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Viscosimetric behaviour of hyaluronic acid in different aqueous solutions

A. García-Abuín, D. Gómez-Díaz, J.M. Navaza*, L. Regueiro, I. Vidal-Tato

Department of Chemical Engineering-ETSE, University of Santiago de Compostela, Galicia, Spain

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ABSTRACT

Rheological properties of aqueous solutions of hyaluronic acid (HA), of interest for the medical and cosmetics industry, have been studied. The value of the intrinsic viscosity of hyaluronic acid has been determined using Huggins and Kramer equations, and the value of molecular weight of this polymer has been calculated on the basis of intrinsic viscosity. The apparent viscosity value and the influence of the shear rate upon this physical property and the polymer concentration in aqueous solution have been determined. The effects caused by temperature, the concentration and nature of alcohols, as well as the electrolyte presence on the rheological behaviour have been also studied. These results are expected to offer enough information to obtain the rheological behaviour and to have a better understanding of HA-based mixtures for different purposes.

1. Introduction

A lot of areas such as food, textile, pharmaceutical or cosmetics use non-Newtonian fluids in their processes (Feddersen & Thorp, 1993). In some cases, the presence of small quantities of polymers produces important effects on the behaviour of the liquid phase.

Hyaluronic acid (HA) is a linear polysaccharide that consists of repeating disaccharide units of β-D-glucuronic acid and N-acetyl-β-D-Glucosamine, linked together via alternating 1,4 and β-1,3 bonds. It is a potent humectant that absorbs 1000 times its weight in water. HA is present at the extracellular matrix, synovial fluid, vitreous humour, rooster comb and umbilical cord, and it takes part in many functions in vivo, such as joint lubrication, tissue hydration and wound healing. Thanks to its unique rheological properties and incomparable with those of any another natural or synthetic polymers, HA has a wide variety of cosmetic and pharmaceutical applications; for instance, to fill soft tissue defects such as facial wrinkles or to treat articulary disorders in horses. The HA solutions rheological properties are very important for these applications; for example, ophthalmic viscosurgical devices are classified according their rheological behaviour.

Previous studies regarding the viscosity of the systems where HA is used (Fouissac, Milas, & Rinaudo, 1993; Mendichi, Olts, & Giacometti-Schieroni, 2003) have observed a complex rheological behaviour, and have carried out studies related to the intrinsic viscosity value and its relation to the molecular weight value, with the aim of obtaining the value of Mark–Houwink equation constants, as well as confirming the results obtained by other researchers.

The aim of this work has been studying the influence of the polymer concentration, temperature, electrolyte presence and the use of co-solvents (used in cosmetics formulations) on the rheological behaviour of HA water solutions, in order to make the properties of mixtures HA-based better understandable. To fit the experimental data, the power law (Ostwald de Waele model) has been used. This model was chosen for its simplicity and good fit to the experimental data.

2. Materials and methods

Hyaluronic acid was supplied by Aldrich with a purity ≥99%. The solutions were prepared by mass using a balance with a precision of $\pm 10^{-7}$ kg. Distilled water was used for these solutions. Also, ethanol and 2-propanol have been supplied by Sigma–Aldrich with a purity ≥99.5% in both cases.

The average molecular weight determination was achieved via intrinsic viscosity calculation by means of Huggins and Kramer equations (Rao, 1999). The experimental set up to carry out the intrinsic viscosity determination was a capillary viscometer (Schott Gerate AVS 350) that was immersed in a thermostated bath with a precision of ±0.1 °C. The solutions were prepared in NaCl 0.1 M, since Mark–Houwink constants (Milas, Rinaudo, Fouissac, & Launay, 1993) were determined using this solvent to prevent the polyelectrolyte expansion in solution when the size exclusion chromatography was used to determine these constants. The kinematic viscosity (ν) was determined from the transit time of the liquid meniscus through a capillary viscosimeter supplied by Schott (Cap

* Corresponding author at: Department of Chemical Engineering-ETSE, University of Santiago de Compostela, Rúa Lope Gómez de Marzoa s/n, E-15782, Santiago de Compostela, Galicia, Spain.
E-mail addresses: diego.gomez@usc.es (D. Gómez-Díaz), josemanuel.navaza@usc.es (J.M. Navaza).

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No 0c, 0.46 ± 0.01 mm internal diameter, $k = 0.003201\ mm^2\ s^{-1}$). The density and sound speed of HA aqueous solutions were measured with an Anton Paar DSA 5000 vibrating tube densimeter and a sound analyser.

An Anton Paar DV-1P digital thermostated rotational viscosimeter with two coaxial cylinders has been used to carry out the rheological measurements. The hyaluronic acid concentration range employed to prepare the aqueous solutions employed in this work was between 0 and 500 mg L^{-1}. The temperature values analysed in the present work have been covered between 10 and 50 °C. Studies were expanded until 50 °C to explore processes away from physiological conditions in order to obtain new media HA-based.

The present work has analysed the influence of the NaCl presence upon the rheological behaviour of aqueous solutions of hyaluronic acid and, in order to perform this study, the value of the shear rate applied to the liquid phase has been varied. The electrolyte concentration was varied between 0 and 0.15 mM. The effect of alcohol use as a co-solvent upon rheological behaviour has also been analysed using two short chain alcohols in the concentration range of 0–50 weight percentage.

3. Results and discussion

The molecular weight of the polymer employed in this study was unknown so, in order to characterize this polymer, the average molecular weight has been calculated. To do this, it was necessary to determine the intrinsic viscosity obtained by a combined application of Huggins and Kramer equations (Eqs. (1) and (2)). A similar procedure has been employed by different authors (Chuah, Lin-Vien, & Soni, 2001; Ma & Pawlik, 2007).

$$\frac{\eta_{sp}}{C_{HA}} = [\eta] + k_1 \cdot [\eta]^2 \cdot C_{HA} \tag{1}$$

$$\frac{\ln \eta_r}{C_{HA}} = [\eta] + k_2 \cdot [\eta]^2 \cdot C_{HA} \tag{2}$$

where $\eta_{sp} = (\eta - \eta_s)/\eta_s$, $\eta_r = \eta/\eta_s$, η and η_s are the viscosities of the solution and the solvent, respectively. To calculate the viscosity it is necessary to know the kinematic viscosity (ν) and density (ρ). The absolute viscosity has been calculated using Eq. (3).

$$\eta = \nu \cdot \rho \tag{3}$$

Fig. 1 shows the representation of Huggins and Kramer equations for aqueous solutions of hyaluronic acid.

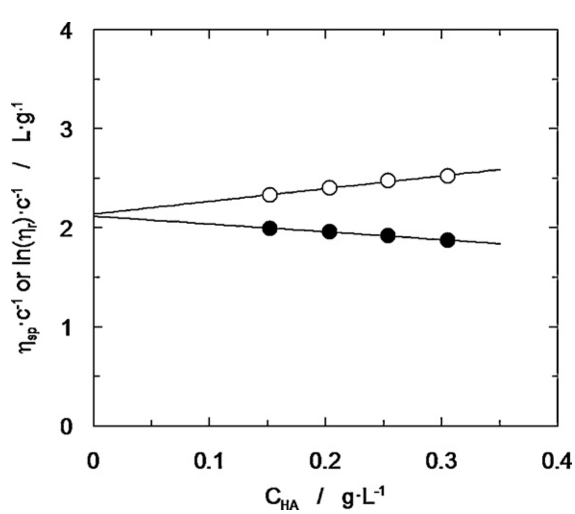

Fig. 1. Determination of intrinsic viscosity of hyaluronic acid using Huggins and Kramer equations. (○) $\eta_{sp}\ c^{-1}$ and (●) $\ln(\eta_r)\ c^{-1}$.

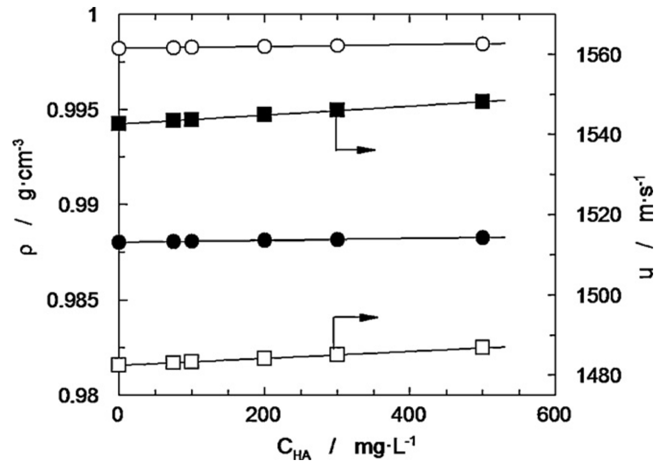

Fig. 2. Influence of HA concentration upon density and speed of sound. Density: (○) $T = 20$ °C and (●) $T = 50$ °C. Speed of sound: (□) $T = 20$ °C and (■) $T = 50$ °C.

Different concentrations of hyaluronic acid in NaCl 0.1 M, were prepared to obtain relative viscosities in a range of 1.2–1.6 to assure a good accuracy and linearity of extrapolation to zero concentration. The intrinsic viscosity corresponding to the hyaluronic acid has been obtained from the value of the intercept by means of Huggins and Kramer equations, and the obtained value was 2.12 L g^{-1}.

The molecular weight was determined using the corresponding Mark–Houwink equation (Eq. (4)). The Mark–Houwink constants for these polymers were obtained from the literature (Milas et al., 1993) with values of $K = 0.0336$ mL g^{-1} and $a = 0.79$ at 25 °C in 0.1 M NaCl.

$$[\eta] = K \cdot M_w^a \tag{4}$$

By means of the Mark–Houwink equation (Eq. (4)) previously commented and the value of the intrinsic viscosity, the hyaluronic acid medium molecular weight has been determined, obtaining a value of 1430 kg mol^{-1}.

The density of aqueous solutions of HA has been settled in order to determine the absolute viscosity from the kinematic viscosity. The sound speed for these aqueous solutions has also been determined. Fig. 2 shows the influence of the composition on the value of these two physical properties. In both cases, since the HA composition is greater in the solution, these properties increase their value, although in a light way in both cases. This behaviour might indicate slight interactions among the polymer–solvent and polymer–polymer molecules. The influence of the temperature on the density value and the sound speed is also shown. In this case, it is observed that its effect is much more significant than the effect caused by the concentration of the polymer, and an increase in the temperature value produces a decrease of the density and an increase of the sound speed, which are behaviours commonly observed when the influence of the temperature is analysed.

The first step of this study has been the characterization of the hyaluronic acid by means of two important characteristics: the intrinsic viscosity and the medium molecular weight. The second part of the study has been centred on the characterization of the rheological behaviour of aqueous solutions of this polymer (HA). In relation to the influence of the shear rate applied to the different liquid phases, an example of the obtained experimental results is shown in Fig. 3. The experimental data shown in Fig. 3 indicate that the variation in the shear rate applied to the liquid phase produces a clear modification in the apparent viscosity value. For the system analysed in this work, an increase in the value of the shear rate produces a decrease in the viscosity but, in the case of shear stress, an increase is observed. This behaviour has been observed

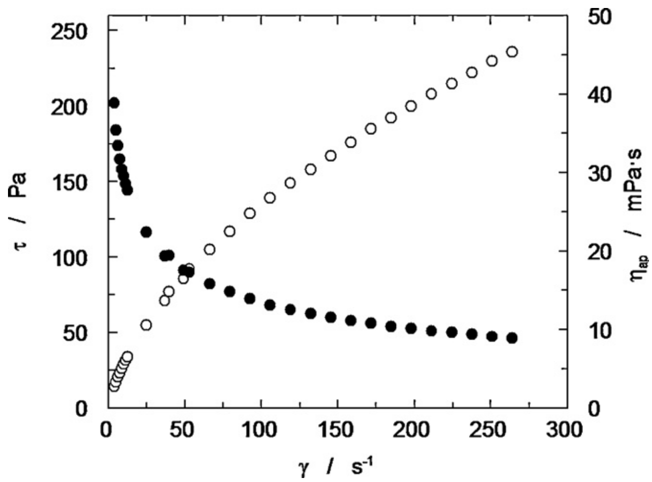

Fig. 3. Influence of shear rate upon shear stress and apparent viscosity. [HA] = 300 mg L^{-1}. T = 10 °C. (○) Shear stress – shear rate data and (●) apparent viscosity – shear rate data.

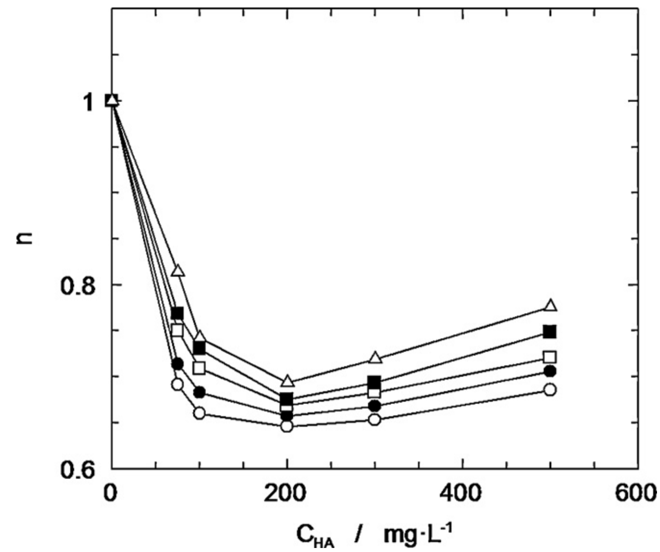

Fig. 5. Influence of HA concentration and temperature upon flow index. (○) T = 10 °C, (●) T = 20 °C, (□) T = 30 °C, (■) T = 40 °C, and (△) T = 50 °C.

under the different experimental conditions (see Fig. 4) in relation to the hyaluronic acid concentration, and an increase in the value of the polymer concentration causes an increase in the value of the apparent viscosity for all the shear rate values.

The experimental results obtained about the influence of the shear rate for all the experimental conditions show that the aqueous solutions of hyaluronic acid are systems included in the non-Newtonian and pseudoplastic fluids. These results are in agreement with previous studies about the influence of the presence of hyaluronic acid sodium salt upon the rheological behaviour (Fouissac et al., 1993; Mendichi et al., 2003).

The experimental data has been fitted with the Ostwald's equations (Eqs. (5) and (6)) and the rheological parameters have been calculated using linearized equation (5). Power law model (Eq. (5)) fits satisfactorily the experimental data and then, the rheological parameters corresponding to Ostwald model (flow and consistency indices) could be determined. It was observed that the behaviour index, n, takes values lesser than 1, so we can conclude that these solutions show a pseudoplastic behaviour.

$$\tau = k \cdot \dot{\gamma}^{n} \tag{5}$$

$$\eta_{app} = k \cdot \dot{\gamma}^{n-1} \tag{6}$$

The experimental values determined for the power law rheological parameters are shown in Figs. 5 and 6 for all the experimental conditions (temperature and hyaluronic acid concentration) employed in the present work.

An interesting characteristic of the aqueous solutions of hyaluronic acid in relation to the value of the flow index is the existence of a minimum in the value of this parameter when the influence of the polymer concentration was analysed (see Fig. 5). At first, a decrease in the behaviour index is observed when the hyaluronic acid concentration increases in the liquid phase. This occurs until a minimum is reached and then, a higher increase in the polymer concentration produces an increase in the value of this rheological parameter up to values near to 1 (Newtonian behaviour). This behaviour is similar to the previous ones observed

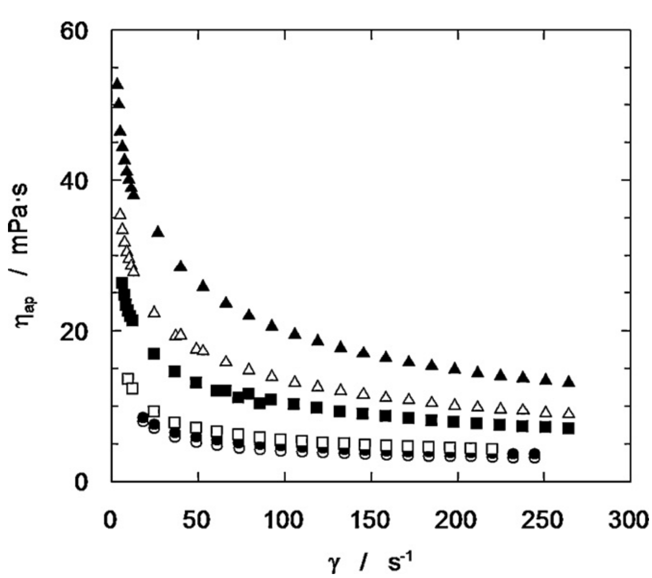

Fig. 4. Influence of shear rate upon apparent viscosity. T = 10 °C. (○) [HA] = 50 mg L^{-1}, (●) [HA] = 75 mg L^{-1}, (□) [HA] = 100 mg L^{-1}, (■) [HA] = 200 mg L^{-1}, (△) [HA] = 300 mg L^{-1}, and (▲) [HA] = 500 mg L^{-1}.

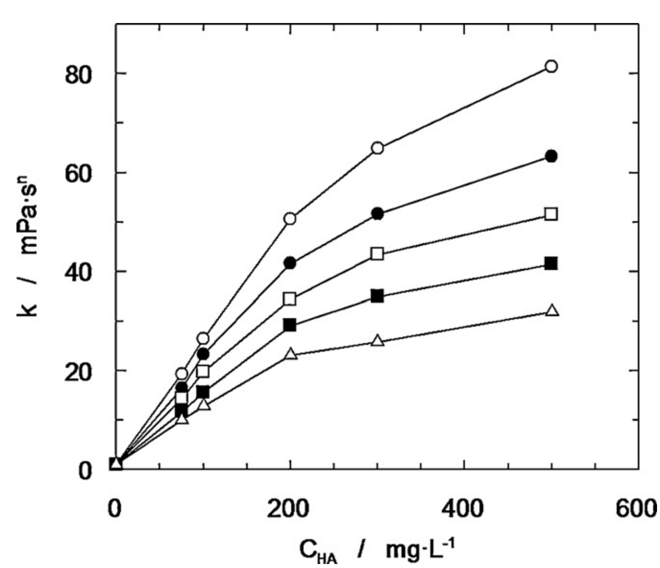

Fig. 6. Influence of HA concentration and temperature upon consistency index. (○) T = 10 °C, (●) T = 20 °C, (□) T = 30 °C, (■) T = 40 °C, and (△) T = 50 °C.

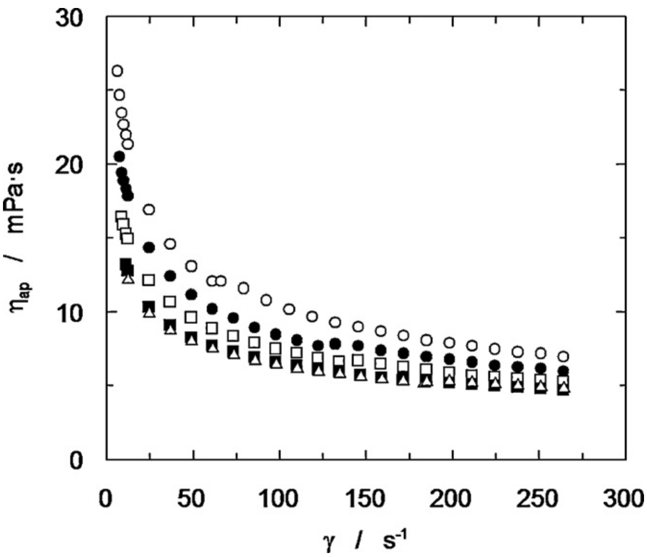

Fig. 7. Influence of temperature upon apparent viscosity value at different shear rate. [HA] = 200 mg L^{-1}. (○) T = 10 °C, (●) T = 20 °C, (□) T = 30 °C, (■) T = 40 °C, and (△) T = 50 °C.

by our research studies and others, for systems constituted by different polymers (Gómez-Díaz & Navaza, 2003; Mothé & Rao, 1999). In relation to the consistency index value (see Fig. 6), a clear and continuous increase in the value of this parameter has been observed when the polymer concentration has been increased in the liquid phase. The behaviour observed for the consistency index is common, as regards data from previous studies that employ different polymers in an aqueous solution (Cancela, Álvarez, & Maceiras, 2005; Gómez-Díaz, Navaza, & Quintáns-Riveiro, 2008).

The effect of temperature on the rheological behaviour of polymers solutions has also been studied and an example of the obtained behaviour is shown in Fig. 7. The experimental results indicate that an increase in the value of temperature produces a continuous decrease in the apparent viscosity value, but the higher effect caused by temperature is observed in the low temperatures values within the studied range. For instance: a slight change is produced in the apparent viscosity by increasing temperature from 40 to 50 °C. On the other hand, when temperature in changed from 10 to 20 °C, the variation observed in the viscosity is high.

The influence of this variable on the Ostwald's parameters for HA aqueous solutions are shown in Figs. 6 and 7. These experimental results show that the solutions have a more Newtonian behaviour when the temperature increases, since the value of flow index increases until values nearer to the unity. The behaviour obtained in relation to the influence of temperature upon the flow index shows a linear trend. In relation to the consistency index, k, the temperature also causes a decrease in its value with a linear trend. The slope of this linear trend is larger when the polymer concentration increases. It was observed that the relation between $\log k$ and temperature based on these experimental results followed a linear equation.

$$\log\ k = A + B \cdot t \tag{7}$$

The average value of the B parameter for HA aqueous solutions was −0.0087. Previous studies (Gómez-Díaz & Navaza, 2003; Muller & Davidson, 1994; Sopade & Kiaka, 2001) have employed Eq. (7) to fit the k values for different polymers.

The effect caused by the presence of an electrolyte upon the rheological behaviour of aqueous solutions of hyaluronic acid has been analysed in the present work. To perform this study, different quantities of NaCl were added to a hyaluronic acid solution of

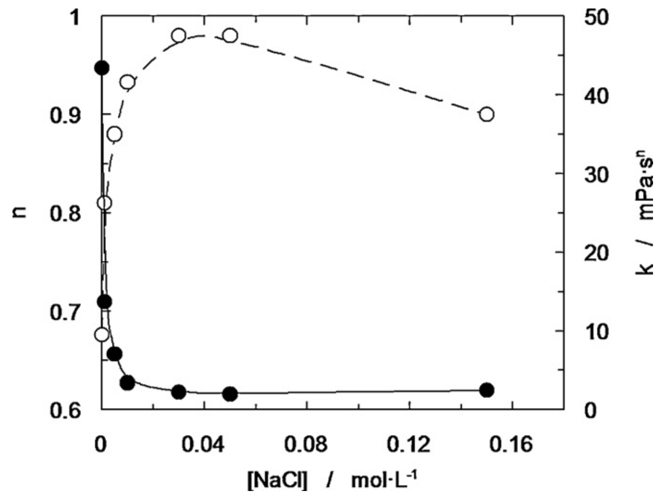

Fig. 8. Influence of NaCl concentration upon power law rheological parameters of HA aqueous solutions. (○) n and (●) k.

300 mg L^{-1}, and the influence of the shear rate upon the apparent viscosity has been analysed. The first effect detected by the electrolyte is a reduction in the value of the apparent viscosity regarding the obtained viscosity value in absence of NaCl. This decrease with the addition of NaCl is due to the charge screening of electrostatic repulsions and then, a more compact conformation is possible, which produces a decrease in the hydrodynamic size of the molecule, producing a decrease in viscosity (Colinet, Dulon, Hamaide, Le Cerf, & Picton, 2009; Khouryieh, Herald, Aramouni, & Alavi, 2007).

Taking into account the flow index value at different shear rate values, the presence of different quantities of NaCl in the hyaluronic acid aqueous solution produce a clear decrease in the pseudoplastic behaviour, since the flow index value is close to the unity (Newtonian behaviour). The experimental values for the flow index and the influence caused by the presence of NaCl are shown in Fig. 8. Fig. 8 also includes the determined values for the consistency index and a reduction in its value is observed. The observed decrease is important and the consistency index reaches values near to the corresponding one to pure water. This decrease in the consistency index is related to the decrease caused in the apparent viscosity produced by the presence of NaCl previously commented.

The present work also analyses the influence of the use of co-solvents commonly employed in different cosmetics and pharmaceutical products, such as low molecular weight alcohols and, more specifically, ethanol and 2-propanol have been employed in this study. Fig. 9 shows an example of the experimental data obtained about the influence of the shear rate upon the apparent viscosity value. This figure shows that the influence of the shear rate, previously obtained in aqueous solutions of HA, remains (there is a decrease in the value of the apparent viscosity when the shear rate increases). Then, a non-Newtonian and pseudoplastic behaviour is observed. In relation to the apparent viscosity magnitude, an increase in this value is observed when the ethanol concentration increases in the mixture. This behaviour is due to the addition of ethanol to pure water, which also produces an increase in viscosity (Pires, Costa, Ferreira, & Fonseca, 2007) until a maximum. For this reason, the increase in viscosity shown in Fig. 9 among between systems with 30 and 50% of ethanol is slight because these systems are close to the maximum. The behaviour corresponding to the systems with 2-propanol is very similar to the corresponding one to the ethanol. Regarding the influence of temperature, a decrease in the value of the apparent viscosity is observed in all the cases analysed for different compositions and shear rates.

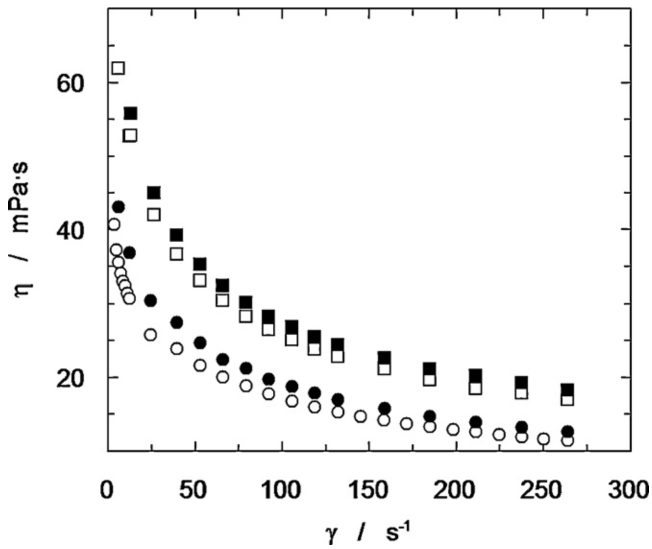

Fig. 9. Influence of shear rate upon apparent viscosity. $T = 20\,^{\circ}C$. [HA] = 300 mg L^{-1}. (■) 50% ethanol, (□) 30% ethanol, (●) 10% ethanol, and (○) 0% ethanol.

Using the same procedure employed for the aqueous solutions of HA, the experimental data of apparent viscosity *versus* shear rate have been fitted using the power law equation to determine the fit parameters (behaviour and consistency index). Fig. 10 compares the calculated values for these parameters and analyses the influence of the alcohols addition. In relation to the behaviour index, the addition of both alcohols produces a decrease in the value of this parameter which shows a higher non-Newtonian behaviour. This behaviour is clearer in the case of the ethanol, since a high decrease in the behaviour index is produced. This decrease is observed in both cases with the addition of a small alcohol quantity, but a slight increase was observed when high quantities of alcohols were added. A similar behaviour has been observed when the influence of polymer concentration was analysed.

On the other hand, the calculated values for the consistency index (Fig. 10) show that an increase in the alcohol concentration produces an increase in the value of this parameter. This behaviour is due to the increase in the value of the apparent viscosity. The maximum in the value of viscosity when the alcohol concentration increases was previously commented, and this behaviour is also observed in the consistency index value.

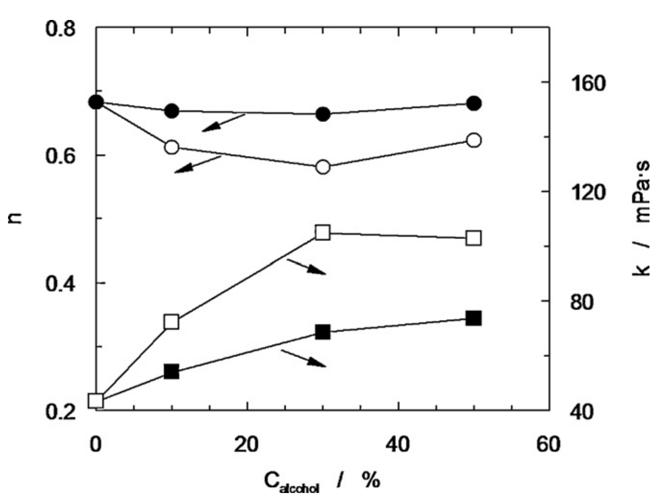

Fig. 10. Influence of concentration and co-solvent nature upon the behaviour and consistency index. $T = 30\,^{\circ}C$ y [HA] = 300 mg L^{-1}. Ethanol (○ and □); 2-propanol (● and ■).

In relation to the influence of temperature upon the fit parameters (behaviour and consistency index), an increase in this variable produces an increase in the behaviour index value but a decrease in the consistency index value. These behaviours are similar to the previous ones obtained for the system formed by water and HA.

4. Conclusions

The aim of this work was relating the conformational characteristics of HA-based mixtures with rheological properties, density and speed of sound.

The rheological characterization of hyaluronic acid aqueous solutions has been carried out, determining the value of the intrinsic viscosity and the average molecular weight. The influence of the polymer concentration, temperature and presence of an electrolyte on the magnitude of the viscosity, density, speed of sound and on the rheological behaviour has been analysed. The presence of HA in an aqueous solution showed a complex rheological behaviour, including this system into the pseudoplastic fluids. Both the increase of temperature and the presence of an electrolyte produced an important decrease on the viscosity magnitude, as well as an approximation to the Newtonian behaviour in relation to its rheology.

In relation to the influence of a co-solvent upon the rheological behaviour, both alcohols produce a decrease in the behaviour index value, which indicates a more pronounced pseudoplastic behaviour. The presence of alcohols also produced an increase in the consistency index value in relation to aqueous solutions of HA.

Density and speed of sound measurements have not allowed us to obtain valuable information in relation to conformational changes in mixtures of HA under the experimental conditions employed in this work; however, the presence of other biomolecules and different experimental conditions could modify the observed behaviour.

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