Polymer Bulletin 50, 373–380 (2003)
DOI 10.1007/s00289-003-0173-9

Polymer Bulletin

Influence of concentration and molecular weight on the photosensitized degradation of alginate in aqueous solutions

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Received: 27 January 2003 / Accepted: 3 May 2003

Summary

Riboflavin (RF), photosensitizer, was used in combination with irradiation with light of wavelengths in the range of 310-800 nm to induce degradation of the biopolymer alginate. The effects of molecular weight and concentration were studied. The viscosity increases as the concentration is raised both for the alginate and alginate-RF solutions but is lower for the solutions irradiated in the presence of RF. Similar to many entangled and/or associated polymer networks this system shows strong shear-thinning effect at higher concentrations. The viscosity change induced by illumination in the presence of RF seems to be most extensive for the solution with the highest molecular weight. This was emphasized by oscillatory shear measurements, which indicate that RF in combination with irradiation has most effect on the rheological parameters of alginate of the highest molecular weight.

Introduction:

In order to develop a matrix based drug delivery system triggered with light [1,2], riboflavin (RF) was used as a photosensitizer to induce degradation of alginate. Alginate is a negatively charged biocompatible copolymer consisting of mannuronic acid and guluronic acid (Figure 1). The polymer will be negatively charged at physiological pH, which is of importance for its bioadhesive properties [3]. Alginate occurs in nature as a structural component in brown algae, mainly harvested from *Laminaria hyperborea*. Alginate is approved by the European Pharmacopoeia and the FDA (Food and Drug Administration) as pharmaceutical excipient and is also commonly used in the food industry. Therefore there is little toxicological risk in the use of alginate [4,5].

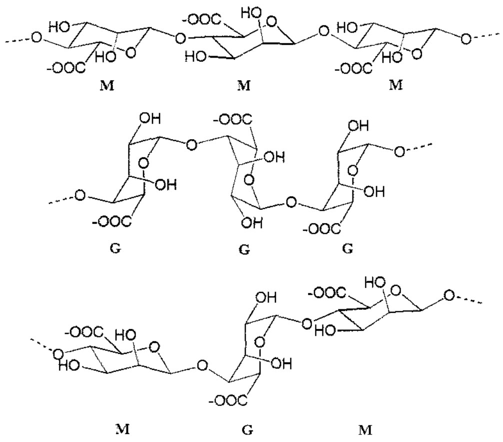

Figure 1. The structure of the chemical units of alginate (M = mannuronic acid and G = guluronic acid).

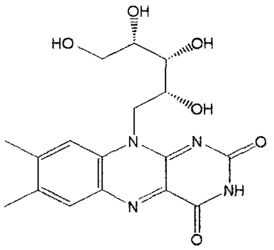

Figure 2. The chemical structure of riboflavin (RF).

Riboflavin (RF) or Vitamin B_2 (Figure 2) is a widely used and approved yellow pigment, which can act as a photosensitizer in the light-induced degradation of polysaccharides [6,7]. RF is a source of active oxygen species and free radicals and can be reversibly reduced/oxidized by accepting or loosing a pair of hydrogen atoms [6,8,9]. RF undergoes photochemical degradation when exposed to irradiation both via photodealkylation and intramolecular photoreduction [10]. During both degradation pathways, under neutral aqueous conditions, lumichrome is a dominant product [10,11]. The mechanism of RF photosensitizer induced alginate degradation is not known but most likely it proceeds through the triplet state of RF and the finale step is free radical induced scission of the glycosidic linkage [12].

In the development process of drug formulations, it is important to get sufficient knowledge about the physicochemical parameters that are of interest for the system. Rheological properties of pharmaceutical products are of importance with respect to formulation characteristics, stability and production. In externally regulated polymeric systems the release of drug molecules will to a large extent be dependent on a change in rheological properties, which can be induced by a change in the polymer network structure. With this in mind, we have studied how RF-induced photochemical scission of polymer chains is affected by molecular weight and polymer concentration. In this work, the photosensitized degradation of alginate is characterized with the aid of rheology, which is a powerful method to survey the viscoelastic properties of the systems.

Materials and methods:

Materials and Solution Preparation.

Alginate from FMC Biopolymers, Drammen, Norway, of three different molecular weights (M_w), given by the manufacturer, of 140 000 (#333629-A), 200 000 (#333629) and 250 000 (#333629-O), was used in this study. According to the specifications from the manufacturer, this type of alginate has guluronic acid to mannuronic acid (G/M) ratio of 0.96. The pK_a values of G and M have been determined to 3.7 and 3.4, respectively [5]. RF was supplied by Sigma and was of analytical grade. The polymer was dissolved in a phosphate buffer (PBS) (pH = 7.4) in the absence or presence of the RF (0.1 mM) photosensitizer. To reduce the influence of trivial molecular weight effects on the rheological parameters, the samples were compared at the same value of the hydrodynamic volume fraction $c[\eta]$, where c is the polymer concentration and $[\eta]$ is the intrinsic viscosity. The measurements were carried out in the regime where the samples before irradiation gave the following values of $c[\eta]$ 0.5, 1.0, 2.0, 5.0, 10.0, and 20.0 ($[\eta]$ is given in Table 1). Most of the solutions are located in the semidilute regime (see the discussion below). All samples were stored in the dark at 6 °C prior to measurement. Great care was exercised to ensure that the samples were homogeneous and freshly prepared. All experiments were conducted at 25 °C.

Table 1. The intrinsic viscosity of alginate (AL) and alginate-riboflavin (AL-RF) samples (PBS, pH=7,4) after light irradiation in the wavelength range of 310-800nm. The intrinsic viscosity of the non-irradiated AL and AL-RF samples is the about the same as the intrinsic viscosity of the irradiated AL samples.

M_w	$[\eta]$ (dl/g)	
	AL	AL-RF
140 000	7.58	6.14
200 000	8.81	7.31
250 000	9.60	8.06

Irradiation Source.

The samples were irradiated in a Suntest CPS (Heraeus GmbH, Hanau) apparatus, equipped with a 1.8 kW xenon lamp and a glass filter transmitting irradiation corresponding to exposure behind window glass (wavelength range 310-800 nm). The light intensity was measured to $1.4 \cdot 10^5$ lux and 15 W/m^2 in the visible and UV range, respectively, using a lux meter (Hagner EC1 Digital luxmeter) in combination with a UV-filter radiometer (Hagner EC1 UV-A). Both the alginate-RF solution and the corresponding alginate solution without RF (blank) were illuminated. Alginate-buffer solutions served well as blanks, because for non-irradiated samples the small amount of added RF had no effect on the rheological results of the solutions. The time of irradiation was 15 min, which is sufficient for a substantial photochemical degradation of the polymer [12]. The alginate-RF samples were kept in the dark after the exposure to light, which caused cessation of the photo-induced degradation of the polymer.

Rheological Experiments.

Oscillatory shear and viscosity experiments were conducted in a Paar-Physica MCR 300 rheometer using a cone-and-plate geometry, with a cone angle of 1° and a diameter of 75 mm. The sample was applied on the plate, and to prevent dehydration from the solution, the free surface of the sample was always covered with a thin layer of a low-viscosity silicone oil (the viscoelastic response of the sample is not significantly affected by this layer). The measuring device is equipped with a temperature unit (Peltier plate) that gives a very good temperature control over an extended time.

Results and discussions:

Viscosity:

In dilute polymer solutions, the molecules act as individual units and the intramolecular interactions are dominant. As the concentration increases, the interactions between molecules become more and more frequent, intermolecular interactions become dominant, and entanglement effects come into play. These entanglement couplings are likely to play an important role for the viscosity at high concentrations. At a certain concentration, the overlap concentration (c^*) (transition from dilute to the semidilute concentration regime), a transient polymer network is formed. The overlap concentration is estimated by the equation $c^* = 1/[\eta]$. Values of $c[\eta]<1$ are in the dilute regime and $c[\eta]>1$ represents the semidilute regime. Figure 3a shows the zero-shear viscosity as a function of $c[\eta]$ for irradiated alginate and alginate-RF solutions. The zero-shear viscosity of both alginate and alginate-RF solutions rise with increasing concentration of alginate. Lower viscosity is detected after irradiating alginate solutions in the presence of RF, and the difference seems to be largest for the solutions of the polymer with the highest molecular weight. This effect is depicted in Figure 3b, where the differences in the zero-shear viscosity (η_0) of irradiated alginate solutions and the corresponding alginate-RF solutions are plotted against $c[\eta]$. The viscosity change induced by RF is very small in the dilute regime but increases significantly in the semidilute regime. This is probably due to the fact that the scission of chains in an individual molecule does not affect the rheological parameters as much as rupture of chains in a network. The inset plot in Figure 3b shows the molecular weight dependence of the difference in η_0 between the AL and the AL-RF samples. In the semidilute regime, the change in the zero-shear viscosity increases as the molecular weight is raised. This is probably caused by a more pronounced loss of entanglement effects as the long polymer chains are cut down to shorter chains by the photo-induced reaction in the presence of RF.

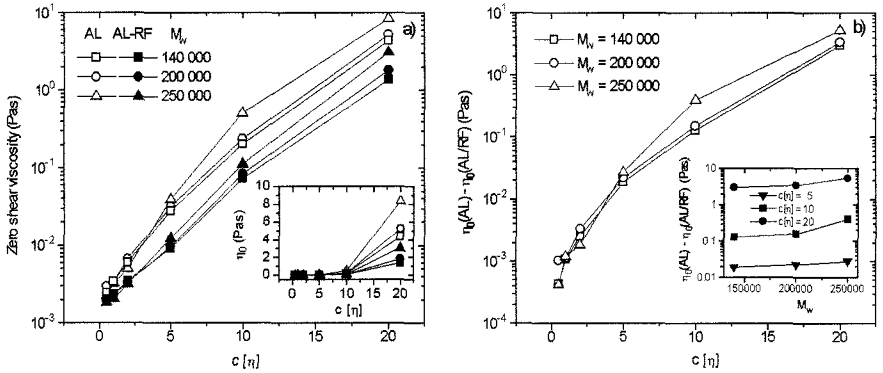

Figure 3. a) Zero-shear viscosity as a function of $c[\eta]$ for irradiated alginate (AL) and alginate-RF solutions. The inset plot shows a better view of the difference at high concentrations. **b)** The difference in zero-shear viscosity (η_0) for alginate solutions irradiated in the presence and absence of RF plotted against the quantity $c[\eta]$.The inset plot shows the molecular weight dependence of this difference for the highest concentrations.

Many entangled and/or associated polymer systems have a strong shear dependence of the viscosity. Shear thinning starts at the shear rates where the shear force is strong enough to disrupt the network by reducing the number of intermolecular bonds and thereby lowering the viscosity. Figure 4 shows the effect of shear dependence on the viscosity for the alginate solutions in the presence and absence of RF. The viscosity is clearly lower for the irradiated alginate-RF solution than for the corresponding alginate solution at all concentrations. Furthermore, the shear thinning effect is less pronounced for the alginate-RF samples. At low concentrations, a Newtonian behavior is observed but as the concentration of alginate increases a stronger shear thinning is detected. The reason for stronger shear thinning at higher concentrations is that entanglements and/or associations of the polymer are enhanced.

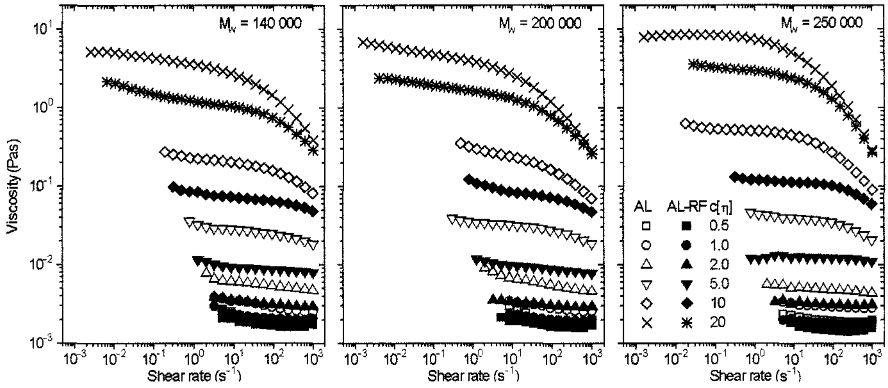

Figure 4. Shear rate dependencies of the viscosity of irradiated alginate (AL) and alginate-RF solutions at the molecular weights and polymer volume fractions indicated.

Oscillation:

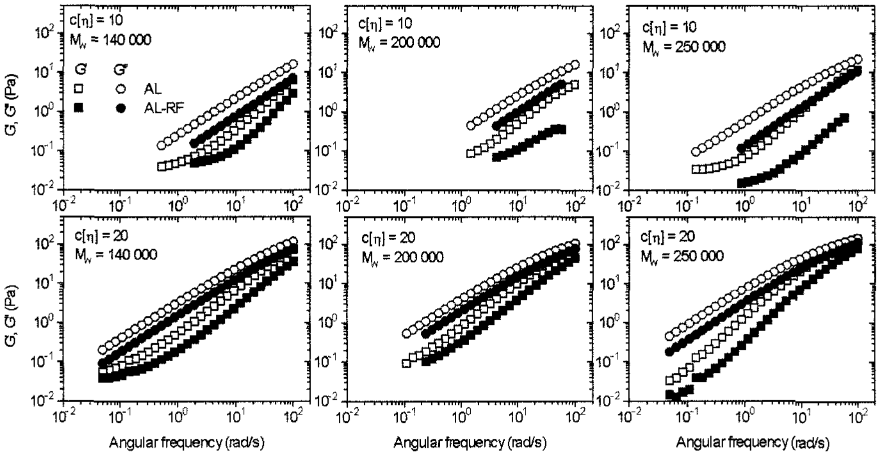

Figure 5. Frequency dependencies of the storage (G') and loss (G'') moduli of alginate (AL) solutions, in the presence and absence of RF, for the considered molecular weights at two values of c[η].

Figure 5 illustrates the frequency dependencies of the dynamic moduli. The loss modulus (G") lies above the storage modulus (G') at all frequencies for all the conditions considered, which indicates that the viscous response of the system is more dominant than the elastic effect. We note that both the dynamic moduli exhibit lower values after illumination in the presence of RF, which probably can be attributed to photo-induced scission of polymer chains. Figure 6 demonstrates the difference between the storage and the loss moduli for the different molecular weights at a constant angular frequency (7.27 rad/s) and a fixed value of c[η]. Both G' and G'' increase with increasing molecular weight, and this effect is more pronounced in the solutions without RF. This suggests that the photochemical scission of chains in alginate-RF solutions is more efficient in the presence of long polymer chains.

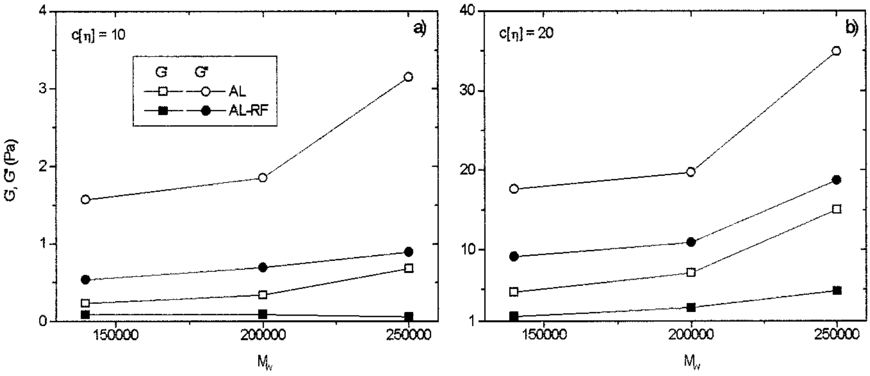

Figure 6. The dynamic moduli (at an angular frequency of 7.27 rad/s) as a function of molecular weight for irradiated solutions of alginate (AL) and AL-RF. The values of c[η] are 10 (a) and 20 (b).

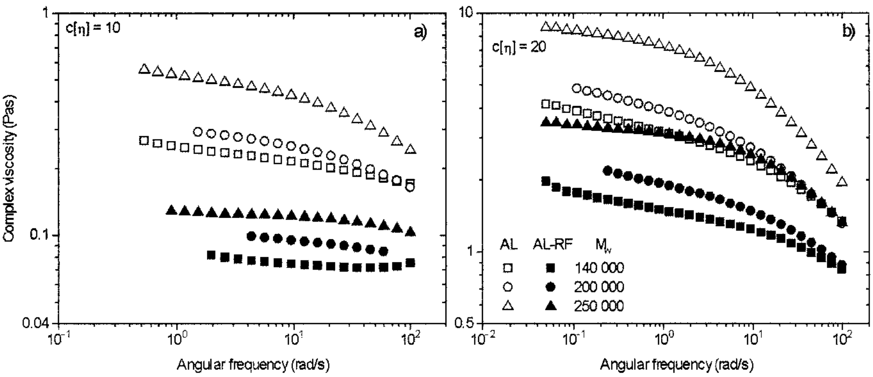

Figure 7. Angular frequency dependencies of the complex viscosity of alginate solutions, in the presence and absence of RF, for all three molecular weights at values of c[η] of 10 (a) and 20 (b).

On the analogy of the observed shear dependency of the shear viscosity (cf. above), the complex viscosity ($\eta^* = (G'^2 + G''^2)^{1/2} / (2\pi\omega)$, where ω is the angular frequency) of the solutions shows an angular frequency dependency as depicted in Figure 7. A strong frequency dependence is typical for systems with intermolecular associations and for entangled chain networks. As can be perceived from Figure 7, the frequency dependence is strongest for the highest concentration, and the addition of RF causes lower values of η* and a reduction of the frequency dependence of η*. In Figure 8a, the power law exponent ν, obtained from the relation $\eta^* \sim \omega^{-\nu}$, is plotted as a function of the weight average molecular weight for the values of c[η] indicated. The results clearly illustrate that the frequency dependency of the complex viscosity increases with concentration and molecular weight, while the addition of RF under irradiation causes a much lower frequency dependency. This suggests that the scission of the chains, caused by illumination in the presence of RF, results in a reduction of the number of entanglements in the sample. In Figure 8b the complex viscosity is plotted at a constant shear rate. From this figure and also from Figure 7, it can be perceived that the complex viscosity increases as the molecular weight is raised. This effect is most pronounced in the absence of RF. This feature is shown in the inset plot of Figure 8b, where the difference in the complex viscosity of the irradiated samples without and with RF is displayed. As can be seen from the figure, addition of RF causes the largest change for the highest molecular weight. This behavior is parallel to that discussed for the dynamic moduli. This again illustrates that the reduction of the viscosity of the alginate solution, induced by the combination of RF and irradiation in the range of 310 – 800 nm, exhibits the most marked change in solutions of the highest molecular weight.

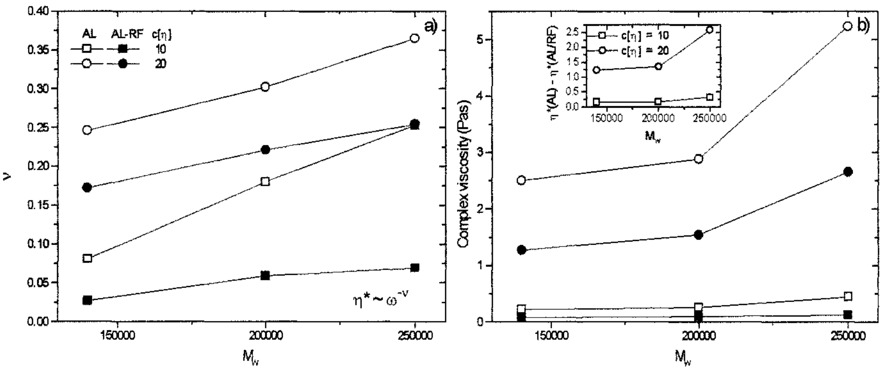

Figure 8. a) The angular frequency dependence of the complex viscosity. **b)** The complex viscosity at a constant angular frequency (7.27 rad/s). The inset plot shows the difference between in the complex viscosity for alginate solutions irradiated in the presence and absence of RF.

Conclusions:

In this work, changes of rheological parameters of alginate solutions induced by the combination of RF and irradiation in the wavelength range of 310-800 nm have been studied. Under aerobic conditions (pH = 7,4) RF causes disruption of the alginate network and the rheological quantities are affected. At higher concentrations, the solutions are more entangled and hence more sensitive to rupture of the chains by photosensitized RF than dilute solutions. In spite of that the alginate samples of different molecular weights were compared at fixed values of c[η], effects of molecular weight were observed. The scission of polymer chains in the semidilute regime was more efficient as the molecular weight of the sample was increased. It seems that entanglement effects play an important role.

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