Carbohydrate Polymers 116 (2015) 117–123

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Rheological study of reinforcement of agarose hydrogels by cellulose nanowhiskers

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ARTICLE INFO

Article history:
Received 31 December 2013
Received in revised form 25 March 2014
Accepted 21 April 2014
Available online 28 April 2014

Keywords:
Agarose hydrogels
Cellulose nanowhiskers
Structure
Rheological behavior

ABSTRACT

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

1. Introduction

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

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

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

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

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http://dx.doi.org/10.1016/j.carbpol.2014.04.085
0144-8617/

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

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

2. Materials and methods

2.1. Materials

2.1.1. Agarose

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

2.1.2. Preparation of agarose solutions

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

2.1.3. Elaboration of cellulose nanowhiskers

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

2.1.4. Preparation of nanowhiskers suspensions

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

2.1.5. Preparation of agarose gels filled with cellulose nanowhiskers

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

2.2. Methods

2.2.1. Transmission electron microscopy (TEM)

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

2.2.2. Atomic force microscopy (AFM)

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

2.2.3. Titration

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

2.2.4. Rheometry

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