Probing the viscoelastic properties of polyacrylamide polymer gels in a wide frequency range

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Polymer gels have been shown to behave as viscoelastic materials but only a small amount of data is usually provided in the glassy transition. In this paper, the dynamic moduli G' and G'' of polyacrylamide hydrogels are investigated using both an AFM in contact force modulation mode and a classical rheometer. The validity is shown by perfect matching of the two techniques.

Measurements are carried out on gels of increasing polymer concentration in a wide frequency range. A model based on fractional derivatives is proposed, covering the whole frequency range. G_N^0, the plateau modulus, as well as n_f, the slope of the G'' modulus, are obtained at low frequencies. The model also predicts the slope a of both moduli in the transition regime, as well as a new transition time λ_T. The whole frequency spectrum is recovered, and the model parameters contain interesting information about the physics of such gels.

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Polymers exhibit interesting rheological behavior in the sense that they can successively behave as liquids, elastic materials showing a rubbery plateau, then undergo a glassy transition before reaching the solid domain [1]. These processes are temperature-dependent. Because of this broad range of properties, polymers are widely used in industrial applications, as well as biological processes. However, it is often difficult to characterize their material properties, as the range of frequencies involved covers several decades [2, 3]. Techniques such as classical rheometry, diffusing-wave spectroscopy, dynamic light scattering [4] or ultrasonic experiments have been used to describe the complex behavior of polymers each in its own range of frequencies [5, 6]. In particular, an important way to extend the linear viscoelastic behavior (LVE) is found by using the time–temperature superposition principle, wherein results obtained at various temperatures are shifted onto a reference temperature master curve [3]. These observations have motivated quite a lot of theoretical studies. Different models providing relaxation spectra have been proposed, ranging from multiple Maxwell models to continuous relaxation spectra [2], involving both liquid and glassy modes. The concept of soft glassy rheology [7, 8] appeared recently and provides another alternative suited for many systems. Indeed it is based on the idea that sub–elements in the microstructure are linked via weak interactions, and are in a disordered metastable state. Based on this concept, many complex fluids can be described thanks to this model, in particular packed colloidal suspensions, the cell cytoskeleton [9] as well as foams or slurries.

Due to their cross-linked network, polymer gels share similar properties [7] with polymers. They can be characterized using modern microrheology techniques [10, 11], as applied in particular for actin networks [12, 13]. The behavior of classical gels is in fact similar in the glassy transition domain, but no modelling attempt has been made so far to characterize the entire frequency domain covered by recent instruments. Therefore, it is interesting to characterize a wide domain of frequency for various polymeric gels, especially biological systems, and develop a model for such behavior. This is the main purpose of the work presented here. In addition, a new AFM–based microrheology method [14–16] will be used allowing to investigate a wide range of frequencies, in combination with classical rheometry. This technique to probe the mechanical properties of biological cells locally using dynamic AFM measurements was developped [14], but has not yet been validated on a model system, like a polyacrylamide gel. Here, we choose to characterize the behavior of polyacrylamide hydrogels due to their interesting mechanical properties depending on the cross-linked network. These gels are known to exhibit a viscoelastic behavior, with an elastic modulus G', and a frequency–dependent viscous modulus G'', usually one decade below in the classical rheology domain [$0.01\,\mathrm{Hz} - 10\,\mathrm{Hz}$] [17]. The elastic modulus (G_N^0) has been investigated and increases with acrylamide concentration [18]. Thus, changing the cross-linked network and measuring the dynamical moduli in a wide range of frequencies can bring forward new data

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related to the physical properties of the gels.

Polyacrylamide gels were synthesized by mixing acrylamide (30% w/w) at four different weight concentrations (5 − 7.5 − 10 − 15%), and N,N–methylene–bisacrylamide 1% w/w at a fixed concentration 0.03% in deionized water. This means that the hydrogels were slightly crosslinked. Polymerization was then initiated by incorporating N,N,N,N-tetramethylethylenediamine (TEMED, Sigma) and ammonium persulfate 10% solution (APS), as described in [19]. Gels of thickness 70 µm were prepared on a pre–treated glass Petri dish for a better adhesion [19]. Gels were always kept in humid conditions, so that they were swollen and in equilibrium. They were set onto an AFM (JPK Instruments, Berlin) equipped with an inverted microscope (Zeiss, model D1, Berlin). The AFM chips (Bruker, MLCT, pyramid shape, tip half–angle $\theta = 20°$) were mounted onto the AFM glass block and calibrated using the thermal fluctuations method. Then an initial indentation δ_0 of the sample was made under a prescribed force F_0 given by Hertz model:

$$F_0 = \frac{3\,E\tan\theta}{4\,(1-\nu^2)}\delta_0^2 \tag{1}$$

where E is Young's modulus, ν is the Poisson ratio (usually assumed to be close to 0.5 for such gels [20]) and θ as defined above. δ_0 is chosen so that the tip penetration depth into the sample is large enough to have a sufficient contact area and not too large to remain within the linear elasticity assumptions corresponding to the Hertz model. In order to carry out microrheology measurements, a small perturbation (frequency f from 1 Hz to 0.5 kHz, and $\omega = 2\pi f$ is the angular frequency) was superposed onto the initial indentation. The perturbation being small, Eq. (1) can be linearized about the equilibrium. By the correspondence principle of LVE, in the ω–domain, one operates with complex quantities. Let δ^*, F^* be the complex indentation and force. Substracting the hydrodynamic drag $i\omega b(0)$ [14], the complex shear modulus $G^*(\omega)$ is given by:

$$G^*(\omega) = \frac{1-\nu}{3\,\delta_0\,\tan\theta}\{\frac{F^*(\omega)}{\delta^*(\omega)} - i\omega b(0)\} \tag{2}$$

where $b(h)$ is a function which contains the geometry of the tip and depends on the height h from tip to sample, and was measured as in [14] by extrapolation of the function at $h = 0$.

Rheometry measurements were carried out on a controlled stress rheometer (Malvern, Gemini 150) at low frequencies [0.001 Hz − 2 Hz] in the linear regime (deformation of 1%). Interestingly, an excellent agreement was found between these measurements and the AFM microrheology experiments for all gels, as seen in Fig. 1 where matching occurs around 2 Hz in the case of the 10% concentration gel.

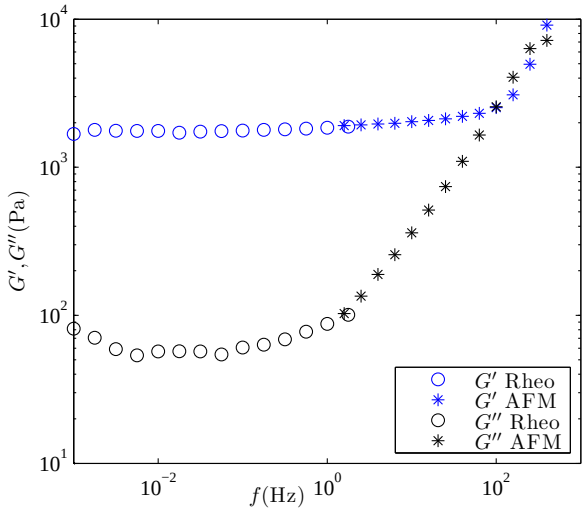

FIG. 1: Superposition of rheometrical and AFM microrheological measurements. The acrylamide content is 10% in this case. Typical error bars (not shown) are around 10%. $T = 25°$C.

The variations of the dynamic moduli (Fig. 1) show a constant elastic plateau modulus (G_N^0) at low frequencies ($G_N^0 \simeq 2300$ Pa in Fig. 1). The gel undergoes a glassy transition in the higher frequency regime as the AFM measurements do show. The slopes of the moduli G' and G'' (around 1.0) are similar above 100 Hz.

To predict the observed behavior, a rheological model was used. The complex modulus $G^*(\omega)$ can be related to a relaxation function $H(\lambda)$ using the general formalism [2] :

$$G^*(\omega) = \int_0^\infty H(\lambda)\frac{i\omega\lambda}{1+i\omega\lambda}\frac{d\lambda}{\lambda} \tag{3}$$

$H(\lambda)$ is the continuous relaxation spectrum, the expression of which is shown in this work to model the LVE response from flow to glassy state. In particular, the flow regime is described with the corresponding function $H_{\rm f}(\lambda)$:

$$H_{\rm f}(\lambda) = \begin{cases} n_{\rm f}\,G_N^0\left(\dfrac{\lambda}{\lambda_{\max}}\right)^{n_{\rm f}} & \text{if } \lambda \le \lambda_{\max} \\ 0 & \text{if } \lambda > \lambda_{\max} \end{cases} \tag{4}$$

This power law behavior will then describe the continuous relaxation time distribution required to model the plateau regime observed in Fig. 1 at low frequencies. This model is unsuitable to describe the high frequency state observed here. Another approach based on the BSW description [2] was found to be insufficient to predict the data accurately. Therefore a fractional derivative model

[21] is added to the previous one, to account for this behavior. The corresponding expression for the dynamic complex modulus $G^*_{\rm g}(\omega)$ is simply given by :

$$G^*_{\rm g}(\omega) = G_1 \frac{(i\omega\lambda_1)^b}{1+(i\omega\lambda_1)^a} \qquad (5)$$

where a and b are the orders of fractional derivatives [21]. Compatibility with thermodynamics requires $0 < a \leq b$ [3]. This type of model accounts for the slopes of the glassy transition, as observed in the current data.

The coupling of the two linear models is insured by the simple relationship $G^*(\omega) = G^*_{\rm f}(\omega) + G^*_{\rm g}(\omega)$, to account for the whole frequency spectrum. The parameters of this global model are G^0_N, $\lambda_{\rm max}$, $n_{\rm f}$, a, b, G_1 and λ_1, . G^0_N appears naturally to be the classical elastic plateau modulus (Fig. 2). $\lambda_{\rm max}$ is the maximum relaxation time corresponding to the flow domain. In the case of gels, this specific time is out of reach since gels do not actually flow and exhibit a plateau even at very low frequencies [22]. $-n_{\rm f}$ is the slope of G'' at low frequencies, in a log–log plot and is found to lie between -0.8 and 0. b represents the slopes of G' and G'' in the glass transition regime (with $b = a$ in Fig. 2). G_1 is the high frequency limit modulus of G', and is far above our data, so complementary rheological experiments would be necessary to reveal such high values of the limiting modulus [3]. λ_1 is a time related to the microstructure and is not shown in Fig. 2. Actually $1/\lambda_1$ is a typical crossover frequency between the glass transition and solid domain. Finally $b - a$ would correspond to the limiting slope of G' and G'' moduli at the highest frequencies, but is not shown in Fig. 2. Here we used $a = b$, which was quite sufficient for describing our data, and led to the optimal fit.

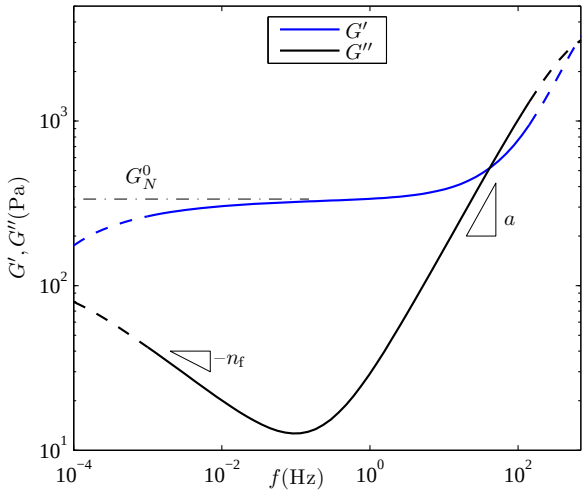

FIG. 2: Significance of the model parameters G^0_N, $n_{\rm f}$, and $b = a$. $\lambda_{\rm max}$ is not shown but should appear at lower frequencies at the intersection of G' and G'' occuring for $\omega \sim 1/\lambda_{\rm max}$. This does not occur in such gels since the flow region is not reached at low frequencies. Similarly G_1 corresponds to a higher plateau modulus in G' but is well above our data, this being also the case for λ_1.

TABLE I: Best-fitting values of parameters used in the model.

Gel	G^0_N(Pa)	$\lambda_{\rm max}$(s)	$n_{\rm f}$	λ_1(s)	G_1(Pa)	$a = b$
5%	336	1.5×10^4	0.73	1.3×10^{-4}	1.0×10^4	0.82
7.5%	710	2.0×10^5	0.18	2.4×10^{-4}	1.0×10^4	0.85
10%	2 307	9.0×10^9	0.08	2.4×10^{-4}	2.0×10^4	1.00
15%	8 801	1.0×10^{10}	0.06	2.0×10^{-4}	5.9×10^4	1.00

Fitting of the data was carried out for the four gels characterized both in rheometry and AFM microrheology. The best-fitting values of the parameters were determined by minimizing a weighted sum of squared residuals. The weights were chosen from the data. Minimization was achieved using the Levenberg–Marquardt method. The initial guesses followed the discussion on the role of each single parameter (see Fig. 2). The best-fitting values of the parameters are reported in Table I and the associated curves are presented below in Figures 3. Very good agreement is shown.

Note that, as expected, the plateau modulus G^0_N increases with c, the acrylamide concentration. These values are shown in Fig. 4 and the slope can be compared to other available data from the literature. For the lower frequency plateau, the relationship is of the kind $G^0_N \sim c^{3.0}$. Previous observations using combined light scattering and mechanical tests [18] were reported, showing an exponent 2.55 using dynamic mechanical measurements (and 2.35 using dynamic light scattering) as compared to the theory of de Gennes giving 2.25 for good solvents [23]. The value of the exponent for G^0_N is also close to the exponent 2.55, found for collagen gels [24, 25] but is larger than the typical exponent of 1.4 obtained for entangled actin solutions [26].

The longest relaxation time $\lambda_{\rm max}$ does not seem to play a significant role, because it is related to a possible crossover of the G' and G'' moduli at low frequencies which does not occur for such gels (in our frequency range) since they do not flow at low frequencies. However, $-n_{\rm f}$, the low–frequency slope of G'', is an important parameter here, and decreases as gel concentration increases. This further emphasizes the fact that high concentration gels exhibit moduli which have almost flat G' and G'' moduli (see in particular Figures 3 at 10% and 15% acrylamide concentration) and do not cross at low frequencies. Note that values of G' and G'' at low frequencies (0.001 Hz) using the classical rheometry setup are difficult to obtain, due to the long experimental times required, therefore a larger uncertainty is unavoidable for $n_{\rm f}$. For the four gels, λ_1 was found to be

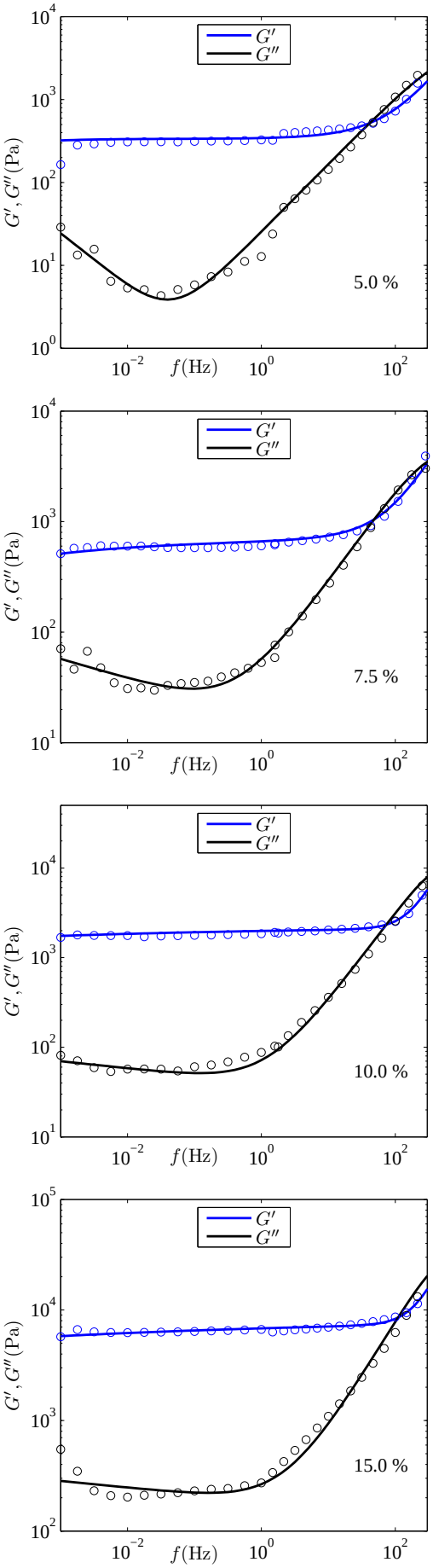

FIG. 3: Gel rheology: 5%, 7.5%, 10% and 15% acrylamide concentrations. Open circles are experimental data whereas solid lines are the model best-fit curves.

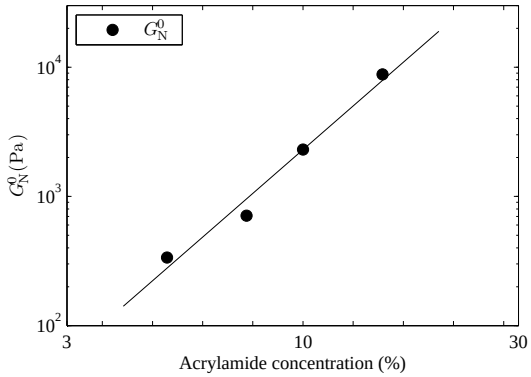

FIG. 4: Evolution of gel moduli G^0_N vs. concentration. The slope of the power law exponent corresponds to 3.0 ± 0.3.

almost constant within experimental error, as a function of concentration c, revealing no clear difference in the crosslinks relaxational processes of the acrylamide gels at very high frequencies. Therefore, crosslinks appear to link the acrylamide network in a regular mesh, and the short relaxation time λ_1 ($\sim 2.0 \times 10^{-4} s$) is gel independant. This short time relaxation process corresponds to a single Maxwellian mode with values of a and b ranging between 0.82 and 1.0. In all cases, the optimal value of $b - a$ was found to be 0, so we used $a = b$ in the optimisation method (see table I). Remember that values of a (or b) are directly related to the fractional derivatives present in the model.

Finally, the onset of the glass transition is an interesting parameter for such gels, and corresponds to a typical transition time $1/\lambda_T$. As can be seen in Figures 3, the corresponding frequency (around 100 Hz) is increasing with polymer content, as curves are slightly shifted towards the right. The angular frequency $\omega_T = 1/\lambda_T$ can be found analytically by investigation of the change of slope of G'. On the plateau $G' \sim G^0_N$ and at the transition $G' \sim G_1\, cos(\pi a/2)(\omega\lambda_1)^a$ since $\omega\lambda_1 << 1$. Therefore the transition (angular) frequency ω_T is given by:

$$\omega_T = \frac{1}{\lambda_1}\left(\frac{G^0_N}{G_1\, cos(\pi a/2)}\right)^{1/a} \qquad (6)$$

This can be checked easily from Table I. Indeed $\frac{G^0_N}{G_1}$ increases with polymer concentration since G^0_N varies faster than G_1, while $cos(\pi a/2)$ decreases, thus the ratio $\frac{G^0_N}{G_1\, cos(\pi a/2)}$ increases and $1/a$ increases as well. Since λ_1 is almost constant, we deduce that ω_T increases with polymer concentration as shown experimentally. ω_T is linked to the ability of polymer crosslinks to move at such frequencies, and its increase shows that such motions are restricted due to the polymer excess at higher concentration (and constant crosslinker concentration). This typical frequency could be an important parameter

to exhibit in future studies on gels.

Further extensions of the model may be considered for other physical (or chemical) gels, as well as the study of biological gels, involving cytoskeleton filaments such as actin, tubulin, and finally living cells [14]. Therefore, this model, coupled with the use of high frequency AFM measurements, allows to investigate different types of filamentous networks in order to determine their behavior in a large range of frequencies, and could be applied to investigate the microstructure of complex materials such as living cells. In particular the determination of the transition time λ_T could be of importance.

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