

Scaling Laws Between Hydrodynamic Parameters and Molecular Weight of Linear Poly(2-ethyl-2-oxazoline)

Xiaodong Ye,^{1,2,*} Jinxian Yang,¹ Jaweria Ambreen¹

1) Hefei National Laboratory for Physical Sciences at the Microscale, Department of Chemical Physics, University of Science and Technology of China, Hefei, Anhui 230026, China;

2) CAS Key Laboratory of Soft Matter Chemistry, University of Science and Technology of China, Hefei, Anhui 230026, China

Abstract: Poly(2-ethyl-2-oxazoline) (PEtOx) as an alternative polymer to poly(ethylene glycol) has potential applications in biomedical field. The hydrodynamic parameters, such as hydrodynamic radius and sedimentation coefficient, are important to understand its dynamics and properties, including its effect on the interactions between proteins and cells. In this study, we have studied the hydrodynamic properties, thermodynamic parameters of a series of narrowly distributed PEtOx with molecular weight ranging from 1.3×10^3 to 3.1×10^5 g/mol and the scaling laws between them by use of a combination of analytical ultracentrifugation and laser light scattering. It has been found that the sedimentation coefficient ($S_{20,w}$) and hydrodynamic radius ($R_{h,0}$) at infinite dilution scale with molecular weight (M_w) as $s_{20,w} = K_s \times M_w^\alpha = 0.0071(S) \times M_w^{0.462 \pm 0.019}$ and $R_{h,0} = K_{R_h} \times M_w^\beta = 0.0179(nm) \times M_w^{0.539 \pm 0.012}$, respectively.

Keywords: analytical ultracentrifugation, hydrodynamic radius, laser light scattering, molecular weight, poly(ethylene glycol)

*To whom correspondence should be addressed

Introduction

Polyethylene glycol (PEG) has received great interest in pharmaceutical industry because PEG is nontoxic, biocompatible and is approved by the FDA.^{1, 2,3, 4} It is well known that covalent attachment of PEG to proteins (i.e., PEGylation) can increase the circulating half-life in blood by increasing the stability of the proteins, as well as by reducing the renal ultrafiltration.^{2,3, 4} Normally, PEGylation can reduce the immunogenicity of some proteins.⁵ Whereas due to the wide use of PEG in biomedical applications, recently more and more anti-PEG antibodies have been reported.^{6, 7,8} Thus, drug companies are eager to find alternative polymers. Among all the alternative polymers, poly(2-ethyl-2-oxazoline) (PEtOx) is a promising candidate because of its low toxicity and immunogenicity.⁹⁻¹⁷ The chemical structures of PEtOx and PEG are shown in Figure 1.

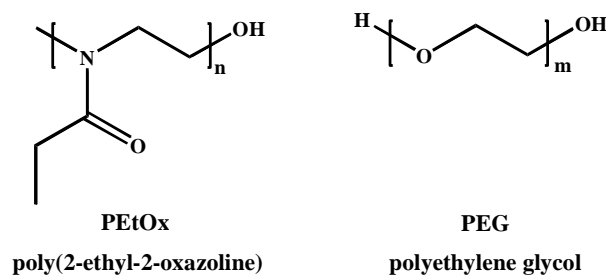


Figure 1. Chemical structures of PEtOx and PEG.

In order to reduce kidney filtration, the hydrodynamic radius (R_h) of PEtOx-protein conjugate should be larger than the size of the glomerular basement membrane, which is in the range of 2.5 – 5 nm.¹⁸ Based on the extensive data from pharmacokinetic studies of PEG-protein conjugates, a possible easy way is to replace PEG by PEtOx which has the same hydrodynamic radius. Recently Armstrong et al. found that the hydrodynamic radii of nonionic polymers and proteins are the principal determinant of their effect on red blood cell aggregation.¹⁹ Moreover, Sim et al. have reported that the precipitation efficiency of PEG is mainly determined by the hydrodynamic radius.²⁰ These studies further indicate that knowing the relationship between R_h and molecular weight of PEtOx can help us to explain the effect of PEtOx on the interaction

between cells and proteins. The solution properties of a series of PEtOx with molecular weight ranging from 6.8×10^4 g/mol to 9.14×10^5 g/mol in THF have been studied by light scattering and viscometry.²¹ While most biomedical applications of PEtOx are in aqueous systems, the aqueous solution properties of PEtOx with a wide range of molecular weights, including hydrodynamic radius, need to be examined systematically.

In this article, a series of PEtOx with molecular weights from 1.3×10^3 to 3.1×10^5 g/mol was obtained by fractionation of a linear commercial PEtOx sample with gel permeation chromatography (GPC) or by cationic ring-open polymerization of monomer 2-ethyl-oxazoline. Both analytical ultracentrifugation (AUC) and laser light scattering were used to characterize PEtOx samples. For two linear PEtOx samples with lower molecular weights, both AUC and LLS give similar results about the R_h and weight-average molecular weight (M_w). However, because of the low scattering intensity, the concentration used in LLS experiments was much higher than that used in AUC to obtain reliable results. Possible due to the same reason, Sung et al. didn't report the hydrodynamic radius of PEtOx samples with molecular weights less than 1.41×10^5 g/mol in THF.²¹ Our objective is to understand the scaling of molecular weight-dependent hydrodynamic parameters of PEtOx in aqueous solutions.

Experimental

Materials: Linear poly(2-ethyl-2-oxazoline) with a weight-average molar mass of 500,000 g/mol was purchased from the Sigma-Aldrich Company, Inc. and used as received as a starting material for fractionation. The commercial linear PEtOx sample was carefully fractionated by GPC. Briefly, 50 μ L of PEtOx tetrahydrofuran solution with a concentration of 20 mg/mL was injected into a stream from a sample loop. According to the principle of GPC, it takes a shorter time for the high-molecular-weight polymer to reach the column outlet. So fractions were collected at 1 mL intervals from the chromatography. The same procedure was repeated ten times to obtain enough samples. Then, the solvent THF was evaporated under reduced pressure.

Each PEtOx fraction aqueous solution was prepared by dissolving PEtOx fraction in 0.6 mL Milli-Q water. Two PEtOx samples with lower molecular weights were synthesized by cationic ring open polymerization of monomer 2-ethyl-2-oxazoline. In brief, 2-ethyl-2-oxazoline (99%, Sigma-Aldrich) was dried over calcium hydride and distilled under reduced pressure prior to use. Methyl tosylate (98%, Sigma-Aldrich) was distilled under reduced pressure. Acetonitrile as reaction solvent was refluxed with and distilled from alkaline KMnO_4 and KHSO_4 , followed by fractional distillation from calcium hydride. Methyl tosylate, EtOx and acetonitrile were added into a glass tube. After three freeze-vacuum-thaw cycles, the tube was sealed under vacuum and then placed in a thermostate at 80 °C for 24 h. The PEtOx acetonitrile solution was added into sodium carbonate aqueous solution. The mixture was stirred for 16 h at 90 °C and extracted three times with chloroform after cooling to room temperature. The organic phases were dried over sodium sulfate and filtered. Then, the solvent was evaporated under reduced pressure and the resultant PEtOx was dried under reduced pressure. $^1\text{H-NMR}$ spectra of these two PEtOx samples were recorded in CDCl_3 using a Bruker 300 MHz spectrometer.

Laser light scattering. A commercial laser light scattering spectrometer (ALV/DLS/SLS-5022F) equipped with a multi- τ digital time correlator (ALV5000) and a cylindrical 22 mW UNIPHASE He-Ne laser ($\lambda_0 = 632.8$ nm) was used. In static LLS,^{22,23} the weight-average molar mass (M_w) and the z -average root-mean square radius of gyration ($\langle R_g^2 \rangle_z^{1/2}$) in a solution from the angular dependence of the excess absolute scattering intensity can be obtained by using

$$\frac{KC}{R_{v,w}(q)} \approx \frac{1}{M_w} \left(1 + \frac{1}{3} \langle R_g^2 \rangle_z q^2 \right) + 2A_2C \quad (1)$$

where $K = 4\pi^2 n^2 (\text{dn}/\text{d}C)^2 / (N_A \lambda_0^4)$ and $q = (4\pi/\lambda_0) \sin(\theta/2)$ with C , $\text{dn}/\text{d}C$, N_A , and λ_0 being concentration of the polymer, the specific refractive index increment, the Avogadro's number, and the wavelength of light, respectively. The refractive index increment [$\text{dn}/\text{d}C = (0.161 \pm 0.001) \text{mL g}^{-1}$] of PEtOx in water was measured with a precise differential refractometer at 20 °C and 633 nm.²⁴ In dynamic LLS,²⁵ the Laplace inversion of a measured intensity-intensity time

correlation function $G^{(2)}(t, q)$ in the self-beating mode can result in a line-width distribution $G(\Gamma)$. For a pure diffusive relaxation, Γ is related to the translational diffusion coefficient D by $\Gamma/q^2 = D$ at $q \rightarrow 0$ and $C \rightarrow 0$, or a hydrodynamic radius $R_h = k_B T / (6\pi\eta D)$ with k_B , T , and η being the Boltzmann constant, the absolute temperature, and the solvent viscosity, respectively. Each PEtOx aqueous solution was repeatedly filtrated by using a peristaltic pump (Masterflex[®], Model 77390-00, Cole-Parmer Instrument Co.) and a 0.45- μm hydrophilic PTFE filter.

Sedimentation Velocity Analysis. Sedimentation velocity assays were performed using a Proteomelab XL-A/XL-I analytical ultracentrifuge (Beckman Coulter Instruments) with an An-60 Ti rotor, one cell assembled by sapphire windows, a double-sector 12 mm length charcoal-filled Epon centerpiece and an interference optics detector. All experiments were conducted at 60,000 rpm and 20 °C. 400 μL of PEtOx fraction solution were loaded for measurement with 410 μL of water as the reference, respectively. Data were collected using the software provided with the instrument and analyzed using the program SEDFIT (version 12p44).^{26,27} The partial specific volume (ν) of PEtOx in aqueous solution was measured by a DMA4500 densitometer (Anto Paar) at 20 °C. The value of ν was 0.85 $\text{mL}\cdot\text{g}^{-1}$, which is close to the value ($\nu = 0.87 \text{ mL}\cdot\text{g}^{-1}$) reported by Chen et al.²⁸ As the partial specific volume usually doesn't have molecular weight-dependence, we used this value for all PEtOx fractions.²⁶

Results and discussion

It is known that narrowly distributed linear poly(2-ethyl-2-oxazoline) with molecular weights ranging from 1 to ~50 kg/mol can be synthesized by conventional thermal heating and microwave assisted cationic ring-open polymerization (CROP).^{11, 29-33} However, it is still difficult to obtain narrowly distributed linear PEtOx with molecular weight higher than 50 kg/mol. Our preliminary results showed that the distribution of PEtOx samples fractionated by the dissolution/precipitation process in a mixture of dry THF and dry *n*-hexane were not narrow enough for AUC experiments.³⁴ Thus, in this study we used GPC to fractionate a commercial

linear PEtOx sample. Owing to the advantage of AUC experiments that small amounts of PEtOx samples (~ 0.2 mg) are needed, here only analytical GPC was used. Two PEtOx samples with molecular weights of 1.3 kg/mol and 3.3 kg/mol were synthesized by conventional thermal heating CROP.

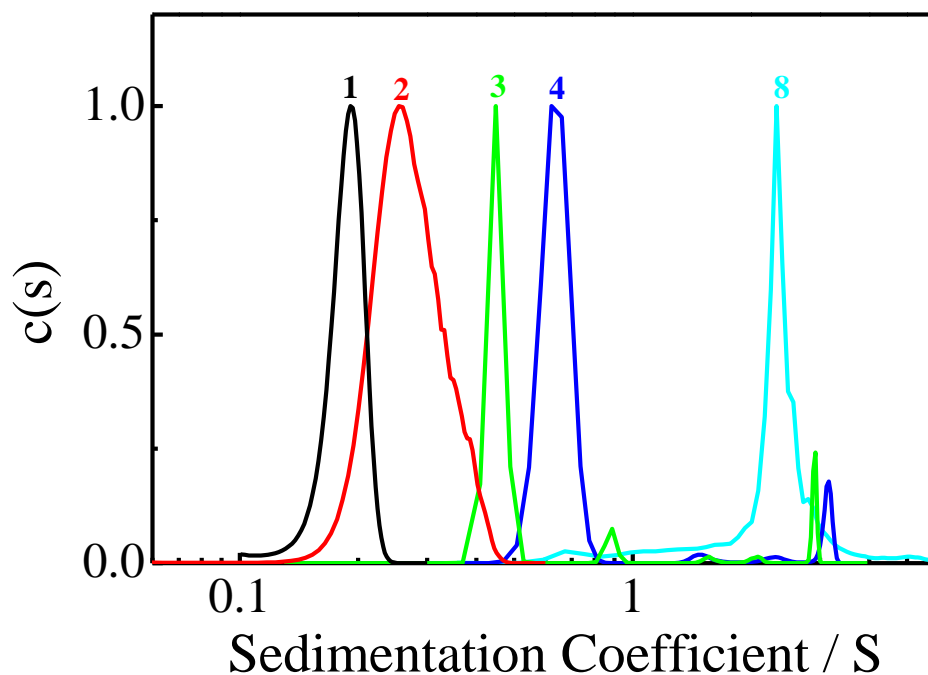


Figure 2. Sedimentation coefficient (s) distributions of PEtOx-1, PEtOx-2, PEtOx-3, PEtOx-4, and PEtOx-8 at 20 °C, where the corresponding concentrations of the samples were 0.6 mg/mL, 0.6 mg/mL, 0.2 mg/mL, 0.3 mg/mL and 0.5 mg/mL, respectively.

Figure 2 shows sedimentation coefficient distributions of five PEtOx fractions in aqueous solutions analyzed with the continuous $c(s)$ distribution model by SEDFIT software. From Figure 2, we know that the distributions of all fractions are narrow. Note that for PEtOx-3 and PEtOx-4 fractions, small amounts of polymers with higher sedimentation coefficient exist in each fraction. It means that the polymers with higher s which may have the same hydrodynamic radius compared with the majority of the fractions can not be fractionated by GPC.

Besides using $c(s)$ model, $c(s,ff0)$ model has also been used to analyze the data.²⁷ Note that from $c(s,ff0)$ model, the polydispersity index (M_w/M_n) can be obtained. The corresponding

results are summarized in Table 1. From Table 1, we know that the difference between the M_w values from these two models are less than 10 % and the distributions of all fractions are narrow.

Table 1. Characterization of PEtOx Samples Used

Sample	M_w (g/mol)		M_w/M_n^a
	$c(s)^b$	$c(s,ff0)$	
PEtOx-1	1300	1190	1.01
PEtOx-2	3230	3470	1.10
PEtOx-3	6890	7470	1.04
PEtOx-4	14300	15300	1.14
PEtOx-5	25300	26850	1.17
PEtOx-6	53600	57620	1.21
PEtOx-7	125600	131200	1.47
PEtOx-8	313100	277000	1.58

^a analyzed by $c(s,ff0)$ model; ^b the relative error of M_w is $\pm 3\%$.

Due to solute-solute interactions and shapes of macromolecules differing from spherical, hydrodynamic parameters (such as sedimentation coefficient and hydrodynamic radius) might have concentration dependence. In order to eliminate this effect, concentration dependence of sedimentation coefficient and hydrodynamic radius has been investigated. Because of the limited amount of the PEtOx fractions which was less than 1 mg, the concentration of PEtOx solutions can not be determined by conventional balance. As we used an interference optics as the detector and a triggerable laser diode as light source, the relationship between the shift of the interference fringes (J) and the concentration of the solution (C) can be described as³⁵

$$J = \frac{a \cdot (dn/dC)}{\lambda} \cdot C \quad (2)$$

where a is the thickness of the centerpiece, dn/dC is the specific refractive index increment of the PEtOx polymer and λ is the wavelength of light in vacuum, respectively. In this study, $a = 12$ mm and $\lambda = 660$ nm. The dn/dC value of commercial linear PEtOx with molecular weight of 500,000 g/mol is (0.161 ± 0.001) mL·g⁻¹, which is the same as the value reported by

Bijsterbosch et al.^{36,37} Based on the fact that dn/dC is independent of molecular weight, this value can be used for all fractions.³⁶ Thus during our experiments, knowing the shift of fringes will help us to determine the concentration of PEtOx, i.e. a concentration of $1 \text{ mg}\cdot\text{mL}^{-1}$ corresponds to 2.93 fringes.

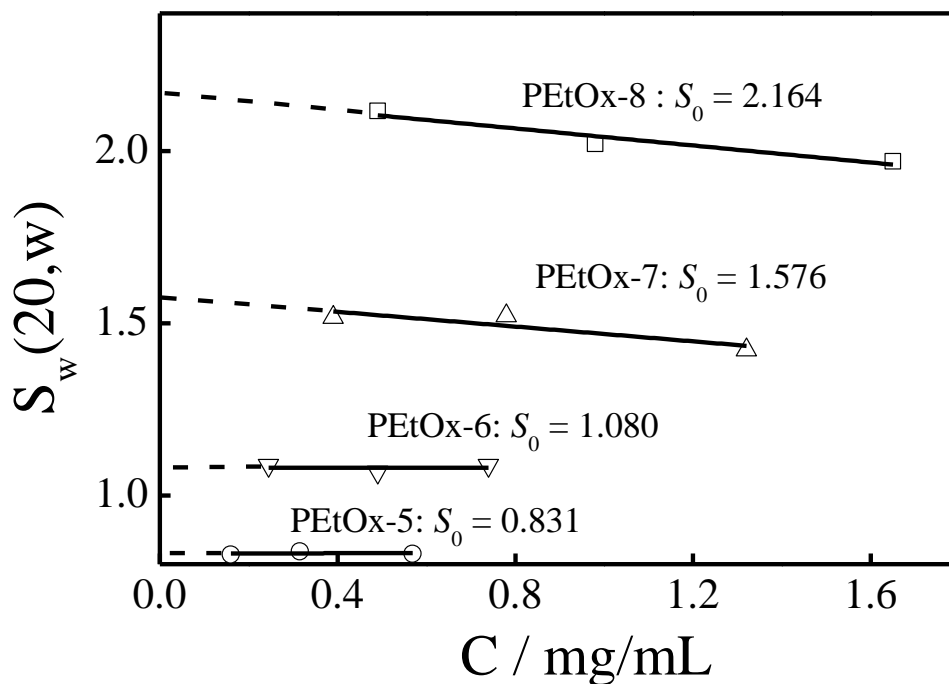


Figure 3. Concentration dependence of sedimentation coefficients of PEtOx-5, PEtOx-6, PEtOx-7 and PEtOx-8 at 20 °C.

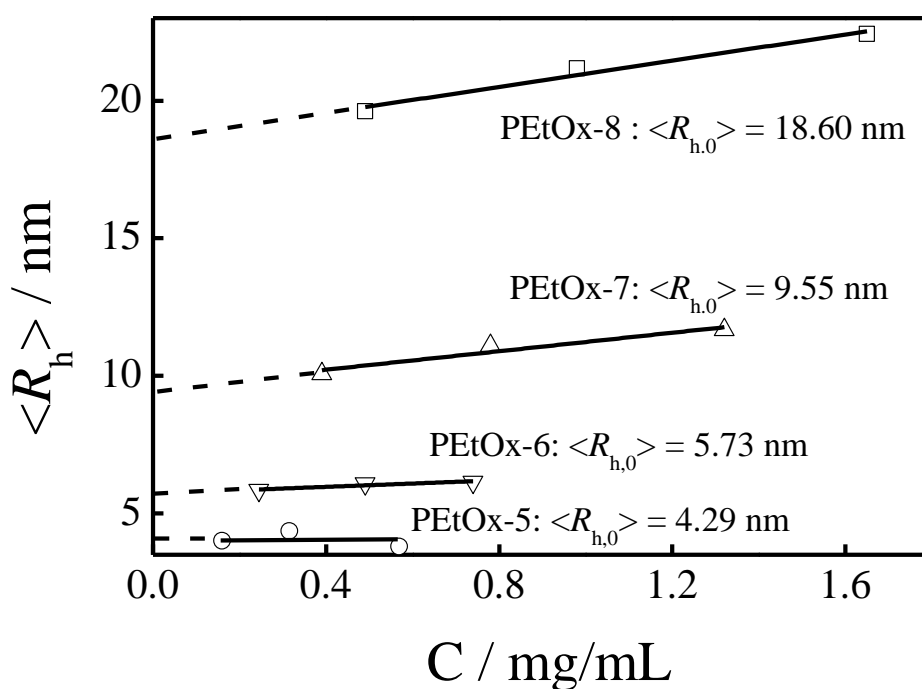


Figure 4. Concentration dependence of hydrodynamic radius of PEtOx-5, PEtOx-6, PEtOx-7 and PEtOx-8 at 20 °C.

From Figure 3 and Figure 4, we know that when molecular weight is below $\sim 5 \times 10^4$ g/mol, both s and R_h do not change with concentration and extrapolation of s and R_h to zero concentration leads to s_0 and $R_{h,0}$ at infinite dilution. Note that Luo et al. reported that for PEG molecules, when molecular weight is less 6×10^3 g/mol, s shows no dependence of molecular weight.³⁸ The difference between these two polymers implies that for the same molecular weight, the interactions between PEG molecules are larger than that between PEtOx possibly due to the larger hydrodynamic volume of PEG.¹²

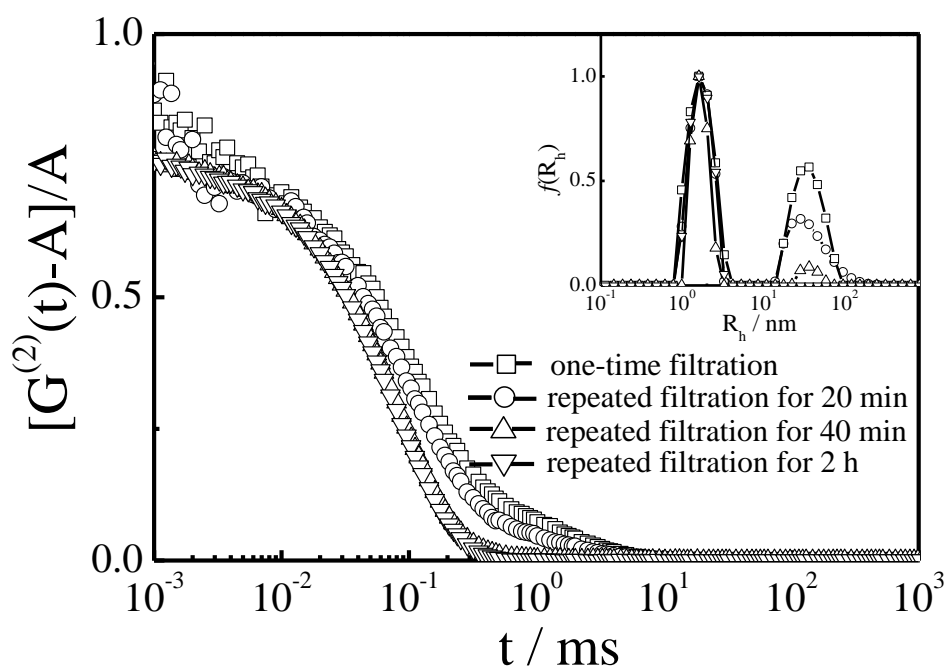


Figure 5. Typical measured intensity-intensity time correlation function $[G^{(2)}(t,q)-A]/A$ for PEtOx-2 fraction aqueous solutions by one-time filtration and repeated filtration for different times. The inset shows corresponding hydrodynamic radius distribution $f(R_h)$.

Besides sedimentation velocity (SV) experiments, we also used a combination of static and dynamic light scattering to characterize PEtOx aqueous solutions. Figure 5 shows that the intensity-intensity time correlation functions of PEtOx-2 aqueous solution contain two relaxation modes. The faster mode corresponds to the pure diffusion of single PEtOx-2 chain

and the slower relaxation mode might be related to the loosely assembled aggregates due to the remained hydrophobic tosyl end group. Our results show that the slow mode can be removed by repeated filtration for 2 hour. Note that it is important to remove the slow mode for measurement of the molecular weight and hydrodynamic radius of PEtOx, especially for PEtOx samples with smaller M_w . In our study, the same process was used for the characterization of two synthesized samples PEtOx-1 and PEtOx-2.

Figure 6 and Figure 7 show typical Zimm plots and hydrodynamic radius distribution of PEtOx-1, respectively. The extrapolation of $[KC/R_{vv}(q)]$ to $C \rightarrow 0$ and $q \rightarrow 0$ leads to the value of $M_w = 1.27 \times 10^3$ g/mol which is close to the value of $M_w = 1.30 \times 10^3$ g/mol determined by SV experiments. The slope for the line plotting $[KC/R_{vv}(q)]_{q \rightarrow 0}$ versus C give the value of $A_2 = (2.2 \pm 0.7) \times 10^{-3}$ mol·mL·g⁻², indicating that water is a good solvent for PEtOx. Due to the small size of the PEtOx-1 in comparison with the wavelength of the laser light, there is no angular dependence of $[KC/R_{vv}(q)]$. Thus it is difficult to obtain reliable $\langle R_g \rangle$ of PEtOx-1. The distribution of hydrodynamic radius shows that the PEtOx-1 is narrowly distributed and $\langle R_h \rangle = 1.02$ nm, which is also consistent with the value determined by SV experiments ($\langle R_h \rangle = 0.95$ nm). Note that the scattering light intensity is proportional to the square of the mass of a single scattering object, in order to obtain enough scattering light intensity, ~ 500 mg of PEtOx-1 was needed to determine the accurate molecular weight and R_h as the molecular weight of PEtOx-1 is as small as $\sim 1.3 \times 10^3$ g/mol.

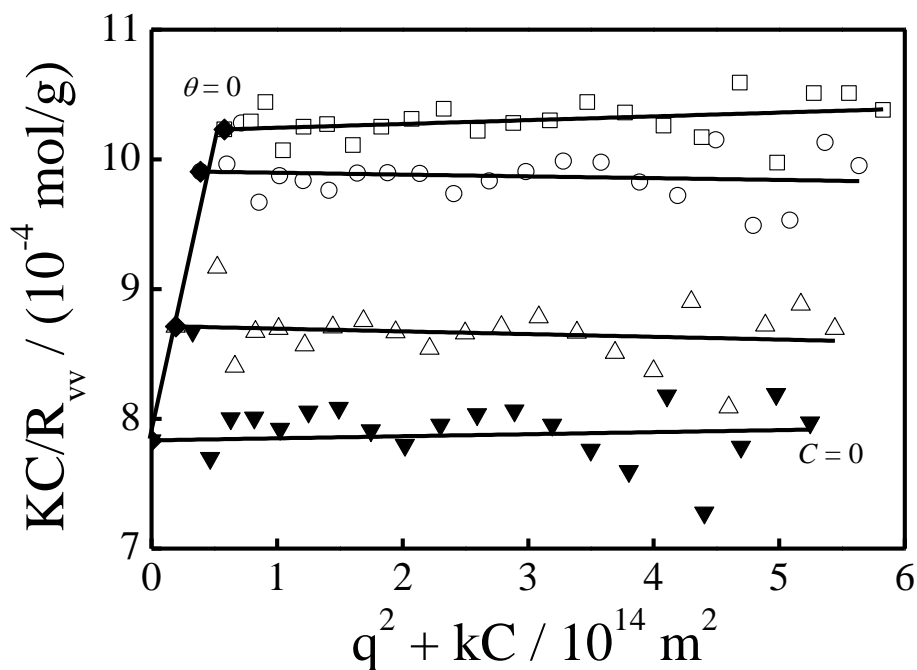


Figure 6. Typical Zimm plot of PEtOx-1 in aqueous solutions at 20 °C, where C were 19.5 mg/mL, 39 mg/mL and 58 mg/mL, respectively.

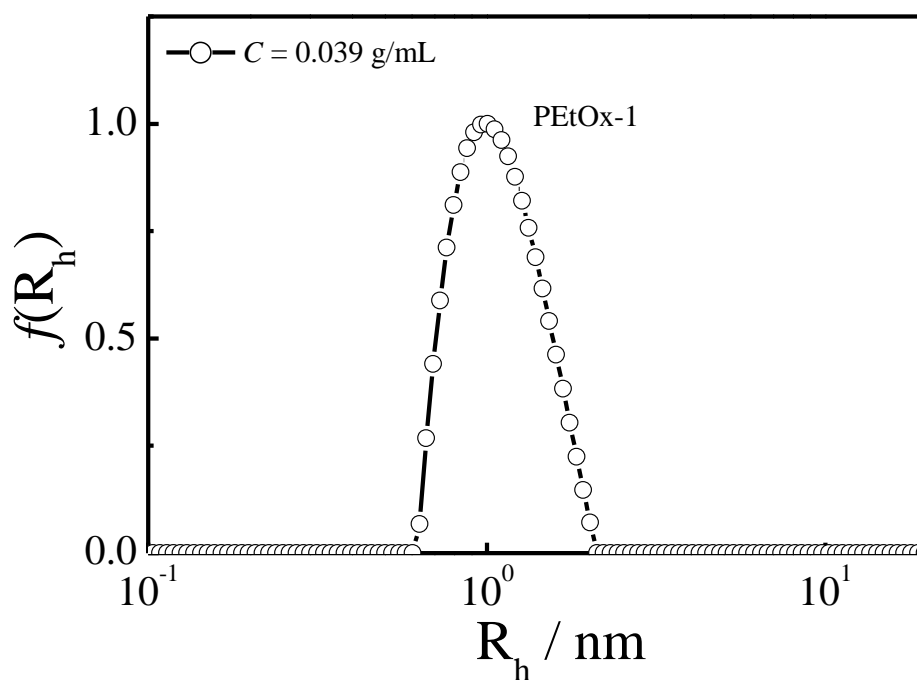


Figure 7. Hydrodynamic radius distribution of PEtOx-1 in aqueous solution, where C were 39 mg/mL.

Figure 8 shows that there is a scaling relationship between $S_{20,w}$ and M_w when M_w is in the range of 1×10^3 g/mol and 4×10^5 g/mol, i.e. $s_{20,w} = K_s \times M_w^\alpha = 0.0068(S) \times M_w^{0.462 \pm 0.019}$. The

scaling index is $\alpha = (0.462 \pm 0.019)$, which is between 0.4 and 0.5, indicating that PEtOx polymers adopt a random coil conformation in aqueous solution.³⁹ Luo et al. reported for PEG, the sedimentation coefficient (S_0) scales to M_w as $s_{20,w} = K_s \times M_w^\alpha = 0.0061(S) \times M_w^{0.469 \pm 0.008}$.³⁸ Scaling index α for PEtOx is similar to that for PEG, implying that both of two polymers adopt the same conformation in water. Figure 8 also shows a transition of the scaling laws. The results show that values of $\alpha_1 = (0.559 \pm 0.016)$ and $\alpha_2 = (0.394 \pm 0.010)$ were obtained for the $M_w < 2 \times 10^4$ g/mol and 2×10^4 g/mol $< M_w < 4 \times 10^5$ g/mol, respectively. It should be noted that scaling index is typically around 0.67 for a compact sphere, 0.4-0.5 for random coil and 0.15 for rigid rod.⁴⁰ The parameter from the five samples with higher M_w is lower than those four samples with lower M_w , indicating that the PEtOx samples with higher M_w adopt a more extended coil conformation.

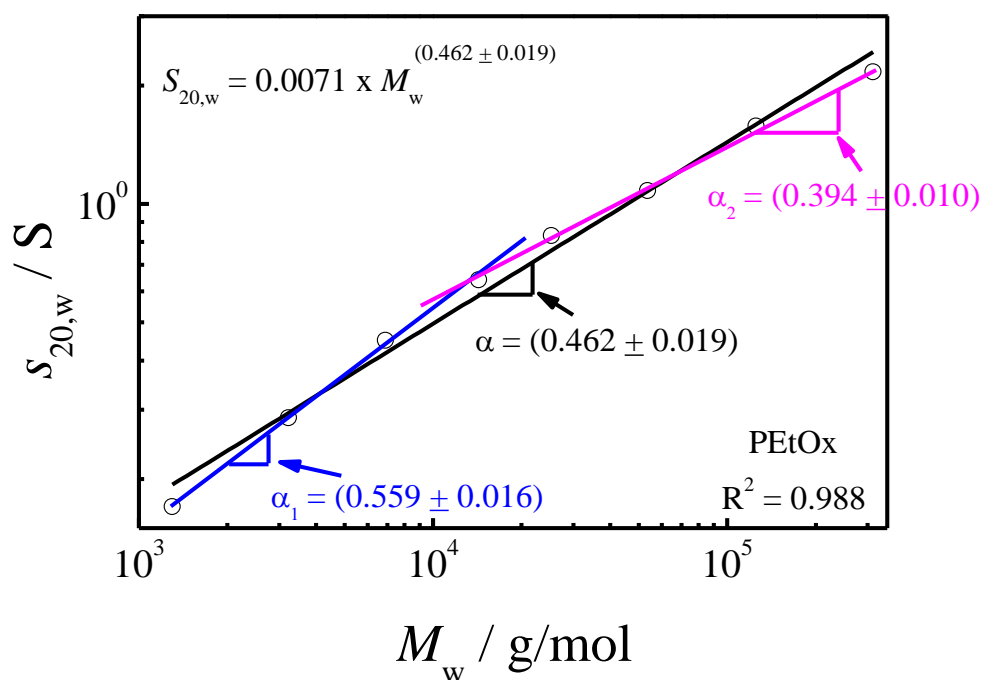


Figure 8. Weight average molecular weight dependence of sedimentation coefficient ($S_{20,w}$) at 20 °C. The relative error of $S_{20,w}$ is $\pm 5\%$.

Both Chen et al. and Jaweria et al. studied phase transition of PEtOx aqueous solution by laser light scattering.^{34,41} Chen et al. reported that the $\langle R_h \rangle$ of PEtOx polymer with a molecular

weight of 1.16×10^5 g/mol was 10.7 nm at 30 °C which was lower than its cloud point.⁴¹ Jaweria et al. characterized a narrowly distributed PEtOx fraction by a combination of static and dynamic light scattering and showed that the $\langle R_h \rangle$ of this fraction with M_w of 8.7×10^5 g/mol was 29 nm.³⁴ So we put their data together with our data in this study in Figure 9 to show molecular weight dependence of R_h . From Figure 9, we know $R_{h,0} = K_{R_{h,0}} \times M_w^\beta = 0.0179(\text{nm}) \times M_w^{0.539 \pm 0.012}$. Note that for real polymer chains in a good solvent and a theta solvent, the scaling indexes are 0.59 and 0.5, respectively.⁴² Recently, using coarse-grained molecular dynamics simulation, Wang et al. reported that the radius of gyration of poly(ethylene terephthalate) scales with degree of polymerization with a scaling index of 0.50, which is very close to the theoretical value. The scaling index obtained in our study is between the values predicted in the good solvent and the theta solvent.⁴³⁻⁴⁵ Moreover, the sum of α and β is 1.001 which is reasonable because from Sverberg equation we know $M^1 \propto s/D \propto s \cdot R_h \propto (K_s \cdot M^\alpha) \times (K_{R_{h,0}} \cdot M^\beta) \propto (K_s \cdot K_{R_{h,0}}) \times M^{\alpha+\beta}$. Figure 9 also shows the weight-average molecular weight dependence of hydrodynamic radius of PEG in water and PEtOx in THF. For the same molecular weight of PEtOx and PEG, the hydrodynamic radius of PEG is higher than that of PEtOx. Based on their structure, as shown in Figure 1, the reason that PEG has a higher hydrodynamic radius may be due to more favorable interaction with water molecules and larger degree of polymerization as the repeating unit of PEG has a smaller molar mass. Moreover, it is obvious that water is better solvent for PEtOx than THF because for the same M_w , the R_h in water is much larger than that in THF. Knowing the scaling law between R_h and molecular weight can help us to choose PEtOx polymers with right molecular weight. For example, Armstrong stated that nonionic polymers with R_h less than 4 nm can inhibit red blood cell aggregation and those with R_h larger than 4 nm can enhance the aggregation.¹⁹ Based on Figure 9, we know that the R_h of a PEtOx polymer with molecular weight of 2.3×10^4 g/mol is 4 nm. Furthermore, if we consider that the interaction between the PEtOx and proteins is similar to

that between PEG and proteins, we can replace PEG with PEtOx with the same hydrodynamic radius according to the scaling laws between R_h and M_w of these two polymers. For instance, for three approved PEG conjugates (PEG-growth hormone receptor antagonist, PEG-interferon $\alpha 2b$ and Pegfilgrastim), PEG polymers with molecular weights of 5×10^3 g/mol, 1.2×10^4 g/mol and 2×10^4 g/mol have been used.⁵ We might choose PEtOx polymers which have the same R_h to replace PEG polymers, that is, the molecular weights of PEtOx are 6.5×10^3 g/mol, 1.7×10^4 g/mol and 2.8×10^4 g/mol, respectively.

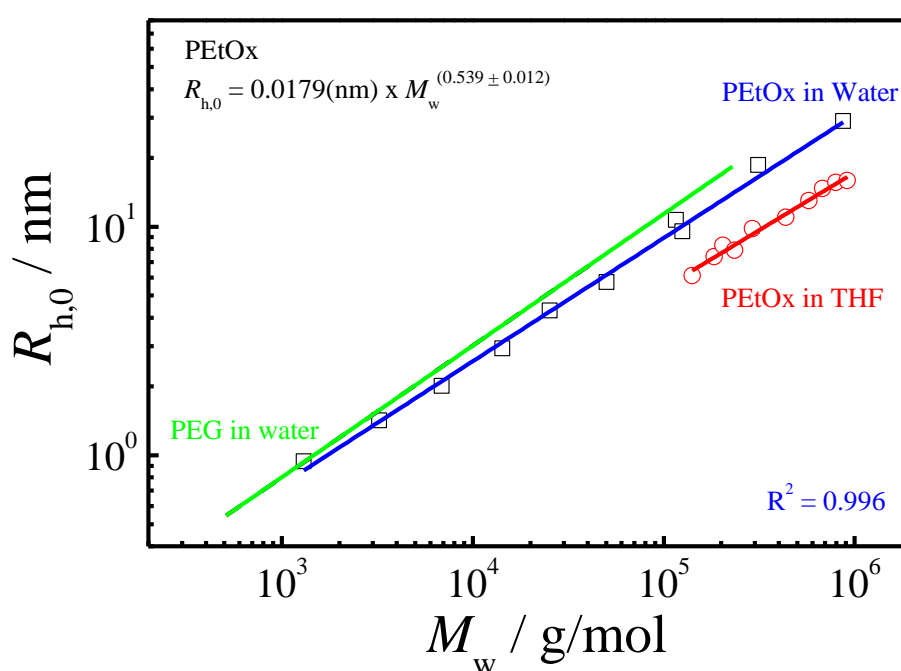


Figure 9. Weight average molecular weight (M_w) dependence of the hydrodynamic radius ($R_{h,0}$) of PEtOx at 20 °C. The hydrodynamic radius of PEG in aqueous solution (green line) and R_h of PEtOx in THF (red line) were taken from references 38 and 21. The relative error of $R_{h,0}$ is $\pm 2\%$.

Conclusion

By use of a combination of analytical ultracentrifugation and laser light scattering, the relationships between hydrodynamic radius ($R_{h,0}$), sedimentation coefficient ($S_{20,w}$) at infinite dilution and molecular weight of a series of linear poly(2-ethyl-2-oxazoline) in aqueous solutions have been studied. Our results show that $R_{h,0}$ and $S_{20,w}$ scale with molecular weight

(M_w) as $R_{h,0} = K_{R_{h,0}} \times M_w^\beta = 0.0179(\text{nm}) \times M_w^{0.539 \pm 0.012}$ and

$s_{20,w} = K_s \times M_w^\alpha = 0.0071(S) \times M_w^{0.462 \pm 0.019}$, respectively, indicating that PEtOx polymers adopt a

random coil conformation in aqueous solution. Our findings will provide a guide for choosing PEtOx polymer with appropriate molecular weight in its pharmaceutical applications.

Acknowledgment. The financial support of the National Natural Scientific Foundation of China (NNSFC) Projects (21274140), the National Program on Key Basic Research Project (2012CB933802), and the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry is gratefully acknowledged.

References and Notes

1. B. Jeong, Y. H. Bae and S. W. Kim, *Macromolecules*, 1999, **32**, 7064.
2. M. J. Roberts, M. D. Bentley and J. M. Harris, *Adv. Drug. Deliver. Rev.*, 2002, **54**, 459.
3. J. M. Harris and R. B. Chess, *Nat. Rev. Drug. Discov.*, 2003, **2**, 214.
4. P. Caliceti, *Adv. Drug. Deliver. Rev.*, 2003, **55**, 1261.
5. F. M. Veronese and G. Pasut, *Drug Discovery Today*, 2005, **10**, 1451.
6. T. C. Fisher, J. K. Armstrong, R. B. Wenby, H. J. Meiselman, R. Leger and G. Garratty, *Blood*, 2003, **102**, 559a.
7. J. K. Armstrong, R. Leger, R. B. Wenby, H. J. Meiselman, G. Garratty and T. C. Fisher, *Blood*, 2003, **102**, 556a.
8. J. K. Armstrong, G. Hempel, S. Kolling, L. S. Chan, T. Fisher, H. J. Meiselman and G. Garratty, *Cancer*, 2007, **110**, 103.
9. S. Zalipsky, C. B. Hansen, J. M. Oaks and T. M. Allen, *J. Pharm. Sci.*, 1996, **85**, 133.
10. S. C. Lee, Y. K. Chang, J. S. Yoon, C. H. Kim, I. C. Kwon, Y. H. Kim and S. Y. Jeong, *Macromolecules*, 1999, **32**, 1847.

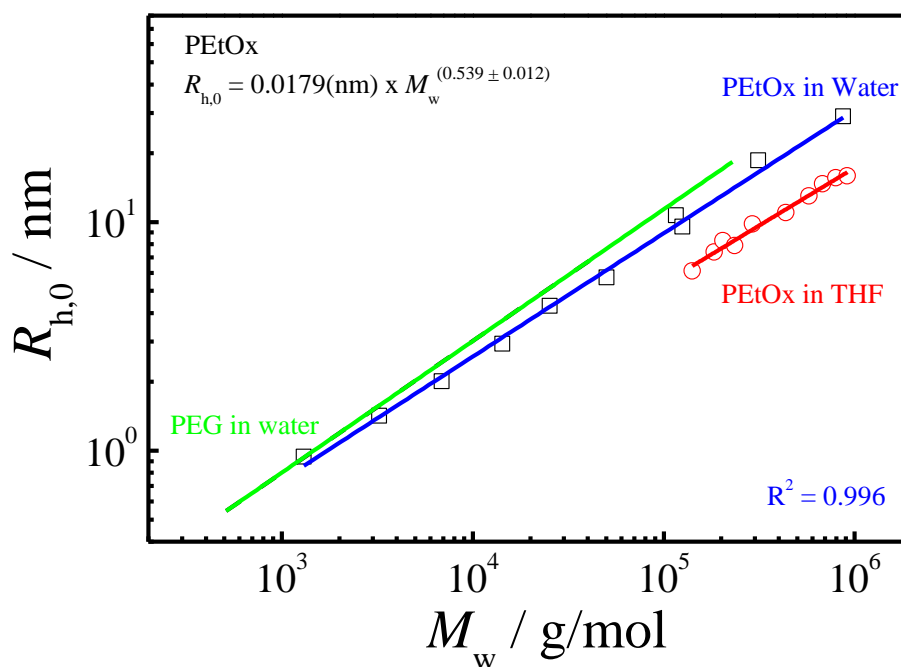
11. F. Wiesbrock, R. Hoogenboom, M. A. M. Leenen, M. A. R. Meier and U. S. Schubert, *Macromolecules*, 2005, **38**, 5025.
12. T. X. Viegas, M. D. Bentley, J. M. Harris, Z. F. Fang, K. Yoon, B. Dizman, R. Weimer, A. Mero, G. Pasut and F. M. Veronese, *Bioconjugate Chem.*, 2011, **22**, 976.
13. R. Hoogenboom, F. Wiesbrock, H. Huang, M. A. M. Leenen, H. M. L. Thijs, S. F. G. M. van Nispen, M. van der Loop, C.-A. Fustin, A. M. Jonas, J.-F. Gohy and U. S. Schubert, *Macromolecules*, 2006, **39**, 4719.
14. N. Adams and U. S. Schubert, *Adv. Drug. Deliver. Rev.*, 2007, **59**, 1504.
15. R. Hoogenboom, *Macromol. Chem. Phys.*, 2007, **208**, 18.
16. A. Mero, G. Pasut, L. D. Via, M. W. M. Fijten, U. S. Schubert, R. Hoogenboom and F. M. Veronese, *J. Control. Release*, 2008, **125**, 87.
17. R. Hoogenboom, *Angew. Chem. Int. Ed.*, 2009, **48**, 7978.
18. Z. Ota, H. Makino, Y. Takaya and T. Ofuji, *Ren. Physiol.*, 1980, **3**, 317.
19. J. K. Armstrong, R. B. Wenby, H. J. Meiselman and T. C. Fisher, *Biophys. J.*, 2004, **87**, 4259.
20. S. L. Sim, T. He, A. Tscheliessnig, M. Mueller, R. B. Tan and A. Jungbauer, *J. Biotechnol.*, 2012, **157**, 315.
21. J. H. Sung and D. C. Lee, *Polymer*, 2001, **42**, 5771.
22. B. H. Zimm, *J. Chem. Phys.*, 1948, **16**, 1099.
23. B. Chu, *Laser Light Scattering*, Academic Press, New York, 1991.
24. C. Wu and K. Q. Xia, *Rev. Sci. Instrum.*, 1994, **65**, 587.
25. B. Berne and R. Pecora, *Dynamic Light Scattering*, Plenum Press, New York, 1976.
26. P. Schuck, *Biophys. J.*, 2000, **78**, 1606.
27. P. H. Brown and P. Schuck, *Biophys. J.*, 2006, **90**, 4651.
28. C. H. Chen, J. Wilson, W. Chen, R. M. Davis and J. S. Riffle, *Polymer*, 1994, **35**, 3587.

29. O. Sedlacek, B. D. Monnery, S. K. Filippov, R. Hoogenboom and M. Hruby, *Macromol Rapid Commun*, 2012, **33**, 1648.
30. F. Wiesbrock, R. Hoogenboom, C. H. Abeln and U. S. Schubert, *Macromol. Rapid Commun.*, 2004, **25**, 1895.
31. F. Wiesbrock, R. Hoogenboom and U. S. Schubert, *Macromol. Rapid Commun.*, 2004, **25**, 1739.
32. R. Hoogenboom and U. S. Schubert, *Macromol. Rapid Commun.*, 2007, **28**, 368.
33. C. Guerrero-Sanchez, R. Hoogenboom and U. S. Schubert, *Chem. Commun.*, 2006, 3797.
34. J. Ambreen, J. X. Yang, X. D. Ye and M. Siddiq, *Colloid Polym. Sci.*, 2013, **291**, 919.
35. W. Machtle and L. Borger, *Analytical Ultracentrifugation of Polymers and Nanoparticles*, Springer, Berlin, Heidelberg, New York, 2006.
36. H. D. Bijsterbosch, M. A. Cohen Stuart, G. J. Fler, P. van Caeter and E. J. Goethals, *Macromolecules*, 1998, **31**, 7436.
37. H. D. Bijsterbosch, M. A. Cohen Stuart and G. J. Fler, *Macromolecules*, 1998, **31**, 9281.
38. Z. L. Luo and G. Z. Zhang, *J. Phys. Chem. B.*, 2009, **113**, 12462.
39. S. E. Harding, *Biophys. Chem.*, 1995, **55**, 69.
40. S. Hokputsa, K. Jumel, C. Alexander and S. E. Harding, *Carbohydrate Polymers*, 2003, **52**, 111.
41. F. P. Chen, A. E. Ames and L. D. Taylor, *Macromolecules*, 1990, **23**, 4688.
42. I. Teraoka, *Polymer Solutions: An Introduction to Physical Properties*, John Wiley & Sons, Inc., New York, 2002.
43. Q. Wang, D. J. Keffer, D. M. Nicholson and J. B. Thomas, *Macromolecules*, 2010, **43**, 10722.
44. Q. Wang, D. J. Keffer and D. M. Nicholson, *J. Chem. Phys.*, 2011, **135**, 214903.
45. Q. Wang, D. J. Keffer, S. Deng and J. Mays, *J. Phys. Chem. C*, 2013, **117**, 4901.

For Table of Contents Use Only

Scaling Laws Between Hydrodynamic Parameters and Molecular Weight of Linear Poly(2-ethyl-2-oxazoline)

Xiaodong Ye, Jinxian Yang, Jaweria Ambreen



Scaling laws between hydrodynamic radius, sedimentation coefficient and molecular weight of linear poly(2-ethyl-2-oxazoline) (PEtOx) have been established by use of a combination of analytical ultracentrifugation and laser light scattering.