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Thixotropic Suspensions of Self-Assembled Steroid Nanotubes: Structures, Kinetics and Rheological Specificities

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Summary: Characteristic structural, kinetical and rheological behaviors observed with alkaline aqueous suspensions of sodium lithocholate nanotubes are presented. The scaling behavior of the elastic shear modulus and yield stress as a function of concentration support the description of the fragile network with degrees of freedom at the hinges. The kinetics of nanotube formation mixes a component of the structural sequence for single aggregates and a collective component describing the relaxation of the distribution of tubes. SLC suspensions are easily shear-thinned (and oriented) in purely Newtonian shearing conditions.

Keywords: gels; nanotubes; rheology; SAXS; self-organization

Introduction

Assemblies of 1D species (dilute, semi-dilute solutions, and gels) are known to exhibit particular viscoelastic properties that depend on the persistence length of the fibers, their energy of scission, a statistical distance between crosslinks and the lifetime of the crosslinks. The description of the flowing properties raises a series of fundamental issues for which recent theoretical guidelines can be used.

Self-assembled rods and fibers are "equilibrium polymers" with an average molecular weight in thermal equilibrium. The length polydispersity is very large while the cross-sections are frequently monodisperse. Tubes with cross-sections in the nanoscale are of special interest due to their potential properties and applications in the field of nanosciences. For instance, release diffusion of active species across the internal cylindrical cavity, replication and functionalisation of the nanostructures are promising routes that need to be explored. The investigation of the fundamental background is also an active field and for instance, the mechanism of tube formation through intermediary species like fibrils, ribbons and helical ribbons remains a challenging question. The structural and rheological characterizations of such suspensions are prerequisite steps before implying the nanotubes in more complex reactions. We present here, characterizations of spontaneous assemblies of steroid nanotubes in alkaline aqueous solutions. Three remarkable features are highlighted such as the monodispersity of the cross-sections, the scaling with concentration of the elastic shear modulus and the kinetics of formation and equilibration of the nanotubes.

Material and Methods

Sodium lithocholate (SLC) nanotubes are formed by neutralizing the lithocholic acid (LCA) by a sodium hydroxyde aqueous solution. Small-angle X-ray scattering (SAXS) and electron microscopy methods are described elsewhere.[1,2]

Results and Analysis

Mature alkaline SLC suspensions contain tubular aggregates. SAXS characterizes the

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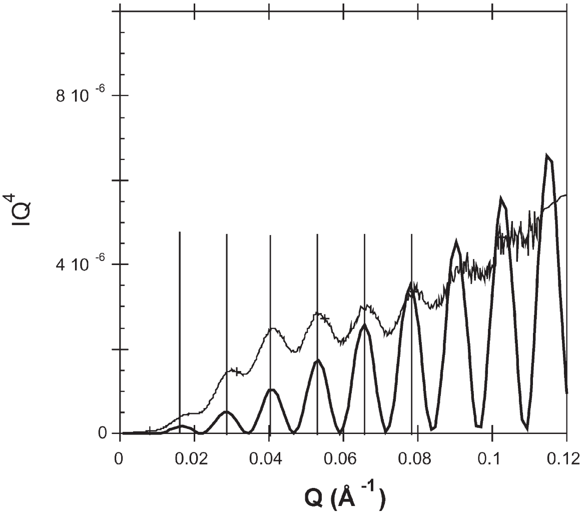

Figure 1.
SAXS (thin full line) for a LCA suspension (3 wt.%) in a sodium hydroxyde solution ($T = 0.25$ M). The representation emphasizes the interfacial scattering and form-factor oscillations whose Q-positions are compared to the theoretical ones (thick full lines and vertical bars).[2]

structures at the nanoscale. Figure 1 shows the interfacial scattering that compares the data to the theoretical scattering function for a long and rigid nanotube (external diameter $D_e = 52$ nm, internal cylindrical cavity with $D_i = 49$ nm). 8 oscillations are clearly seen that demonstrate the high level of cross-sectional monodispersity. The Q-positions at the apexes of the first five oscillations are well reproduced by the theory while beyond, small departures are observed. Small amplitude radial fluctuations of the tubes as well as the presence of a residual scattering from weak proportions of intermediate species (helical ribbons, co-axial tubes)[1] may produce such deviations.

The unidirectional character of the aggregates is confirmed by the electron micrograph of Figure 2 that shows the random organization found in the solid obtained by evaporation of the suspension. It also shows that during the evaporation, the system evolves from a liquid-like suspension ($C \sim 1$ wt.%) to a concentrated gel whose mechanical cohesion is provided by the solid-like 3D network of tubes. The evidence for the tubular structure is supported by the SAXS modeling and previous cryo-transmission electron microscopy studies.[2] Both techniques confirm that single walled nanotubes are formed.

The rheological signature (Figure 3) obtained through a frequency sweep experiment exhibits a G' profile that is more or less sloped depending upon the concentration, the aging time and handling condition. Over long time scales, the system exhibits a flattening of the viscosity (Newtonian pseudo plateau) concomitant with a decrease (by more than one order of magnitude) of the G' indicating the prevalence of slow relaxation processes. At shorter time scales, the mechanical response is more that of a soft visco-elastic solid.

The scaling of G' and the yield stress with the concentration can also inform about how the elastically active components are interacting. Figure 4 shows that power laws can describe the increase of the rheological quantities with C. An exponent of ca. 1.0 is found for G' while 1.4 is found for the yield stress. The great majority of thermoreversible networks made up of self-assembled low-mass molecules as found in molecular gels[3] exhibit a scaling exponent of 2.0.

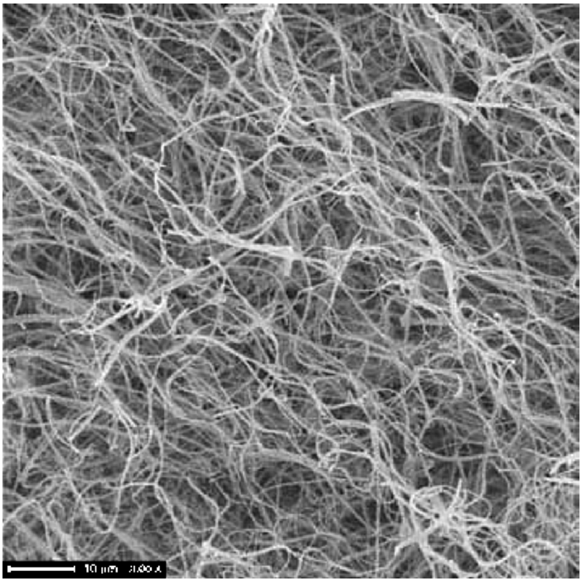

Figure 2.
Scanning electron micrograph of a SLC suspension slowly evaporated to a porous solid. The picture confirms that the network is composed of rod-like species whose persistence length is of the order of the contour length.

Different theoretical frameworks would predict such value from cellular solids[4] to entangled semiflexible networks[5] and rigid polymer network model.[6] Clearly, none of these contexts can be appropriate for SLC systems.

The elastically active elements in SLC suspensions are very rigid nanotubes for which the persistence length is estimated to be of the same order of magnitude than their total contour length (>microns). The way forces are transmitted through a network and which type of deformation of a single fiber gives the dominant contribution to the network elasticity is a difficult theoretical challenge. The deformation field in the network can be either affine or non-affine and such analysis can be done as a function of the length scale and degree of cross-links in the networks.[7–10] In these contexts,

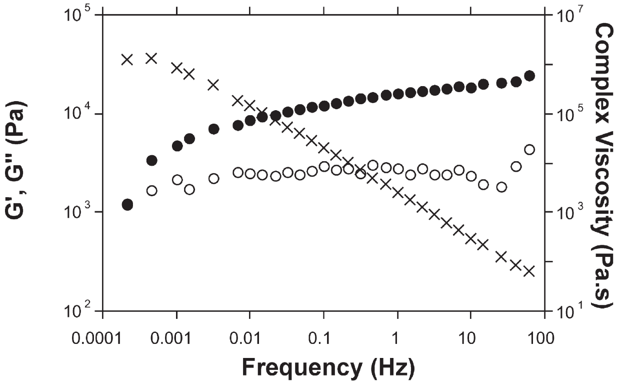

Figure 3.
Frequency sweep experiment (stress $\sigma = 10$ Pa) of a SLC suspension ($C_{LCA} = 2.87$ wt.%, $T_{NaOH} = 0.25$ M). G' (●), G'' (○), *viscosity* (x).

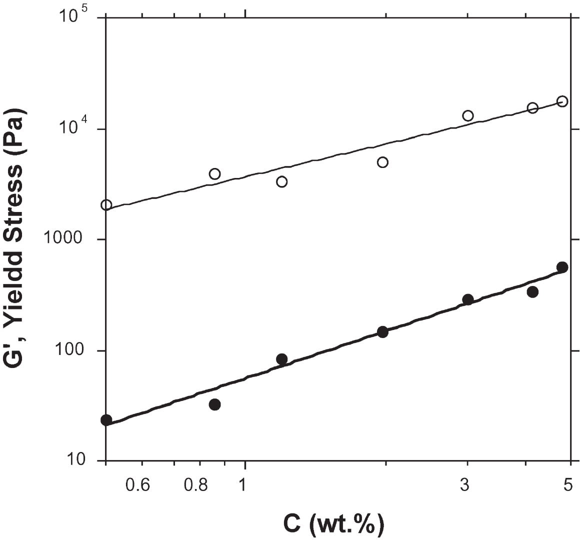

Figure 4.
Scaling of G' (●) and yield stress (○) *versusC*. The exponents for the power laws are respectively 1.0 and 1.4.

affine deformation would correspond to networks for which the energy is stored in the stretching modes of the fibers and a non-affine regime where the energy is stored primarily in the bending of the fibers. The experimental values of the exponents well inferior to 2 suggest that some entropy arguments have to be introduced by assuming either some degree of free rotation of the rod-like species at the crosslinks[6] (theoretical exponent 3/2) or distortions of undulations of the surrounding network (theoretical exponent 4/3)[11] or all bending undulations with a wavelength longer than a critical deflection length being perturbed by the network (theoretical exponent 5/3).[12] At this stage, the length polydispersity of the SLC nanotubes, the proportion of bundles of nanotubes, the accuracy itself of the measurements (exponent value ±0.15) are experimental limitations in the use of such idealized theoretical two-dimensional models. Still, compared to stiff SAFINs for which an exponent of 2 is found that is consistently modeled in a theoretical context of cellular solids (or energetic networks of rigidly connected rod-like fibers), it is clear that motional fluctuations participate to enhance the entropy of the SLC networks. Considering the similar rigidity of SLC tubes and fibers from conventional SAFINs, the most reasonable option is that the crosslinks at tube ends build a transient network with degrees of freedom at the hinges.

The fragility of the elaboration of the supramolecular architecture can be illustrated by two complementary sets of rheological experiments. The elastic shear modulus G' of a suspension of interacting colloidal species depends on their volume fraction, their form-factor, the nature of their interactions: rheometry is thus appropriate to study the kinetics of formation of SLC nanotubes. As for other techniques, there is a minimum time ("dead-time": t_d) required to mix the LCA powder to the NaOH aqueous solution before starting a measurement. Two types of measurements are shown on Figures 5. First, an "equilibration" study measures the equilibration process (at $\nu = 1$ Hz) in the gap of the rheometer of an already formed and stabilized SLC suspension (preparation time $t_p \sim 2{,}3$ days). A second type of experiment measures G' *versus* t (at $\nu = 1$ Hz) of a freshly prepared suspension ($t_p = t_d$) and is referred as a "formation kinetic" experiment in the following. Figure 5A shows three remarkable rheological features. First, G' evolves towards equilibration over a very large period of time

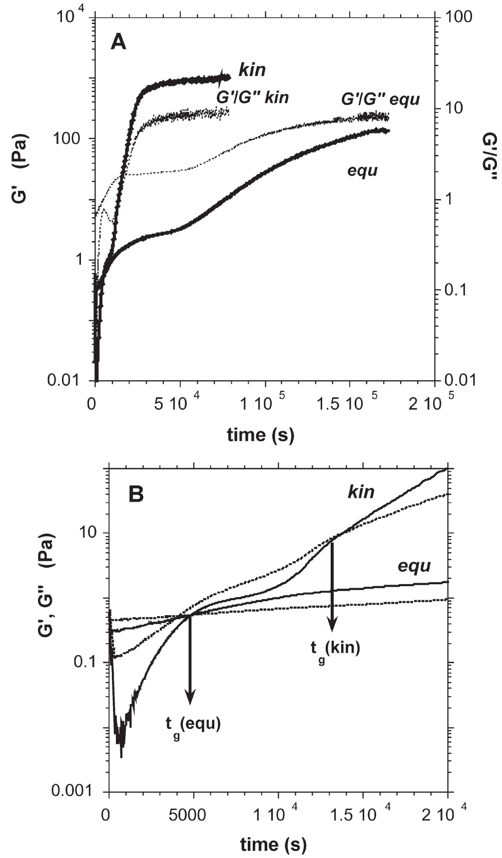

Figure 5.
Kinetic evolution of G' (bold) and G'/G'' (dotted) during the nanotube formation (label "kin") and equilibration of an already formed suspension (label "equ"). G'/G'' is shown to indicate the "gelation" threshold. B: enlargement of the first part of the kinetic to focus on the characteristic time (t_g) for overlap of the nanotubes at $G'/G'' = 1$.

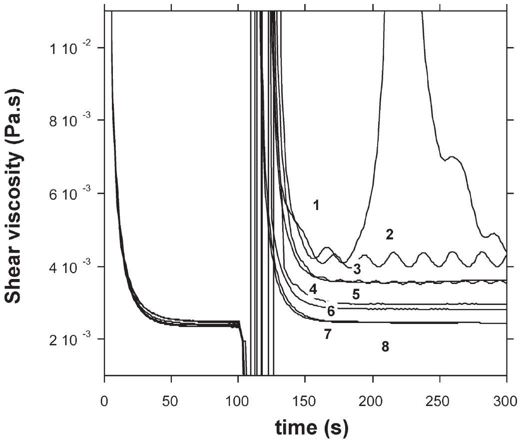

Figure 6.
Shearing experiments of a SLC suspension ($C = 0.57$ wt.%). The sequence of increasing applied shear stresses is (from top to bottom): 1,2: 0.05 Pa, 3: 0.075 Pa, 4: 0.1 Pa, 5: 0.2 Pa, 6: 0.3 Pa, 7: 1 Pa, 8: 5 Pa.

(typically a few thousands of minutes), the two types of experiments showing similar behaviors in this range. Second, the initial part of the kinetic curves shows that G' already starts at significant values (depending on the volume fraction) and suggests that a master piece of information concerning the first stages of the nucleation-growth of the self-assembled species cannot be reached. Thirdly, the cross-over between G' and G'' defines the kinetic region in which the interactions between the tubes (or growing tubes) is such that a solid-like transient network is formed. It appears that the equilibrated situation is reached much sooner with the "kinetic" experiments where tubes are directly generated within the gap of the rheometer (see also enlargement Figure 5B). The profile in a kinetic experiment is more complex with 3 parts: (i) a first G' decrease related to the shear-thinned very first short aggregates; (ii) a second increasing part appearing as a secondary maximum that reveals morphological changes during the structural sequence; (iii) a long increasing process towards equilibration.

These observations, suggest that, two types of kinetics can be more or less mixed in such rheological experiments: the kinetic variation of the number of elastically active species in the system and their orientation distribution within the gap. In an equilibration experiment, the specimen of dispersed mature tubes is loaded in the gap and oriented in a non-controlled way (depending on the protocol for the introduction): the kinetics is a relaxation processof the orientation correlated species. In a real kinetic experiment, despite the limitation of t_d, most of the species grow directly in the gap, the resulting specimen is not disturbed (oriented) by the introduction protocol in the gap and the equilibration plateau is reached sooner with a more complex profile at the first stages probably revealing the morphological changes in the system. On the contrary, the equilibration kinetics is much longer to reach equilibrium but the "gelation" time (at $G'/G'' \sim 1$) is faster reached since the mature tubes already exist and easily overlap. The increase of the

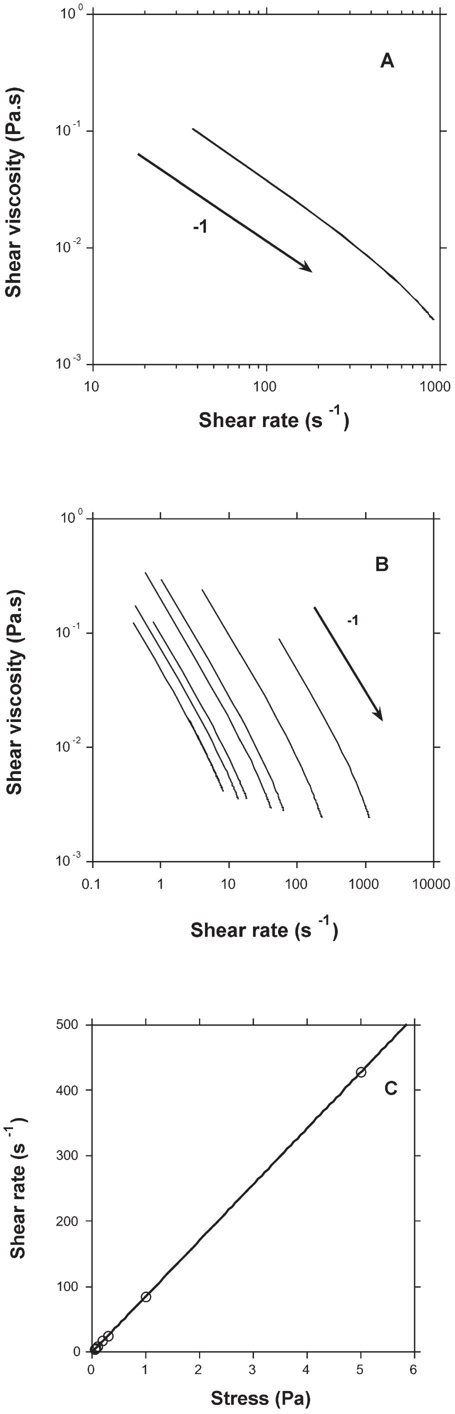

Figure 7.
Flow curves corresponding to Figure 6. A: preshear step. B: shearing at various stresses. C: shear rate *versus* stress curve.

storage modulus as a function of time is related to the randomization at rest of the shear-sensitive suspension as observed for other thixotropic suspensions.[13] The combination of a kinetic component due to the aggregate growth and a relaxation component related to the network distribution can produce complex profiles. Dilute suspensions and semi-dilute regimes of concentrations can produce simplified signatures whose profile analysis will be presented in a forthcoming work.

It remains that the suspensions can be easily shear-thinned. Their propensity to develop a transient network generating specific viscoelastic features is illustrated in Figure 6 describing a two-segment experiment with a suspension at $C = 0.573$ wt.% and $T_{NaOH} \sim 0.1$ M.

The first segment is a pre-shearing step (100 s at $\sigma = 4$ Pa) and the second one consists in shearing the system at increasing values of stress (and shear rates). Figure 6 shows that the equilibration value of the shear viscosity is naturally dependent on the applied stress (or shear rate) and that for the minimum applied value ($\sigma =$ 0.05 Pa), a transient network tends to form (with a sharp viscosity increase) and is rapidly destroyed (with a sharp viscosity decrease) in the shear flow (curve 1). The corresponding shear viscosity *versus* shear rate flow curves are shown in Figures 7. The first pre-shear segment lies in the single master flowing curve of Figure 7A while the set of flow curves in Figure 7B correspond to the different equilibrium stress (or shear rate) values. It comes out that at the horizontal line for a viscosity of 10^{-2} Pa.s, the corresponding set of shear rate-stress couples can be extracted providing a linear function $\dot{\gamma} = 85.02\sigma$. The observation demonstrates that the system behaves a purely Newtonian shear-thinned suspension of nanotubes with a viscosity $1/85.02 \sim 10^{-2}$ Pa.s. The absence of convexity/concavity confirms that no non-linear effects are involved and that no yield stress is allowed to develop in such shearing conditions.

In the semi-dilute range of concentrations, the SLC nanotubes form a transient network with fragile ‘‘crosslinks’’. These crosslinks can be mechanical ‘‘entanglements’’, electrostatic interactions by several tubes at their ends, swollen lyotropic hexagonal local organizations. In any case, the networks do not contain extended ‘‘junction zones’’ with high cohesive energy responsible of large values of the yield stress as found in usual SAFINs (self-assembled fibrillar networks) of molecular gels.[3,14] The above shear-thinning experiments in purely Newtonian suspensions of tubes suggest that the tubes are oriented along the shear direction. SAXS is convenient to identify such orientational effects as it is sensitive to the long-range fluctuations of electron density. As long as the distribution of the scatterers is random, the scattering intensity collected on a bidimensional detector will be isotropic with circular isointensity contour plots.

Conversely, with oriented systems, rods are position and/or orientation correlated and the scattering is dependent upon the angular position of the sample. If the direction of orientation is orthogonal (and vertical for simplicity) to the incident beam, non-zero contributions are obtained only for $\gamma = \pi/2$ (γ is the angle between $\vec{Q}$ and the rod axes) and a horizontal ‘‘equatorial’’ diffraction line is observed. Figure 8 illustrates the high ability of the SLC system

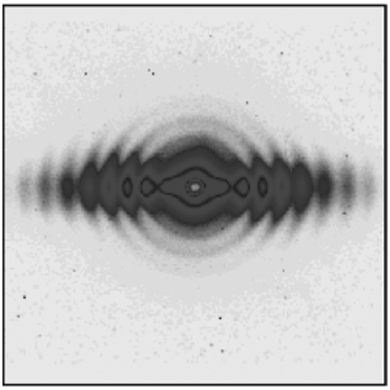

Figure 8.
Anisotropic X-ray low-angle scattering pattern of a SLC suspension under an elongational stress ($C = 7.9$ wt.%). The direction of elongation is vertical.

to be oriented under any external stress: a highly anisotropic scattering pattern is observed. Spots along the equatorial line combine both organizational features (intertube spacings but also apexes of the cross-sectional form-factor oscillations for very monodisperse nanotubes.[15]

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