

Study on the Shear Viscosity Behavior of Keratin/PEO Blends for Nanofibre Electrospinning

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ABSTRACT: Graessley's theory has been applied to keratin/PEO concentrated aqueous solutions giving qualitative insight to the rheology of these polymer blends in electrospinning. The shear rate dependent viscosity of different blends was compared with that of pure polymer solutions. The characteristic time τ_η was calculated by the minimum value of $\dot{\gamma}$ at the beginning of the non-Newtonian viscosity behavior. Flow curves of PEO (at concentration from 1.0 to 7.0 wt %) reduce to a single curve by plotting η/η_0 against $\dot{\gamma}\tau_\eta$. Moreover, PEO solutions exhibit a linear proportionality between zero-shear viscosity and the characteristic time $\eta_0 \propto \tau_\eta$. Keratin/PEO blend solutions follow the same proportionality at very high and low keratin content, whereas linearity drops when the keratin content range from 50 to 70%. The departure from the theory has been interpreted as a sign of some interaction between the macromolecules of keratin and PEO. It was supposed that keratin displaces solvent molecules and expands the PEO chain coils increasing the relaxation time of the polymer solution. This behavior was correlated with changes in the morphology of the nanofibres produced by electrospinning from these polymer blends. Finally, additive rules to zero-shear viscosity were applied to keratin and PEO solutions, indicating that the experimental η_0 values were higher than the theoretical ones for all the proportions of the blends, especially for high keratin amount. © 2008 Wiley Periodicals, Inc. *J Polym Sci Part B: Polym Phys* 46: 1193–1201, 2008

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INTRODUCTION

Many theories have been developed on the relationships between polymer structure, macromolecular interaction, and viscoelastic properties of polymer blend solutions. They are, however, restricted to very low concentration ranges, where experiments are difficult to perform and where practical applications are scant. In concentrated solutions and undiluted polymers, viscoelastic

properties are easier to measure and very important in many technological applications. In this work, molecular theories are applied to two-component systems made of concentrated aqueous solutions of wool keratin and poly(ethylene oxide) (a water-soluble synthetic polymer) blended in different proportions, giving qualitative insight into rheology behavior and filament formation during electrospinning.

Interest in natural polymers is increasing because of the development of policy guidelines about the use of renewable sources. Keratin is considered to be a nearly inexhaustible natural polymer with a fascinating structure and properties suitable for meeting the remarkable

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demand for environmentally friendly and biocompatible products. It is known that keratin is a highly stable protein. It is insoluble in solvents and soluble in few solvent systems, which leads to a limitation in its reactivity and processability for further utilization. Keratin can be extracted from wool by cleavage of the disulfide bonds and hydrogen interchain bonds. Several extraction methods have been reported in literature and most of them involve oxidants or reducing agents, namely peroxides or thiols.^{1–3} However, these solvent systems are harmful, difficult to handle and environmentally dangerous. In our laboratory, a solvent system, *m*-bisulfite/sodium dodecylsulfate/urea aqueous solution, has been successfully tested on wool⁴ producing *S*-sulfo-nated keratins. Keratins are the main constituents of the spindle-shaped long cortical cells making-up 30–60% by weight of the wool fibers. Keratins are distinguished from collagen, silk fibroin and other structural proteins because of their high content of cystine (7–20 mol % of all amino acid residues), which is responsible of the high environment stability.⁵ Keratin extracted from wool has broad molecular weight distribution, with three main fractions: the “low-sulfur content” proteins ranging from 45 to 65 kDa, the “high-sulfur content” proteins ranging from 11 to 28 kDa, and the “high glycine and tyrosine content” protein fraction, ranging from 11 to 9 kDa.⁶

Poly(ethylene oxide) (PEO) is an amphiphilic, water-soluble, and non degradable polymer with good biocompatibility and low toxicity. Its polymer chains are flexible in water due to the ether linkages in the backbone and the absence of bulky side groups. To produce nanofibres of keratin, PEO is added in different ratios to keratin solutions to improve the processability of the keratin itself.

In the electrospinning process,^{7–9} membranes composed of thin (usually nanometric or submicron sized) fibers are produced applying a high voltage to a polymer solution droplet. The polymer solution is fed to a capillary connected to a high voltage generator. The droplet is stretched by the electrostatic field to a jet that, traveling from the capillary tip to the grounded collector, solidifies in the form of a continuous filament, which is collected as a randomly oriented mat.

The study of the influence of the process parameters on the morphology of nanofibres produced via electrospinning is a complex matter, which involves electro-fluidynamic aspects.¹⁰ In particular, low surface tension and high viscos-

ity solutions produce fibers free from the so-called bead defects. The surface tension of polymer solutions depends both on the polymer and the solvent characteristics, and high surface tension values favor the formation of beads.¹¹ A high density of the charged species in the jet, not only promotes the formation of fibers without beads, but also produces fibers with thinner diameter.¹² Moreover, the morphology is determined by the non uniformity of the electrostatic field in the trajectory of the jet. A jet of viscoelastic fluid, initially strongly stretched, is subjected to stress relaxation when the electrostatic field becomes weaker and the polymer solution is not yet completely solidified. Therefore, bending instabilities arise when the jet is thin enough and sufficient stress relaxation of the viscoelastic stress has taken place, but relaxation times of viscoelastic solutions have not yet been studied enough to explain all the relationships with the morphology of electrospun filaments.

To mimic *in vivo* topography of the native tissue created by extra cellular matrix components, the surface features of scaffolds should be on the nanoscale dimension.¹³ The texture of electrospun membranes is similar to the structure of the extra cellular matrix of the skin. Therefore, in the past decade, considerable efforts have been made in the attempt to develop scaffolds for tissue regeneration based on electrospun membranes. More recently, natural polymers have been used with the aim of enhancing biological interactions with cells and increasing the cell growth rate.¹⁴

Many works deal with the electrospinning of natural polymer (e.g., silk fibroin, chitosan, collagen). Most of them were principally aimed at improving the electrospinnability of natural polymers by blending them with biocompatible synthetic polymers, such as PEO and PLLA.^{15–18} Keratin well supports fibroblast cell attachment and proliferation¹⁹ and degrades both *in vitro* (by trypsin) and *in vivo* (by subcutaneous embedding in mouse)²⁰; therefore it is an ideal candidate for biomedical applications in a similar manner to collagen,²⁰ but there is a lack of information about viscoelastic properties and processability of keratin-based blend solutions.

THEORY

The non-Newtonian viscosity behavior in concentrated polymer solutions is very similar to

that of undiluted polymers. The decrease in viscosity with increasing shear rate (called shear thinning) can be understood qualitatively as due to a decrease in the entanglement density and to an increase in the orientation of three-dimensional macromolecular coils during flow. At high shear rates, the η versus $\dot{\gamma}$ logarithmic plot becomes linear and the slope becomes less negative with decreasing concentration, due to the low degree of coil overlap.²¹

Shear thinning behavior could be explained supposing that, at rest, each single macromolecule stands in the state of minimum energy as a three-dimensional coil that could be ideally enclosed in a sphere. Moreover, each coil is entangled many times with neighboring macromolecules. During the shear process, the macromolecules disentangle to a certain extent and the shape of the coils deforms in the direction of the flow.²²

At low shear rates a non-Newtonian fluid has a Newtonian plateau, which corresponds to the limiting vanishing-shear viscosity or zero-shear viscosity (η_0). The magnitude of η_0 decreases with decreasing polymer concentration. The zero-shear viscosity of polymer solutions can be attributed primarily to intermolecular effects, since the viscosity is dominated by the longest relaxation times that are associated with entanglements between different molecular chains.²¹ As pointed out by Graessley,²³ this conclusion is supported by the minimum value of $\dot{\gamma}$ at which the non-Newtonian viscosity behavior appears. The onset of non-Newtonian behavior is established when the ratio η/η_0 falls to a characteristic value significantly smaller than unity (usually ~ 0.8). The reciprocal of $\dot{\gamma}$ is a characteristic time τ_η , which is found to be similar in magnitude to the longest relaxation time in the terminal zone.²¹ The onset of the shear thinning behavior is related to the time required by an entangled polymer solution for reaching the complete configurational rearrangement.

Very good agreement with experimental data for undiluted polymers with sharp molecular weight distribution is obtained for Graessley's theory. Similar features have been observed also for concentrated polymer solutions, therefore the theory could be extended.^{24,25} The theory is based on the presence of a dynamic steady state in the entanglement's formation and breaking. With increasing shear rate, the entanglement concentration is diminished mainly because the time required for a macromolecule to escape

from its topological restraints becomes smaller compared with the time required to form an entanglement and the time required to form an entanglement is proportional to the viscosity at the existing shear rate. The decrease in viscosity with increasing shear rate is more gradual for a polymer with a broad distribution of molecular weights than for a sharply fractionated polymer. The feature of a curve η against $\dot{\gamma}$ can be evaluated using the theory of Graessley²⁴ for any arbitrary molecular weight distribution and a characteristic time τ_η can still be identified by matching experimental results and theory.

For a binary polymer blend in which the molecular weights differ significantly, the final relaxation processes involves only the component of higher molecular weight.²¹ In general, the longest relaxation time for the component with the higher molecular weight will be smaller than the corresponding value for this component in the pure state. Similarly, the longest relaxation time for the component with the lower molecular weight will be larger in the blend than in the pure state. Finally, the terminal relaxation time is impossible to define for a polymer with continuous molecular weight distribution.²¹

In this work, the interaction between keratin and PEO in aqueous solutions was investigated by means of the additive rule of viscosity. There are no generally accepted and universally validated mixing rules for the viscosity of polymer blends in solution. Furthermore, experimental data on the viscosity of polymer blends in concentrated solutions are often inadequate and not always consistent.

Because of the non-Newtonian behavior of the solutions, the viscosity at low shear rate (i.e., zero-shear viscosity) has been chosen for this study.

There is a difference of about three order of magnitude in the viscosities of keratin and PEO solutions; therefore, the linear mixing rule is not useful in this case. For this reason, the theoretical zero-shear viscosity of the blends $\eta_{0,b}^{\text{theor}}$ was calculated using the following eq 1:

$$\eta_{0,b}^{\text{theor}} = \eta_{0,1}(c_1) + \eta_{0,2}(c_2) \quad (1)$$

where $\eta_{0,i}(c_i)$ is the zero-shear viscosity of the pure i polymer at the concentration c_i for $i = 1, 2$. The c_1 and c_2 concentrations conform to the equation $c_1 + c_2 = 7\%$. The viscosity of the solvent is negligible for concentrated solutions.

The result of the eq 1 is practically equivalent to the following logarithmic mixing rule^{26–28} adapted for polymer blend solutions:

$$\log \eta_{0,b}^{\log} = w_1 \log \eta_{0,1}(c_{\max}) + (1 - w_1) \log \eta_{0,2}(c_{\max}) \quad (2)$$

where w_1 is the weight fraction of the component 1, and $\eta_{0,i}(c_{\max})$ is the zero-shear viscosity of the pure i polymer for $i = 1, 2$ at the maximum concentration (c_{\max}) equal to 7 wt %.

EXPERIMENTAL

Materials and Methods

Keratin was extracted from wool by sulfitolysis. The wool fibers were cleaned by Soxhlet extraction with petroleum ether, washed with distilled water, and conditioned at 20 °C and 65% R.H. for at least 24 h. Then, 5 g of cleaned and conditioned fibers were cut and placed in 100 mL of an aqueous solution containing urea (8 M), *m*-bisulfite (mBS, 0.5 M), and sodium dodecyl sulfate (SDS, 0.05 M), which was adjusted to pH 6.5 with 5 N NaOH. The extracting process was carried out at 65 °C under shaking using the Original Hanau Linitest apparatus for 2.5 h. The resulting solution was filtered through a stainless steel mesh (50 mesh) and dialyzed against distilled water for 3 days, using a cellulose tube with a molecular weight cut-off of 12–14 kDa. The insoluble material was removed by vacuum filtration with 5 μ m pore sized filter (Millipore). The solution was concentrated in a Büchi Rotavapor R-205 rotary vacuum evaporator. The keratin concentration was measured by the Bradford protein assay method (with bovine serum albumin as a standard).²⁹

PEO powder with a viscosity-average molecular weight of 4×10^5 g mol⁻¹ (from Sigma-Aldrich) was dissolved in distilled water at room temperature for about 12 h in a magnetic stirred vessel.

The keratin/PEO blend solutions were prepared at room temperature in a magnetic stirred vessel in about 12 h by the simple addition of PEO powder to the keratin aqueous solution. The solutions of the keratin/PEO blend had a total polymer concentrations of 7 wt % with a keratin/PEO weight ratio of 10/90, 30/70, 50/50, 70/30, and 90/10.

Shear rate-dependent viscosity measurements were carried out in an Anton Paar Physica MCR 301 rheometer, equipped with a PTD 200 Peltier temperature control device at 25.0 (± 0.1) °C, using a cone-plate geometry (75 mm diameter, 1° angle and 45 μ m truncation) in controlled shear rate mode. The shear rate was logarithmically increased from 0.1 to 10,000 s⁻¹. Data were acquired and elaborated with the Rheoplus v2.66 (Anton Paar GmbH) software.

Pure PEO and keratin/PEO solutions were electrospun under the same conditions. A positive voltage of 20 kV was applied to a metal capillary with 0.2 mm of internal diameter by means of a high voltage generator (HVA from b2 Electronics GmbH). A constant flow rate of 0.60 mL h⁻¹ of solution was ensured by a metering pump (KDS 200 from KD Scientific). The grounded collector (a metal disk of 55 mm of diameter) was placed 20 cm below from the capillary.

Scanning Electron Microscopy (SEM) investigation was performed with a Leica Electron Optics 435 VP SEM (LEO Electron Microscopy) instrument at an acceleration voltage of 15 kV, with a 50-pA current probe, at a working distance of about 20 mm. An aluminum sheet with the nanofibre mat was cut and mounted on aluminum specimen stubs with double-sided adhesive tape. The samples were sputter-coated with a gold layer in rarefied argon with an Emitech K550 (EM Technologies) sputter coater with a current of 20 mA for 180 s.

RESULTS AND DISCUSSIONS

In molecular theories for non-Newtonian viscosity, the reciprocal of a characteristic time τ_η is a measure of the shear rate above which non-Newtonian behavior is observed, usually where η/η_0 falls to about 0.8. For a small departure from uniform distribution, the characteristic value of η/η_0 corresponds to about 0.78.^{22,30} From η versus $\dot{\gamma}$ curves of both pure PEO and keratin/PEO solutions (not reported here), τ_η has been obtained from the reciprocal of $\dot{\gamma}$ ($\tau_\eta = \dot{\gamma}^{-1}$) corresponding to $\eta/\eta_0 = 0.78$. Under condition of high entanglement, τ_η is found to be proportional to the terminal relaxation time. At lower concentrations, τ_η is related to the relaxation time in the absence of entanglements, as given by the Rouse theory.^{21,25}

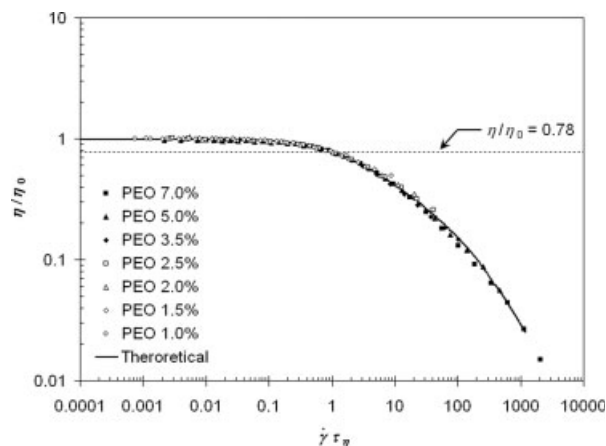


Figure 1. Non-Newtonian viscosity ratio η/η_0 versus $\dot{\gamma}\tau_\eta$ for poly(ethylene oxide) aqueous solutions at different concentrations. Characteristic time τ_η calculated from the reciprocal of $\dot{\gamma}$ value corresponding to $\eta/\eta_0 = 0.78$. Solid line from Graessley's theory.

Viscosity curves from pure PEO solutions with concentrations from 1.0 to 7.0 wt % can be reduced to a single curve by plotting η/η_0 logarithmically against $\dot{\gamma}\tau_\eta$, as shown in Figure 1. A similar behavior is observed also for the keratin/PEO solutions with keratin concentration up to 50%, as Figure 2 shows. Blend solutions with 70/30 and 90/10 keratin/PEO ratio differ in shape from the theoretical curves. In particular, the decrease in η with increasing $\dot{\gamma}$ is more gradual. This is due to the broadening of the molecular weight distribution caused by the presence of large amount of keratin with lower molecular weight with respect to PEO.

From the theory, the plots of $\log \eta$ versus $\log \dot{\gamma}$ become practically linear at rather high shear rates, with the viscosity proportional to $\dot{\gamma}^{-9/11}$ (power-law shear thinning).^{21,22,24} The final slope β (exponent of the power-law) is a measure of the degree of coil overlap and it is found that, at high concentrations, it approaches the theoretical value. From the experimental data of pure PEO solutions, the power-law index of the curves converges to -0.8351 , that is close to the theoretical value ($\beta = -0.818$) for concentrated solutions of high molecular weight polymers.

For keratin/PEO solutions, the power-law index becomes less negative with increasing keratin content, as Figure 2 shows. This is not due to the dilution of PEO macromolecules because pure PEO solutions with low concentration still lie on the theoretical curve (see Fig. 1), on the

contrary of 70/30 and 90/10 keratin/PEO solutions. Therefore, keratin seems to change the three-dimensional arrangement of PEO chains diminishing the degree of coil overlap and expands the macromolecular chains in solution.

As Graessley pointed out, the characteristic time τ_η increases with increasing polymer concentration. Moreover, τ_η is found to be proportional to η_0 . From Figure 3, where τ_η is plotted versus η_0 , it is found that $\eta_0 = 37.9\tau_\eta^{1.019}$ for pure PEO solutions. This practically perfect linearity is confirmed also for keratin/PEO solutions with low keratin content. With increasing keratin content, the linearity drops when the keratin amount is between 50 and 70%, as can be seen by the departure of the dotted line from the solid line. It is worth noting that the linear proportionality between τ_η and η_0 is again established for further increase of the keratin content above 70% (the dotted line becomes parallel to the solid line). In fact, for a binary blend in which the molecular weights differ significantly (as is in this case) the relaxation time is dominated only by the component with the higher molecular weight (i.e., PEO).²¹ Moreover, keratin aqueous solutions behave like a Newtonian fluid (without shear thinning) in the shear rates range considered in this work. In addition, wool keratin has different typical molecular weights with broad molecular weight distributions and

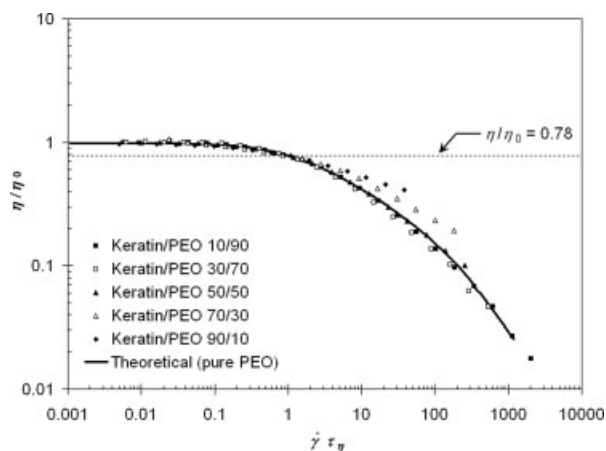


Figure 2. Non-Newtonian viscosity ratio η/η_0 versus $\dot{\gamma}\tau_\eta$ for keratin/poly(ethylene oxide) aqueous solutions with different blend ratios and total polymer concentration 7 wt %. Characteristic time τ_η calculated from the reciprocal of $\dot{\gamma}$ value corresponding to $\eta/\eta_0 = 0.78$. Solid line from the Graessley's theory.

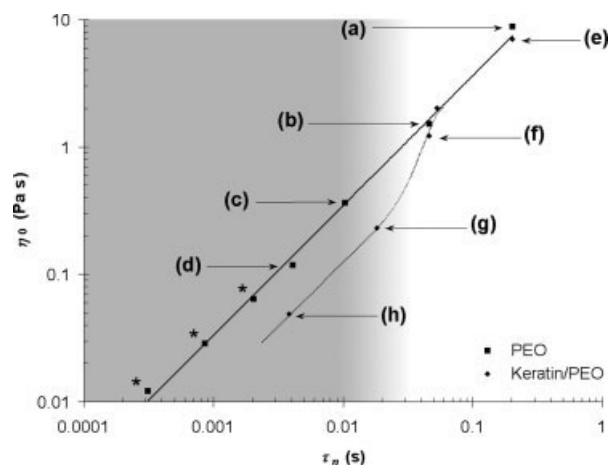


Figure 3. Plot of τ_e versus η_0 for pure poly(ethylene oxide) solutions with concentrations: (a) 7 wt %, (b) 5 wt %, (c) 3.5 wt %, (d) 2.5 wt %; and 7 wt % keratin/poly(ethylene oxide) solutions with blend ratios: (e) 10/90, (f) 50/50, (g) 70/30, (h) 90/10. Gray background delineates the conditions at which beads-on-string structures were produced. The asterisks (*) highlight the unspinnable PEO solutions. Labels (a)–(g) correspond to the pictures in Figure 4.

consequently, the terminal relaxation time is impossible to define.

From the findings illustrated above, it seems that *S*-sulfonated keratin interacts with PEO, increasing τ_e when the keratin content exceeds 50%. Because of the existing relation between the magnitude of τ_e and the relaxation time from the Graessley's theory, it could be supposed that the relaxation time increases, too. Intuitively, the result could be understood assuming that in keratin/PEO solutions each polymeric chain unit of PEO is surrounded with solvent, keratin molecules and other polymeric segments, and the solvent molecules can be displaced in flow motion much more easily than keratin, as well as keratin molecules can be displaced more easily than other PEO segments. The *S*-sulfonated keratin macromolecules displace the molecules of solvent and interact with the PEO chain; in fact the only effect of changing the surrounding environment from solvent molecules to other macromolecules is the change (ordinarily, increase) of the relaxation times.²¹ This is in good agreement with the hypothesis that keratin changes and expands the random coil of PEO chains in solution. In the keratin/PEO solutions with keratin content higher than 70%, the effect of keratin on the characteristic time reaches the "saturation," and the η_0 -de-

pendence of τ_e becomes again perfectly linear. This is further evidence that some interaction between *S*-sulfonated keratin and PEO takes place, in agreement with the findings observed for these blends in the solid state.⁴

It is worth noting that keratin/PEO solutions with high keratin content have τ_e higher than pure PEO solutions at a fixed η_0 . This has repercussions both on the morphology of electrospun nanofibres and on the electrospinnability of the solutions. In particular, solutions of PEO with low concentration (highlighted by asterisks in Fig. 3) are impossible to electrospin, unlike keratin/PEO solution with low PEO content.

As illustrated by the SEM pictures shown in Figure 4, nanofibres produced from the higher viscous solutions (pure PEO (a) and (b), and keratin/PEO (e) and (f)) show regular diameters and are free from bead defects. Notwithstanding the low η_0 value, also the 70/30 keratin/PEO solution has been electrospun with few defects [Fig. 4 (g)], but nanofibres from 2.5 and 3.5 wt % solution of pure PEO, that have the same magnitude of η_0 , show a beads-on-string structure of drops connected by thin liquid filaments [Fig. 4 (c,d)]. The 90/10 keratin/PEO solution also produces beads-on-string morphology [Fig. 4 (h)] because of the very low values of both η_0 and τ_e . Below the concentration of 2.5 wt % PEO the solutions, due to insufficient viscosity and short relaxation times, produce beads not connected to each other and they are not electrospinnable at all. This finding suggests also that relaxation times are significant rheological factors in determining the morphology of the electrospun nanofibres from viscoelastic fluids; in fact the electrospun jet of polymer solution is subject to stress relaxation when the electrostatic field becomes weak. This is in good agreement with the observations of Yu et al. who recently pointed out that the relaxation time is the essential property controlling the morphology of electrospun fibers.³¹

Finally, the interaction between *S*-sulfonated keratin and PEO in aqueous solutions was also investigated by means of additive rules to zero-shear viscosity using the eqs 1 and 2. The zero-shear viscosity of pure polymers in water solutions was measured for different polymer concentrations and reported in Figure 5 against both the keratin and the PEO concentrations. The experimental data were fitted (thin solid lines) in the whole range of the concentration studied here (from 0 to 7 wt %). The dashed line

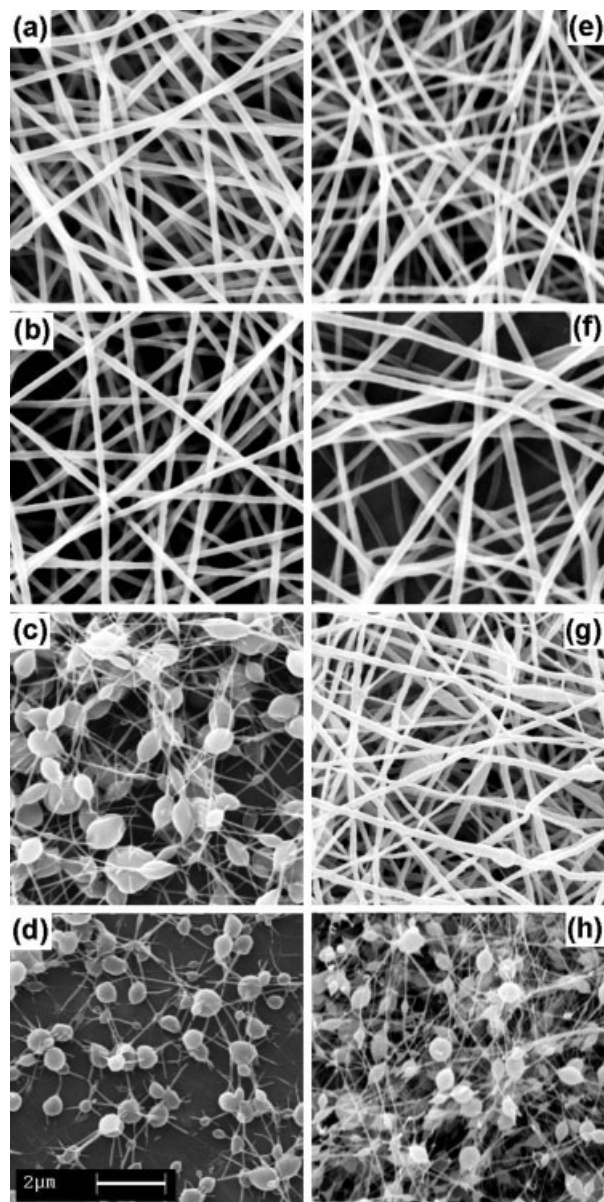


Figure 4. SEM pictures of the nanofilaments obtained electrospinning the solutions under the following conditions: +20 kV applied voltage, 20 cm distance, 0.60 mL h^{-1} flow rate, 0.2 mm internal diameter of the capillary. Pure poly(ethylene oxide) solutions with concentrations: (a) 7 wt %, (b) 5 wt %, (c) 3.5 wt %, (d) 2.5 wt %, and 7 wt % keratin/poly(ethylene oxide) solutions with blend ratios: (e) 10/90, (f) 50/50, (g) 70/30, (h) 90/10.

of keratin data is an extension of the fitting based on the existing experimental results. This is necessary because it is difficult to produce aqueous keratin solution above the concentration of 5 wt % avoiding gelation. The results of the eqs 1 and 2 are shown in Figure 5 as the

thick solid line and dash-dotted line, respectively. In this case, it is clear that the eq 1 mixing rule is in qualitative agreement with the eq 2 mixing rule. The measured η_0 values of keratin/PEO blends are always higher than the calculated values. In particular, when the keratin concentration is high, the η_0 values of the blend are at least three times larger than the theoretical values, whereas for high concentration of PEO the experimental values approach the theoretical results. Therefore, there is a positive deviation of the viscosity-composition curve of keratin/PEO blend with respect to the theoretical mixing rules (eqs 1 and 2). Usually, this feature suggests that molecular chains of polymers are associated together in a common solvent.³²

CONCLUSIONS

S-sulfonated keratin was extracted from wool by sulfitolysis. PEO was added to keratin aqueous solution with the aim of enhancing electrospinnability. Both pure PEO solutions and keratin/PEO blend solutions exhibit a shear thinning behavior. The theory of Graessley has been applied to the studied systems translating the η versus $\dot{\gamma}$ curves on a single curve by plotting η/η_0 logarithmically versus $\dot{\gamma} \tau_\eta$, and τ_η is found to be proportional to the terminal relaxation time.

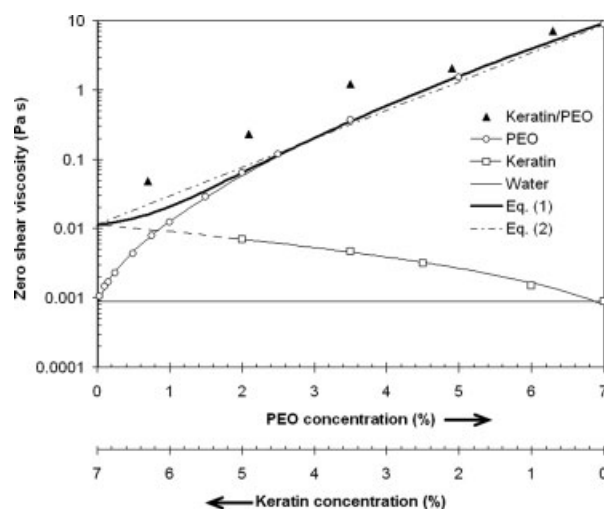


Figure 5. Plot of η_0 of keratin and poly(ethylene oxide) solutions and their blends versus keratin and poly(ethylene oxide) concentrations. Thick solid line is the theoretical curve of η_0 obtained by eq 1 and dash-dotted line is the theoretical curve of η_0 obtained by eq 2 mixing rule.

The theory is perfectly followed by the pure PEO solutions with concentrations ranging from 1.0 to 7.0 wt % and keratin/PEO solutions with keratin concentration up to 50%, whereas, 70/30 and 90/10 keratin/PEO blend solutions differ in shape from the theoretical curves for high shear rates. This is due to the broadening of the molecular weight distribution caused by the presence of large amount of keratin with lower molecular weight with respect to PEO.

The degree of coil overlap is measured by the final slope β (exponent of the power-law). From the experimental data of pure PEO solutions, the power-law index of the curves converges to -0.8351 , that is near to the theoretical value ($\beta = -9/11$) for concentrated solutions of high molecular weight polymers. For keratin/PEO solutions, β becomes less negative with increasing keratin content. Because pure PEO solutions at low concentration still lie on the theoretical curve, it is supposed that the decrease of the slope in keratin/PEO solutions is due to the presence of S-sulfonated keratin that changes the three-dimensional arrangement of PEO chains, diminishing the degree of coil overlap.

From pure PEO solutions τ_η is found to be linearly proportional to η_0 in the considered concentration range, whereas for keratin/PEO blend solutions, there is a departure from the linearity between 50 and 70% keratin. It seems that keratin interacts with PEO increasing τ_η when the keratin content exceeds 50% with respect to PEO. It could be supposed that keratin macromolecules displace the molecules of solvent and bond with the PEO chain.

It is worth noting that keratin/PEO solutions with a high keratin content have τ_η higher than pure PEO solutions at a fixed η_0 . This has repercussions on the morphology of the nanofibres obtained from the same solutions and on the electrospinnability of the solutions themselves. In particular, solutions with low PEO concentration are impossible to electrospin, unlike keratin/PEO solution with low PEO content.

Finally, the interaction between keratin and PEO in aqueous solutions was also investigated by means of the additive rules. The measured η_0 values of keratin/PEO blends are always higher than the theoretical values, in particular for the higher keratin contents. Therefore, the positive deviation from the theoretical mixing rules suggests the existence of some interactions between the polymers in the aqueous medium.

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