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RHEOLOGICAL BEHAVIOUR OF SONOCHEMICALLY PREPARED BACTERIAL CELLULOSE AQUEOUS DISPERSIONS

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Abstract.*Bacterial cellulose (BC) is an environmentally-friendly polymeric material that has high purity, high crystallinity, a high degree of polymerisation, high tensile strength and strong biological adaptability,all of whichproperties make it interesting for various applications. Two different approaches in purifying BC are demonstrated, water and alkaline methods, and in the alkaline case followed by ultrasoundtreatment. The different approaches in BC purification and sonochemical processingare shown to affect drastically the morphology of the BC fibrils, as seen from films by optical methods. Rheological investigation shows that there is a difference in manifestation of particle shape/crystallinity response and that sonicated samples show a rheological behaviour similar to that of liquid crystals in bothlinear and non-linear regimes. This opens up new possibilities for novel application of BCin the preparation of self-oriented composite materials.*
Keywords*:microbial cellulose, ultrasound, liquid crystals, aligned composites, self-assembly*

1. INTRODUCTION

Bacterial cellulose (BC)is being used in a broad range of fields, includingbiomedical and smart material applications, rheological modifiers in food, cosmetics and chemical products, foams, aerogels, and as reinforcement in a range of polymer matrices. Novel industrial applications of nanofibrillar cellulose (NFC), of which BC can be considered a subset, include production of electrically conductive all-polymer batteries, magnetic nanopaper, additive in paper coatings, and in the cellulose nanocrystal form (CNC) as transparent films for electronic display applications[9, 17]. The rheological behaviour and structure of complex materials define the nature of their mechanical properties[3, 7]. This is more apparent withbio-polymeric materials, whose unique combination of mechanical properties derives from an architectural design that spans from nanoscale to macroscopicdimensions [13,17].

Macromolecules, in particular cellulose, control the intricate nano and microstructures of biocomposites, which can be probed rheologically in the process toward achieving functional material design concepts. By application of shear structuration conditions, BC suspensions can be tuned, making it possible to create controlled structure orientation and, thus, opening up new possibilities with clear industrial application potential in products and processes[6].

Polymer liquid crystals form a class of materials whose molecular structure and macro-scale arrangement are very diverse. The rheology of liquid crystals composed of small molecules has been already described by many authors [1,9, 11, 14]. Important aspects in related phenomena take into account anisotropy, where the viscosity along the direction is different from the viscosity perpendicular thereto [14]. Polymer liquid crystals all possess the usual characteristics of polymers, since they are composed of long chains that lead to a complex viscoelastic behaviour [2, 4, 5, 6]. Yet, their intrinsic orientation also makes them follow theconstraints of that orientation in relation to the direction and of elasticity due to its distortion [5].

The influence of the orientation leads to a set of six viscosity coefficients (a Newtonian fluid only has one) [14]. The rheology of nematic liquid crystals provides a very rich behaviour in respect to their viscosity. Entropic elasticity components originating from the polymer, as well as anisotropic viscosities and elasticity of distortion of the direction originating from its intrinsic orientation, are characteristics of thisnematicmesophaseorder [6, 10, 11, 15].

Rheological behaviour of cellulose suspensions could influence the properties of final dried films [16].The main reason for enthusiasm in obtaining liquid crystalline order within suspensions is that,once dewatered, these materials have excellent mechanical properties due to very high levels of molecular orientation in the solid phase [8, 11, 14].

The aim of this work is to determine the rheological behaviour of alkaline purified, sonochemically treated BC aqueous dispersions prior to their solvent evaporative drying process to obtain BC films.

2. MATERIALS AND METHODS

2.1*BC aqueous dispersions*

BC was purchased from a commercially available product, nata de coco gel cubes, distributed by PT Cocomas, Indonesia [9, 13, 17]. BC aqueous dispersions were prepared as reported by Tsalagkas *et al*. [17]. Briefly, BC was washed and soaked in distilled water until neutral pH (5-7) to remove the citric acid and other components added to nata de coco syrup. Then, a portion of the water purified BC was alkaline treated to remove bacterial cell debris and other soluble polysaccharides existing in nata de coco. Purification treatment was conducted by warming water purified nata de coco cubes in 0.01 M NaOH at 70 ºC for 2h under continuous stirring and rinsed at neutral pH.Subsequently, both water and alkaline purified nata de coco cubes wereblended, poured into silicone trays and dried at 50 ºC. After drying, BC films were cut and redispersed (0.1% w/w immersed in 80 cm^3 distilledwater). Water purified BC aqueous dispersions (W BC) were used as control. Alkaline treated BC dispersions were further submitted to ultrasonication (US BC). Sonication was directly applied at low frequency (20 kHz) in a cold water bath for 30 min using an ultrasonic probe (25 Wcm^{-2}).

Characterization of samples

The crystallinity was recorded with X-ray diffraction patterns at room temperature in the 5-80 °C 2Θ angle using an MDP Pro Panalyticaldiffractometer equipped with Xcelerator linear detector.

FE-SEM micrographs were obtained using a Zeiss ULTRA Plus (Oberjochen, Germany) instrument at an acceleration voltages of 1 and 2 kV. The suspensions were filtered through a gilded PC membrane and dried for 1 h at room temperature. All samples were coated with a highly conductive film of gold by Bal-Tec SCD 500.

Transmission electron microscopy (TEM) was applied in a morphological study conducted using a JEOL JEM 2100 at 100Kv. BC samples for TEM were sonicated for 1 min at ambient temperature, at 80 W. Then, a drop of diluted supernatant containing dispersed BC was deposited onto carbon-coated Cu grids, following removal of excess liquid by a piece of filter paper. Finally, the samples were dried at room temperature.

Zeta potential, ζ, of W BC and US BC aqueous dispersions were determined with a Zetasizer Nano ZS90 (Malvern Instruments) at room temperature. Smoluchowski's approximation was used to convert the electrophoretic mobility into aζ potential value.

2.2*Rheological measurements*

The rheological characterisations were performed using an Anton Paar 300 (Anton Paar Germany GmbH) equipped with a bob and cup geometry, with an operating gap of 1 mm.For oscillatory measurements,a pre-shear protocol was applied at angular frequencyω = 10 $rad.s^{-1}$ and strain amplitude, γ = 0.01 % for 100 s, followed by a rest time of a further 100 s. The linear viscoelastic range of the sample (LVE) was obtained from an amplitude sweep oscillatory test using constant angular frequency (ω = 1 $rad.s^{-1}$) with varying strain amplitude (γ = 0.01 - 500 %). Dynamic moduli, G' and G'', and complex viscosity (η^*) of BC dispersions were measured as a function of angular frequency (ω) over a downward frequency range (ω = 100 - 0.01 $rad.s^{-1}$) in frequency sweep oscillatory tests, with logarithmic spread of data points.Hysteresis of steady state flow measurements was obtained with a pre-shear protocol of 10 s^{-1} for 2 min and rest time of 2 min. In addition toη^* (ω), also steady state flow curves as a function of shear rate $\dot{\gamma}$ were used to investigate differences in flow behaviour between W BC and US BC. Hysteresis of flow curves were measured in downward and upwardshear rate sweeps$\dot{\gamma}$= 1000-0.01 s^{-1} and 0.01 – 1000s^{-1} with logarithmic spread of data point duration from 1 to 100 s.Structure recovery measurements were performed with the 3ITT test, consisting of three intervals: oscillation/rotation/oscillation. Once again a pre-shear protocol was applied at$\dot{\gamma}$= 10 s^{-1} for 20 s and rest time of 2 min. In the first interval of oscillation, the test was performed at constant angular frequency ω = 10 $rad.s^{-1}$and constant direct strain (DSO) strain γ = 0.01%, which has a value in the linear viscoelastic range (LVE) determined previously by an amplitude sweep test. During the second interval, a deformation was applied to the sample under a rotational regime with a medium to high shear rate of 500 s^{-1}.

Each type of measurement was performed five times, including the pre-shear protocol, and an average value with deviation was calculated.

3. RESULTS AND DISCUSSION

Crystallinity index (Cr. I) and maximum degradation temperature (T_{dmax}) values of dried, alkaline purified, sonochemically treated BC films were found to be around 78 % and 374 ºC, respectively [17]. ζpotential values of W BC and US BC were measured anionic at -35.9 ±3.14 and -21.8 ±1.05 mV, respectively.

FE-SEM and TEM images presented in Fig.1 displayed differences in the homogeneity, transparency, size and number of aggregated BC microfibrils after ultrasound treatment [17].

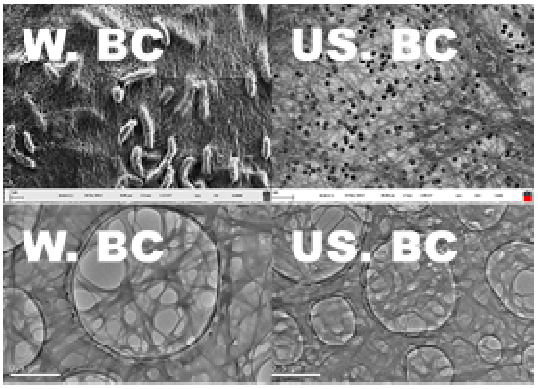

Fig. 1 FE-SEM and TEM images of W. BC and US. BC aqueous dispersions.

Once the linear region was specified with an amplitude sweep oscillatory rheological measurements, a frequency sweep was used to characterise the material's linear viscoelastic response to small amplitude deformations [3,7]. Results from oscillatory measurements are presented in Fig. 2a-band reveal that both BC suspensions, being at very low solid content range of 0.1%, are like those of a low aggregated liquid crystalline form [2, 9, 14]. The cross-over point of G' and G'' is at lower strain rates for US than for W samples, probably due to re-ordering of the liquid crystalline structure. Fig. 2bshows the frequency sweep measurement, which reveals a behaviour of G' typical for liquid crystals, with a cross-overlapping of $G'$and G'' occurring two times, at distinct values of ω. G'' is seen to increase with increasing frequency, which is typical for low aggregated networks, as presented schematically in Fig. 3.The liquid crystalline nature of the BC suspensions and the associated observed presence of change in orientation during flow, is obvious from the curves presented in Fig. 4. This is demonstrated by the presence of two distinct hysteresis loops, defining*bistability*,typical for liquid crystals [10, 14, 15]. Evident also is the distinct difference between the two BC treatments, i.e. between (i) greater shear thinning and (ii)larger hysteresis loops each in the case of the US samples, indicating structuration during shear flow [5, 10, 11]. This bistability is also present in the W BC samples, but to a lesser extent, as, due to the lower energy less dispersing pre-treatment of water washing (W), the rods are not as free to move as in the case when they were alkali washed and sonicated (US).

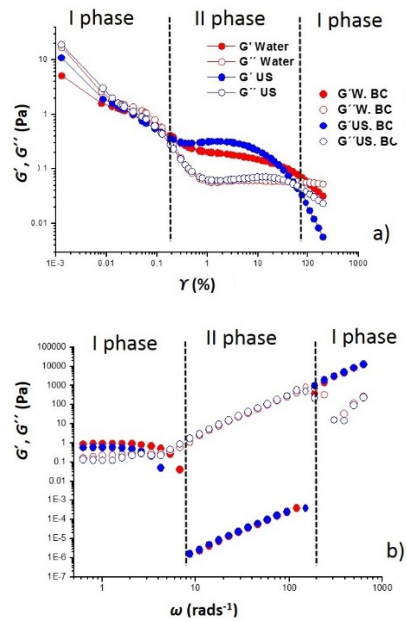

Fig 2Viscoelastic measurements for US BC and W BC suspensions at 0.1 % solids content: a) amplitude sweep, b) frequency sweep. Elastic modulus (G') closed symbols, loss modulus (G'') open symbols.

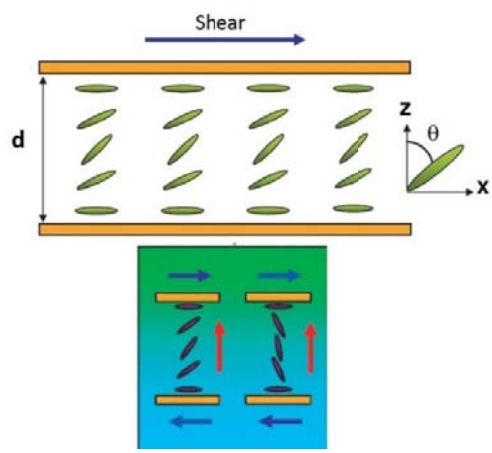

Fig. 3 Schematics of the behaviour of crystalline BC rods that show *bistability* in orientationwhen subjected to shear flow, similar to liquid crystals [11].

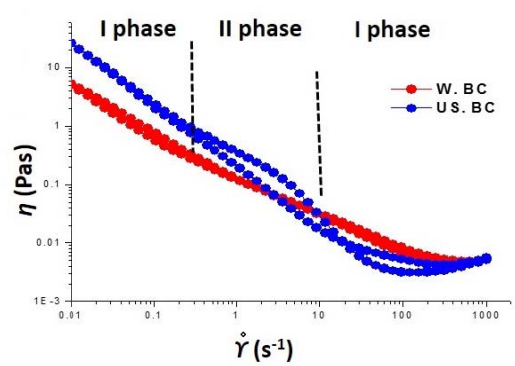

Fig. 4 Dynamic viscosity $\eta$for US BC and W Csuspensions at 0.1 % solids content as a function of shear rate. Two hysteresisloops are present in the flow

curves of both samples, with the US.BC samples having more pronounced hysteresis loops and greater shear thinning behaviour.

Structure recovery thixotropic 3ITT tests reveal the break-up of elastic structure during the middle high shear interval, as presented in Fig.5. The recovery path of liquid crystalline structures is seen through the overshooting character of G', and the slow recovery towards the initial value ofG'_0 [14]. Overshoot is higher for the US BC samples, which correlates well with data in Figs. 3 and 4. The structure recovery 3ITT measurements also indicate the faster recovery of the liquid crystalline structure in the US BC case,after the high shear field, due to the easier orientation of the better dispersed crystalline rods and the potential energy well "spring effect" typical for liquid crystal structures[1, 2, 14] .

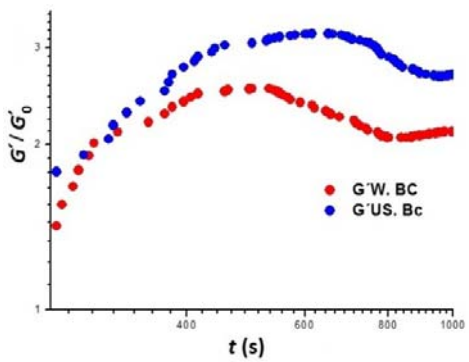

Fig.5 Elastic modulus (G') during the third interval in the oscillation – shear- oscillation protocol of the 3ITT experiment, and the normalised elastic modulus (G'/G'_0).

4. CONCLUSIONS

The BC aqueous dispersions tested showed to be highly crystalline bio-polymers, which after being treateddisplayed a complex and richrheological behaviour, by borrowing their viscosity and entropic elasticity characteristics from polymers and their anisotropic viscosity, as well as elastic distortion, from liquid crystals. Alkaline purification increased the surface charge and removed BC debris contamination. The following ultrasound mechanical treatment, fibrillated and restructuredthe aggregated BC microfibrils, enhanced further dispersion, and increased the freedom of movement of the rod-like particles, such that they could readily develop a liquid crystalline behaviour.

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