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Unusual reversible elastomeric gels from *Nostoc commune*

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ABSTRACT

Nostoc commune cyanobacteria grow in extreme conditions of desiccation and nutrient-poor soils. Their colonies form spherical gelatinous bodies are composed of a variety of polysaccharides that allow them to store water and nutrients. In this paper, we study this type of biological gel that shows characteristics of both chemical and physical gels. The structure of this gel was assessed by means of scanning electron microscopy, plate-plate rheometry, Fourier transform infrared spectroscopy and absorption/desorption tests. The storage modulus of this gel was found to be frequency independent, as is usual for chemical gels. The stress sweeps showed a reversible stress softening behaviour that was explained in terms of the physical nature of the interactions of this network. The high density of physical crosslinks probably allows this physical network to behave as a highly elastomeric chemical network, limiting the relaxation of individual chains. On the other hand, reversibility is associated with the physical nature of its bonds.
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1. Introduction

Natural and synthetic polymers can be physically or chemically cross-linked in order to produce gels. Polysaccharides have been used for the preparation of new gels and gel-based systems, including contact lenses, hygiene products, tissue engineering scaffolds, drug delivery systems and wound dressings [1–5].

A wide range of bacteria synthesize and secrete extracellular polymeric substances (EPS). The extracellular polymeric substances of cyanobacteria from the Nostocaceae family are usually composed of a mixture of high-molecular-mass heteropolysaccharides, proteins, nucleic acids and lipids [6–8]. The heteropolysaccharides contain an array of sugar units including glucose, galactose, xylose and uronic acids [7]. EPS form gels that serve as protection against desiccation, antibacterial agents, or predation by protozoans [9]. *Nostoc* bacteria grow in colonial spherules (Fig. 1) formed by an internal EPS gel and an outer skin. These colonies are typical in many regions of the tropics and subtropics, which are characterized by their erratic wetting-drying cycles and high levels of solar radiation [10].

This study is focused on the gel of the *Nostoc commune* spherules. As far as the authors are concerned, there is still a lack of information regarding the exact components of *Nostoc commune* gels and the nature of interactions between their EPS. According to Lembre et al. [11], gels produced by bacterial colonies form networks based on physical interactions between polysaccharide chains. According to Or [12], EPS networks absorb water by surface, osmotic and capillarity forces. It has also been proposed that these gels may retain water entropically (repulsive osmotic pressure between counterions increase the configurational entropy and EPS remain water-saturated) rather than through specific water-binding mechanisms.

The nature of these interactions determines the rheological properties of the EPS gels. In fact, gels can be classified into two main categories according to the types of cross-linking. Chemical gels are networks crosslinked by covalent bonds while physical gels are formed by physical associations between molecules, fibers or particles. These physical associations include physical entanglements between polymer chains and weak interactions such as hydrogen bonds, van der Waals forces, London dispersion forces and electrostatic interactions. Physical gels are reversible as their physical cross-links can be broken in response to changes in temperature, pH, and pressure, among others. In contrast, chemical cross-links are permanent and, thus, chemical gels are not reversible.

As seen in Fig. 1, the colonies of *Nostoc commune* are formed by spheres of 5–10 mm in diameter which encases a gel. We have found that this natural gel displays characteristics of both physical and chemical gels. The aim of this paper is to unveil the

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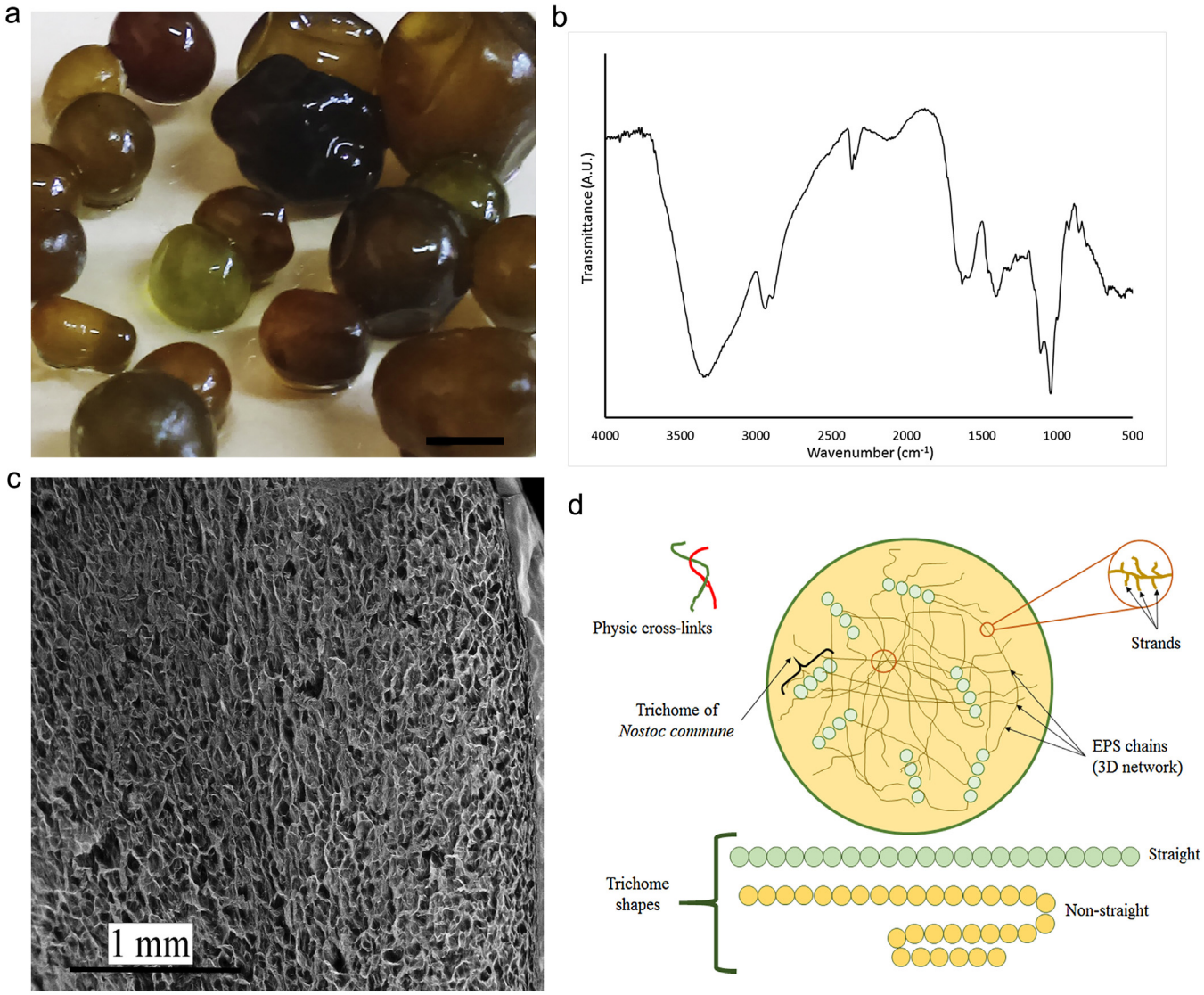

Fig. 1. *Nostoc commune* bacteria as found in their natural environment. The EPS gel is found inside these spherules (a). EPS are a diverse collection of secreted macromolecules, including different types of proteins and polysaccharides. The FTIR spectra (b) show signals typical of polysaccharides. The freeze dry *Nostoc commune* gel is a porous structure which reminds the morphology of freeze dry porous scaffolds (c). A schematic illustration of the physical network formed by the EPS gel is shown (d).

structural characteristics that provide this unusual gel with properties of chemical and physical networks. This would potentially contribute to the development of novel gels bioinspired with combined properties from chemical and physical networks.

2. Materials and method

2.1. Materials

Nostoc commune colonial spherules were obtained from Lake Querococha (Ancash, Perú, 9°42′55.9”S, 77°19′27.6”W). The spherules were washed, air dried and stored at room temperature prior to use.

2.2. Characterization techniques

2.2.1. Morphology

Freeze-dry samples were analysed with a FEI Quanta200 scanning electron microscope in high vacuum with a voltage of 25 kV and a working distance with a range of 9.9–10.7 mm. Samples were mounted onto stubs and were observed without any coating.

2.2.2. Thermal analysis

Thermogravimetric measurements were performed with a Perkin Elmer TGA 4000. Tests were carried out in the range 30–700 °C with a heating rate of 10 °C/min and purged with N_2 at a flow rate of 20 mL/min.

2.2.3. Fourier transform infrared spectroscopy (FTIR)

IR analysis of the freeze-dry samples was performed in a Lambda Scientific FTIR analyser. A mixture of the powdered samples and KBr were compressed into pellets. For each test, an average of 8 scans at a resolution of 4 cm^{-1} in a range from 4000 to 650 cm^{-1} were recorded.

2.2.4. Rheological tests

The outer skin of the *Nostoc commune* spherules was removed and discs (diameter ≈ 5 mm, thickness ≈ 2 mm) were cut from the internal gel for rheological tests. An AR 550 rheometer (TA Instruments, New Castle, DE) equipped with control software (Rheology Advantage Data Analysis Program, TA, New Castle, DE) was used. A parallel plate attachment was used. The AR 550 was supplemented with a Peltier temperature control system and the tests were

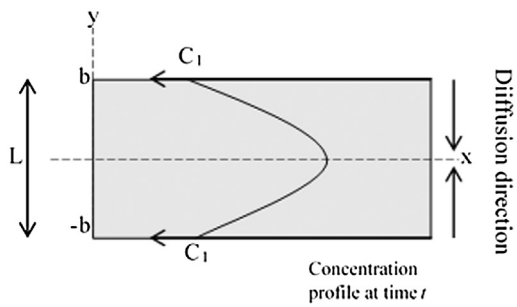

Fig. 2. Schematic illustration of the diffusion process in a film of thickness L = 2b.The region −b < x < b is initially at a uniform concentration (C_0), and the external surfaces of the film are kept at a concentration (C_1).

carried out at 25 °C. Storage modulus (G'), loss modulus (G") and phase angle (tan δ) were obtained directly from the software.

Frequency scans and stress scans were carried out. Each type of scan was performed at least five times. For the frequency scans, a frequency sweep from 0.1 to 40 Hz was used with a constant stress of 5 Pa. This oscillation stress was selected based on linear part of the viscoelastic range determined at a strain sweep.

The stress scans were performed using two steps. In the first step, the stress varied from 0.1 Pa to 100 Pa. Immediately after the first stress finished, the second step started with a stress of 100 Pa until 0.1 Pa was reach. For both steps, frequency was kept constant at 1 Hz.

2.2.5. Water absorption and desorption

For water absorption, square pieces of dry *Nostoc commune* spherules (10 × 10 × 0.5 mm) were cut and immersed in water at room temperature for 3 days. During this process the samples were removed from the bath every hour, blotted dry with a cloth, weighed and immediately place back into the bath.

For water desorption, square pieces of fresh *Nostoc commune* spherules (10 × 10 × 2 mm) were cut and stored at room temperature for 3 days. Similarly, the samples were weighed every hour until they maintained a constant weight. The dried samples were used for a new water absorption test.

Diffusion equations were used to describe the transport of water. When a flat material is in contact with a diffusant-rich environment, penetration occurs towards the midplane of the flat material. The concentration of diffusants throughout the thickness is time dependent and can be modeled using Fickís laws. A schematic representation of the samples used is shown in Fig. 2. For a sample with thickness L = 2b. Assuming that the region −b < x < b is initially at a uniform concentrationC_0, and the surfaces are kept at a concentration C_1, the concentration profile can be described by Eq. (1)

$$\frac{C - C_0}{C_1 - C_0} = 1 - \frac{4}{\pi}\sum_{0}^{\infty}\frac{(-1)^n}{2n+1}\exp\left[-\frac{D(2n+1)^2\pi^2 t}{4b^2}\right] x\cos\frac{(2n+1)\pi x}{2b} \quad (1)$$

If M_t denotes the total amount of diffusing substance that has penetrated the sheet at time t, and M_{eq} represents the mass uptake at equilibrium (given an infinite diffusion time), then:

$$\frac{M_t}{M_{eq}} = 1 - \sum_{0}^{\infty}\frac{8}{(2n+1)^2\pi^2}\exp\left[-\frac{D(2n+1)^2\pi^2 t}{4b^2}\right] \quad (2)$$

According to Karimi [13], for processes that take place within a short amount of time, Eq. (2) can be written for a thickness of L = 2b. This equation is useful to deduce an average diffusion coefficient from the initial gradient of the sorption curve when M_t/M_{eq} is plotted against the square root of time for a sheet.

$$\frac{M_t}{M_{eq}} = \frac{4}{\pi^{1/2}}\left(\frac{Dt}{L^2}\right)^{1/2} \quad (3)$$

3. Results and discussion

Fig. 3 shows a representative frequency sweep of a *Nostoc commune* gel sample. The storage modulus had a constant value (∼11,000 Pa) throughout the entire range of frequencies tested. This behavior is typical of cross-linked chemical gels. In contrast, the storage modulus of physical gels scales with frequency following a power law (G'(ω)–ω^n). This is due to the fact that the mechanical response of physical networks is influenced by the relaxation of individual polymer chains while reptation is probably the main relaxation mechanism in entangled polymer chains [14]. In chemical gels, covalent cross-linking prevents the relaxation of polymeric chains [15].

Gels from cyanobacteria are thought to be based on physical interactions of the EPS chains [8]. The fact that the storage modulus of *Nostoc commune* gels is frequency independent, as in typical chemical gels, is an unusual result. EPS gels are formed by weak physical interactions with binding energies ranging 10–30 kJ mol^{-1} [16]. Such binding energy is small compared to a covalent C—C bond (335 kJ mol^{-1}). It has been suggested that the large number of functional groups of EPS gels result in an overall binding energy equivalent to the energy of several covalent C—C bonds [17]. The density of physical cross-links on *Nostoc commune* gels should be high enough to allow their total binding energy to be in the range of the total binding energy of covalent cross-links.

Cordier et al. [18] showed that rubber elasticity theory has been used to characterize networks that are either covalently cross-linked [19,20] or connected by physical associations including ionic aggregates or multiple hydrogen bonds [21–23]. Theoretical networks parameters can be calculated using the storage modulus (G') from the frequency sweep tests. According to Clasen et al. [24], the induced energy of the small amplitude oscillatory shear experiment is stored elastically by the polymer strand between two entanglement or network points, acting as an entropic spring. Therefore, the number density of crosslinks (ν) can directly be calculated using the constrained junction model of Flory and Erman [25] which predicts that, for an affine network, G' is given by:

$$G' = \nu \cdot k_B T \quad (4)$$

where k_B is the Boltzman constant and T is the temperature of the network. It is assumed that the junctions of the *Nostoc commune* network do not fluctuate and transform affinely (linearly) with the macroscopic deformation. The molecular weight between crosslinks (Mc) and the distance between sequential crosslinking points (ξ) can be calculated according to the following equations [26,27]:

$$M_C = \frac{\rho}{\nu} \quad (5)$$

$$\xi = \left(\sqrt[3]{\nu}\right)^{-1} \quad (6)$$

where ρ is the density of the network in the swollen state. The crosslink density found was 32.5 × 10^{23} m^{-3} and the mesh size was 6.75 nm. This crosslink density is in the same order of magnitude of the one reported for other crosslinked gels such as methyl- and hydroxypropylmethyl-cellulose gels (4.9 × 10^{21}–3.9 × 10^{23} m^3) and poly(N-isopropylacrylamide)/alginate gels (22.5 × 10^{23} m^3).

Fig. 4 depicts the variation of storage modulus (G') and loss modulus (G") as function of the applied stress. The storage modulus is almost stress independent until the sample reaches a high

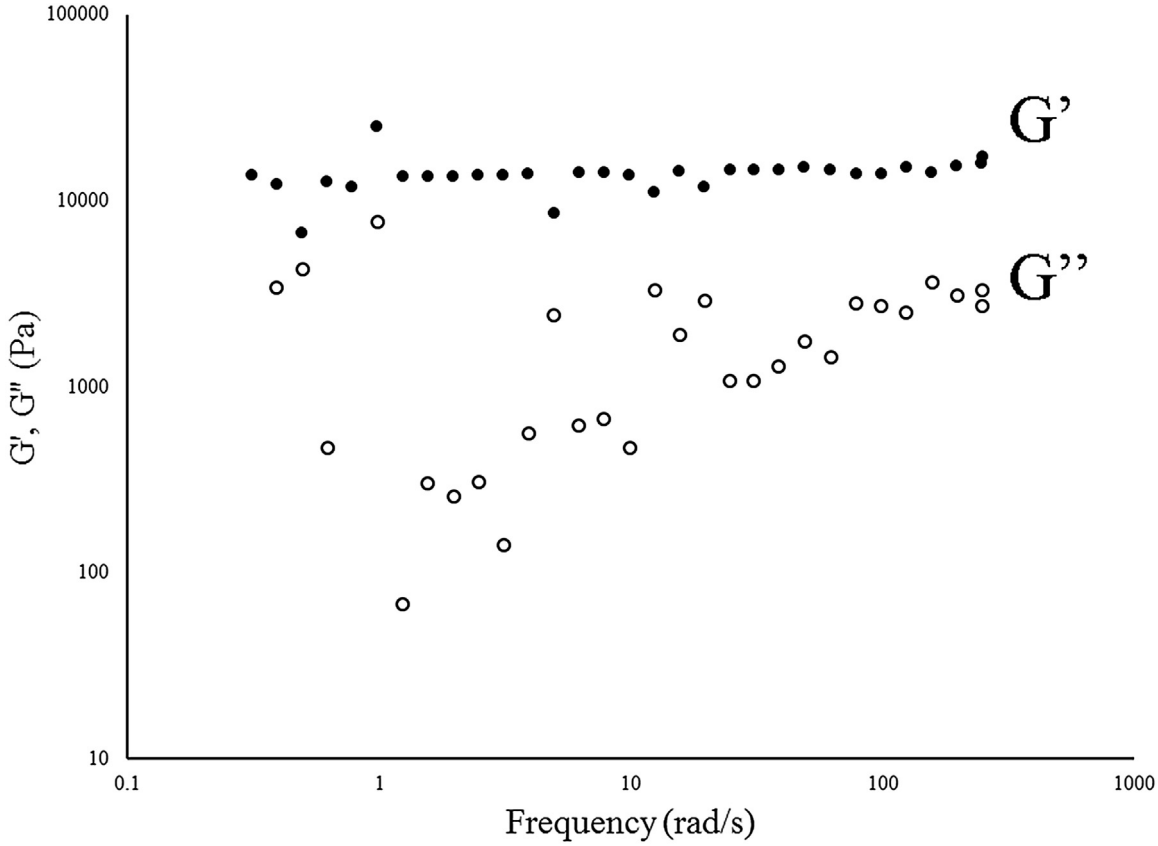

Fig. 3. Frequency dependence of the storage modulus (●) and loss modulus (○) of a representative *Nostoc commune* gel sample.

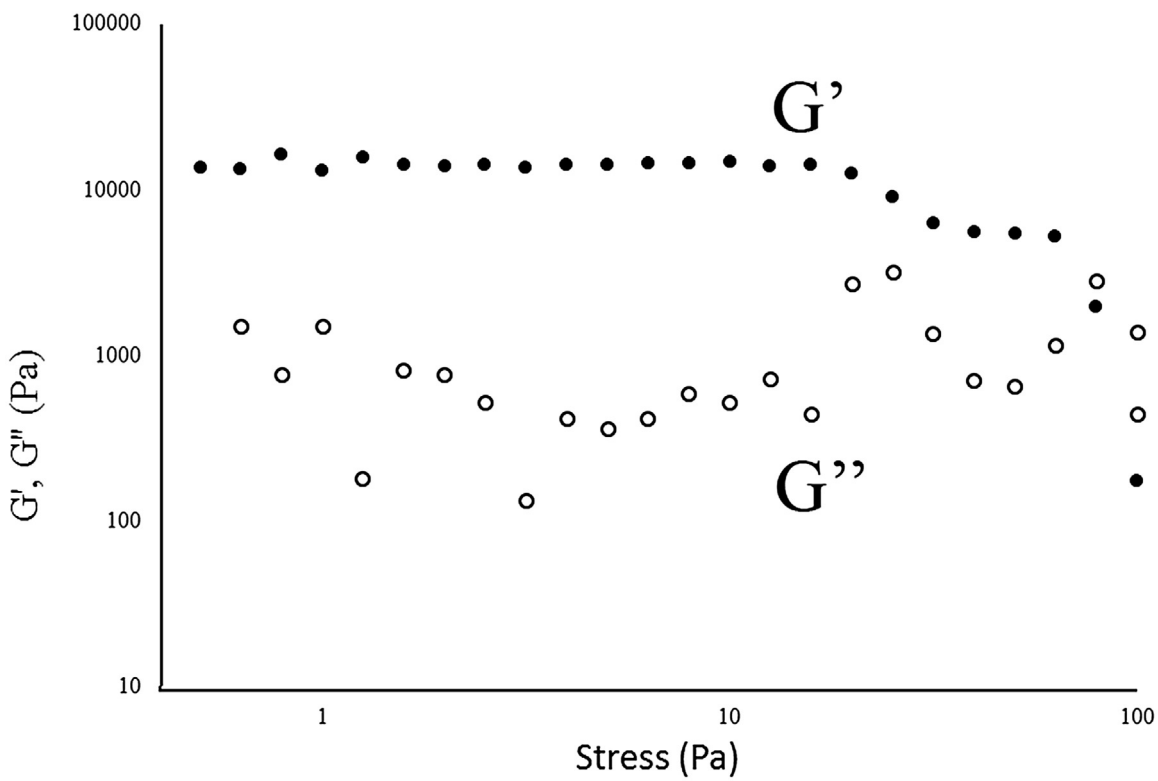

Fig. 4. Stress dependence of the storage modulus (●) and loss modulus (○) of a representative *Nostoc commune* gel sample.

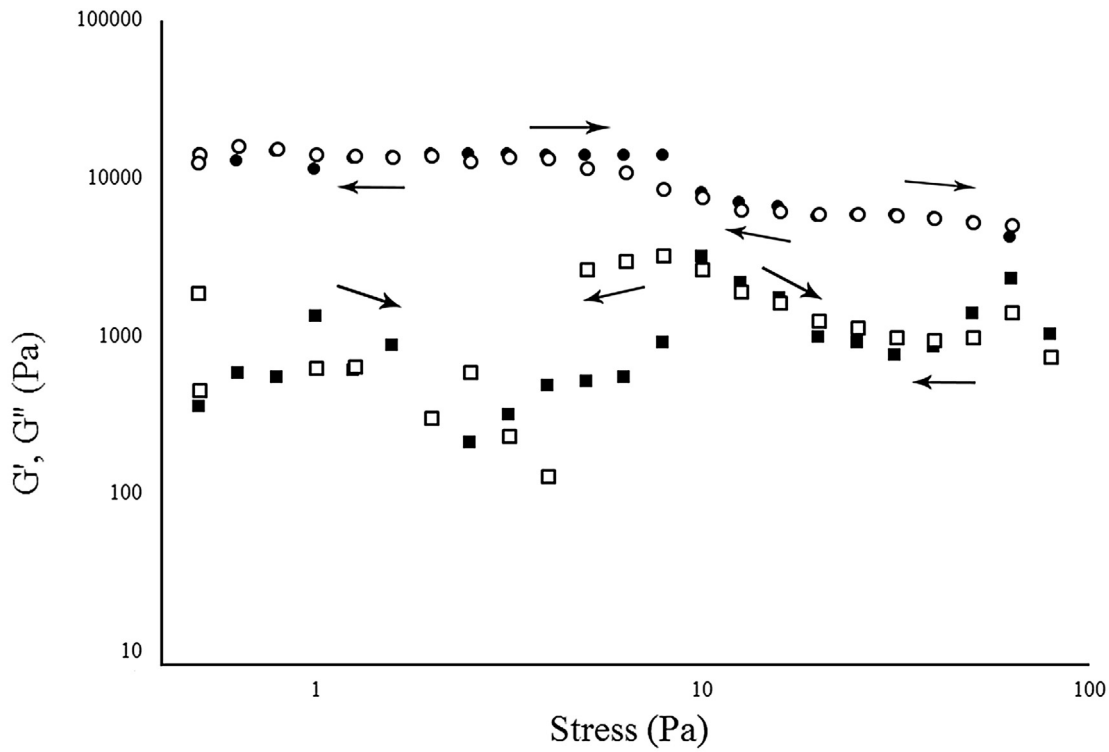

Fig. 5. Storage modulus (●) and loss modulus (■) of a representative *Nostoc commune* gel sample during a two-step stress sweep. Filled symbols are used to plot the first step (increasing stress) while empty symbols are used to plot the second step (decreasing stress).

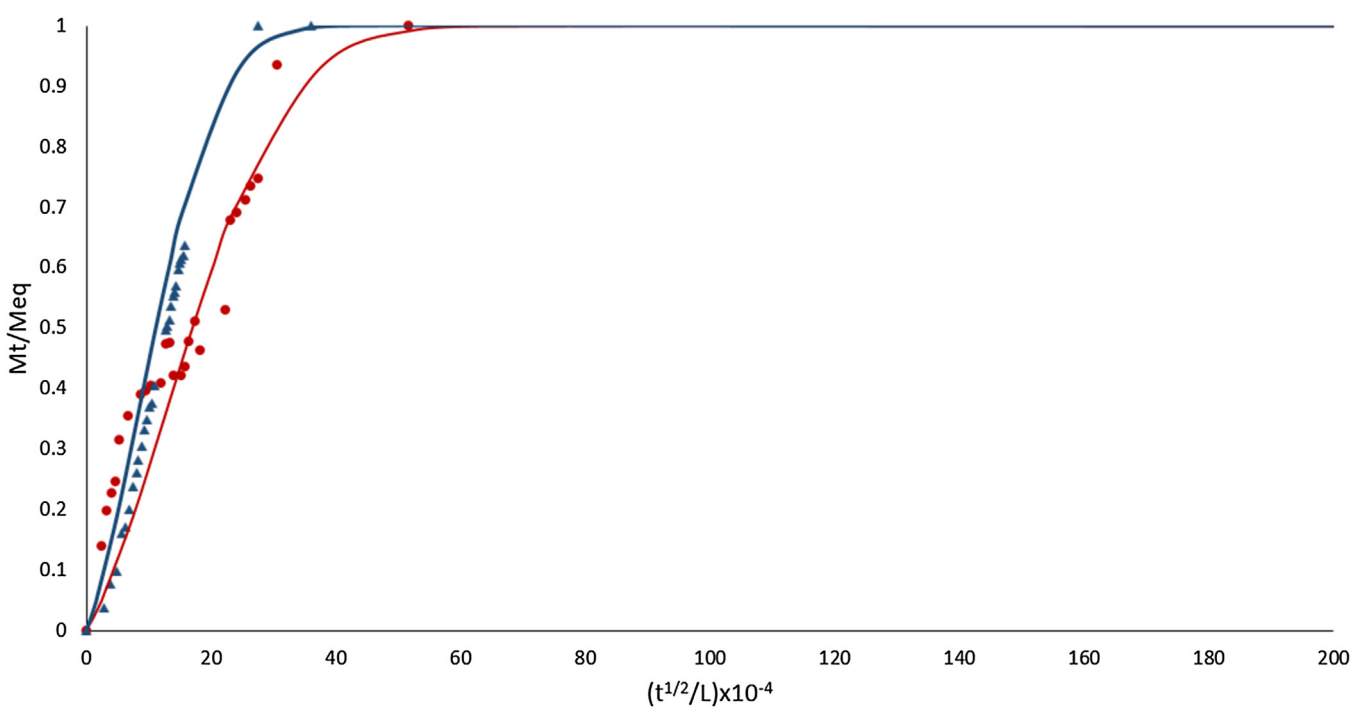

Fig. 6. Representative absorption (●) and desorption (▲) curves of a *Nostoc commune* gel sample.

stress level (14,000 MPa), where the storage modulus value drops. The drop in the mechanical properties of a gel is referred to as stress softening. The stress softening behavior has been explained in terms of a disruption of the bonds that form gels. These gels are destroyed when a certain stress level is achieved. For instance, in rigidly crosslinked actin networks, stress softening has been associated with the breakage of crosslinks, followed by a drastic decrease in elasticity [28–30].

However, cyclic tests showed that, in the case of *Nostoc commune* gels, stress softening is reversible. Fig. 5 shows the dependence of G' and G" under an oscillatory stress load cycle. During the first cycle, the storage value drops but it totally recovers its

value when the oscillatory stress is removed during the second cycle. This phenomenon is known as reversible stress softening, and it has been reported for other biopolymer networks, such as collagen in jumbo squid mantle, cellulose and actin networks. The reversible nature of the stress softening behavior of *Nostoc commune* gels showed that the non-permanent physical crosslinks that form this network can be destroyed and formed again very quickly so the storage modulus value is recovered as soon as the imposed stress is withdrawn.

As other physical gels, *Nostoc commune* gels loose solvent almost completely by water evaporation when kept in air at room temperature. This process is also reversible, as dry samples are able to re-swell again after water immersion. TGA tests showed that fresh *Nostoc commune* samples had a water content of around 97.4% while rehydrated samples had a water content of 99%. Water absorption and desorption tests were carried out in order to assess the kinetic of these processes. Fig. 6 shows the normalized mass of the samples as function of time. The experimental results are plotted together with the Fickian diffusion predictions.

Fresh samples were first dried for the desorption test. Then, the same samples were immersed in water for the absorption tests. Both, absorption and desorption are fairly well described by the Fick's law. The diffusion coefficient found for the water desorption process was 2.83×10^{-11} m^2/s while the coefficient for absorption was 8.18×10^{-15} m^2/s, showing that the samples dry more quickly than they rehydrate.

In the case of absorption and desorption of gels, Fick's law fit the experimental measurements when the absorbed solvent does not interact with the porous gel and, thus, the diffusion is only governed by the solvent concentration gradient. Otherwise, the influence of polymer relaxation upon solvent imbibition should be taken into account to model the kinetics of absorption/desorption of gels [31,32]. The fact that, both absorption and desorption of *Nostoc commune* gels follows Fick's law, could be accounted for a high density of physical crosslinks that would prevent polymer relaxation. This result is in agreement with rheological tests in which the storage modulus was independent from frequency, showing that the effect of polymer relaxation was not considerable.

4. Conclusion

The original structure of *Nostoc commune* gels as they are found in nature was studied. Similar to other biopolymeric structures, *Nostoc commune* gels are composed mainly of water (97% w/w). The polymeric network is formed by polysaccharides chains hold together by physical cross-links. Oscillatory rheological tests showed that this gel displays characteristics of both chemical and physical gels. The storage modulus was found to be independent from frequency as in the case of chemical gels. On the other hand, stress sweeps showed that the storage modulus decreases when the oscillatory stress achieves a limit value, a phenomenon known as stress softening. The reversibility of this stress softening was associated with the physical nature of the crosslinks which can be created and destroyed at a short time scale. The high density of physical crosslinks allows this physical network to behave as a highly elastomeric chemical network preventing relaxation of individual chains. Rubber elasticity theory was used to calculate and confirm the high density of crosslinks in the network. The absorption/desorption tests confirm the absence of mechanisms of relaxation on the polymeric chains of *Nostoc commune* networks.

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References

[1] P.C. Nicolson, J. Vogt, Soft contact lens polymers: an evolution, Biomaterials 22 (2001) 3273–3283.
[2] K. Kabiri, H. Omidian, M.J. Zohuriaan-Mehr, S. Doroudiani, Superabsorbent hydrogel composites and nanocomposites: a review, Polym. Compos. 32 (2011) 277–289.
[3] J.L. Drury, D.J. Mooney, Hydrogels for tissue engineering: scaffold design variables and applications, Biomaterials 24 (2003) 4337–4351.
[4] Y. Qiu, K. Park, Environment-sensitive hydrogels for drug delivery, Adv. Drug Deliv. Rev. 53 (2001) 321–339.
[5] J.S. Boateng, K.H. Matthews, H.N. Stevens, G.M. Eccleston, Wound healing dressings and drug delivery systems: a review, J. Pharm. Sci. 97 (2008) 2892–2923.
[6] S. Pereira, A. Zille, E. Micheletti, P. Moradas-Ferreira, R. De Philippis, P. Tamagnini, Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly, FEMS Microbiol. Rev. 33 (2009) 917–941.
[7] S. Jensen, B.O. Petersen, S. Omarsdottir, B.S. Paulsen, J.O. Duus, E.S. Olafsdottir, Structural characterization of a complex heteroglycan from the cyanobacterium *Nostoc commune*, Carbohydr. Polym. 91 (2013) 370–376.
[8] H.C. Flemming, J. Wingender, The biofilm matrix, Nat. Rev. Microbiol. 8 (2010) 623–633.
[9] R. De Philippis, M. Vincenzini, Exocellular polysaccharides from cyanobacteria and their possible applications, FEMS Microbiol. Rev. 22 (1998) 151–175.
[10] L.P. Brüll, Z. Huang, J.E. Thomas-Oates, B.S. Paulsen, E.H. Cohen, T.E. Michaelsen, Studies of polysaccharides from three edible species of Nostoc (cyanobacteria) with different colony morphologies: structural characterization and effect on the complement system of polysaccharides from Nostoc commune, J. Phycol. 36 (2000) 871–881.
[11] P. Lembre, C. Lorentz, P. Di Martino, P. Di Martino, Exopolysaccharides of the biofilm matrix: a complex biophysical world, in: D.N. Karunaratne (Ed.), The Complex World of Polysaccharides, INTECH Open Access Publisher, Croatia, 2012, pp. 372–392.
[12] D. Or, S. Phutane, A. Dechesne, Extracellular polymeric substances affecting pore-scale hydrologic conditions for bacterial activity in unsaturated soils, Vadose Zone J. 6 (2007) 298–305.
[13] M. Karimi, Diffusion in polymer solids and solutions, in: J. Markoš (Ed.), Mass Transfer in Chemical Engineering Processes, INTECH Open Access Publisher, Croatia, 2011, pp. 18–40.
[14] M.E. Cates, Reptation of living polymers: dynamics of entangled polymers in the presence of reversible chain-scission reactions, Macromolecules 20 (1987) 2289–2296.
[15] D.T.N. Chen, Q. Wen, P.A. Janmey, J.C. Crocker, A.G. Yodh, Rheology of soft materials, Annu. Rev. Condens. Matter Phys. 1 (2010) 301–322.
[16] P.W. Atkins, Physical Chemistry, fifth ed., W.H. Freeman & Co., New York, 1994.
[17] A.E. Ehret, M. Böl, Modelling mechanical characteristics of microbial biofilms by network theory, J. R. Soc. Interface 10 (2013) 20120676.
[18] P. Cordier, F. Tournilhac, C. Soulié-Ziakovic, L. Leibler, Self-healing and thermoreversible rubber from supramolecular assembly, Nature 451 (2008) 977–980.
[19] D. Tabor, Gases, Liquids and Solids: and Other States of Matter, third ed., Cambridge University Press, 1991.
[20] J.L. Rubinstein, J.E. Walker, R. Henderson, Structure of the mitochondrial ATP synthase by electron cryomicroscopy, EMBO J. 22 (2003) 6182–6192.
[21] R. Stadler, L. de Lucca Freitas, Thermoplastic elastomers by hydrogen bonding 1. Rheological properties of modified polybutadiene, Colloid Polym. Sci. 264 (1986) 773–778.
[22] R.P. Sijbesma, F.H. Beijer, L. Brunsveld, B.J. Folmer, J.K. Hirschberg, R.F. Lange, E.W. Meijer, Reversible polymers formed from self-complementary monomers using quadruple hydrogen bonding, Science 278 (1997) 1601–1604.
[23] R.F. Lange, M. Van Gurp, E.W. Meijer, Hydrogen-bonded supramolecular polymer networks, J. Polym. Sci. A Polym. Chem. 37 (1999) 3657–3670.
[24] C. Clasen, T. Wilhelms, W.M. Kulicke, Formation and characterization of chitosan membranes, Biomacromolecules 7 (2006) 3210–3222.
[25] J.E. Mark, B. Erman, Rubberlike Elasticity: A Molecular Primer, John Wiley & Sons, New York, 1988.
[26] M. Balk, M. Behl, U. Nöchel, A. Lendlein, Shape-memory hydrogels with switching segments based on oligo (ω-pentadecalactone), Macromol. Mater. Eng. 297 (2012) 1184–1192.
[27] C. Clasen, T. Wilhelms, W.M. Kulicke, Formation and characterization of chitosan membranes, Biomacromolecules 7 (2006) 3210–3222.
[28] J. Xu, Y. Tseng, D. Wirtz, Strain Hardening of actin filament networks regulation by the dynamic cross-linking protein α-actinin, J. Biol. Chem. 275 (2000) 35886–35892.
[29] M.L. Gardel, J.H. Shin, F.C. MacKintosh, L. Mahadevan, P. Matsudaira, D.A. Weitz, Elastic behavior of cross-linked and bundled actin networks, Science 304 (2004) 1301–1305.

[30] C. Storm, J.J. Pastore, F.C. MacKintosh, T.C. Lubensky, P.A. Janmey, Nonlinear elasticity in biological gels, Nature 435 (2005) 191–194.
[31] J. Siepmann, N.A. Peppas, Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC), Adv. Drug Deliv. Rev. 48 (2001) 139–157.
[32] P. Baglioni, N. Bonelli, D. Chelazzi, A. Chevalier, L. Dei, J. Domingues, M. Martin, Organogel formulations for the cleaning of easel paintings, Appl. Phys. A 121 (2015) 857–868.