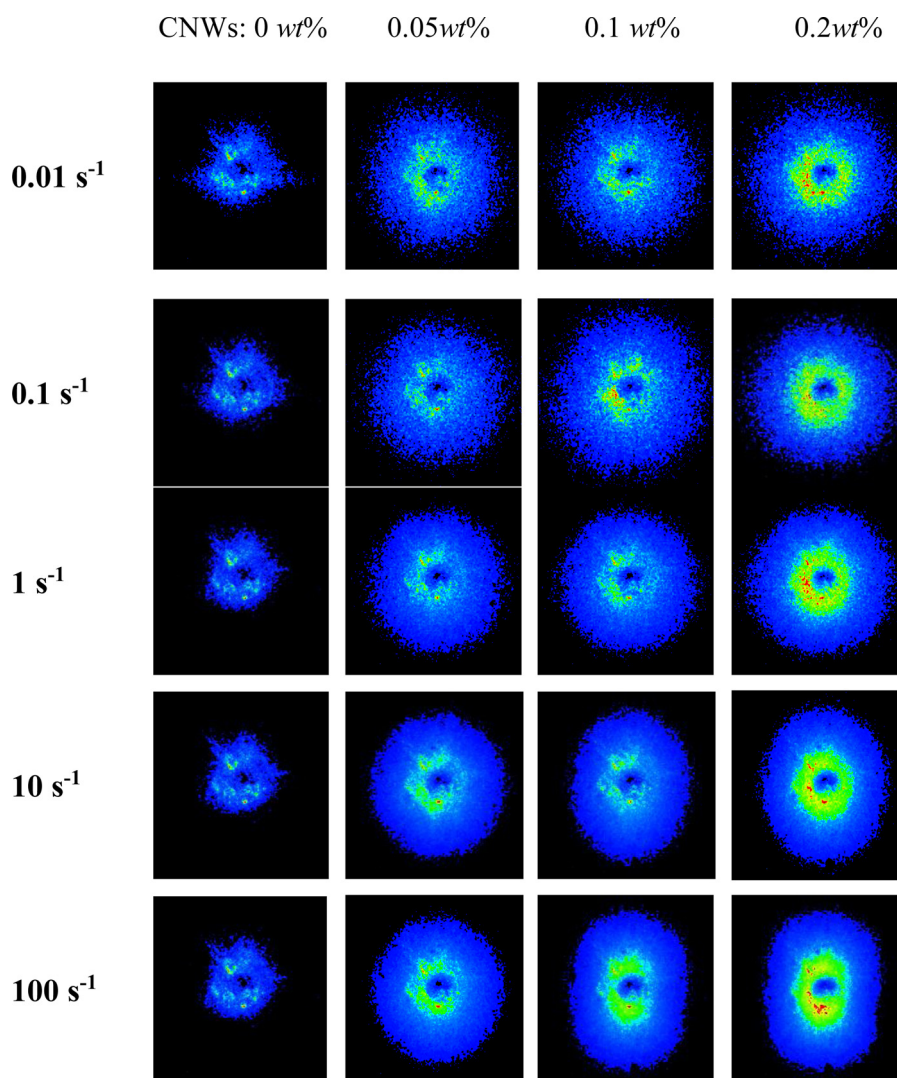


Fig. 6. 2D-SALS images of EHEC(2wt%)/SDS(4mM) aqueous solutions comprising 0, 0.05, 0.1, and 0.2 wt% cellulose nanowhiskers at 25 °C and at the indicated shear rates (0.01, 0.1, 1.0, 10, 100 s⁻¹).

surfactant interactions play an important role. For the EHEC/CNW system, the 2D intensity scattering profiles are isotropic, suggesting no major structural changes on the probed dimensional scale, whereas for the EHEC/CNW/SDS system, the profiles become anisotropic (elliptical) at high shear rates and augmented levels of whiskers, indicating enhanced concentration fluctuations and shear-induced structural alterations, which probably can be traced to alignments of CNWs. We therefore conclude that addition of CNWs can to a large extent control the viscoelastic and gelling properties of EHEC-based hydrogels, giving properties that are in several respects different from those obtained using SDS. These results should be highly relevant for future work concerning fine-tuning of the properties of such hydrogels, possibly including *in vivo* tests of hydrogel stability as well as of release dynamics of incorporated pharmaceutical compounds. An interesting finding is that SDS interacted differently with EHEC than with CNWs, but the gel point is virtually not affected by the additives.

Author statement

Prof. Mirzadeh has initiated the project and discussed the results. Dr. E. Dashtimoghdam and R. Nasseri have produced most of the data and helped in the analyses of the results. Dr. K.D. Knudsen has carried out the SANS experiments and analyzed the data and also contributed in the writing of the manuscript. Prof. B. Nyström and Dr. H. Salimi-

Kenari have contributed in the interpretation of the results and written large parts of the manuscript.

Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could influence the work reported here.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.colsurfa.2020.124489>.

References

- [1] B. Nyström, B. Lindman, Dynamic and viscoelastic properties during the thermal gelation process of a nonionic cellulose ether dissolved in water in the presence of ionic surfactants, *Macromolecules* 28 (1995) 967–974.
- [2] A.-L. Kjøniksen, B. Nyström, B. Lindman, Dynamic viscoelasticity of gelling and nongelling aqueous mixtures of ethyl (hydroxyethyl) cellulose and an ionic surfactant, *Macromolecules* 31 (1998) 1852–1858.
- [3] X.-Z. Zhang, D.-Q. Wu, C.-C. Chu, Synthesis, characterization and controlled drug release of thermosensitive IPN-PNIPAAm hydrogels, *Biomaterials* 25 (2004) 3793–3805.
- [4] J. Jagur-Grodzinski, Polymeric gels and hydrogels for biomedical and pharmaceutical applications, *Polym. Adv. Technol.* 21 (2010) 27–47.

- [5] Y. Hu, J.-O. You, D.T. Auguste, Z. Suo, J.J. Vlassak, Indentation: a simple, non-destructive method for characterizing the mechanical and transport properties of pH-sensitive hydrogels, *J. Mater. Res.* 27 (2012) 152–160.
- [6] A. Galperin, T.J. Long, S. Garty, B.D. Ratner, Synthesis and fabrication of a degradable poly (N-isopropyl acrylamide) scaffold for tissue engineering applications, *J. Biomed. Mater. Res. A* 101 (2013) 775–786.
- [7] N.K. Khorshid, K. Zhu, K.D. Knudsen, S. Bekhradnia, S.A. Sande, B. Nyström, Novel structural changes during temperature-induced self-assembling and gelation of PLGA-PEG-PLGA triblock copolymer in aqueous solutions, *Macromol. Biosci.* 16 (2016) 1838–1852.
- [8] J.E. Nielsen, K. Zhu, S.A. Sande, L. Kováčik, D. Cmarko, K.D. Knudsen, B. Nyström, Structural and rheological properties of temperature-responsive amphiphilic triblock copolymers in aqueous media, *J. Phys. Chem. B* 121 (2017) 4885–4899.
- [9] B. Nyström, H. Walderhaug, F. Hansen, B. Lindman, Rheological behavior during thermoreversible gelation of aqueous mixtures of ethyl (hydroxyethyl) cellulose and surfactants, *Langmuir* 11 (1995) 750–757.
- [10] B. Cabane, K. Lindell, S. Engström, B. Lindman, Microphase separation in polymer-surfactant systems, *Macromolecules* 29 (1996) 3188–3197.
- [11] I. Nahrungbauer, Polymer – surfactant interaction as revealed by the time dependence of surface tension. The EHEC/SDS/water system, *Langmuir* 13 (1997) 2242–2249.
- [12] E. Hoff, B. Nyström, B. Lindman, Polymer – surfactant interactions in dilute mixtures of a nonionic cellulose derivative and an anionic surfactant, *Langmuir* 17 (2001) 28–34.
- [13] R. Lund, R.A. Lauten, B. Nyström, B. Lindman, Linear and nonlinear viscoelasticity of semidilute aqueous mixtures of a nonionic cellulose derivative and ionic surfactants, *Langmuir* 17 (2001) 8001–8009.
- [14] M.T. Calejo, A.-L. Kjøniksen, E.F. Marques, M.J. Araújo, S.A. Sande, B. Nyström, Interactions between ethyl (hydroxyethyl) cellulose and lysine-based surfactants in aqueous media, *Eur. Polym. J.* 48 (2012) 1622–1631.
- [15] K. Lindell, B. Cabane, Structures of physical gels in the EHEC – SDS – water system, *Langmuir* 14 (1998) 6361–6370.
- [16] Q. Dai, J.F. Kadla, Effect of nanofillers on carboxymethyl cellulose/hydroxyethyl cellulose hydrogels, *J. Appl. Polym. Sci.* 114 (2009) 1664–1669.
- [17] T. Abitbol, T. Johnstone, T.M. Quinn, D.G. Gray, Reinforcement with cellulose nanocrystals of poly (vinyl alcohol) hydrogels prepared by cyclic freezing and thawing, *Soft Matter* 7 (2011) 2373–2379.
- [18] D.G. Martínez, M. Stading, A.-M. Hermansson, Viscoelasticity and microstructure of a hierarchical soft composite based on nano-cellulose and κ -carrageenan, *Rheol. Acta* 52 (2013) 823–831.
- [19] R. Dash, M. Foston, A.J. Ragauskas, Improving the mechanical and thermal properties of gelatin hydrogels cross-linked by cellulose nanowhiskers, *Carbohydr. Polym.* 91 (2013) 638–645.
- [20] K.J. Le Goff, C. Gaillard, W. Helbert, C. Garnier, T. Aubry, Rheological study of reinforcement of agarose hydrogels by cellulose nanowhiskers, *Carbohydr. Polym.* 116 (2015) 117–123.
- [21] Y. Chen, W. Xu, W. Liu, G. Zeng, Responsiveness, swelling, and mechanical properties of PNIPAA nanocomposite hydrogels reinforced by nanocellulose, *J. Mater. Res.* 30 (2015) 1797–1807.
- [22] M.A.S. Azizi Samir, F. Alloin, A. Dufresne, Review of recent research into cellulose whiskers, their properties and their application in nanocomposite field, *Biomacromolecules* 6 (2005) 612–626.
- [23] S.J. Eichhorn, Cellulose nanowhiskers: promising materials for advanced applications, *Soft Matter* 7 (2011) 303–315.
- [24] Y. Habibi, L.A. Lucia, O.J. Rojas, Cellulose nanocrystals: chemistry, self-assembly, and applications, *Chem. Rev.* 110 (2010) 3479–3500.
- [25] M.A. Hubbe, P. Tayeb, M. Joyce, P. Tyagi, M. Kehoe, K. Dimic-Misic, L. Pal, Rheology of nanocellulose-rich aqueous suspensions: a review, *BioResources* 12 (2017) 9556–9661.
- [26] S. Shafiei-Sabet, W.Y. Hamad, S.G. Hatzikiriakos, Rheology of nanocrystalline cellulose aqueous suspensions, *Langmuir* 28 (2012) 17124–17133.
- [27] R. Nasser, N. Mohammadi, Starch-based nanocomposites: a comparative performance study of cellulose whiskers and starch nanoparticles, *Carbohydr. Polym.* 106 (2014) 432–439.
- [28] M.M. Hasani-Sadrabadi, E. Dashtimoghdam, R. Nasser, A. Karkhaneh, F.S. Majedi, N. Mokarram, P. Renaud, K.I. Jacob, Cellulose nanowhiskers to regulate the microstructure of perfluorosulfonate ionomers for high-performance fuel cells, *J. Mater. Chem. A* 2 (2014) 11334–11340.
- [29] K. Zhu, H. Jin, A.-L. Kjøniksen, B. Nyström, Anomalous transition in aqueous solutions of a thermoresponsive amphiphilic diblock copolymer, *J. Phys. Chem. B* 111 (2007) 10862–10870.
- [30] E. Dashtimoghdam, G. Bahlakeh, H. Salimi-Kenari, M.M. Hasani-Sadrabadi, H. Mirzadeh, B. Nyström, Rheological study and molecular dynamics simulation of biopolymer blend thermogels of tunable strength, *Biomacromolecules* 17 (2016) 3474–3484.
- [31] E. Dashtimoghdam, H. Mirzadeh, F.A. Taromi, B. Nyström, Thermoresponsive biopolymer hydrogels with tunable gel characteristics, *RSC Adv.* 4 (2014) 39386–39393.
- [32] A. Izuka, H.H. Winter, T. Hashimoto, Molecular weight dependence of viscoelasticity of polycaprolactone critical gels, *Macromolecules* 25 (1992) 2422–2428.
- [33] J.C. Scanlan, H.H. Winter, Composition dependence of the viscoelasticity of end-linked poly (dimethylsiloxane) at the gel point, *Macromolecules* 24 (1991) 47–54.
- [34] H.H. Winter, F. Chambon, Analysis of linear viscoelasticity of a crosslinking polymer at the gel point, *J. Rheol.* 30 (1986) 367–382.
- [35] D.F. Hodgson, E.J. Amis, Dynamic viscoelasticity during sol–gel reactions, *J. Non. Solids* 131 (1991) 913–920.
- [36] M. Muthukumar, Screening effect on viscoelasticity near the gel point, *Macromolecules* 22 (1989) 4656–4658.
- [37] P. Terech, L. Chazeau, J. Cavaille, A small-angle scattering study of cellulose whiskers in aqueous suspensions, *Macromolecules* 32 (1999) 1872–1875.
- [38] F. Tanaka, W.H. Stockmayer, Thermoreversible gelation with junctions of variable multiplicity, *Macromolecules* 27 (1994) 3943–3954.
- [39] F. Tanaka, Phase formation of associating polymers: gelation, phase separation and microphase formation, *Adv. Colloid Interface Sci.* 63 (1996) 23–40.
- [40] F. Tanaka, Thermoreversible Gelation With Multiple Junctions in Associating Polymers, *Hydrocolloids*, Elsevier, 2000, pp. 25–34.
- [41] Y. Liu, R. Pandey, Sol–gel phase transitions in thermoreversible gels: onset of gelation and melting, *J. Chem. Phys.* 105 (1996) 825–836.
- [42] M. Muthukumar, Dynamics of polymeric fractals, *J. Chem. Phys.* 83 (1985) 3161–3168.
- [43] C. Yamane, T. Aoyagi, M. Ago, K. Sato, K. Okajima, T. Takahashi, Two different surface properties of regenerated cellulose due to structural anisotropy, *Int. Polym. Process. J. Polym. Process. Soc.* 38 (2006) 819–826.
- [44] B. Lindman, B. Medronho, The subtleties of dissolution and regeneration of cellulose: breaking and making hydrogen bonds, *BioResources* 10 (2015) 3811–3814.
- [45] H.S. Kenari, M. Imani, E. Dashtimoghdam, A. Maleki, B. Nyström, A. Nodehi, Oscillatory rheometric tracing of dextran crosslinking reaction in aqueous semidilute solutions—Effects of formulation on the gelation properties, *Polymer* 54 (2013) 2999–3007.
- [46] E. Helfand, G.H. Fredrickson, Large fluctuations in polymer solutions under shear, *Phys. Rev. Lett.* 62 (1989) 2468.
- [47] X.-L. Wu, D. Pine, P. Dixon, Enhanced concentration fluctuations in polymer solutions under shear flow, *Phys. Rev. Lett.* 66 (1991) 2408.