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Rheological behavior of anionic collagen injectable gels in the presence of rhamsan for plastic surgery applications

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Abstract The present paper describes the rheological properties of anionic collagen gels and anionic collagen:rhamsan composites gels in the concentration of 0.7, 4 and 6%, estimated to be used as injectable biomaterials for plastic reconstruction. Rheological studies of these gels showed that independently of pH, composition and concentration the viscoelastic behavior was dependent on the frequency, with the storage modulus always greater than the loss modulus ($G' > G''$ and $\delta < 45°$). Creep experiments showed that anionic collagen:rhamsan composites equilibrated at pH 7.4 were less elastic and more susceptible to deformation in comparison to gels equilibrated at pH 3.5. Flow experiments indicated that the force needed for the extrusion of anionic collagen:rhamsan composites, in comparison to anionic collagen, was significantly smaller and with a smoother flow, suggesting the association with rhamsan may be a good alternative in the replacement of glutaraldehyde to stabilize the microfibril assembly of commercial collagen gel preparations. Finally, on the basis of dynamic viscosity profiles found for different preparations, some of these composites are potential candidates to be utilized in laryngology.

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Introduction

The biological properties of collagen, the most abundant protein in the animal kingdom [1] have been known for a long time. Its capacity of reconstruction from diluted solutions has taken to a range of applications of biomedical interest [2]. Different collagen formulations (e.g. collagen films and sponges) have been developed in recent years for various biomedical devices targeting specific clinical needs [3]. Aiming at minimally invasive surgical procedures, more focus has been placed on injectable implants. In the form of injectable biomaterials, collagen is generally prepared in the form of a microfibril suspension for plastic corrections [4]. This application form is preferable to invasive surgical techniques, as showed in the treatment of urinary incontinence [5], increase of vocal folds [6] and vesicourethral reflux [7]. The objective of these applications is to provide a mechanical function as required by the indication.

To reduce the solubility of these suspensions in physiologic conditions, and at the same time to give a better fluidity to the gel, the glutaraldehyde has been used [8]. These preparations have presented problems, such as high cytotoxicity effect of glutaraldehyde [9] and precipitation of fibers in the needle [10], which make difficult both flow and correction. To minimize those problems the use of glycosaminoglycans has been proposed for the preparations of collagen, not only as a means of microfibril suspension stabilization, but also to give better flow properties to injectable collagen preparation minimizing fiber precipitation by a lubrificating effect. Nevertheless this use has been commercially limited as the extraction and purification procedures from tissues are quite troublesome [11].

In order to contribute in this sense, the use of rhamsan [12], a bacterial polysaccharide produced by *Alcaligenes ssp ATCC 3196* has been proposed. Its structural features

consist of a linear structure with a repetitive unit in the form of a tetrasaccharide, presenting carboxylic groups in its structure. The existence of branching in the polymeric chain seems to have deep effects on the interactions. Although rhamsan does not form gels in aqueous solutions, it creates very viscous solutions with high degree of thermal stability, pH variations and ionic force.

Previous results obtained with anionic collagen in the presence of this polysaccharide showed that besides good injectability characteristics [13], it is highly biocompatible [14].

A way to evaluate the flow characteristics is the study of the rheological properties of the fiber dispersions of reconstituted collagen or the composites formed with rhamsan. Studies of the rheological properties in this area have not been extensive. Limited previous researches included creep compliance measurements [15], extrusion forces in the suspensions of collagen and increase in the rigidity of the collagen fibers [16]. Therefore, an experimental study of rheological parameters becomes important, as they can help to establish a relation among the several structural characteristics of these fibrilar gels, once gels with defined structural geometry respond when subjected to shear stress.

The objective of the present paper is to investigate the rheological properties of collagen and collagen:rhamsan composites, to obtain injectable collagen gels for plastic corrections replacing the commercial preparations of those stabilized with glutaraldehyde.

Experimental

Collagen preparation: polyanionic collagen

In an attempt to hydrolyse amide groups selectively in collagen-rich, 50 g of Porcine Intestinal Submucosa (PIS) were treated at 20 °C for 72 h with an alkaline solution (3 mL of solution/g of tissue) containing 6% per volume of dimethylsulfoxide, salts (chlorides and sulfates), bases of alkaline (K^+, 1.19 M and Na^+, 1.74 M) and alkaline earth metals (Ca^{++}, 0.86 M) [17]. The resulting materials were equilibrated with a solution containing Na_2SO_4, NaCl, KCl and $CaSO_4$ (6 mL of solution/g of tissue) for a period of 12 h, and the excessive salts were removed by washes with the following solutions: H_3BO_3 3% (3 × 2 h, 250 mL), EDTA 0.3%, pH 11 (3 × 2 h, 250 mL) and deionized water (3 × 6 h, 250 mL).

Polyanionic collagen gels (CA)

To swell the tissues, PIS treated as described above was suspended in deionized water and the pH was adjusted to 3.5 with pure acetic acid. The mixture was homogenized in a blender and the concentration of collagen gels was adjusted to 0.7%(w/w), as determined by hydroxyproline assay [18]. The polyanionic collagen obtained was purified by precipitation by the addition of NaCl 0.9% and centrifugation of the precipitate in 15,000 rpm at 20 °C for 30 min (Hitachi-CR20B2). After some cycles, the centrifugation precipitate was arranged in dialysis bag (exclusion limit of 1000 Da) and exhaustively dialyzed against phosphate buffer (TF) 0.13 M, (pH 7.4) until the solution conductivity had become the same of the buffer. The concentration of this collagen preparation was adjusted to 0.7%(w/w).

Preparation of anionic collagen:rhamsam composites (CAR)

These composites in gels were prepared by the addition of a calculated volume of rhamsan solution (3.0 mg/mL solubilized in acid acetic pH 3.5) to 0.7%(w/w) anionic collagen pH 3.5 to produce anionic collagen:rhamsan composites with mass ratios between 1:100 to 1:25. Part of each composite gel was dialyzed against TF until constant conductivity similar to the TF solution achieved. The resulting material was centrifuged eight times at 17,000 rpm for 45 min at 20 °C and its concentrations were adjusted to 4 and 6%(w/w) as determined by the hydroxyproline assay [18]. The adjustment of these concentrations was performed by the evaporation of the water contained in gels in graduated plastic tubes, with dimensions of 1.4 cm diameter and 10 cm height, inside a desiccator containing KOH.

Rheological measurements

These determinations were performed with a controlled stress rheometer by TA INSTRUMENTS, Model AR 1000 N, with a stainless steel parallel plate geometry (40 mm diameter with 150 μm gap). As recommended by Barnes [19], the gap width between the two parallel plates was set to be at least an order of magnitude larger than the largest particle in the suspension. The normal force was reset to zero prior to the start of the test. The temperature control was via Peltier plate using the peltier effect to control heating and cooling both rapidly and accurately. The peltier plate temperature range is from –10 °C to 99 °C with resolution of 0.01 °C. The other specifications are: torque variation from 0.1 μNm to 100 mNm; angular displacement resolution of 0.62 μrad s^{-1}; angular velocity range (controlled stress) from 0.628 mrad/s to 628rad/s; shear stress range for used geometry from 7.9×10^{-3} to 7.958 Pa; shear range for used geometry from 0.1 to 10000 s^{-1}. Data reported represent mean from three replicates for each formulation.

Oscillation measurements

At first, the linear viscoelastic region was determined with a stress sweep between 0.1 and 80 Pa at constant frequency of 10 rad s^{-1}. All the subsequent frequency sweeps measuring storage modulus (G′), loss modulus (G″), complex modulus (G^*) and phase angle (δ = arc tan G″/G′) were performed at constant deformation in the linear viscoelastic region. Measurements of dynamic viscosity were performed at frequency range of 1.0–60 rad s^{-1}, covering 32 frequencies over three decades. All the measurements were taken at 25 °C.

Creep measurements

To obtain results independent of the pre-existing deformation of the samples, these measurements were taken after the set up of the linear viscoelastic region (2% of strain). This strain was determined to be well within the linear viscoelastic limit as identical spectra were recorded for strain of <2%. The determinations were based on the application of constant stress to the gel and the strain compliance ($J(t) = \gamma(t)/\sigma$) was monitored in function of the time. The applicable shear stress scanning was between 5.0 Pa and 30.0 Pa for 300 s and the recovery was monitored over 600 s [15] to verify in which shear stress applied a maximum recuperation of the materials would occur. This determined shear stress was 10.0 Pa. The experiments were performed with approximately 3.0 mL of CAR in concentrations of 0.7, 4 and 6%(w/w) with mass ratios of 100:1 to 25:1 at pH 7.4 for CAR. The geometry used was the same used in viscoelastic properties. The measurements were taken at 25 °C.

Fluidity assay

CA and CAR gels at 0.7%(w/w) in the proportion of 75:1 and 25:1 equilibrated at pH 3.5, and CAR gels 4 and 6%(w/w) in the proportion of 75:1 and 50:1 equilibrated at pH 7.4 were put in a syringe of 5.0 mL and extruded through a needle of 20 mm of length and inner diameter of 0.55 mm. An INSTRON, model TTDML-3241, with a traction cell of 50 Kg induced the extrusion at a rate of 1.0 mL min^{-1}. Force versus syringe plunger was recorded for mean of three replicates for each formulation.

Results and discussion

The viscoelastic data were obtained after strain loop at a frequency of ω = 10 rad s^{-1} to set up the linear region of the viscoelastic behavior of the CA gels and CAR composites gels equilibrated at pH 3.5 and 7.4. The linear region was determined at a constant strain of 2%. This strain was determined to be within the linear viscoelastic limit as identical spectra were recorded for strain of <2%, i.e., G′ and G″, independently of strain. In this region, the storage modulus, which describes the elasticity of the materials, whereas the loss modulus ($G'' = \eta \cdot \omega$), which reflects the dissipated energy as characteristic of the viscous properties and the phase angles (δ = arc tan G″/G′) were determined for CA gels and CAR composite gels. The results showed that the addition of rhamsan caused significant effects on the elastic modulus, with values of G′ considerably larger than G″ (Fig. 1). Although G′ and G″ varied in magnitude, the elastic modulus was always predominant on the viscous modulus (G′ > G″ and δ < 45)—Table 1—independently of the pH, concentration and rhamsan proportion in the composites (Table 1).

Table 1 shows values of G′, G″, G* and δ, for all preparations. Comparing collagen with its composites with rhamsan at pH 3.5, it shows a storage modulus larger than the one of its composites, as at this pH the collagen

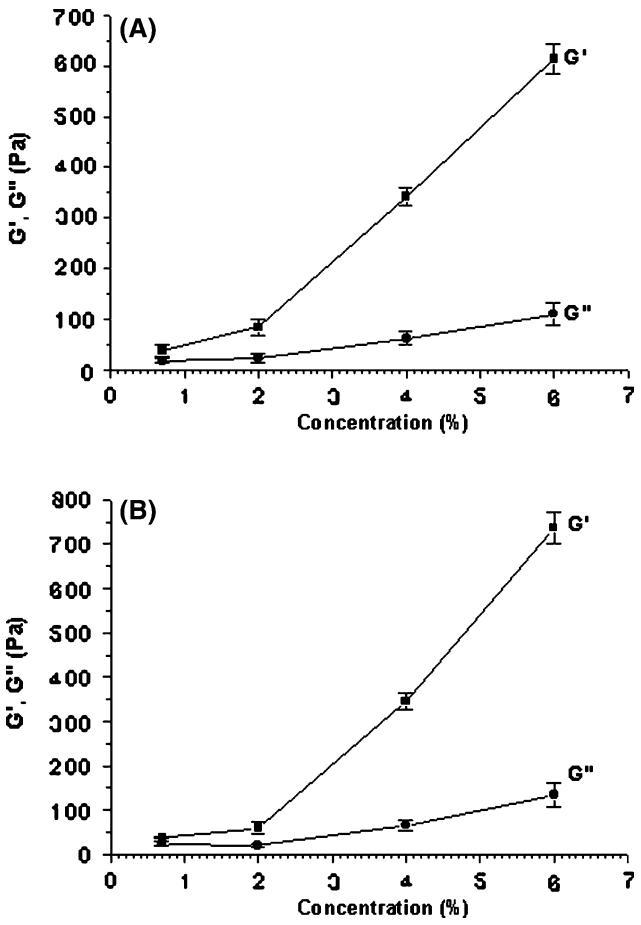

Fig. 1 Elastic and Viscous Modulus in function of the concentration of anionic collagen:rhamsan composites gels at pH 7.4: (**A**) anionic collagen:rhamsan 75:1; (**B**) anionic collagen:rhamsan 50:1

Table 1 Parameters of dynamic shear stress obtained at $\omega = 1$ rad s^{-1} for anionic collagen gels and their composites with rhamsan at pH 3.5 and 7.4

PH	Parameter	Anionic collagen	CAR 100:1	CAR 75:1	CAR 50:1	CAR 25:1
3.5	G′ (Pa)	80.34 ± 1.36	41.92 ± 1.85	41.89 ± 1.81	42.64 ± 1.62	60.51 ± 1.98
	G″(Pa)	32.30 ± 0.88	18.59 ± 1.15	18.92 ± 1.01	19.49 ± 0.75	24.63 ± 0.60
	G*(Pa)	86.59 ± 1.22	45.85 ± 1.44	45.96 ± 1.38	46.89 ± 1.10	65.33 ± 1.33
	δ (degrees)	21.90 ± 1.01	23.92 ± 0.94	24.56 ± 0.96	24.31 ± 0.78	22.15 ± 0.84
7.4	G′(Pa)	18.99 ± 0.62	24.31 ± 0.97	19.57 ± 0.84	18.07 ± 0.76	18.50 ± 0.88
	G″(Pa)	8.26 ± 0.22	12.21 ± 0.43	11.14 ± 0.39	8.99 ± 0.31	9.10 ± 0.12
	G*(Pa)	19.78 ± 0.48	25.28 ± 0.71	22.8 ± 0.62	20.18 ± 0.54	19.83 ± 0.59
	δ (degrees)	23.68 ± 0.39	24.64 ± 0.47	30.87 ± 0.41	26.39 ± 0.48	26.87 ± 0.44

Mean of three replicates

hydrolyzed for 72 h has 163 positive charges [20], which yields a great solvatation. When rhamsan is added (polyeletrolyte with negative charge) this solvatation decreases and therefore the storage modulus is smaller.

At pH 7.4 the behavior is different, due to the predominance of negative charge, reaching approximately 91 charges [20] for hydrolyzed collagen for 72 h. However, when rhamsan is added (negative polyeletrolyte), this amount of negative charges increases in such a way that the collagen solvatation in the presence of rhamsan is larger in relation to the pure collagen, (G′ composite > G′ collagen).

When only anionic collagen:rhamsan composites are compared in the two values of pHs, the effect of the amount of existent charge on the collagen can be better observed. For pH 3.5, the collagen has positive charge and at pH 7.4 the charge is negative. However, the amount of charge at pH 3.5 is much larger (163 charges) in relation to negative charges (91 charges) at pH 7.4 [19], suggesting that the existent solvatation at pH 3.5 is larger than pH 7.4 and therefore, the rigidity at pH 3.5 should also be superior than pH 7.4, as observed by the data of G* (Table 1).

The evaluation structural level of these data suggests that more ordered fibers occur at pH 7.4, resulting in a narrower range of viscoelastic deformation, independently of the presence of rhamsan. Such behavior can be explained starting from the progressive fibrilar orientation in the direction of the drainage, resulting in a decrease in the deformation of the hydration layer of the protein fibers.

To gain further insight into the origin of the elastic response of both anionic collagen gels and composites with rhamsan, creep experiments were performed using the AR-1000 controlled-stress rheometer. For the accomplishment of the creep measurements, it was necessary to determine the shear stress maximum recovery. This shear stress was determined, subjecting the gels to induced strain at variable shear stress, followed by recovery monitoring in a time interval of 600 s [15]. Under these conditions, larger recoveries of the induced deformation for gels equilibrated at pH 3.5 and 7.4 were 10.0 Pa.

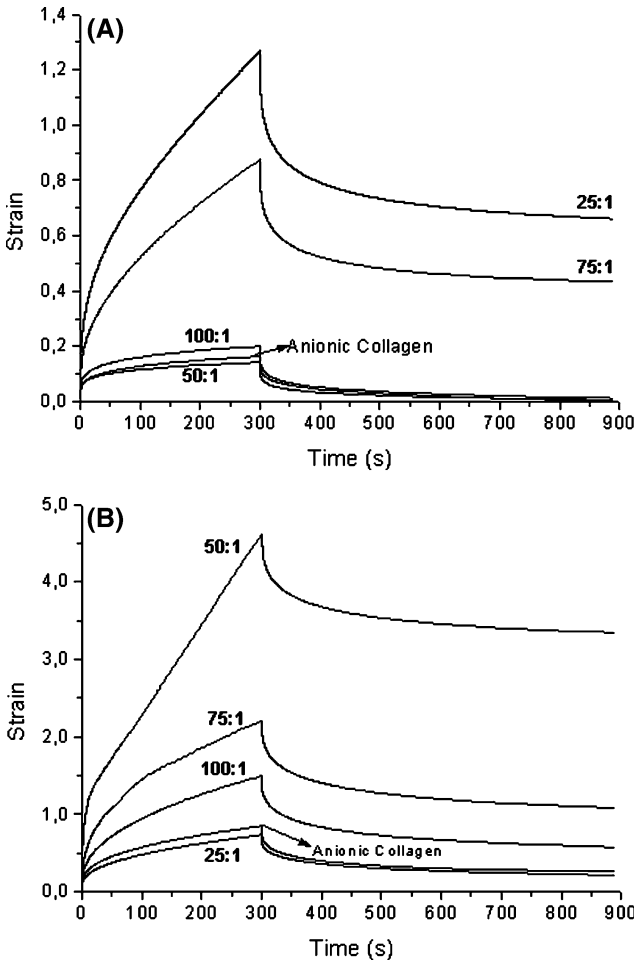

Fig. 2 Creep results for anionic collagen and anionic collagen:rhamsan composites at 0.7% in the proportions of 100:1, 75:1, 50:1 and 25:1(w/w), obtained after the application of a shear stress of 10 Pa (three replicates): (**A**) pH 3.5; (**B**) pH 7.4

The recovery results (Fig. 2) for CA and CAR gels at 0.7% in the compositions between 100:1 and 25:1 at pH 3.5 (Fig. 2a) showed that they recovered with larger efficiency than those equilibrated at pH 7.4 (Fig. 2b) and with lower values when rhamsan is added. While the recovery percentage for CA at pH 3.5 was 98.44 ± 1.16% (mean ± s.d.), in the presence of rhamsan in the composite at 25:1 the recovery was 47.93 ± 0.33%. At pH 7.4 these values were respectively, 68.35 ± 2.06% and 27.67 ± 0.24% for CA and CAR at 25:1 (Table 2). The agreement is good. Tests of repeatability on identical samples, the agreement was similar with differences in strain at identical times differing by <5% [15].

These differences observed in the preparations at different pHs confirm the oscillation results, showing that at pH 3.5 the tropocollagen molecules are more solvated than at pH 7.4. Although the preparations at pH 3.5 show a better viscoelastic behavior than the preparations at pH 7.4, they are less resistant to deformation, which is not viable for the type of application these preparations are destined for.

The 4 and 6% CAR composites gels equilibrated at pH 7.4 (Fig. 3) showed similar behavior to the 0.7% gel, that is, a larger recovery capacity as the amount of rhamsan in the composite increases, which is an important property for injectable gels destined for plastic correction.

The flow property is an important characteristic of any product candidate to be injectable. The viscoelastic behavior observed in the creep measurements should be maintained in a syringe during the extrusion, because to obtain a good application for the correction of soft tissue, the gels to be injected should resist to high stress and present a behavior similar to an elastic solid in the place of the application.

The fluidity assay of CA and CAR composites gels at 0.7%(w/w) in the proportions of 75:1 and 25:1 equilibrated at pH 3.5 and extruded through a needle of 20 × 0.55 mm

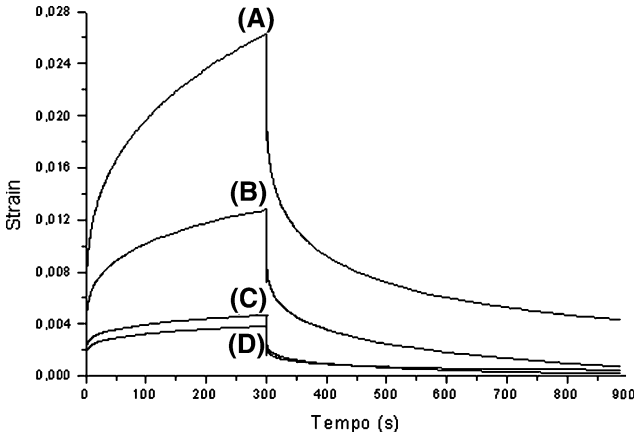

Fig. 3 Experiments of creep anionic collagen:rhamsan composites equilibrated at pH 7.4 obtained after the application of a stress of 10.0 Pa (three replicates): (**A**) CAR 4% (50:1); (**B**) CAR 4%: (75:1); (**C**) CAR 6%: (50:1); (**D**) CAR 6%: (75:1)

(Fig. 4a) showed the influence of rhamsan on the behavior of these gels and that gels with this polysaccharide are easily extrudable through fine-gauge needles. While the necessary force for the extrusion of CA gels was 21.08 ± 2.42 N (mean ± s. d.), for the CAR composite gels at 75:1 and 25:1 the forces were significantly smaller, i.e., 13.14 ± 1.51 N and 11.96 ± 1.37 N, respectively.

Another characteristic observed for the extrusion of CAR composites gels in relation to CA gel was the lower frequency of against-pressure spikes, probably due to similar problems already described for stabilized gels with glutaraldehyde [10]. Results of fluidity for CAR composites gels 4 and 6% (w/w) in the proportion of 75:1 and 50:1 equilibrated at pH 7.4 (Fig. 4b) showed a similar behavior to those obtained at pH 3.5 in the concentrations of 0.7%(w/w). The advantage is that these preparations had not been stabilized with GA.

Table 2 Percentage of collagen gels recovery with or without rhamsan when subjected to a shear stress of 10 Pa

Preparations	% Recovery	
	pH 3.5	pH 7.4
Sample 0.7%		
Anionic:collagen 72 h	98.44 ± 1.16	68.35 ± 2.06
Anionic:collagen:rhamsan (100:1)	93.30 ± 2.19	61.42 ± 2.13
Anionic:collagen:rhamsan (75:1)	92.07 ± 1.63	70.81 ± 1.28
Anionic:collagen:rhamsan (50:1)	50.40 ± 0.41	50.78 ± 0.22
Anionic:collagen:rhamsan (25:1)	47.93 ± 0.33	27.67 ± 0.24
Sample 4 and 6% at pH 7.4		
Anionic:collagen:rhamsan 6% (75:1)	88.18 ± 1.75	
Anionic:collagen:rhamsan 6% (50:1)	96.14 ± 1.21	
Anionic:collagen:rhamsan 4% (75:1)	83.52 ± 1.36	
Anionic:collagen:rhamsan 4% (50:1)	94.44 ± 2.34	

Mean of three replicates

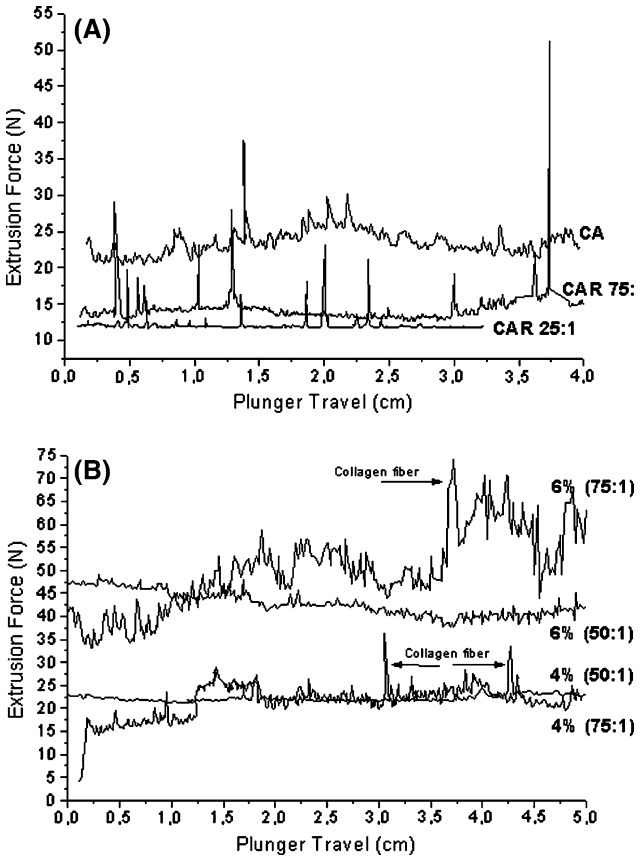

Fig. 4 Flow curves of anionic collagen gels and their composites with rhamsan, through a needle (20 × 0.55 mm) and flow constant speed of 1.0 mL/min (three replicates): (**A**) anionic collagen gel (CA) at 0.7%(w/w) and rhamsan proportions of 25:1 and 75:1(w/w), pH 3.5; (**B**) anionic collagen:rhamsan composites in the concentrations of 4 and 6%, in the proportions of 75:1 and 50:1(w/w), pH 7.4

The effects observed with rhamsan addition at pH 3.5 in the concentration of 0.7%(w/w) and at pH 7.4 with gels of 4 and 6%(w/w) are very important and significant, as extrusion forces below 40 N are easily achieved by manual muscular force [10], making it possible for the surgeon to manipulate the material more easily during the surgical procedure. In addition, the force spikes achieved in the presence of rhamsan are below those found in the commercial preparations [8], indicating that, besides excluding the problem of citotoxicity associated to glutaraldehyde, rhamsan facilitates the surgeon's work during the extrusion.

A structural evaluation of these data together with those of creep and viscoelasticity suggests that bunches of more ordered fibers (pH 7.4) result in a lower frequency of against-pressure spikes and a narrower range of viscoelastic deformation, independently of the presence of rhamsan. Such behavior can be explained starting from the progressive fibrilar orientation in the direction of the drainage as that force is applied, resulting in a decrease in the deformation of the hydration layer of the fibers protein.

Dynamic viscosity

The viscosity of a material is the measurement of resistance to the shear stress and is always associated with the dissipation of internal energy, typically as heating. A material with low viscosity slides easily, with little internal energy being dissipated in the shear stress process. A highly viscous material, however, slides more slowly and dissipates more energy in the process. That resistance is quantified by dynamic viscosity. Few data about this parameter related to biomaterials, mainly those used in urology and laringology, are available in the literature. Particularly in laringology, where these data are more difficult to be found, a study of the dynamic viscosity of collagen can be a good contribuition, as this parameter characterizes the internal friction of the material during oscillatory deformation around an equilibrated position that can be defined in the context of the sinusoidal oscillatory shear.

The dynamic viscosity is a very important parameter for vocal fold augmentation surgery, because when a sinusoidal oscillatory shear stress is applied to a sample of viscoelastic material like collagen [13], a steady-state condition in which the sinusoidal shear stress has a constant relation to the sinusoidal shear strain at a given frequency of oscillation can be reached [21]. The strain amplitude will be proportional to the stress amplitude, and a constant phase lag will exist. Therefore, the dynamic viscosity can be derived from this phase lag and the ratio of stress-to-strain amplitude. Mathematically, the dynamic viscosity is defined as the real part of complex dynamic viscosity and can be described by the analytic relationship between shear stress, strain and strain rate for Kelvin-Voigt viscoelastic model [22]:

$$\tau = \mu\gamma + \eta \cdot \dot{\gamma} \tag{1}$$

where τ is shear stress (Pa), γ is shear strain (rad), $\dot{\gamma}$ is strain rate (s^{-1}), μ is elastic shear modulus (Pa) and η is dynamic viscosity (Pa s). For time, the dynamic viscosity is related to the viscous contribution or the dissipation of energy in each cycle, and can be represented as

$$\eta' = \frac{G''}{\omega} \tag{2}$$

where G″ is loss shear modulus (Pa) and ω is oscillation frequency (s^{-1}).

Figure 5 shows the dynamic viscosity as a function of oscillation frequency for anionic collagen gels and anionic collagen:rahamsan composites gels at 0.7%(w/w) in the

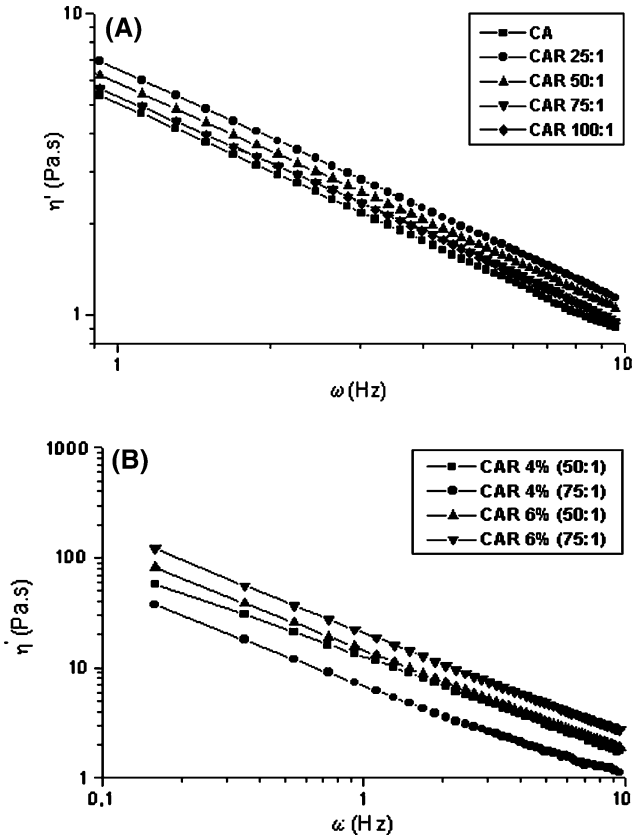

Fig. 5 Dynamic viscosity of anionic collagen gels and anionic collagen:rhamsan composites at pH 7.4. (**A**) samples at 0.7%(w/w); (**B**) samples to 4 and 6%(w/w)

proportions between 100 and 25:1 (Fig. 5a) and anionic collagen:rhamsan composites gels at 4 and 6%(w/w) in the proportions of 75 and 50:1 (Fig. 5b). As G″ and η are viscoelastic linear properties obtained in small amplitude oscillatory conditions, a small variation in the rhamsan concentration in the composite causes significant effects on the viscosity of the gels (Fig. 5a).

Figure 5 shows the shear thinning effect, where the dynamic viscosity decreases in function of frequency and which is observed for most biomacromolecules and polymeric materials [22]. The viscosity decrease is approximately linear on the log-log scale, independently of the rhamsan concentration, indicating that the relationship between dynamic viscosity and frequency can be modeled by a power law:

$$\eta = k f^n \tag{3}$$

where (is frequency, k and n are constants.

The data for the anionic collagen gels and anionic collagen:rhamsan composites in different proportions and concentrations shown in Fig. 5 were fitted to Eq. 3 by simple linear least-squares regression and are showed in Table 3 with the respective values of k, n and correlation coefficient r. The data obtained are very well matched by the regression equation, presenting correlation coefficients above 0.99. The dynamic viscosity decreased with the frequency at approximately the same rate for different preparations with very similar slopes (values of n), as clearly shown in Fig. 5. The main difference between the materials can be described by the vertical separation of the curves in Fig. 5 or by the differences of values of k in Table 3.

If we compare the results obtained with the anionic collagen:rhamsan composites with those presented by Chang and Titze [23] and Finkellor [21] who compared the viscosity of different biomaterials used for the correction of vocal fold, including collagen, we can suggest the use of anionic collagen:rhamsan composites in the concentrations of 4 and 6%(w/w). They can also be used in phono-surgery (e.g., repair of vocal fold scarring or atrophy), because gels in higher concentrations have shown to be more efficient in the appropriate production of elevation and persistence of volume resulting in the easiness of phonation. These properties together with those related to fluidity are essentially important in the initial stage of the surgical procedure that involves implant and must be considered together with medical and other concerns (in particular, short and long term biologic tissue response) for complete applications in vocal fold augmentation surgery.

Table 3 Results of linear least-squares regression curve fitted (Eq. 3) for anionic collagen gels and anionic collagen:rhamsan composites at pH 7.4

Concentration	Proportion	η'(Pa s)	k	n	$r^{(*)}$
0.7%	CA	0.33 ± 0.02	1.91 ± 0.04	–0.78 ± 0.01	–0.99
	100:1	0.37 ± 0.01	2.16 ± 0.01	–0.78 ± 0.01	–0.99
	75:1	0.37 ± 0.01	2.17 ± 0.01	–0.78 ± 0.02	–0.99
	50:1	0.40 ± 0.03	2.47 ± 0.04	–0.80 ± 0.02	–0.99
	25:1	0.43 ± 0.02	2.68 ± 0.04	–0.81 ± 0.01	–0.99
4%	50:1	1.76 ± 0.04	12.45 ± 0.04	–0.86 ± 0.02	–0.99
	75:1	1.12 ± 0.03	6.89 ± 0.02	–0.83 ± 0.01	–0.99
6%	50:1	1.88 ± 0.04	14.45 ± 0.04	–0.91 ± 0.01	–0.99
	75:1	2.72 ± 0.02	20.87 ± 0.06	–0.99 ± 0.01	–0.99

Mean of three replicates

(*)There was not significant variation

Conclusions

The results described above showed that, independently of pH, concentration and proportions, CA and CAR gels were characterized by storage modulus (G′) always larger than loss modulus (G″). The creep results together with the fluidity assay showed that CAR gels to 0.7% equilibrated at pH 7.4, although less elastic than the equilibrated gels at pH 3.5, are more susceptible to deformation when subjected to relatively high stress. The 4 and 6% gels showed larger recovery capacity as the amount of rhamsan in the composite increases. This behavior was confirmed by the preliminary studies of flow, suggesting that CAR gels are compatible with their use in plastic corrections, mainly because glutaraldehyde is not used as a stabilization agent of microfibrilar suspensions.

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