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A thixotropic hydrogel from chemically cross-linked guar gum: synthesis, characterization and rheological behaviour

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ABSTRACT

Polysaccharide guar gum (GG) was cross-linked in an alkaline solution with polyethylene glycol diglycidyl ether (PEGDGE) to create a new hydrogel. The GG hydrogel was examined by FT-IR spectroscopy, AFM analysis and SEM analysis. The water uptake of the GG hydrogel was measured at different pHs, and rheological studies were performed to verify the thixotropic nature of the material. Rheological studies revealed the pseudoplastic behaviour of the GG hydrogel and its thixotropic nature. AFM analysis on a sample which was subjected to shear stress showed the presence of nanoparticles in the hydrogel. When the sample was left to settle, the gel surface returned to its original homogenous morphology. The thixotropic and injectable nature of the GG hydrogel suggest its possible use in biomedical applications.

1. Introduction

The field of natural polysaccharides along with their applications is becoming more and more important due to their low cost, low environmental impact and non-toxicity to humans. Guar gum (GG) is a hydrophylic, non-ionic polysaccharide extracted from the endospermic seed of the plant *Cyamopsis tetragonalobus*. GG belongs to the large family of galactomannans and consists of a D-galacto-D-mannan. The formula of the GG is shown in Figure 1. GG hydrates in cold water to form a highly viscous solution in which the single polysaccharide chains interact with each other in a complex way.[1]

As the GG polymer is a low-cost, easily available and non-toxic polysaccharide, it is widely applied in many industrial fields.[2] Thanks to its property of producing highly viscous aqueous solutions, it is commonly used as a thickening agent in cosmetics and in sauces, salad dressings and ice creams in the food industry. Sometimes instead of the native guar-gum, chemically modified products can be used with the objective of changing its intrinsic characteristics of solubility, viscosity and rheological behaviour. Hydrossilalchyl derivatives are often used for the formulation of cements and plasters,[3] whereas carboxymethyl derivatives are employed as thickening agents for inks for fabric materials.[4,5] The physically cross-linked product is used when the aim is to obtain a water insoluble compound. On the other hand these types of bonds are weak and strongly influenced by the pH of the mixture; thus by adding acidic or basic solutions to the cross-linked product it easily dissolves.[6] The cross-linked products with solubility sensitive to pH changes can be useful in the formulation of paints, but in other applications such as the biomedical fields a more stable compound is required. In the last few decades, polysaccharide-based hydrogels have aroused much interest in pharmaceutical fields as 3D scaffolds for cell culture, fillers for tissue engineering and as carriers for drugs.[7,8] The chemical cross-linking through a spacer arm between the polymer chains allows the obtainment of an insoluble compound in a wide range of pHs with a good mechanical stability. The chemical cross-linking of GG has been performed with glutaraldehyde and phosphating agents[9,10] or in combination with polyacrylic acid, in order to obtain polyelectrolyte hydrogels with mouldable mechanical properties and swelling degree.[11]

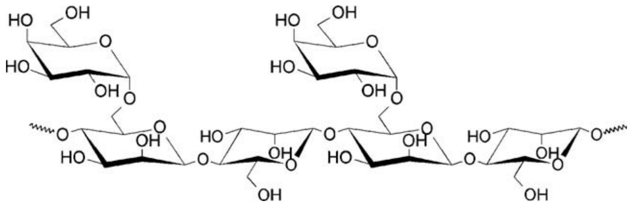

Figure 1. Formula of the GG polymer.

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The aim of this study is the synthesis of a new GG-based hydrogel using polyethylene glycol diglycidyl ether ($CH_2OCH–(CH_2CH_2O)_n–CHOCH_2$, $n = 10$, PEGDGE)[12] as the cross-linking agent. We chose PEDGE as cross-linking agent, thanks to its low toxicity. Moreover, this choice allows us to carry out the cross-linking reaction of GG in water. Particular attention was devoted to the investigation of the hydrogel's thixotropic behaviour, infrared spectroscopy characterization and swelling behaviour.

2. Results and discussion

2.1. Synthesis of the GG hydrogel and physico-chemical characterization

The preparation of the GG-based hydrogel was achieved by the insertion of a cross-linker between the polymer chains. Figure 2 shows the reaction route of the synthesis of the GG hydrogel. The GG polymer repeating unit has two primary hydroxylic groups on the Galactoside unit and one on the Mannoside unit. PEGDGE is a bifunctional cross-linker with two epoxide rings linked at the head and at the tail of a PEG chain. The cross-linking of GG with PEGDGE, through the formation of an ether bond, is conducted in alkaline solution (NaOH, pH >12).[12] The amount of PEGDGE added was in excess (molar ratio GG/PEGDGE = 1/3) to avoid the formation of a loop on the same GG macromolecule by the PEGDE chain and to obtain the best hydrogel yield. However, lower amounts of PEGDE demonstrated to be ineffective for the obtainment of the hydrogel.

A comparison of the FT-IR spectrum of the GG hydrogel (black spectrum) and the spectrum of the native GG polymer (grey spectrum) is shown in Figure 3a. The two spectra (GG polymer and GG hydrogel) are very similar because no new additional functional groups were introduced by the cross-linking reaction. The band at 1026 cm^{-1} and the one centred at 1148 cm^{-1} were relative to the hydroxylic C–O single bond stretching and to the vibrations of the C–O–C bonds, respectively.[13] The only difference between the two spectra concerned the intensity of the peak at about 1127 cm^{-1} relative to the asymmetric ether bonds stretching,[14] which is higher after the introduction of the PEGDE even if it should be taken into consideration that the PEGDE itself contains ether groups. As a matter of the fact, the deconvolved spectrum of GG hydrogel (see Fig. 3b) showed that the shoulder centred at 1127 cm^{-1} is the real peak which is not marked in the spectrum

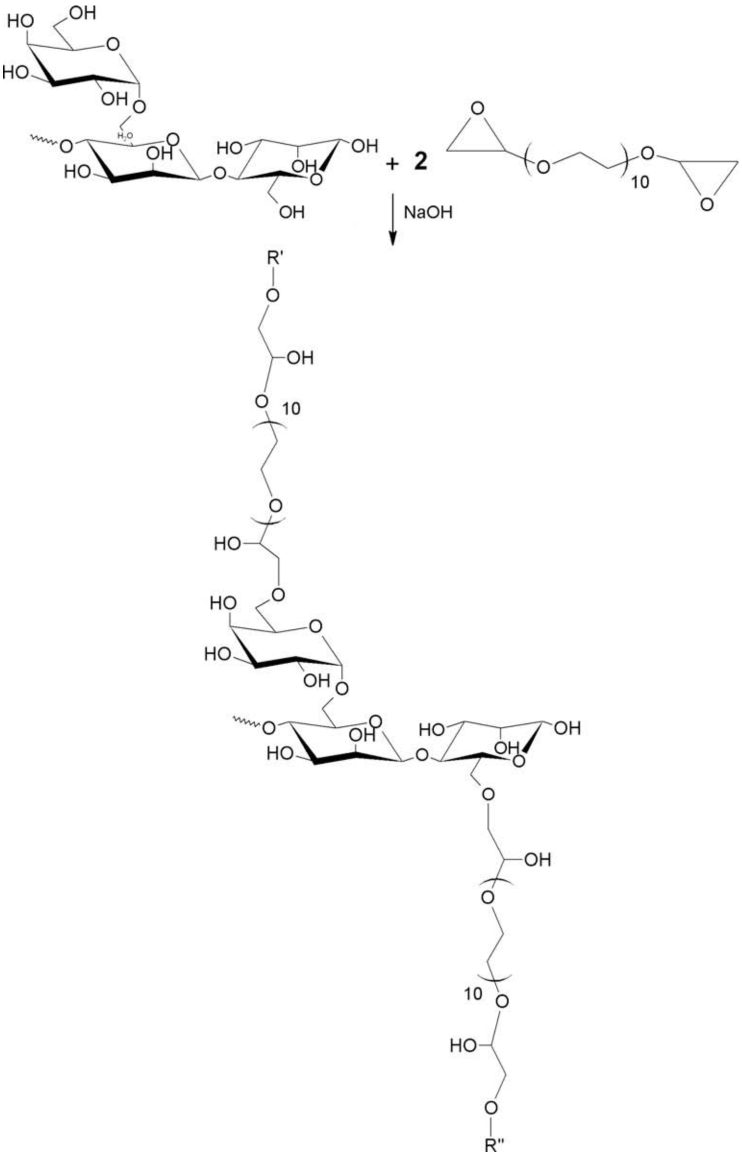

Figure 2. Diagram of the synthesis of the GG hydrogel.

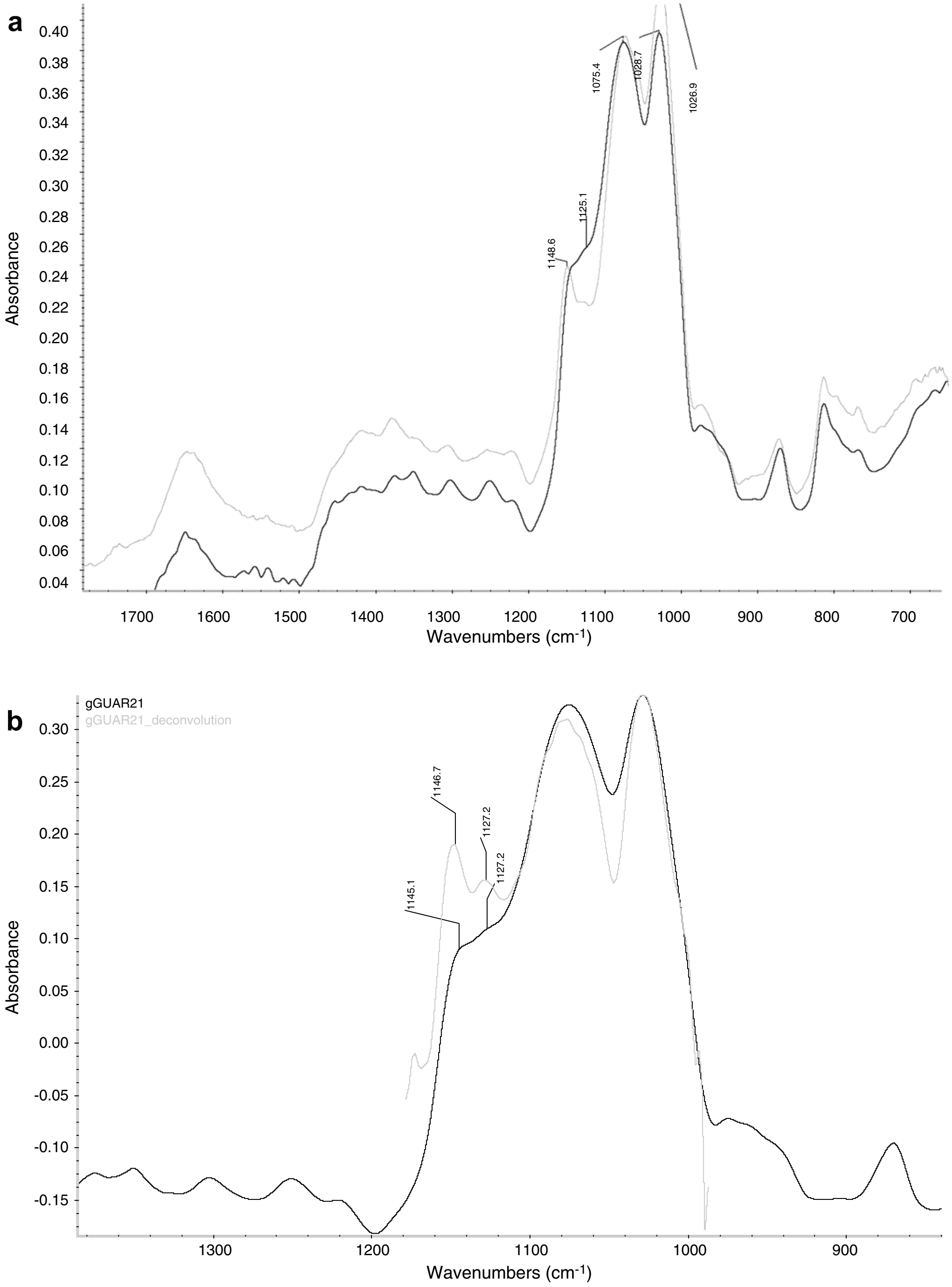

Figure 3. (a) IR spectrum of the GG polymer (grey line) and the GG hydrogel (black line) in the region between 1800 and 750 cm^{-1}. Both the polymer and the hydrogel are in dry state. (b) Comparison between the original spectrum (black line) and the deconvolved spectrum (grey line) of the GG hydrogel. Parameters of the deconvolution: FMHW 15, *K* factor 2.5.

of the GG polymer. These data are consistent with previous findings reported in the literature.[14]

Figure 4 summarizes the water content values for the swelling of the GG hydrogel at different pHs. The data reported indicated that the water content is not affected by the pH of the solution in which the hydrogel is made to swell (the swelling degree is the same within the experimental errors). This trend could be explained taking into consideration that protonable groups like carboxylic or aminic groups are not present along the GG polymer chains, thus the pH value of the solution does not influence the water content inside the hydrogel.

2.2. Rheological studies

Thixotropy is a characteristic of some pseudoplastic materials that leads to a progressive decrease in their viscosity when these

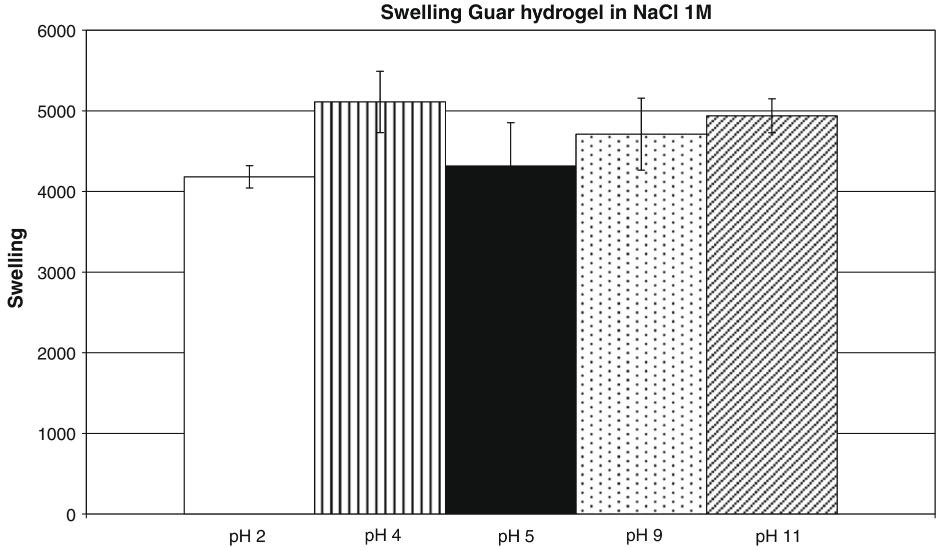

Figure 4. Water content of the GG hydrogel at different pHs at constant ionic strength after 4 days.

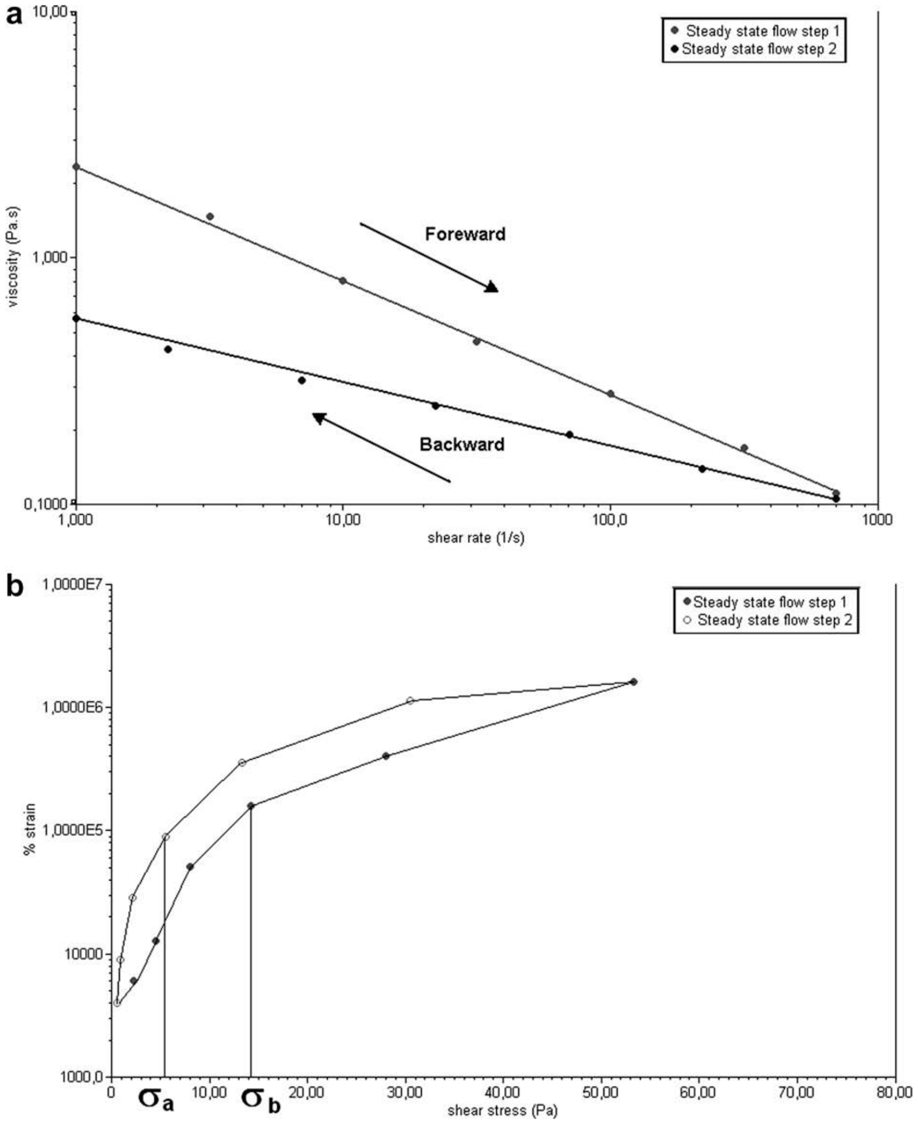

Figure 5. (a) Viscosity versus shear rate for the GG hydrogel in the steady state flow test. (b) Strain percentage versus shear stress for the GG hydrogel in the steady state flow test (step 1 forward curve and step 2 backward curve: the direction of the curves is indicated by the arrows).

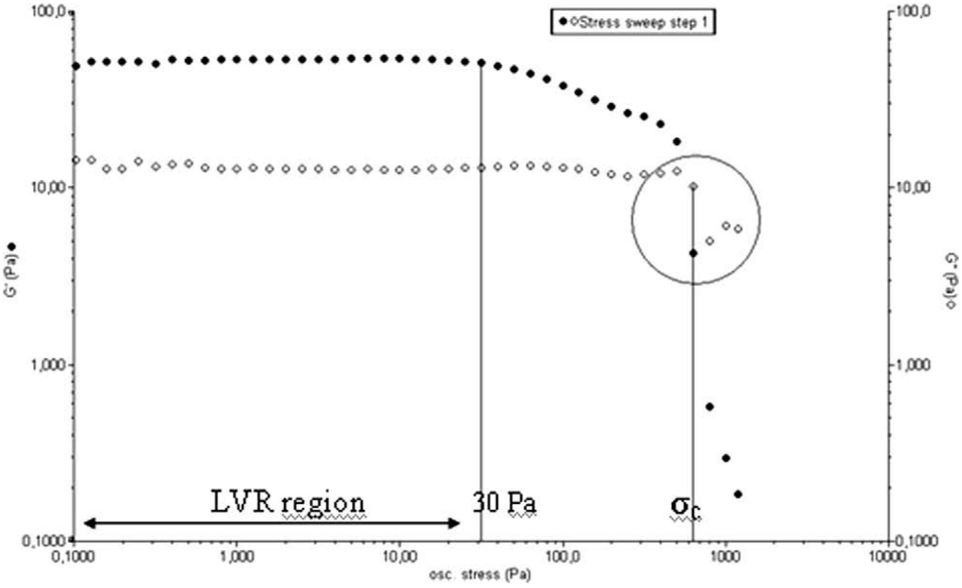

Figure 6. Elastic and viscous moduli versus oscillation stress for the GG hydrogel in the stress sweep test. The LVR (linear viscoelastic region) is between 0 and 30 Pa and the cross-over point (σ_c) (the point at which the sol–gel transition occurs) is 801 Pa.

materials are subjected to a specific mechanical stress, followed by the recovery of *its* rheological properties after a more-or-less prolonged period of rest.[15] Under stress conditions, an infinitesimal layer of fluid slides onto the adjacent layer, leading to the settling of a laminar flux. Hence the fluid transforms from the gel state to the liquid state, representing a reversible process of gel–sol isothermal transformation. This characteristic of the material is time dependent and is shown by a double rheological curve, in which a rising and a descending curve can be distinguished.

Figure 5a shows the viscosity trend vs shear rate of the GG hydrogel in a ‘steady state flow test’. The two curves (forward and backward) were not overlapped, meaning that the material morphology had changed after the rheometer stress. The area within the two curves is called hysteresis loop and it represents the energy loss required to obtain the sol–gel transition. The hysteresis loop also represents the time necessary for the rearrangement of the material. Since the energy loss, which is measured as the area of the hysteresis loop, changes linearly with the logarithm of the recovery time[16], the greater the area is the longer it takes for the rearrangement of the material. The presence of the hysteresis-loop demonstrates the thixotropic nature of the GG hydrogel. The same test performed on the GG polymer (aqueous solution at a concentration of 1.6% w/vol) demonstrated that the GG polymer is not a thixotropic material.

Figure 5b shows how the percentage strain is influenced by shear stress on the native GG hydrogel during one single rheometer test (forward curve and backward curves). The value at which the slope of the curve changes (reported in the figure as σ_a in the forward curve and as σ_b in the backward curve) is called the yield stress. At this point under a specific applied load, the morphology

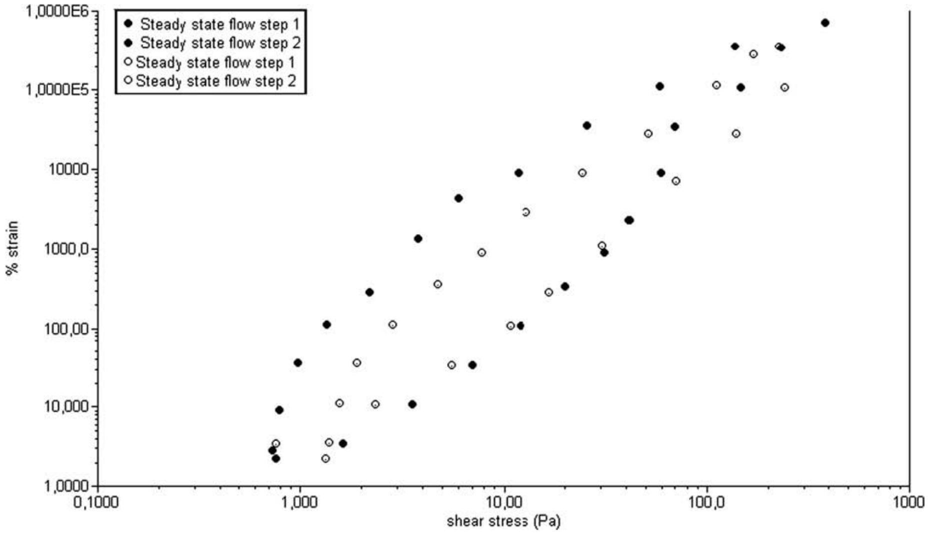

Figure 7. Influence of storage time on the shear stress versus strain relationship of the GG hydrogel. Black points correspond to the trend (step 1 forward curve and step 2 backward curve) for the hydrogel immediately after the rheometer test. White points correspond to the trend (forward and backward curves) for the hydrogel after 24 h of rest.

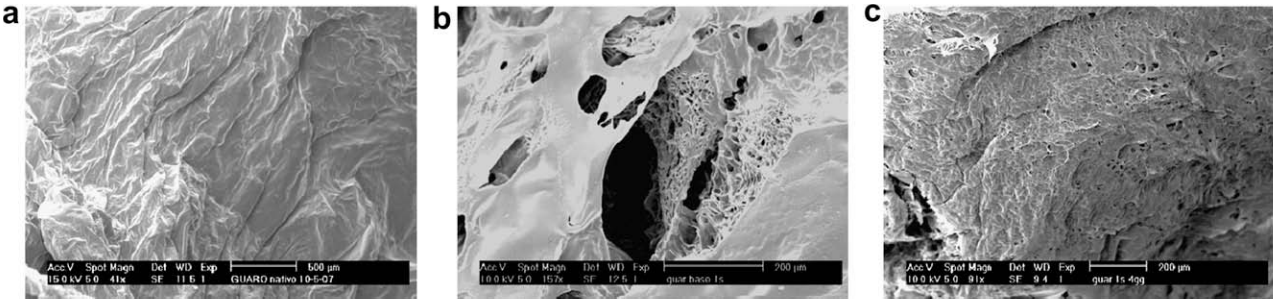

Figure 8. SEM images of (a) the native GG hydrogel, (b) the GG hydrogel after the mechanical stress and (c) the GG hydrogel after 4 days of settling.

of the material begins to modify. The yield stress values of the forward and backward curves are different, indicating that the consistency of the material has changed due to mechanical stress.

The G' and G'' moduli trend versus oscillation stress is reported in Figure 6. Within the linear viscoelastic region (LVR), the G' values were always greater than the G'' values, indicating that the GG hydrogel showed more elastic character than the viscous one. G' and G'' did not change within the range of the LVR, suggesting that the structure of the material was not broken. As a further test, Ft IR analysis of the same sample before and after the mechanical stress was performed. IR analysis demonstrated that the two spectra are practically overlapped: none of the GG hydrogel groups was altered after the mechanical rheometer-induced stress (data not shown).

Outside the LVR, for stress values between 30 and 801 Pa, the trend of G' began to decrease, while G'' values remained more or less constant, meaning that the viscosity of the material increased with respect to its elasticity. At the crossover point (also called critical stress σ_c) (801 Pa)—the point at which the G' and G'' curves intersected—the viscosity dropped drastically because the material collapsed and the transition from gel to sol occurred. After four days of settling, the G' and G'' regained the same values of the native hydrogel, demonstrating the thixotropic nature of the GG hydrogel and the recovery of its original morphology.

Figure 7 shows the trend of strain percentage versus the shear stress of the GG hydrogel immediately after the rheometer test (black points) and of the same gel after 24 h of settling (white points). The two loops are almost overlapped, meaning that after

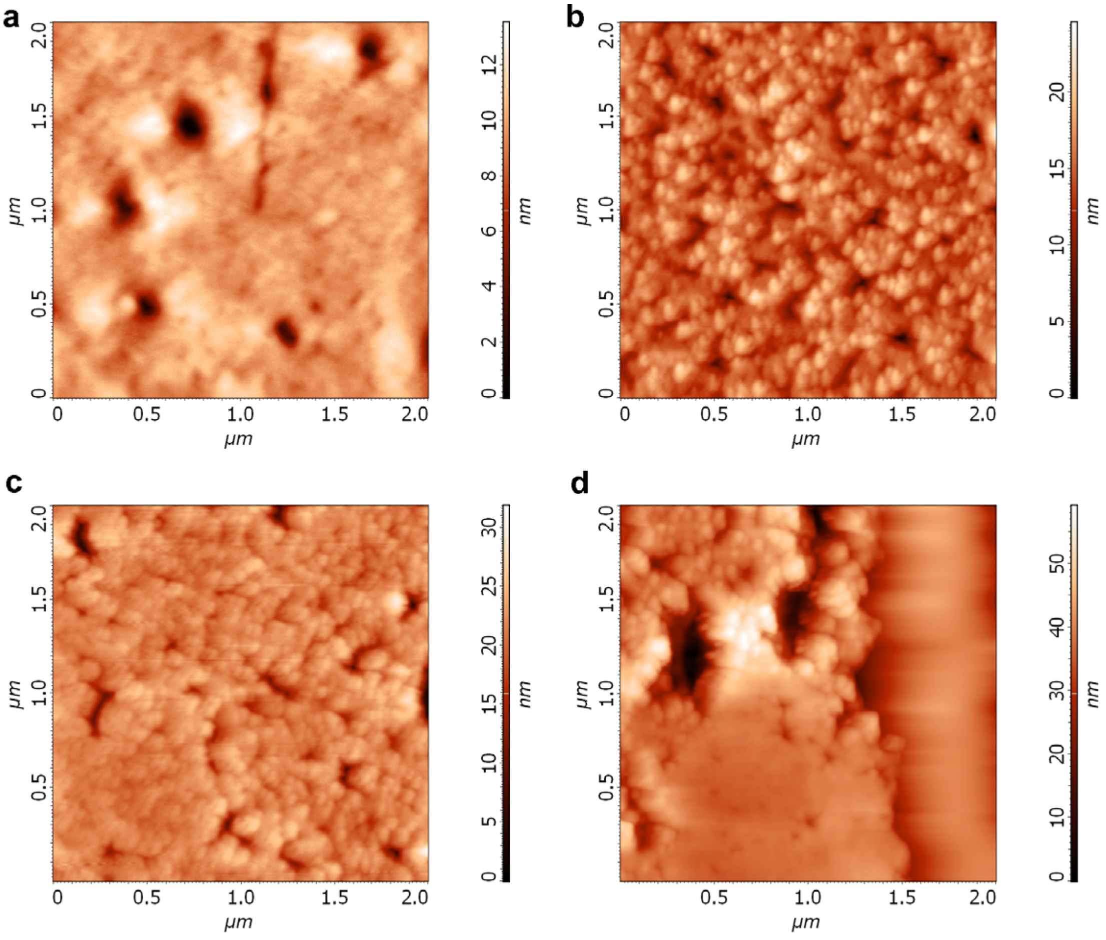

Figure 9. AFM topography scans (scan size 2×2 µm^2) of the GG hydrogel under different conditions: (a) native state, (b) 30 min, (c) 2 h and (d) 24 h after the steady state flow test. The region analyzed is always the same.

the stress the hydrogel should have regained its consistency and structure at least partially, and that the material possesses a thixotropic nature.

2.3. Morphological characterization: SEM and AFM analyses

The GG hydrogel was morphologically characterized by SEM and AFM to investigate the effect of the applied mechanical stress on the material structure. SEM analysis was performed immediately after subjecting the hydrogel to the mechanical stress and after four days of rest, in order to verify visible morphological changes in the structure of the material and to verify an eventual rearrangement of the structure. SEM analysis performed immediately after the mechanical stress showed the porous structure of the GG hydrogel with respect to the native structure of the same hydrogel, which was more compact (Fig. 8a and b). After four days of settling, the gel regained its typical native structure (see Fig. 8c). This behaviour was confirmed by the stress sweep tests, which showed a decrease in the G' and G'' after the mechanical stress in comparison with the values of the native GG hydrogel. After four days of settling the G' and G'' regained the same values as the native hydrogel, demonstrating the thixotropic nature of the GG hydrogel.

AFM analysis was performed in order to obtain high resolution images of small areas (5×5 or $2 \times 2\ \mu m^2$). Previous findings in the literature showed that a thixotropic material is always made up of nanometric particles that, under quiescent conditions, make the material stiff, thanks to the numerous points of contact among the particles.[17] When a mechanical stress is applied, the particles begin to slide towards each other until the final collapse of the structure, leading to the sol–gel transition. Once the external mechanical stress is removed, the material rearranges itself because of the Brownian motion of the nanoparticles and returns to its original morphology.[17] The morphology of the native guar is reported in Figure 9a. The appearance of the GG hydrogel was typical of a soft material with different non-homogeneous areas. Figure 9b shows the morphology of the same GG hydrogel after being subjected to a shear rate of $700\ s^{-1}$ during the rheometer measurement. At the same scan size the surface morphology of the hydrogel was completely different. The presence of nanoparticles (average diameter of 27.0 ± 5.0 nm) with no particular orientation was observed all over the surface of the gel. The same area was viewed after the hydrogel was left in a quiescent condition for 2, 3 and 24 h. It was observed that the particle diameter increased (52.0 ± 9.0 nm) as time elapsed and the particles started to interpenetrate each other, taking the hydrogel back to its original morphology (see Fig. 9c). After 24 h, the nanoparticles were visible in only a few parts of the surface, while the other regions were completely rearranged into a homogenous structure (see Fig. 9d). These observations lead us to the conclusion that the GG hydrogel behaves like a thixotropic material. In other words, when it is exposed to an external mechanical stress it loses its structure and consistency, but once the mechanical stress is removed it is able to rearrange itself, regaining its original appearance. After 24 h of settling, the structure of the GG hydrogel was only partially recovered: SEM analysis demonstrated that at least four days of settling are required for complete rearrangement.

3. Experimental

3.1. Materials

The guar gum (M_w = 220 kDa), the polyethylene glycol diglycidyl ether (PEGDGE) and all other chemicals and solvents were purchased from Sigma–Aldrich (Switzerland) and used without further purifications.

3.2. Synthesis of the GG hydrogel

The GG was dissolved in 0.1 M NaOH at a concentration of 1.6% w/v under magnetic stirring. Prior to the addition of the cross-linking agent, the temperature of the mixture was brought up to 40 °C. The molar ratio of GG and PEGDGE was 1:3, to obtain the best yield. The reaction was maintained under stirring for 15 h; at the end of the reaction, the product was washed in water and neutralized with proceed 1 M acetic acid. The hydrogel was washed with plenty of distilled water and freeze-dried.

3.3. Infrared spectroscopy and swelling measurements

IR spectra of the GG polymer and the GG hydrogel (dry state) were recorded using a Thermo Nicolet 6700 FT-IR spectrometer (Thermo Fisher Scientific, USA) equipped with a ZnSe crystal. Sixty-four scans at a resolution of $4\ cm^{-1}$ were averaged for each spectrum. Spectra were deconvolved according to the following parameters: K factor 2.5. and a FMHW 15.

For swelling measurements, 10 mg of lyophilized GG hydrogel was immersed in five different solutions with different pH values and similar ionic strength. The solution consisted of 1 M NaCl whose pH was adjusted to the required values (2, 4, 5, 9, 11) by adding 1 M HCl or 1 M NaOH. All samples were maintained at room temperature. The swollen hydrogel was weighed every 24 h, paying attention to remove all the water in excess until an equilibrium was reached (after 4 days). The water content of the hydrogel was calculated according to the equation

$$\%\mathrm{H_2O} = \frac{w_s - w_d}{w_d} \cdot 100$$

where w_s is the weight of the swollen hydrogel and w_d is the weight of the dried hydrogel.

3.4. Rheological measurements

An AR2000 controlled stress dynamic rheometer (TA Instruments, Leatherhead, UK) was used in the oscillation mode to measure the viscoelasticity of the GG hydrogel. A parallel plate (PP 20 cell model, 2 cm diameter) was fitted to the instrument, which was then controlled electronically. All experiments were conducted at 25 ± 1 °C.

Stress sweep, frequency sweep and steady state flow tests were performed to investigate the rheological behaviour of the GG hydrogel. A dynamic stress sweep test was conducted on the material to determine its linear viscoelastic region (LVR). During a stress sweep test, the sample is subjected to an increasing stress, while constant frequency and temperature are maintained. A frequency of 1 Hz or 10 Hz was used.[18] The sample's response to the stress was displayed and the LVR was determined. The LVR is the stress range in which the G' and G'' values are constant, before they drop off due to the deformation of the material. For the GG hydrogel the LVR was between 0 and 30 Pa. Test samples were subjected to a frequency sweep (from 0.1 to 10 Hz)[19] at a constant stress (6 Pa) within the LVR, and their viscoelastic parameters were measured. The frequency sweep is used to define the time dependence of the sample's deformation. The steady state flow test is used to study changes in viscosity with the increasing shear rate and gives an idea of how viscous or elastic the material is under shear stress. Steady-state flow properties were measured by subjecting the sample to an increasing shear rate ramp ranging from 1 to $700\ s^{-1}$. One/two cycles (two/four steps) were generally carried out in order to investigate the morphologic changes of the GG hydrogel, its eventual degradation and the hypothesised rearrangement.

3.5. Morphological characterization (SEM and AFM)

Native and mechanically stressed GG hydrogels were analyzed by SEM (XL20, Philips, Eindhoven, NL). The mechanical stress was induced by rheometer tests and by passing the hydrogels through a syringe, with the aim of assessing whether the mechanical stress was able to induce morphological changes in the structure of the material. Samples were freeze-dried, then gold sputtered with an automatic sputter coater (BALTEC SCD 050, Balzer, Germany) and finally viewed by SEM at an accelerating voltage of 10 kV.

The surface morphology of native and mechanically stressed GG hydrogels was analyzed by atomic force microscopy (AFM) (Solver Pro, NT MDI Instruments, RUS). The hydrogels were viewed in dry state because if the hydrogel was hydrated the surface roughness would be too high for the maximum extension of the scanner in the *Z*-direction. The procedure for preparing the hydrogels for AFM analysis was similar to that reported in the literature.[20] Briefly, after the mechanical stress the GG hydrogel was left to dry at room temperature until it was possible for the tip to approach the surface. This time was estimated to be around 30 min and was strictly dependent on the amount of hydrogel. The surface topography was analyzed after 30 min, 2 h, 3 h and 24 h in the same area of the samples to evaluate the recovery effect on the surface morphology. AFM images were acquired in air in non-contact mode on five different areas per sample (three samples per type) using a sharpened gold coated silicon tip with a spring constant of 2.5–10 N/m and a nominal resonance frequency of between 120 and 180 kHz.

4. Conclusions

A new GG hydrogel with a thixotropic character was synthesized by cross-linking the polymer chains with PEGDE. Other GG hydrogels chemically cross-linked with different agents such as glutaraldehyde, and phosphated compounds do not show the same thixotropic nature. Moreover, it has been shown how atomic force microscopy can be a useful tool to investigate the thixotropic behaviour of a 3D matrix.

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