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Synthesis of a New Hydrogel, Based on Guar Gum, for Controlled Drug Release

G. Panariello,[1] R. Favaloro,[1] M. Forbicioni,[2] E. Caputo,[2] R. Barbucci[1,2]*

Summary: Guar gum (GG) polymer was crosslinked in order to obtain a new hydrogel. The GG hydrogel has been characterized by means of FT-IR spectroscopy, the determination of the water content, at different pH values, rheological measurements and in vitro release studies. The GG hydrogel shows a maximum water uptake at acid and basic pH values. The mechanical properties are investigated in order to verify the thixotropic behaviour of the material. In vitro release studies are conducted to evaluate the application of the GG hydrogel as a matrix for controlled drug release.

Keywords: drug release; guar gum; hydrogel; thixotropy

Introduction

Guar gum (GG) is an hydrophylic and nonionic polysaccharide extracted from the seed endosperm of the plant Cyamopsis Tetragonalobus. GG belongs to the wide family of galactomannans and consists of a $(1 \rightarrow 4)$ linked β-D-mannopyranosyl backbone partially substituted at O, linked at C6, with α-D-galactopyranosyl side groups.

Taking into consideration that GG polymer is a cheap, easily available and non toxic[1] polysaccharide, it is widely used in many industrial fields. Thanks to its characteristic property to produce highly viscous aqueous solutions, it is commonly used as a thickening agent in cosmetics and in the food industry. In pharmaceutical sector, for example, its properties are of fundamental importance for the controlled release of drugs in the gastrointestinal tract, as well as carrier for colon targeted drugs[2] in the treatment of colorectal cancer.[3] The GG polymer is also used in the transdermal drug delivery system[4] and as supplementing agent to increase the viscosity for osteoarthritis treatments.[5]

In literature are reported different strategies to obtain GG based hydrogel for biomedical applications and in particular for the release of drugs in the gastrointestinal tract. The chemical cross-linking of GG with glutaraldehyde and phosphate[6,7] produces hydrogels to be used as carrier for colon targeted and transdermal drug delivery. Other papers proposed a GG hydrogel based formulation which employed the borate as cross-linking agent.[8]

The aim of this research is the generation of a new GG-based hydrogel[9] suitable to be used as a scaffold for tissue engineering and drug release.

The crosslinking agent used for the preparation of GG hydrogel is the poly ethylene glycol diglycidyl ether (CH_2OCH-$(CH_2CH_2O)_n$-$CHOCH_2$, $n = 10$, PEGDGE) which has been already used as cross-linking agent for hyaluronic acid[10] with proven non toxic properties. A particular attention was devoted to the study of mechanical properties and the in vitro drug release.

Materials and Methods

Materials

The guar gum ($\overline{M}_w = 220$ KDa), the poly ethylene glycol diglycidyl ether (PEGDGE) and all other chemicals and solvents were purchased from Sigma-Aldrich (Switzerland).

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[1] Biosuma s.r.l. Via Cassia Nord 625, 53014 Monteroni d'Arbia (Si)
E-mail: panariello@biosuma.it
[2] C.R.I.S.M.A. University of Siena, Via Aldo Moro 2, 53100 Siena

The Metronidazole (MZ) was purchased from Bioindustria L.I.M. (Italy).

Syntesis of GG Hydrogel

The GG was slowly dissolved in NaOH 0.1 M 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 raised 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 dispersed in water and neutralized (pH = 7) with acetic acid (96%). The hydrogel was washed with plenty of distillated water.

Water Content

10 mg of lyophilized GG hydrogel were put into a Petri dish and immersed in four different solutions with different pH values: (i) HCl (pH 1.0), (ii) HCl (pH 2.6), (iii) MES buffer (pH 4.5), (iv) Phosphate Buffer Solution (PBS, pH 7.4), (v) and buffer solution pH = 10 (0.013 M sodium tetraborate, 0.018 M sodium hydroxide) at room temperature. The swollen hydrogel was weighed every 24 h paying attention to remove all the water in excess.[11] 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 swollen hydrogel and w_d is the weight of dried hydrogel.

FT-IR Spectroscopy

IR spectra of GG polymer and GG hydrogel (dry state) were recorded using FT-IR spectrometer Thermo Nicolet 6700 FT-IR (Thermo Fisher Scientific, USA) equipped with a ZnSe crystal. Sixty-four scans at a resolution of 4 $\mathrm{cm^{-1}}$ were averaged for each spectrum. The FT-IR spectroscopy was interfaced to a personal computer with the Omic software version 7.3.

Rheological Measurements

An AR2000 controlled stress dynamic rheometer (TA-Instruments, Leatherhead, United Kingdom) was used to measure the viscoelasticity of the GG hydrogel in the oscillation mode. A parallel plate (PP 20 cell model diameter 2 cm) was fitted to the instrument which was then electronically controlled.

The shear costant ($K\gamma$) is given by R/H (plate radius/plate gap). The shear rate γ ($\mathrm{s^{-1}}$) is then obtained by $K\gamma$ (ω) (shear costant × angular velocity).[10] The shear stress (σ) is the product of stress costant and torque ($K\sigma \times M$).[10] The stress ($K\sigma$), which is dependent on gravitational force and the plate geometry is given by $Gc/\pi(R/10)3$.[10] Each gel sample was gently loaded onto the peltier plate using a tablespoon. A particular care was taken to minimize shearing during sample removal and sample loading. All experiments were conducted at 25 ± 1 °C.

In a frequency sweep, the frequency is varied while the stress and the temperature are maintained constant. The frequency sweep is run at a stress value (6 Pa) within the LVR, as determined during the previous stress sweep. Frequency is a measure of the time needed to complete one oscillation and is defined as the inverse of time. The frequency sweep is used to the time dependency of the sample's deformation. Steady flow is used to study the viscosity change with the increasing shear stress. It also gives an idea of how viscous or elastic the material is under shear stress.

"Stress sweep", "frequency sweep" and "steady state flow" tests were performed to investigate rheological behaviour of GG hydrogel. Tests were conducted in controlled stress mode to obtain steady-state flow curves. In the stress sweep test samples were subject to a stress sweep (from 0.01 to 1200 Pa) at 1 Hz and their viscoelastic parameters (G',G'') measured. In the frequency sweep test samples were subject to a frequency sweep (from 0.1 to 10 Hz)[12] at a costant stress (6 Pa) and their viscoelastic parameters measured. Steady-state flow properties were measured by submitting the sample to an increasing shear rate rampe in the range from 1 to 700 $\mathrm{s^{-1}}$.

One/two cycles (two/for steps) were generally carried out to investigate the morphologic changes of GG hydrogel, its eventual degradation and the hypothesised rearrangement.

Drug Release Studies

A sample of 20 mg of GG hydrogel lyophilized was loaded with a MZ solution (5 mg/ml). The tests were conducted in this way: the GG hydrogel loaded with MZ was subject to a flow ($0.18\ \mathrm{ml} \cdot \mathrm{s}^{-1} \cdot \mathrm{cm}^{-2}$) of physiological solution. The amount of drug released was measured at time intervals and was then spectrophotometrically determined at 320 nm.

Results and Discussion

Synthesis and FT-IR Analysis of GG Hydrogel

GG polymer has two primary alcoholic groups linked at Carbon C6: the first on the galactosidic unit, the latter on the mannosidic unit. PEGDGE is a bifunctional crosslinker with two epoxide rings linked at the head and tail of the PEG chain. The crosslinking of GG with PEGDGE, through the formation of an ether bond, was conducted in alkaline solution (NaOH, $pH > 12$).[9] The amount of PEGDGE added was in excess compared with amount of GG (molar ratio GG/PEGDGE = 1/3): this allowed to obtain the best yield. The reaction was conducted at 40 °C, overnight. The product was dispersed in distilled water, neutralized with acetic acid (96%), washed with distilled water and freeze-dried. GG hydrogel is non-cytotoxic for contact and for release.

The FT-IR spectra of GG hydrogel and GG polymer show that the two spectra are very similar because no new additional functional groups were introduced with the cross-linking reaction. The only difference was the peak at 1125 cm^{-1}, due to the introduction of PEG in the polymer chain. This peak is ascribable to the C–O–C stretching bonds.

Water Content

The measurements of the water content indicated that the GG hydrogel maximum water uptake occurred at $pH = 1.0$ (5200 ± 260) and at $pH = 10$ (4300 ± 220). A possible explanation for this behaviour can be found taking into consideration that at acid pH most of the OH groups may be protonated and hence the polymer chains are kept away by the electrostatic repulsions among the positively charged groups which are very hydrophilic and hence allow the entrance of water inside the gel structure. On the contrary, at basic pH, most of the OH groups are deprotonated inducing an electrostatic repulsion between the negative charges along the polymer chains and thus the entrance of the water is promoted for the same reason. Hence in both situations (acid and basic pH) a big amount of water was allowed to enter inside the hydrogel. All that does not occur at intermediate pH values, in PBS (3000 ± 150) or MES buffer (3350 ± 170).

Reology and Thixotropy

Thixotropy involves a progressive decrease in viscosity, and thus of the shear stress, due to the agitation produced on submitting the systems to continuousus shear deformation, followed by the recovery of the rheological properties after a more-or-less prolonged rest period.[13]

These fluids are time-dependent and are characterized by a duplex rheological curve, in which it is possible to distinguish an ascending and a descending branch. The area within the two branches, hystheresis-loop, represents the energy required to obtain the sol-gel transition. The hystheresis-loop represents the time necessary for the rearrangement of the material. The wider is the area, the longer is the time necessary for the rearrangement of the material.

Figure 1a shows the viscosity trend vs shear rate of GG hydrogel in a ''steady state flow test''. The two curves were not overlapped, meaning that the material morphology had changed after rheometer stress. When the yield stress (Figure 1b) values of the first and the second curves were compared, it was noticeable how the shear stress decreased when the system had been stressed. The

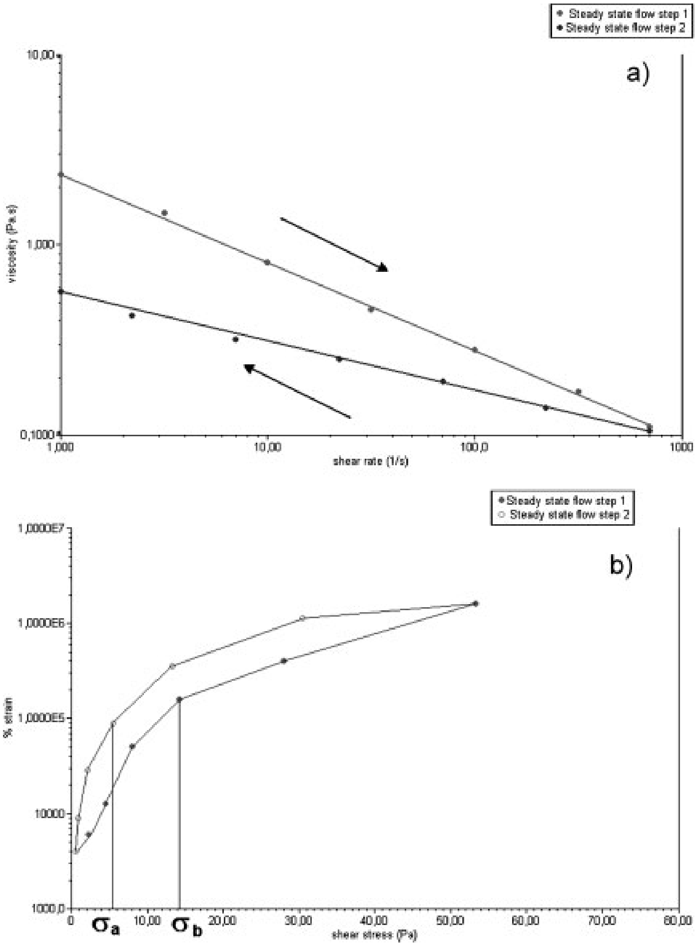

Figure 1.
a) Viscosity trend vs shear rate of GG hydrogel in a "steady state flow test"; b) Yield stress of GG hydrogel.

stressing, however, did not effect the shear thinning behaviour, since the power law index values were equivalent for the first and the second curves.

The viscosity value changes depending on the shear rate and does not follow the rheological path backwards. This means that the mechanical stress on the only-GG hydrogel induces a change in the morphology of the material, which, however, regains more or less fastly its original structure.

From all measurements of GG hydrogels it resulted that G′ is higher than G″ during the entire measured frequency range and G′ changes a little in function of frequency. This can be seen in the dynamic mechanical spectra (frequency sweep test) in Figure 2.

Release Behaviour of GG Hydrogel

The release profiles of MZ from GG hydrogel subject to a flow of a physiological solution is shown in Figure 3.

In this test, the 50% of the MZ loaded was released in 70 min; the drug release from the GG hydrogel, subject to a flow, continued for 8 h and the maximum amount of MZ released was the 80% of the MZ loaded.

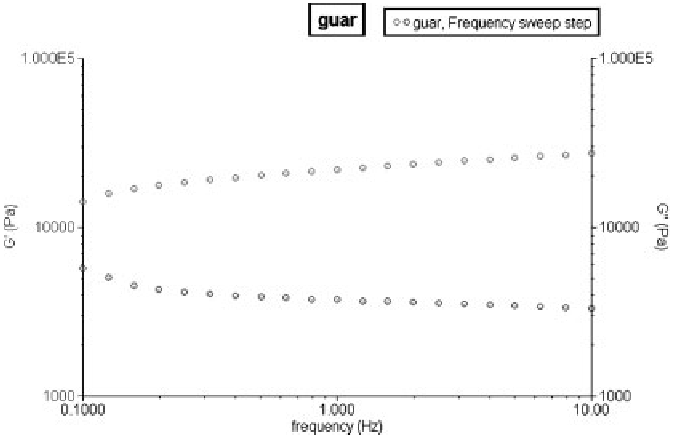

Figure 2.
Frequency sweep test for GG hydrogel.

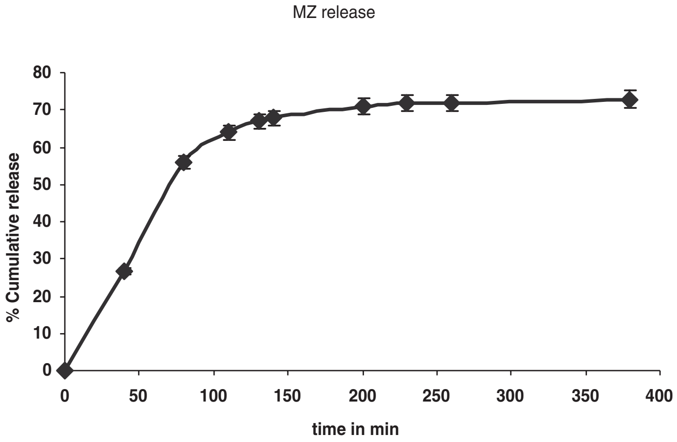

Figure 3.
Experimental data for time-dependent release of MZ from GG hydrogel subject to a flow of physiological solution.

Conclusions

A new hydrogel based on GG was synthesised by cross-linking the polymer chains with PEGDGE. The hydrogel swelling was strictly dependent on the pH of the solution in which the hydrogel was made to swell. The rheological analysis demonstrated that the GG hydrogel can be considered as a thixotropic material. In the in vitro release studies the MZ was used as a prototype drug due to its excellent therapeutic effects against a broad spectrum of bacterial infection. In vitro release studies were performed in presence and in absence of physiological solution flow. The present results suggest that GG hydrogel could be used as a controlled release device.

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