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Viscoelastic properties modulation of a novel autocrosslinked hyaluronic acid polymer

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Autocrosslinked polysaccharide (ACP) polymers are inter- and intra-molecular esters of hyaluronan (HA) in which part of the carboxyl group is esterified with hydroxyl groups of the same and/or different molecules of the polysaccharides. Suspensions of ACPs in bidistilled water, with relative levels of crosslinking varying from 5% to 20% and different weight concentrations, were obtained to analyse their viscoelastic properties. The steady shear tests showed the pseudoplastic behaviour of ACP, viscosity having a higher value at higher concentrations and degrees of crosslinking. The viscoelastic properties showed an essentially elastic behaviour in the examined frequency range, and they can be modulated by varying both the degree of crosslinking and the weight concentration.

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1. Introduction

ACP (autocrosslinked polysaccharides) are a new class of hyaluronic acid (HA) derivatives obtained through a crosslinking process that does not introduce foreign bridge molecules.

This crosslinked polymeric system has potential use in all medical applications where there is a need for a biocompatible, biodegradable, viscoelastic material with improved mechanical properties compared to unmodified HA solutions of the same molecular weight. ACP has in fact a solid-like weak gel behaviour and does not flow easily once put in place, whereas HA solutions present a viscoelastic behaviour intermediate between solid and liquid [1].

Moreover, ACP maintains the shear thinning behaviour of HA solutions, i.e. its viscosity decreases as shear rate increases. The improved viscoelastic and gelling properties of crosslinked HA give rise to an increased residence time of these molecules in the body when compared to unmodified HA, thus extending the range of biomedical applications [2].

Therefore the field of application of ACP could be defined as matrix engineering, in particular viscosurgery and viscosupplementation [1]. ACP can also be processed into products for use in areas such as tissue repair and drug delivery.

The viscoelasticity of ACP can be modulated as required for the different applications, by varying both the concentration and the degree of crosslinking, as verified by the rheological analysis carried out in the present study.

2. Materials

ACP polymers, manufactured by FAB (Fidia Advanced Biopolymers Srl, Abano Terme, Italy) are inter- and intra-molecular esters of HA in which part of the carboxyl groups are esterified with hydroxyl groups belonging to the same molecule and/or different molecules of the polysaccharide thus forming a mixture of lactones and inter-molecular ester bonds [3].

The level of crosslinking can be varied by modulating the reaction conditions. The crosslinking reaction can be carried out on HA of the desired molecular weight. The unique feature of FAB's crosslinking technology with respect to alternative approaches to crosslinking HA [4] is that no bridge molecules are present between the crosslinked HA chains. This ensures that only the natural components of HA are released upon degradation of ACP.

In the present study, the following derivatives, synthesized from HA of 200 kDa, are considered: ACP-5, ACP-10, ACP-15, ACP-20. The numbers reflect the relative level of crosslinking although they are not a direct measure of the percentage of carboxyl groups bound to hydroxyl residues. These ACP's are white powders and give rise, upon hydration, to water adsorbing transparent gels.

Bidistilled water was added to the powders supplied by FAB in order to obtain three different weight concentrations (1, 5, 10 mg/ml) for every degree of crosslinking.

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3. Methods of measurement

The rheological properties of ACP's were evaluated on a Bohlin VOR Rheometer (Bohlin Reologi A B, Lund, Sweden) at a controlled temperature of 25 °C. The measuring system was cone and plate (CP 5/30 cell). The lower plate was forced to rotate or oscillate, whereas the stress transferred from the fluid to the upper plate was measured by means of a Linear Variable Displacement Transducer (LVDT) system.

The non-linear flow properties of the investigated materials were evaluated through steady shear measurements to determine the viscosity η as function of shear rate $\dot{\gamma}$, while the small-amplitude oscillatory shear experiments allowed the measurement of the unsteady response of the samples and hence the determination of their linear viscoelastic properties. Moreover this technique is very useful for the determination of the relationships between structure and mechanical properties [5, 6].

In this dynamic experiment the material is subjected to a sinusoidal shear strain:

$$\gamma = \gamma_0 \sin(\omega t)$$

where γ_0 is the shear strain amplitude, ω is the oscillation frequency and t is the time. The mechanical response expressed as shear stress τ of viscoelastic materials is intermediate between an ideal pure elastic solid (obeying to Hooke's law) and an ideal pure viscous fluid (obeying to Newton's law) and therefore is out of phase respect to the imposed deformation as expressed by:

$$\tau = G'(\omega)\gamma_0 \sin(\omega t) + G''(\omega)\gamma_0 \cos(\omega t)$$

where $G'(\omega)$ is the shear storage modulus and $G''(\omega)$ is the shear loss modulus. G' gives information about the elasticity or the energy stored in the material during deformation, whereas G'' describes the viscous character or the energy dissipated as heat.

Strain sweep tests at fixed oscillation frequency (consisting in monitoring the viscoelastic properties while logarithmically varying the strain amplitude γ_0) were previously performed on these materials to determine the strain amplitudes at which linear viscoelasticity is valid.

4. Results and discussion

4.1. Steady shear viscosity

Steady shear experiments were performed in order to evaluate the effect of the degree of chemical crosslinking and weight concentration on the shear viscosity of ACP.

ACP viscosity shows a pseudoplastic (shear thinning) behaviour, because it decreases as shear rate increases. Moreover the flow curve, i.e. the curve of η as function of $\dot{\gamma}$, shows higher values at higher concentrations and degrees of crosslinking.

Example flow curves of ACP are shown in Fig. 1 at different degrees of crosslinking and a fixed concentration of 10 mg/ml, and in Fig. 2 at different concentrations and a fixed degree of crosslinking (ACP 20). It can be noted that the variation in concentration has a larger effect on viscosity than the variation in degree of crosslinking.

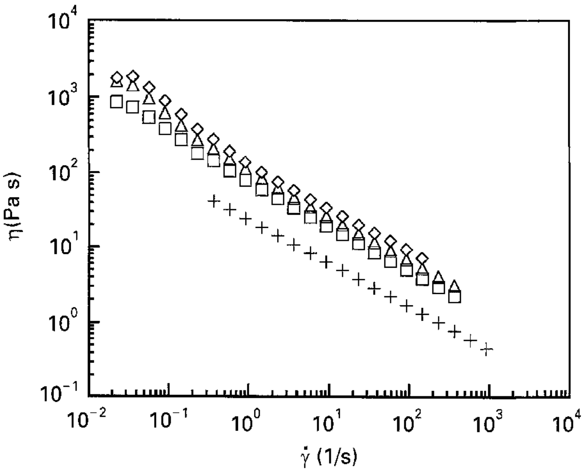

Figure 1 Flow curve of ACP at different degrees of crosslinking and fixed concentration of 10 mg/ml: + ACP 5; □ ACP 10; △ ACP 15; ◇ ACP 20.

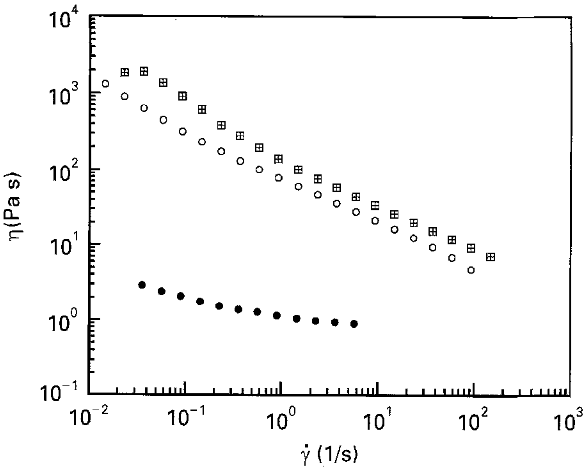

Figure 2 Flow curve of ACP-20 at different concentrations: ● 1 mg/ml; ○ 5 mg/ml; ⊞ 10 mg/ml.

4.2. Viscoelastic properties

The analysed ACP were formed by a suspension of chemically crosslinked hyaluronic acid particles swollen in bidistilled water at different weight concentrations. Rheological analysis allows the quantitative evaluation of the viscous and elastic response of these materials and the correlation of these values to concentration and degree of crosslinking.

The chemical crosslinking of hyaluronic acid is a possible way, without losing biocompatibility, to increase the elasticity of HA and to make it insoluble, both properties being very useful in many medical applications [1, 7].

ACP, at concentrations above 1 mg/ml, shows an essentially elastic behaviour; in fact frequency sweep tests show that the dynamic elastic modulus G' is higher than the dynamic viscous modulus G'' in the examined frequency range (10^{-2}–10^1 Hz). The

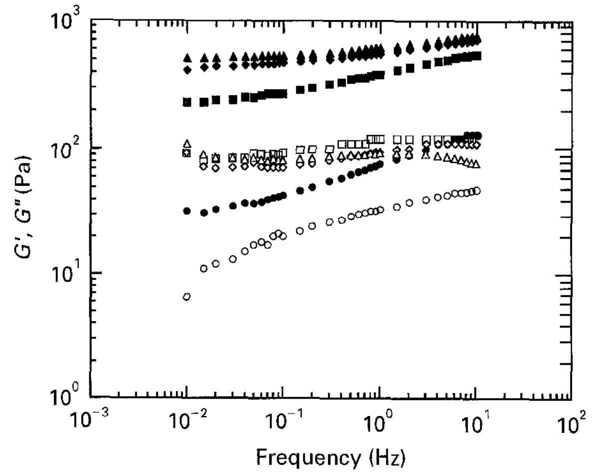

Figure 3 Mechanical spectra of ACPs at fixed concentration of 10 mg/ml: ● G' 5; ○ G'' 5; ■ G' 10; □ G'' 10; ◆ G' 15; ◇ G'' 15; ▲ G' 20; △ G'' 20.

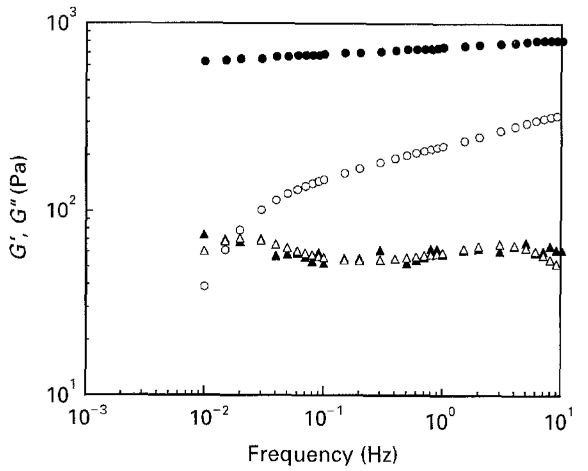

Figure 4 Experimental mechanical spectrum (G' exp (●) and G'' exp (▲)) for ACP-20 at 10 mg/ml compared with transient network mechanical spectrum from "weak gel model" (G' trans (○) and G'' trans (△)).

mechanical spectra of G' and G'' are presented in Fig. 3 as a function of oscillation frequency at a fixed concentration of 10 mg/ml, and at different degrees of crosslinking.

This solid-like behaviour is typical of 'weak gel' because the elastic and viscous moduli are slightly frequency dependent; in fact, especially at higher degrees of crosslinking, G' and G'' maintain a constant value and are parallel to each other all over the frequency range [8, 9].

This mechanical behaviour is different from that typical of HA solutions where at low frequencies the viscous character prevails, whereas at higher frequencies the elasticity overcomes the energy dissipation [1].

Moreover, ACP's show higher elasticity than HA solutions and do not flow easily under moderate stresses, so permitting higher residence times. In the case of ACP's the overall elastic response of the material is due to the contributions from covalent crosslinks obtained through chemical reaction and from transient crosslinks, mainly due to physical interactions and entanglements formed by free polymer chains.

The 'weak gel' model [10] was used in order to separate the contributions from the chemical and the transient network. The viscoelastic behaviour of a transient network at equilibrium can be expressed by the following integrals:

$$G_t'(\omega) = \int_{-\infty}^{+\infty} H(\lambda)\frac{\omega^2\lambda^2}{1+\omega^2\lambda^2}\,\mathrm{d}\ln\lambda$$

$$G_t''(\omega) = \int_{-\infty}^{+\infty} H(\lambda)\frac{\omega\lambda}{1+\omega^2\lambda^2}\,\mathrm{d}\ln\lambda$$

Where ω is the oscillation frequency, λ the relaxation time and $H(\lambda)$ the relaxation times spectrum.

The chemical network can be taken into account through an elastic modulus G_e.

Therefore the experimental dynamic elastic modulus G' can be obtained by the sum of the two contributions:

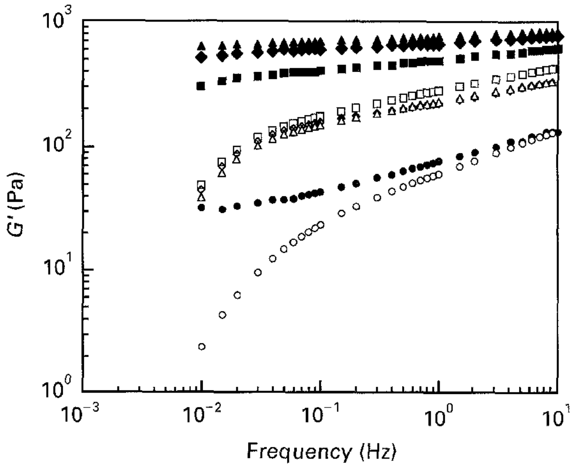

Figure 5 Experimental dynamic elastic modulus (G' exp) compared with transient network dynamic elastic modulus (G' trans) for ACPs at fixed concentration of 10 mg/ml: ● G' exp ACP 5; ○ G' trans ACP 5; ■ G' exp ACP 10; □ G' trans ACP 10; ◆ G' exp ACP 15; ◇ G' trans ACP 15; ▲ G' exp ACP 20; △ G' trans ACP 20.

$$\begin{aligned} G'(\omega) &= G_e + G_t'(\omega) \\ &= G_e + \int_{-\infty}^{+\infty} H(\lambda)\frac{\omega^2\lambda^2}{1+\omega^2\lambda^2}\,\mathrm{d}\ln\lambda \end{aligned}$$

The transient dynamic modulus G_t' was evaluated using the expression of $H(\lambda)$ by Nynomye and Ferry [4] and was compared with the experimental values of G' for all ACP's.

An example is shown in Fig. 4 for ACP-20 at 10 mg/ml. The transient network G' (G' trans) curve is very frequency dependent and is similar in shape to unmodified HA solutions, while the experimental G' (G' exp) is almost completely constant with frequency.

It is noteworthy that, at fixed concentration, the contribution from the permanent network, i.e. G_e, increases as the relative level of chemical crosslinking of ACP's increases, as can be easily seen in Fig. 5, where the curves of experimental G' and transient network G' for ACP's at fixed concentration of 10 mg/ml are presented.

5. Conclusions

ACP is a biocompatible and biodegradable polymer because it is pure HA with no bridge molecule thus ensuring the release of only the natural components of HA upon degradation. Moreover the viscoelastic properties of ACP are substantially improved with respect to unmodified HA of the same molecular weight and can be modulated both by varying the degree of crosslinking and the weight concentration.

In particular, at fixed concentration, the elasticity can be increased by increasing the level of chemical crosslinking substantially without increasing the viscous dissipation.

It has been shown that the contribution to overall elasticity from the transient and the permanent network can be quantitatively evaluated through an appropriate model.

ACP's will improve the physico-chemical properties of HA in its already known orthopaedic and ophthalmologic applications and will find possible application in tissue repair and drug release.

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