Carbohydrate Polymers 112 (2014) 753–757

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Rheological study of chitosan acetate solutions containing chitin nanofibrils

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ARTICLE INFO

Article history:
Received 14 April 2014
Received in revised form 6 June 2014
Accepted 11 June 2014
Available online 26 June 2014

Keywords:
Rheology
Chitosan solutions
Chitin nanofibrils

ABSTRACT

Rheological properties of chitosan acetate solutions containing chitin nanofibrils (n-chitin) and biocompatible plasticizers intended for preparation of biodegradable films are reported in the steady, oscillatory and transient shear flow. The experiments were performed on slurries with an optimum proportion of 65/35 wt.% between chitosan and n-chitin in the films which was determined from our results of mechanical properties and absorption of water vapor.

The time-dependent dynamic experiments revealed the chitin nanofibrils as an effective "gelling agent" of chitosan phase. The phenomenon is explained by a chitosan-like surface of n-chitin and by the interactions inducing orientational cooperativity of chitosan molecules dissolved in close neighborhood of the anisotropic chitin nanofibrils.

Additions of glycerol or poly(ethylene glycol), improving mechanical properties of the films, delay significantly the onset of gelation of chitosan/n-chitin slurries. The effect is induced by an increase in viscosity of the slurries and by their enhanced chaotropic character.

1. Introduction

Nanocomposite biomaterials based on chitosan and n-chitin are widely investigated for their antimicrobial activity, biocompatibility and biodegradability. They are already successfully used in medicine, e.g. in wound healing (Muzzarelli et al., 2007). The ability of these natural materials to suppress microbial growth is a promising also for their application as biodegradable food packaging (Morganti et al., 2013).

Nanoparticles with a high aspect ratio and a highly developed surface area, such as clays or carbon nanotubes, serve as reinforcing agents of various polymers including chitosan (Casariego et al., 2009; Sriupayo et al., 2005). In recent studies, a growing rigidity of chitosan nanocomposites with increasing content of incorporated n-chitin was demonstrated (Morganti et al., 2013; Yudin et al., 2014). The reinforcing effect of n-chitin on chitosan phase is excellent due to good reciprocal compatibility of n-chitin and chitosan, both being linear cationic (1-4)-2-amino-2-deoxy-β-D-glucans, differing only in terms of degree of acetylation (DA) and physical form (Muzzarelli, 2012). Since the enhanced rigidity of chitosan/n-chitin films leads as a rule to a decrease of their elasticity, the proper component proportion in the slurry, including a plasticizer, must be previously defined to ensure the balance between the strength and elasticity of the films.

In preparation of chitosan/n-chitin films by the casting technique from solution, the study of flow properties gets a special importance. Rheological measurements give necessary recommendations for forming a uniform and stable layer of the slurry on a support. It has been reported that rheological properties of moderately concentrated chitosan solutions (Cho et al., 2006 , Desbrieres, 2002, Hwang and Shin, 2000) reflect interactions between chains of chitosan dissolved in acetic acid solutions even at acidic pH, at which the most amino groups were protonated. A tendency of chitosan macromolecules to interact with each other was also detected in acidic aqueous solutions with very low chitosan concentrations by means of static and dynamic light scattering (Anthonsen et al., 1994; Korchagina and Philippova, 2010). The conditions necessary for formation of intermolecular reversible bonds, the nature of forming aggregates and their stabilization are still under discussion. Montembault, Viton, and Domard (2005) and Philippova et al. (2012), have concluded that the balance between the intermolecular interactions through both hydrogen bonds and hydrophobic bindings depends on the content of acetylated glucosamine groups in chitosan chains. In the case of low degree of acetylation (DA), the

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http://dx.doi.org/10.1016/j.carbpol.2014.06.043
0144-8617/

content of sites in chitosan chains interacting through hydrophobic bindings is low and, therefore, hydrogen bonds prevail in the reversible chains aggregation. Shamov, Bratskaya and Avramenko (2002), has concluded that acetate ions screen the protonated amino groups in chitosan chains in a great extent and decrease the cationic property of chitosan having low DA.

In this work, new information about rheological properties of chitosan/n-chitin slurries with and without bioplasticizers such as glycerol and poly(ethylene glycol) was obtained for optimizing the preparation of chitosan/n-chitin films. The effects of chitin nanofibrils and plasticizers on gelation of the chitosan phase due to its self-assembly are discussed in details.

2. Experimental

2.1. Materials

The commercial chitosan (HMC$^+$ GmbH, Germany), molecular weight M_w 374 kDa, degree of acetylation (DA) 11%, aqueous dispersion of chitin nanofibrils (Mavi Sud S.r.l., Italy) with DA 95% were used. The n-chitin dispersion was previously dialyzed against distilled water through a 3 kDa-SpectraPor dialysis membrane to adjust pH to neutral values. After dialysis the n-chitin dispersion was concentrated by vacuum evaporation to n-chitin concentration 0.042 g/g, pH 5.12. Biocompatible plasticizers such as poly(ethylene glycol) (PEG-600) and glycerol (Aldrich, Germany) were used as purchased.

2.2. Preparation of solutions

The chitosan flakes (0.8 g) were dispersed in distilled water (40 ml), acidified with 99% acetic acid (0.8 ml) and mixed during 8 h by mechanic stirring at room temperature to prepare chitosan solutions. The homogeneous filled chitosan solutions with chitosan/n-chitin proportion 65/35 wt.% were prepared by mixing the homogeneous chitosan solution with the aqueous n-chitin dispersion during 2 h. The amount of plasticizers was equal to 30 wt.% of total content of chitosan and n-chitin in the slurry. The component proportions (wt.%) in the measured solutions are summarized in Table 1.

2.3. Rheological characterization

Rheological properties of the pure and filled chitosan solutions were measured at room temperature using a rheometer Physica MCR 501 (Anton Paar GmbH, Austria) equipped with anti-slipping parallel-plate geometry. The free surface of samples between the plates was covered by a micro-layer of silicone oil to avoid evaporation. In the steady shear flow, the strain- and stress-controlled experiments were carried out. In the oscillatory shear flow, the viscoelastic properties were measured in the linear viscoelasticity region confirmed with strain tests at the frequency 1 Hz. The frequency- and time-dependent oscillatory tests were performed at the small amplitude (2% strain) to minimize disturbances of the internal structure in the studied solutions. The measurements with pre-shearing have been started after 3 s of the steady shear at the rate 0.03 s^{-1}. Three independent measurements were carried out for each studied solution.

3. Results and discussion

Rheological properties of chitosan solutions containing chitin nanofibrils with and without the biocompatible plasticizers were measured in the steady, oscillatory and time-dependent shear flow and compared with rheology of the pure chitosan solution. Our previous results on absorption of water vapor in chitosan/n-chitin films (Morganti et al., 2013) revealed very small change of sorption, 2 wt.% only if the concentration of chitin nanofibrils in the films increased from 24 wt.% to 36 wt.%. Based on these results, we conclude that an optimal n-chitin content in chitosan films should be about 30–40 wt.%. The 35 wt.% concentration of n-chitin in chitosan films was chosen for the study of rheological properties of slurries using for films solution casting. Effects of 20 wt.% additions of glycerol and poly(ethylene glycol) on mechanical and barrier properties of prepared films with the content 35 wt.% of n-chitin were measured and the results will be presented in a separate paper.

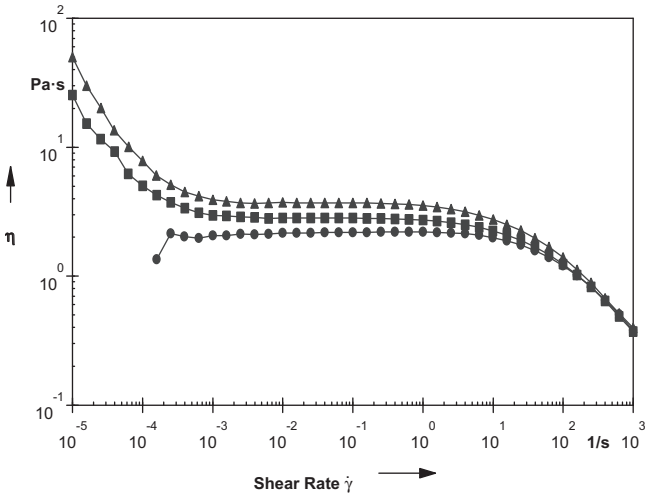

Fig. 1. Steady shear viscosity as a function of the shear rate of chitosan 2 wt.% aqueous acetic acid solution measured at the various rest times after pre-shearing. The pre-sheared solution (●), after the rest time 15 min (■) and 30 min (▲).

The addition of n-chitin into chitosan solution accelerates the process of self-assembly of chitosan macromolecules resulting in faster gelation of the slurry. The solid-like behavior is typical for filled chitosan solutions. It was characterized by values of the yield stress in the steady shear and by appearance of a low frequency plateau of the dynamic moduli in oscillatory flow. The rate of the self-assembly process until the formation of reversible gel was considered as an increase in elasticity of the studied solutions with time.

3.1. Steady shear flow properties of pure chitosan solution

The pre-sheared 2 wt.% chitosan solution exhibited a Newtonian behavior at low shear rates. The shear thinning (non-Newtonian) region appeared at higher shear rates where the polymer chains were oriented in the direction of flow. The flow curve of the pre-sheared solution is plotted in Fig. 1 as the dependence of steady shear viscosity η on the shear rate. The tendency of the chitosan chains to association was found when the solution was kept in the rheometer upon the cessation of flow and then the measurements were carried out repeatedly after 15 and 30 min. After the rest, the growth of viscosity appeared in the flow curves (Fig. 1). The increase in viscosity at very low shear rates (yielding) correlates with the content of chitosan aggregates in the solution. The marked shear-thinning character at this region indicates that the aggregates begin to deform and break down in the shear. At the shear rates higher 10 s^{-1}, the viscosity becomes independent on the rest time due to the breakage of aggregates. The used chitosan solution behaves as an associating polyelectrolyte. The obtained flow curve did not fit the frequency dependence of the complex viscosity obtained from oscillatory tests at low shear rates, i.e. the frequently used Cox-Merz

Table 1
Composition (wt.%) of chitosan solutions under study.

Solution	Chitosan	CS/n-chitin	CS/n-chitin + glycerol	CS/n-chitin + PEG
Chitosan (CS)	1.9	1.5	1.5	1.5
Q water	96.1	96.0	95.0	95.0
Acetic acid	2.0	1.6	1.6	1.6
n-Chitin	–	0.8	0.8	0.8
Glycerol	–	–	1.0	–
PEG-600	–	–	–	1.0

rule (Cox & Merz, 1958) is not valid for the chitosan solution. The observed rheological behavior corresponds to the results published by Cho et al. (2006).

3.2. Steady shear flow properties of chitosan solutions containing chitin nanofibrils

A small addition of chitin nanofibrils into chitosan solutions resulted in a reversible gelation of the slurry at rest. The viscosity increased about 3–4 decades, a slip of the filled solutions between the plates of rheometer at low shear rates as well as flow instabilities at higher shear rates occurred. The slipping effects excluded standard measurements in the mode with increasing shear rate (step-up rate mode) until the critical shear rate about 0.03 s^{-1}. At the shear rates higher than this value, a physical structure between chitosan chains and chitin nanofibrils was disturbed and at very high shear rates, the non-Newtonian behavior became similar to the pure chitosan solution. The experiments performed in the opposite mode with decreasing shear rate (step-down rate mode) showed a self-recovery of the nanofibrils-chitosan structure from the disturbed state into a gel structure. The similar behavior (slipping and structure recovery) was observed also in the shear rate measurements of the filled solutions modified by glycerol or PEG plasticizers.

3.3. Determination of the yield stress in solutions containing n-chitin

The experiments in the steady shear flow revealed a solid-like behavior of filled chitosan solutions at rest. The solutions started to flow at critical shear rate. This typical phenomenon of medium and concentrated suspensions is characterized with the yield stress – the minimum stress needed to initiate flow. Stress controlled experiments were carried out to determine these flow characteristics. Fig. 2 shows the results as a dependence of the shear viscosity on shear stress. The filled solution behaves as an elastic solid below the yield stress and a sharp drop in viscosity after reaching the yield stress reflects the destruction of a physical network. The yield stress was evaluated as the stress corresponding to the maximum value of shear viscosity. For the filled solution, the value of the yield stress equal to 6.8 Pa was determined.

The reversible physical network formed in the n-chitin filled chitosan acetate solutions is induced by strong intermolecular interactions between chitosan chains and the interactions among chitosan molecules and chitin nanofibrils. The nature of interactions lies in mechanical entanglements of chitosan chains, and formation of temporary hydrogen bonds between the hydrogen bond donors and remaining acceptors. Excellent solubility of chitosan in an acidic solution is well known. However, the properties of n-chitin fibrils in aqueous solutions are more complex. The interior of chitin nanofibrils is formed by a rigid α-chitin crystalline phase formed by antiparallel chitin molecules well stabilized by intermolecular H-bonds between acetyl groups of neighboring chains. The degree of deacetylation of the used n-chitin, determined by NMR, is very low (DA ≃ 95%). Supposing that the deacetylation agents cannot easily penetrate into the depth of the α-chitin crystalline phase, we can conclude that several layers of chitin nanofibrils near the surface are partly deacetylated. The strong hydrogen bridges between acetyl groups of neighboring antiparallel chitin molecules are responsible for insolubility of the chitin crystalline phase. The acetyl groups of chitin chains exposed to solvent which were converted to amino groups (chitosan-like surface) determine the excellent stability of aqueous dispersion of huge elongated rigid chitin nanofibrils for many months. Each nanofibril is composed of thousands of macromolecules safely anchored by their chitin segments in the crystalline core of nanofibrils. The deacetylated parts of all these molecules near the fibril surface form numerous hydrogen bonds with water in acidic solutions via their charged amine groups.

Addition of the chitin nanofibrils into the chitosan solution results in a gel formation. In the absence of an external force, the system is in a deep potential minimum, because the intermolecular interactions between chitosan chains and chitosan-nanofibrils are strong. The strong interactions of each highly anisotropic n-chitin nanoparticle with many thousands of chitosan molecules uniformly distributed in a large volume of solution ensure its stability and solid-like behavior under static conditions. Thus, the following model can be used to explain the observed rheological behavior of the system. The sparse polycation matrix of chitosan molecules homogeneously occupies the whole volume of the solution. It is connected by strong attractive and repulsive forces with a network of equally uniformly distributed chitin nanoparticles with high capacity for accumulation of kinetic energy.

The plasticizers PEG-600 and glycerol added to the filled chitosan solutions decreased the yield stress value by about one half. The modification of solutions with glycerol and PEG reduced their yield stresses similarly to the values 3.9 and 3.1 Pa, respectively. Effect of the plasticizers, at their concentration comparable with of

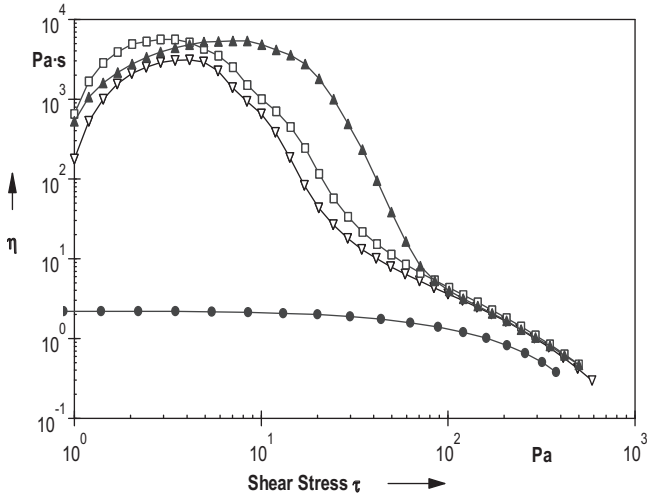

Fig. 2. Effects of the plasticizers on the yield stress value of the chitosan/n-chitin slurries. Steady shear viscosity vs. shear stress of the pure chitosan solution (●) and the slurries: chitosan/n-chitin (▲), chitosan/n-chitin/glycerol (▽), chitosan/n-chitin/PEG (□).

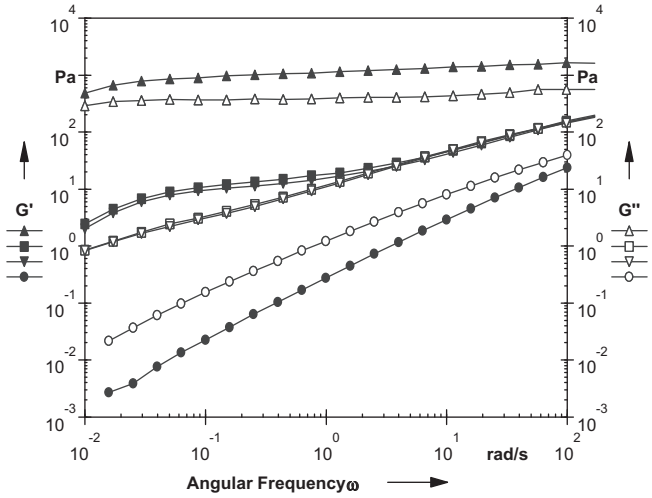

Fig. 3. Storage (G′) and loss modulus (G″) as a function of the angular frequency of the pure chitosan solution and the chitosan/n-chitin slurries. The full marks are used for the storage modulus values. The pure chitosan solution (●,○), and the slurries: chitosan/n-chitin (▲,△), chitosan/n-chitin/glycerol (▼,▽), chitosan/n-chitin/PEG (■,□).

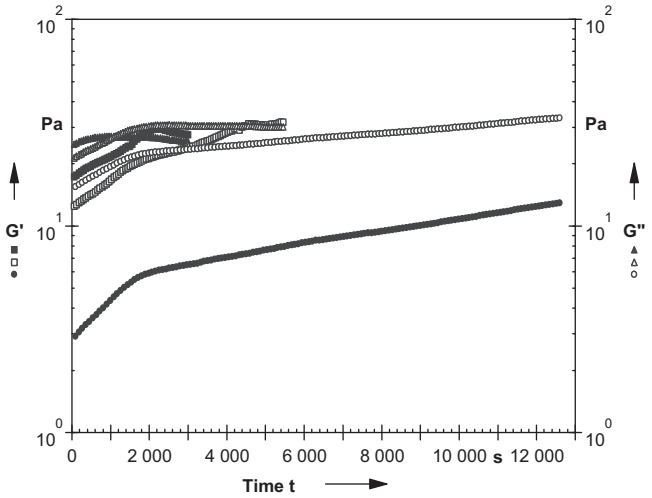

Fig. 4. Physical gelation of the chitosan/n-chitin slurries: time dependence of the dynamic moduli of the pure chitosan solution – (G′ (●), G″ (○)) and the slurries: chitosan/n-chitin – (G′ (■), G″ (▲)), chitosan/n-chitin/PEG – (G′ (□), G″ (△)).

n-chitin, on the filled solution is explained by shielding the electrostatic interactions between positively charged segments, shifting a character of solvent to the chaotropic side and by lowering a number of free water molecules in solution.

3.4. Linear viscoelastic behavior of chitosan solutions containing n-chitin (small-amplitude oscillatory shear tests)

The rheological investigation of the filled solutions in a small-amplitude oscillatory shear has a great advantage against the steady shear measurements. These experiments are performed at very low deformations and therefore no slip of the filled chitosan slurries is observed. The deformation amplitude during the oscillatory experiments is located inside the linear viscoelastic region. This region defines maximum deformation that can be applied to the filled solution without destructing the internal structure. The highly sensitive characteristic of the network structure in solution is the storage modulus G′, which is the measure of elasticity. Rheological properties of the filled chitosan solutions in oscillatory flow are shown in Fig. 3 where the linear viscoelastic characteristics, the storage modulus G′, and loss modulus G″ are plotted against angular shear frequency ω. Similar to the steady shear flow, the added nanofibrils into the chitosan solution moved the both dynamic moduli up to 3–4 decades higher. However, more interesting is the principal change in the rheological behavior of the slurries. The pure chitosan solution behaves as a viscoelastic fluid because of G″ > G′. On the other hand, in the case of filled solution, the storage modulus overwhelms the loss modulus (G′ > G″), the elasticity is dominated and the solution shows the properties as a soft solid. This solid-like phenomenon is manifested also with the frequency independent G′ and G″, the typical feature of strong gels (rubber-like behavior). The both phenomena are attributed to a physical network, formed due to chitin-chitosan interactions in the filled solutions and, as a rule, indicate an improvement of mechanical and barrier properties of the chitosan/n-chitin nanocomposites.

The effects of the used plasticizers on the physical gel formation in chitosan/n-chitin slurries are shown in Fig. 3. As accepted, the elasticity of the modified slurries drops and the rubber-like region is observed at low frequencies only. The impact of both PEG and glycerol on the viscoelastic characteristics was similar and the obtained results are confirmed by the reduced yield stresses. Otherwise, the modified filled chitosan solutions showed the convenient properties for films processing because of the decreased values of slurries viscosity and elasticity.

3.5. Observation of the self-assembly gelling process in chitosan solutions containing n-chitin (sol–gel transition)

A self-assembly gelling process in chitosan/n-chitin slurries was examined using time-dependent experiments in the oscillatory shear flow. To avoid a destruction of the internal structure of slurries, the measurements were carried out at very small deformation. The results of the tests are depicted in Fig. 4 where the time dependences of the storage (G′) and loss (G″) moduli are plotted. The dynamic moduli increase with time for both the pure and filled chitosan solutions. In the case of chitosan/n-chitin solutions and those modified with plasticizers, both moduli intersect in a crossover point (gel point) where the state of slurries was changed from a viscoelastic fluid to a viscoelastic solid phase. The gel point indicated the formation of a physical network between chitosan macromolecules and chitin nanofibrils due to the self-assembly process described in Section 3.3. The added plasticizers retarded the formation of gel that was confirmed by the results of yield stress and oscillatory tests. The chitosan/n-chitin and chitosan/n-chitin/PEG slurries reached the gel point in 28 min and 80 min, respectively, after pre-shearing. In pure chitosan solution, the increase in both moduli with time reflected networking between chitosan macromolecules. Nevertheless, in the absence of large rigid stabilizing particles such as n-chitin, the long-distance structure was not achieved even after 3.5 h. The similar slopes of the elasticity increase (see modulus G′ in Fig. 4) show that kinetics of the self-assembly in the pure and filled chitosan solutions should be analogous at the beginning of the process. This fact encourages us to suppose that the formation of aggregates between chitosan macromolecules in solution is the primary process also in the presence of chitin nanofibrils. The real reason of gelation after n-chitin addition is the ability of each single n-chitin particle to influence simultaneously thousands of chitosan molecules penetrating homogeneously the whole volume of the solution.

4. Conclusions

The incorporation of the highly anisotropic chitin nanofibrils into chitosan solutions changed their rheological properties significantly. The chitosan/n-chitin slurries revealed a solid-like behavior

and their steady shear flow was limited due to the slipping on the plates of rheometer and the flow instabilities. The independence of the dynamic moduli on frequency showed that the rubber-like behavior is typical for the chitosan/n-chitin solutions under study. The self-assembly processes in both the pure and filled chitosan solutions were elucidated by the time-dependent measurements of both dynamic moduli up to reaching the gel-point.

The chitin nanofibrils were recognized as a strong "gelling agent". It was found that at the beginning of the self-assembly, the kinetics is similar in both the pure and the filled solutions. The gelling time is prolonged and the yield stress values decrease when plasticizers are added into chitosan/n-chitin slurries.

The rheological behavior of the system is explained by a tough and elastic interconnection between two physical networks – the network of chitosan chains homogeneously penetrating the whole volume of sample and the homogeneous network of chitin nanofibrils with enormous capacity of kinetic energy.

The plasticizer molecules dissolved in the aqueous phase of chitosan/n-chitin solution increase the viscosity of slurry and protect water to form polarized structures around the charged surfaces, i.e. they give the solution more chaotropic character. The observed decrease of the yield stress values after plasticizers addition into chitosan/n-chitin slurries is thus explained both by an increase in viscosity and a decrease in repulsive forces between polycation segments.

Acknowledgement

The authors gratefully acknowledge the financial support of the European Union (grant No. 315233).

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