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Flow properties of haloarchaeal polysaccharides in aqueous solutions

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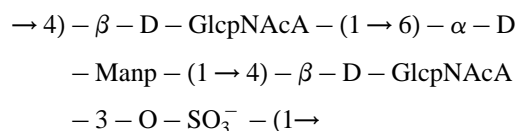
The flow properties of the polysaccharides produced by *Haloferax mediterranei*[T] ATCC 33500 and its super mutant *Haloferax mediterranei* EPS[++], R4 and EPS[++] respectively, were investigated. The R4 repeating unit is a trisaccharide containing mannose and 2-acetamido-2-deoxyglucuronic acid, ester sulphate groups are also present. In order to compare the properties of both polymers viscosimetric measurements were carried out at different integration times and using different concentrations of polymer. Under the assayed experimental conditions no effect of the time applying stress is detected for either polymer. A strong pseudoplastic behaviour is observed which is increased as the polymer concentration rises. Differences in viscosity between both polymers are due to the different molecular weights. The correlation of the experimental data using a modification of the Cross model has given very good results. © 1998 Elsevier Science Ltd. All rights reserved.

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INTRODUCTION

The halophilic Archaea are extreme halophiles that belong to the Archaea Domain[1]. The members of this domain are prokaryotic organisms and phylogenetically are at least as distant from bacteria as they are from eukarya. They require extremely high concentrations of NaCl (1–5.2 M) to grow and can usually be isolated from marine solar salterns[2]. *Haloferax mediterranei* is a member of the Halobacteriaceae family that produces considerable amounts of an exocellular polymeric substance (EPS), which gives the colonies a typical mucous aspect. This extracellular mucilaginous material is a high molecular weight sulphated polysaccharide composed of a regular repeating trisaccharide unit containing one mannose and two 2-acetamido-2-deoxyglucuronic acid moieties. In addition, ester sulphate is present in 1 mol per mol of the trisaccharide repeating unit[3]. The chemical repeat unit is:

$$\rightarrow 4) - \beta - D - GlcpNAcA - (1 \rightarrow 6) - \alpha - D$$
$$- Manp - (1 \rightarrow 4) - \beta - D - GlcpNAcA$$
$$- 3 - O - SO_3^- - (1 \rightarrow$$

Previous data show that even at low polymer concentrations this polysaccharide yields highly viscous aqueous solutions with rheological properties comparable with those of xanthan and thus suitable for technological applications[4]. The viscosity of these solutions remains quite constant over wide range of pH, temperature and salinity values, making this EPS potentially useful in various applications[4]. As with other organisms which live in extreme environments, the cultures are very difficult to contaminate, which permits these organisms to be grown with few sterile precautions if the inoculum is large enough. From this point of view this exopolysaccharide offers a number of advantages for industrial purposes. In the work described in this paper we have examined the flow properties of aqueous dissolutions of two haloarchaeal polysaccharides as a function of the mass fraction of polysaccharide. Experimental results have been correlated simultaneously using the Cross model[5], with some modifications proposed by the authors.

EXPERIMENTAL

Production of polysaccharides

Bacterial strains and growth conditions. Two strains were used in this study, *Haloferax mediterranei*[T] ATCC 33500 and *Haloferax mediterranei* EPS[++], a mutant of the wild type isolated by single-colony cloning[6] that exudes a high amount of EPS. A Braun Biostat ED fermentor with an effective volume of 15 l was used. During growth, temperature (45°C), aeration ($\simeq$100% O_2 saturation), pH (7.2) and stirring (150 rpm) were maintained constant[4]. The growth medium contained a mixture of marine salts[7] at a total concentration of 25% with 1% glucose as carbon source and 0.1% NH_4Cl and 0.015% KH_2PO_4. After autoclaving for 20 min at 120°C the salts, sterile sources of phosphate (KH_2PO_4) and glucose were separately added.

EPS isolation and purification. On reaching the stationary phase, CH_2O (0.15%, v/v) was added to the culture to prevent degradation of the EPS and occasional contamination. The culture was centrifuged (23 000 g, 1 h, 4°C), the supernatant solution was subjected to tangential filtration using a Millipore Pellicon system (0.45 μm) to remove

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any remaining cells, and the EPS was then concentrated and dialysed exhaustively against distilled water (1×10^5 MW cut off) using the same system. The polysaccharide was precipitated from the solution by the addition of CH_3CO_2Na and 2–3 vol of cold ethanol[8], redissolved and reprecipitated and finally freeze-dried. The lyophilised samples were passed through Amberlite IR-120 (Na^+) cation-exchange resin and used for analytical work.

Preparation of polysaccharide dissolutions. The freeze-dried exopolysaccharide samples obtained from *Haloferax mediterranei*[T] ATCC 33500 (strain R4) and *Haloferax mediterranei* EPS[++] (hereafter called R4 and EPS[++], respectively) were dissolved in Milli-Q water with a mass fraction between 0.125 and 1.5%, by prolonged gentle stirring. Samples were stored at 4°C to prevent eventual contamination until used.

Molecular weight determination

The molecular weight distribution of purified polysaccharides was established by gel permeation chromatography on a dextran calibrated sephacryl S-400 column using 0.1 M sodium acetate (pH 5.0) as eluent. Detection of carbohydrates was done by refractive index. The data were very polydisperse centring at aproximately 1.7 million for R4 and over 2 million for EPS[++] (data not shown).

Flow properties

The viscosities of the polymer dissolutions were measured with a Bohlin CS 50 rheometer (Bohlin Instruments) in the viscosimetry test equipped with a parallel plate (40 and 0.5 mm gap) geometry. In order to study its influence in the flow properties several times of stress application to the sample were chosen (i.e. 15, 30, 40 and 50 s). Samples were allowed to stand at room temperature for several hours before testing so that any stresses in the material were able to relax[9]. All the measurements were made at 25°C.

RESULTS

Influence of the time of shearing

As shown in *Figures 1* and *2*, no dependence of the integration time in the viscosity behaviour was observed for the studied samples. In these figures it is possible to observe the behaviour of solutions with the higher concentration (i.e. 1.5%) of both polysaccharides at different integration times. In both polymers all the results show a high coincidence, except at the lowest shear rates where results are somewhat different, especially in the case of the EPS[++]; however, in this zone it is not possible to observe any trend with respect to the integration time. That all seems to indicate that this factor hardly affects the flow properties of the dissolutions, other factors like experimental error or setting of the sample in the measuring system being responsible for those differences. In the rest of the concentrations studied no effect caused by the integration time was observed.

Influence of the shear rate

Four viscometry tests of each concentration for both polysaccharides were run observing a good reproducibility of the results. In *Figures 3 and 4* the behaviour of the different concentrations of both polysaccharides are shown.

First of all it can be pointed out the effect produced by both polysaccharides in the water, causing important increases in the viscosity of the dissolution even at the lowest concentration tested (0.125%); at this concentration the viscosity of the dissolutions can increase up to 100 times, with respect to that of the water ($\sim$1 mPa s).

As expected, dissolutions of both polysaccharides show a strong pseudoplastic behaviour as a consequence of the presence of the polysaccharides, and this behaviour is more marked when increasing the polysaccharide concentration.

On the other hand it is also noticeable that viscosities of the EPS[++] solutions are slightly higher than those of the R4, as a consequence of the higher molecular weight of the former.

DISCUSSION

In order to correlate the experimental data, the mathematical model proposed by Cross[5] was used; this model, based on the formation/breakage of links between the dissolved polymer chains, has been widely applied to fit experimental flow curves of several polysaccharide dissolutions[10,11], and
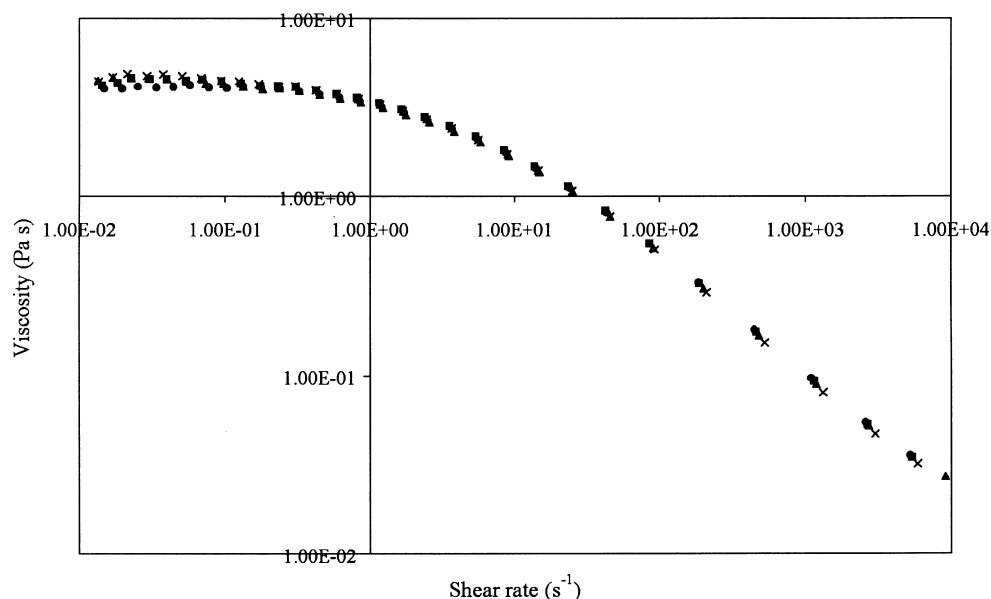

Figure 1 Behaviour of solutions with 1.500% of EPS[++] at the different integration times (in seconds): ▲ 15; ● 30; ■ 40; × 50

assumes the following relation between the shear rate and the viscosity:

$$\frac{\eta - \eta_\infty}{\eta_0 - \eta_\infty} = \frac{1}{1 + (K\dot{\gamma})^m} \tag{1}$$

where:

$\dot{\gamma}$	=	shear rate (s^{-1})
η	=	viscosity at the shear rate $\dot{\gamma}$ (Pa s)
η_0	=	viscosity at zero shear rate (Pa s)
η_∞	=	viscosity at infinite shear rate (Pa s).
K	=	constant of the theoretical model associated with the breakage/formation of links between the polymer chains (s^{+m}).
m	=	constant of the theoretical model that in the majority of the systems studied has a value very approximatly equal to 2/3

This model has four parameters (i.e: η_0, η_∞, m and K) to be calculated from the correlation of the experimental data. This fact leads to the calculation of 44 parameters when considering 11 different experimental conditions used. Thus, a modification of the model including a function to consider the influence of the concentration in η_0, η_∞ and K has been suggested and applied, leading to a lower number of parameters which are capable of simultaneously correlating all the data corresponding to both polymers.

The viscosity of any dissolution is related to its molecular weight by means of the following equation:

$$\eta_0 = c1 \times M_w^{c2} \tag{2}$$

where the $c2$ is approximately 1 for concentrated dissolutions of low molecular weight and 3.5 for concentrated dissolutions of high molecular weight[12]. In this equation M_w is the apparent molecular weight of the dissolution, and $c1$ and $c2$ are common parameters for a wide range of solutions and polymers.

With respect to the high shear rates, no similar equations have been found in the literature. For this reason, and considering that the high shear rate viscosity should increase when increasing the apparent molecular weight of the dissolution, a similar type of equation to equation (2) has been used for both the low shear rates viscosity (η_0) and high shear rates viscosity (η_∞) calculations:

$$\eta_0 = P1 \times M_w^{P2} \tag{3}$$

$$\eta_\infty = P3 \times M_w^{P4} \tag{4}$$

In our case, the apparent molecular weight of the dissolution can be calculated from the molecular weights of the water and that of the polysaccharide (M_p) corresponding in each case to the EPS^{++} and to the R4 and considering the mass fraction of the polysaccharide (w):

$$M_w = (1 - w)M_{H_2O} + wM_p \tag{5}$$

Regarding the parameter K associated with the breakage of links between molecular chains of the polymer, an increase when using higher concentrations of the polymer could be expected. In this way a function similar to that used for the viscosities at zero and infinite shear rates has been employed, obtaining good results:

$$K = P5 \times M_p^{P6} \tag{6}$$

The parameter m has been considered as a constant according to the Cross model:

$$m = P7 \tag{7}$$

Finally the viscosity can be calculated by means the Cross equation:

$$\eta = \eta_\infty + \frac{\eta_0 - \eta_\infty}{(1 + K(\dot{\gamma})^m} \tag{8}$$

The simplex flexible method proposed by Himmelblau[13] has been used in order to calculate the constant which minimise the following objective function:

$$OF = OF_{EPS++} + OF_{R4} \tag{9}$$

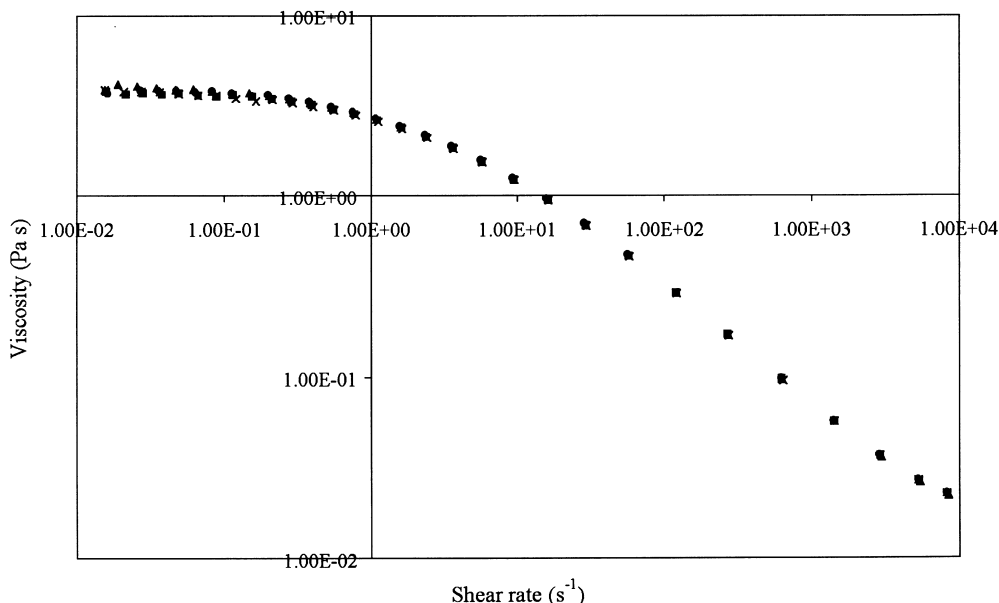where OF_{EPS++} and OF_{R4} are the objective functions

Figure 2 Behaviour of solutions with 1.500% of R4 at the different integration times (in seconds): ▲ 15; ● 30; ■ 40; × 50

corresponding to the experimental data obtained from the polymers EPS^{++} and R4, respectively, and which can be calculated by means of the following equation:

$$OF = \sum_{i=1}^{n} \sum_{j=1}^{k} \left(\frac{\eta e_{ij} - \eta c_{ij}}{\eta e_{ij} + \eta c_{ij}} \right)^2 \qquad (10)$$

where ηe_{ij} is the experimental viscosity j of the curve i, obtained from each concentration of polymer; n is the total number of curves and k is the number of experimental data of the curve i.

On the other hand, it is also possible to calculate the variation coefficient of all the experimental data corresponding to both polysaccharides:

$$VC\,(\%) = \sqrt{\frac{\left(\sum_{i=1}^{n} \sum_{j=1}^{k} \left(\eta e_{ij} - \eta c_{ij}\right)^2 \right)_{EPS^{++}} + \left(\sum_{i=1}^{n} \sum_{j=1}^{k} \left(\eta e_{ij} - \eta c_{ij}\right)^2 \right)_{R4}}{\left(N_{data} - N_{parameters}\right) \times \left(\eta_{average}\right)^2}} \times 100 \qquad (11)$$

where:

N_{data}	=	number of total experimental data
$N_{parameters}$	=	number of parameters to be calculated
$\eta_{average}$	=	average viscosity of all the experimental data (Pa s)
VC	=	variation coefficient (%)

Results obtained with the theoretical model used are shown in *Figures 5 and 6*. Parameters calculated, the variation coefficient and the objective function calculated are shown in *Table 1*.

As can be observed in *Figures 5 and 6* a good correlation with a low variation coefficient has been obtained, especially when considering that all curves have been simultaneously correlated with the same set of seven parameters.

It is also worth noting the values obtained for the parameters *P2* and *P7*. In the first place, the parameter *P2*

should be expected to be 1–3.5 (depending on the molecular weight of the polymer used) as has been stated previously; however, a transition value (1.77) has been obtained indicating that the polymers used are not low molecular weight polymers nor high molecular weight polymers, but medium molecular weight polymers. On the other hand, the parameter *P7* (0.68) is very close to the value that Cross found in a high number of dissolutions (0.66)[5].

Furthermore, as could be expected from the type of function used, the parameters obtained for the function relating the low shear rate viscosity with the concentration yield values at zero shear rate which agree with the experimental trends observed; for example, for the case of the EPS^{++} dissolutions, the following zero shear rate viscosity can be obtained from the model for the different concentrations used: 0.05 Pa s (0.125%), 0.18 Pa s (0.25%), 0.62 Pa s (0.50%), 1.25 Pa s (0.75%), 4.22 Pa s (1.50%). On the other hand, at high shear rate limit all the curves tend to

Table 1 Parameters, objective function and variation coefficient obtained from the correlation of the experimental data

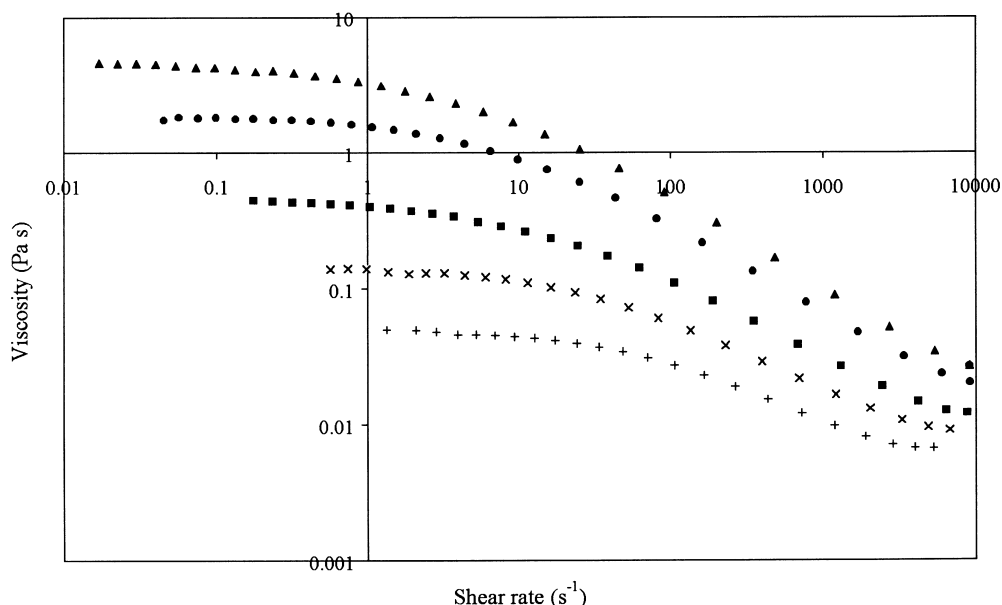| Parameter	Value of the parameter
P1 (Pa s)	5.26×10^{-8}
P2	1.77
P3 (Pa s)	2.65×10^{-3}
P4	7.05×10^{-2}
P5 (s^{+P7})	3.71×10^{-4}
P6	0.677
P7	0.686
OF	0.952
VC (%)	22.8%

Figure 3 Flow properties of the EPS^{++} dissolutions with the following mass percent concentration: ▲ 1.50%; ● 0.75%; ■ 0.50%; × 0.25%; + 0.125%
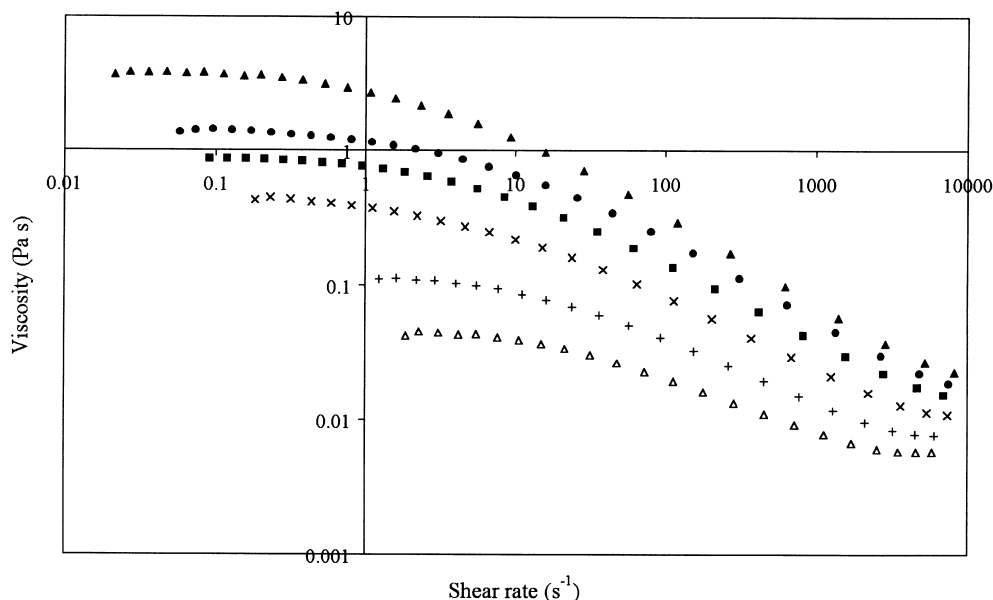
Figure 4 Flow properties of the R4 dissolutions with the following mass percent concentration: ▲ 1.50%; ● 1.00%; ■ 0.75%; × 0.50%; + 0.25%; △ 0.125%
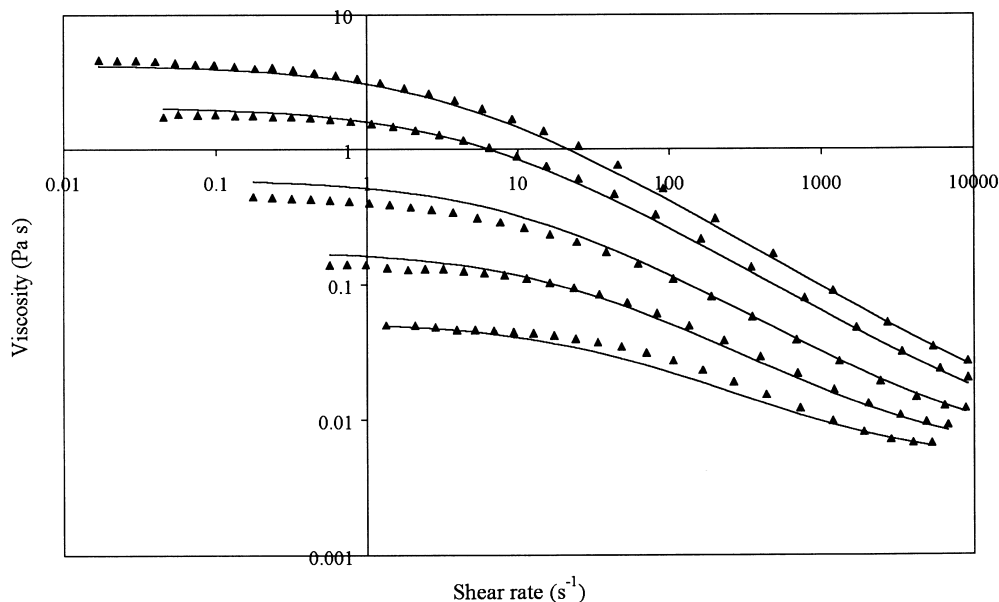

Figure 5 Results obtained from the correlation of the EPS^{++} dissolutions: ▲ experimental–calculated

approximate and the model provides, in accordance with the experimental data, viscosity values which are very close together.

CONCLUSIONS

The viscosity of the water is notably increased by the presence of both EPS^{++} and R4^{++} polysaccharides, even at the lowest concentrations studied (0.125%), presenting a more marked pseudoplastic behaviour when increasing the polymer concentration. No effect of shearing time has been observed. On the other hand, differences between the viscosity observed in dissolutions with the same polymer concentrations for each polymer are due to the different molecular weights of the two polysaccharides. With respect to the correlation of the experimental data, very

good results have been obtained using the Cross model considering the relations between the parameters of the model and the apparent molecular weight of the dissolutions to simultaneously correlate all experimental data obtained.
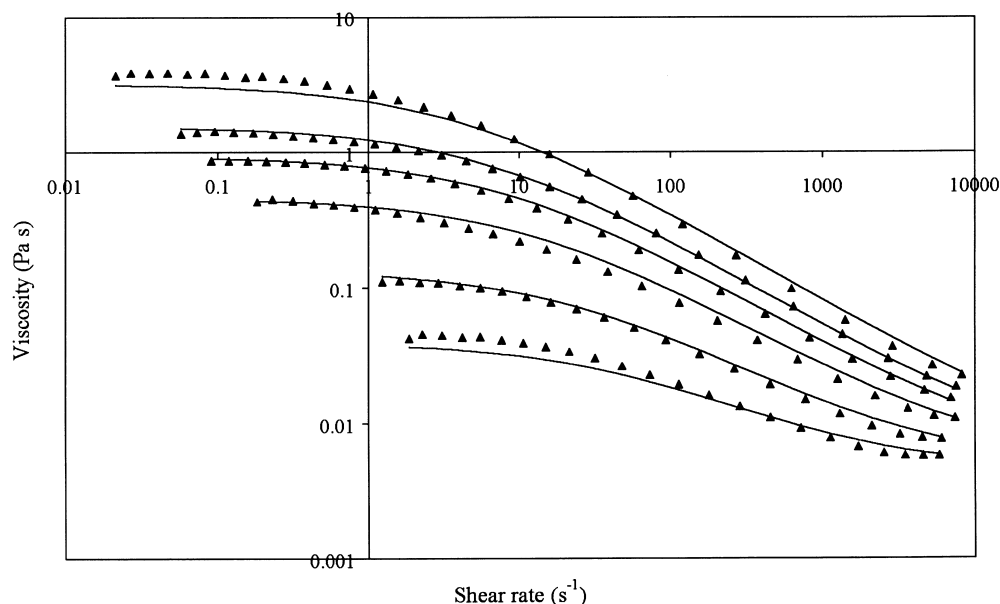
Figure 6   Results obtained from the correlation of the R4 dissolutions: ▲ experimental–calculated

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