

Urea/NaOH Aqueous Solution as New Solvent of *Aeromonas* Gum

Xiaojuan Xu, Lina Zhang, Yuying Zhang

Department of Chemistry, Wuhan University, Wuhan 430072, China

Received 1 April 2004; accepted 2 November 2004

DOI 10.1002/app.21726

Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: *Aeromonas* (A) gum, an acidic heteropolysaccharide, formed aggregates easily in NaCl aqueous solution. A novel solvent of the A gum, which can prevent aggregation, was found to be 0.20M urea/0.25M NaOH aqueous solution. The weight-average molecular weight (M_w), radius of gyration ($\langle s^2 \rangle^{1/2}$), and intrinsic viscosity ($[\eta]$) of the samples were determined in 0.20M urea/0.25M NaOH aqueous solution at 25°C by light scattering (M_w , $\langle s^2 \rangle^{1/2}$) and viscometry ($[\eta]$). The values of M_w , $\langle s^2 \rangle^{1/2}$, and $[\eta]$ were close to those in 0.20M lithium chloride/dimethylsulfoxide, in which the A gum exists as a semiflexible single chain, implying the same conformation for the A gum in

0.20M urea/0.25M NaOH aqueous solution. The results revealed that 0.20M urea/0.25M NaOH aqueous solution is a good solvent, which effectively avoids the aggregates of the A gum in aqueous solution. Moreover, it can be used to investigate the solution properties and chain conformation of water-insoluble polysaccharides or the polysaccharides that are easily aggregated in aqueous systems. © 2005 Wiley Periodicals, Inc. *J Appl Polym Sci* 97: 1710–1713, 2005

Key words: aeromonas gum; urea/NaOH aqueous solution; chain conformation; solvent of polysaccharide; light scattering

INTRODUCTION

Natural polysaccharides are among the richest renewable sources and have potential applications in many fields such as food, cosmetics, drug delivery, oil drilling, and so forth.^{1–3} The conformation of polymers in solutions has much importance in industrial applications, which were characterized successfully by static light scattering and dynamic light scattering.^{4–5} Therefore, fundamental research and industrial applications drive interest in polysaccharides in aqueous solution systems. However, some polysaccharides are difficult to completely dissolve in water. In particular, polysaccharides have a general tendency to aggregate in aqueous solution because of the abundance of inter-chain hydrogen bonds, leading to uncertainty in molecular characterization.^{6–10} Therefore, it is essential to develop a suitable solvent, which not only dissolves polysaccharides, but also prevents aggregation in aqueous systems. In our laboratory, a new safe solvent of cellulose, which is a 6 wt % sodium hydroxide (NaOH)/4 wt % urea aqueous solution, has been developed, which can break strong intermolecular hydrogen bonds to dissolve the cellulose.¹¹

Moreover, the molecular weight and conformation of a water-insoluble α -(1 → 3)-D-glucan from *Lentinus edodes* in 0.5M urea/0.5M NaOH aqueous solution have been successfully determined.¹² However, the polysaccharides have been degraded to different extents when the concentration of the urea and NaOH is relatively high. If an appropriate ratio of the two components has been chosen, is it possible to dissolve polysaccharides and reduce degradation to a maximum extent?

Heteropolysaccharide aeromonas (A) gum from *Aeromonas nichidenii* 5797, which is composed of mannose, glucose, xylose, galactose, and uronic acid, has been investigated for its application as a thickening agent and stabilizer in the field of food research.¹³ Recently, we have confirmed that the A gum forms aggregates in a salt aqueous solution^{14–16} and the aggregates are broken into single chains in cadoxen¹⁴ and dimethylsulfoxide (DMSO) containing 0.20M lithium chloride (LiCl).^{17–18} However, cadoxen is prepared with cadmium oxidation and it is poisonous. Therefore, it is urgent to find an aqueous solution to take the place of cadoxen as a solvent for A gum. In this work, we attempt to use urea/NaOH aqueous solutions with different concentrations of urea and NaOH to dissolve A gum and determine the solubility and degradability of the polysaccharide solution. Moreover, the conformation of the A gum in the suitable solvent is determined and discussed.

Correspondence to: L. Zhang (lnzhang@public.wh.hb.cn).

Contract grant sponsor: National Natural Science Foundation of China; contract grant number: 20074025.

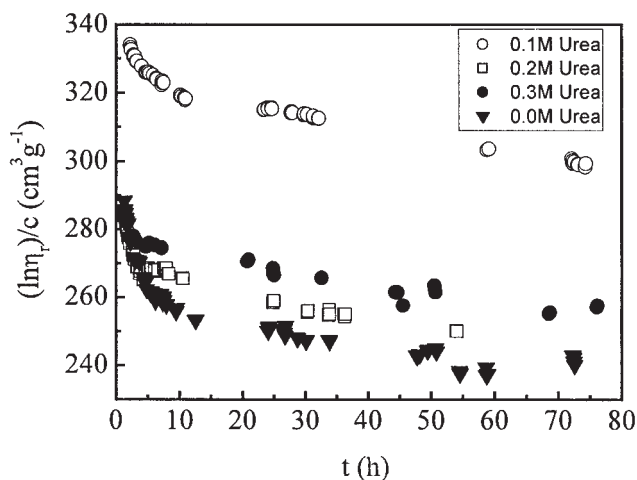


Figure 1 The time dependence of the inherent viscosity for A-5 at different urea concentrations in 0.5M NaOH aqueous solution at 25°C.

EXPERIMENTAL

Sample

A gum was a gift from Asahi Chemical Industry Co. The previous A gum fraction (A-5),¹⁴ which was fractionated by reprecipitation from pure water as a solvent to isopropanol as a precipitant, was used for the present work. The weight-average molecular weight (M_w) and intrinsic viscosity ($[\eta]$) values of the A-5 samples were determined to be 1.28×10^7 and $995 \text{ cm}^3/\text{g}$ in 0.5M NaCl aqueous solution and 6.18×10^5 and $254 \text{ cm}^3/\text{g}$ in cadoxen by static light scattering and viscometry at 25°C, respectively.¹⁴

Viscosity measurement

The viscosities of the A-5 sample in aqueous solution with the desired urea and NaOH concentration and in 0.20M LiCl/DMSO at 25°C were measured with Ubbelohde capillary viscometers. Equations (1)–(3) were used to determine the $[\eta]$ of A-5 in different solvents.

$$\eta_{sp}/c = [\eta] + k'[\eta]^2c \quad (1)$$

$$(\ln \eta_r)/c = [\eta] - (1/2 - k')[\eta]^2c \quad (2)$$

$$[2(\eta_{sp} - \ln \eta_r)]^{1/2}/c = [\eta] + (k' - 1/3)[\eta]^2c \quad (3)$$

where k' is constant for a given polymer at a given temperature in a given solvent, η_{sp}/c is the reduced specific viscosity, and $(\ln \eta_r)/c$ is the inherent viscosity. The measurement for the dependence of the viscosity for the A-5 in urea/NaOH aqueous solution on the storage time was carried out immediately once the sample in the solvent was dissolved with continuous

stirring and the solution became transparent. The storage time was calculated from the time when the sample was placed in the solvent.

Static light scattering

Static light-scattering measurements were carried out in urea/NaOH aqueous solution and 0.20M LiCl/DMSO after the sample solution stood for 30 h. The intensities of the light scattered from the A-5 solution at 25°C were measured at different concentrations on a DAWN DSP light-scattering photometer (Wyatt Technology Co.) using vertically polarized incident light with a 632-nm wavelength. The refractive index increments (dn/dc) at 25°C for the A-5 solutions were determined to be $0.065 \text{ cm}^3 \text{ g}^{-1}$ in 0.20M LiCl/DMSO (dialyzed) and $0.117 \text{ cm}^3 \text{ g}^{-1}$ in 0.20M urea/0.25M NaOH aqueous solution (not dialyzed) at 632 nm. Test solutions were made optically clean by filtration through a sand filter, followed by 0.45- and 0.20- μm Millipore filters directly into the scattering cell. A K5 cell was used for the 0.2M LiCl/DMSO solution, but an SV cell was used for the urea/NaOH aqueous solution. The scattering intensity data obtained as a function of the scattering angle and polymer mass concentration were analyzed by using square-root plots of $(Kc/R_\theta)^{1/2}$ versus c and $(Kc/R_\theta)^{1/2}$ versus $\sin^2(\theta/2)$ according to the following equations:

$$(Kc/R_\theta)^{1/2} = M_w^{-1/2}[1 + (8\pi^2 n_0^2 / 3\lambda_0^2) \langle s^2 \rangle \sin^2(\theta/2) + A_2 M_w c + \dots] \quad (4)$$

$$K = 4\pi^2 n_0^2 (dn/dc)^2 / (\lambda_0^4 N_A) \quad (5)$$

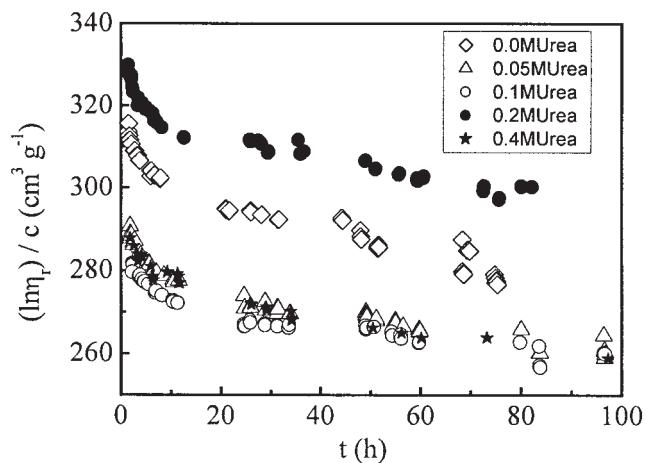


Figure 2 The time dependence of the inherent viscosity for A-5 at different urea concentrations in 0.25M NaOH aqueous solution at 25°C.

TABLE I
Results of Viscosity and Light Scattering for A5 in Different Solvents at 25°C

Solvent	$[\eta]$ (cm^3/g)	$M_w \times 10^{-4}$ (g/mol)	$\langle s^2 \rangle^{1/2}$ (nm)	$A_2 \times 10^4$ ($\text{mol cm}^3/\text{g}^2$)	Reference
0.5M NaCl	995 ^a	1280	99	—	Ref. 14
0.20M urea/0.25M NaOH	320	85.2	43.8	2.6	This work
0.20M LiCl/DMSO	351	108.3	50	2.5	This work

^a Zero shear rate viscosity.

where K is the optical constant, n_0 is the refractive index of the solvent, $\langle s^2 \rangle^{1/2}$ is the radius of gyration, N_A is the Avogadro number, c is the polymer mass concentration, λ_0 is the wavelength of a laser in a vacuum, and R_θ is the excess reduced scattering intensity at the scattering angle (θ). Astra software was used to collect the signals and analyze the data to attain the M_w and $\langle s^2 \rangle^{1/2}$.

RESULTS AND DISCUSSION

Figure 1 shows the time dependence of $(\ln \eta_r)/c$ for A-5 at different urea concentrations in 0.5M NaOH aqueous solutions at 25°C. Obviously, the $(\ln \eta_r)/c$ decreased with time, showing the complex dissolution process of polysaccharides. Usually, relatively high viscosity values for polymers with the same molecular weight reflect a better solvent. The $(\ln \eta_r)/c$ in 0.10M urea/0.5M NaOH aqueous solution is the highest and decreased slowly, compared to those in the other three mixtures, implying a better solvent than others. Moreover, the viscosity in different solvents changed much more slowly after standing 10 h.

Figure 2 shows the time dependences of $(\ln \eta_r)/c$ for A-5 at different urea concentrations in 0.25M NaOH aqueous solutions at 25°C. Interestingly, the $(\ln \eta_r)/c$ in the 0.20M urea/0.25M NaOH aqueous solution is

the highest. Similarly, the viscosity in different mixtures decreased slowly after 10 h and stayed almost constant. From 10 to 80 h of storage time, the $(\ln \eta_r)/c$ in the 0.20M urea/0.25M NaOH aqueous solution decreased by about 3%, indicating relatively good stability. In view of the results from Figures 1 and 2, the value of $(\ln \eta_r)/c$ for the polysaccharide in the urea/0.25M NaOH system changed more slowly than in the urea/0.5M NaOH system. Therefore, the 0.20M urea/0.25M NaOH aqueous solution was chosen as the solvent to study the molecular weight and conformation of the A gum. The $[\eta]$ values of A-5 in 0.20M urea/0.25M NaOH aqueous solution determined after the solution stood for 30 h and in 0.20M LiCl/DMSO at 25°C are summarized in Table I. Obviously, 0.20M LiCl/DMSO is a good solvent for this polysaccharide. It was noted that there was not much difference between the $[\eta]$ values of A-5 in 0.20M urea/0.25M NaOH aqueous solution and 0.20M LiCl/DMSO, suggesting the existence of single chains as predominant species in this case. Thus, the new aqueous solution was also a good solvent for the polysaccharide.

Figures 3 and 4 show Berry plots of A-5 in 0.20M urea/0.25M NaOH aqueous solution and 0.20M LiCl/DMSO at 25°C, respectively. The values of M_w , $\langle s^2 \rangle^{1/2}$, and A_2 are summarized in Table I. The A_2 value for A-5 in 0.20M urea/0.25M NaOH aqueous solution is

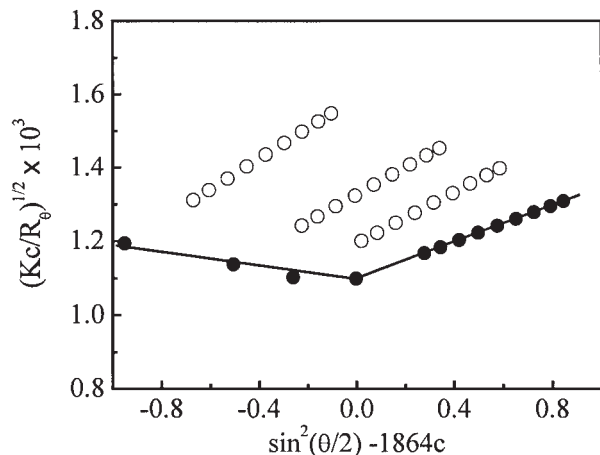


Figure 3 A Berry plot of A-5 in 0.20M urea/0.25M NaOH aqueous solution at 25°C.

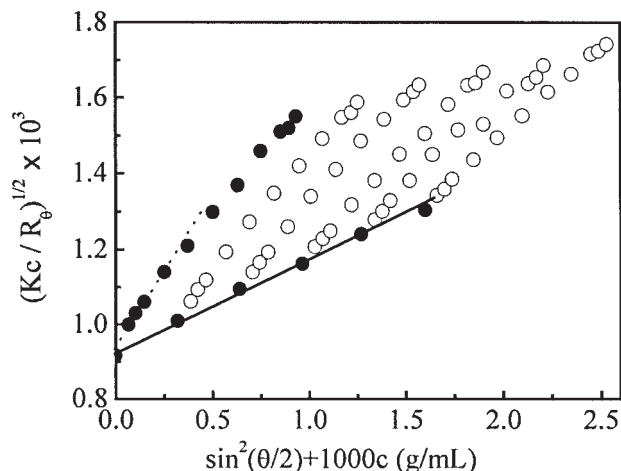


Figure 4 A Berry plot of A-5 in 0.20M LiCl/DMSO at 25°C.

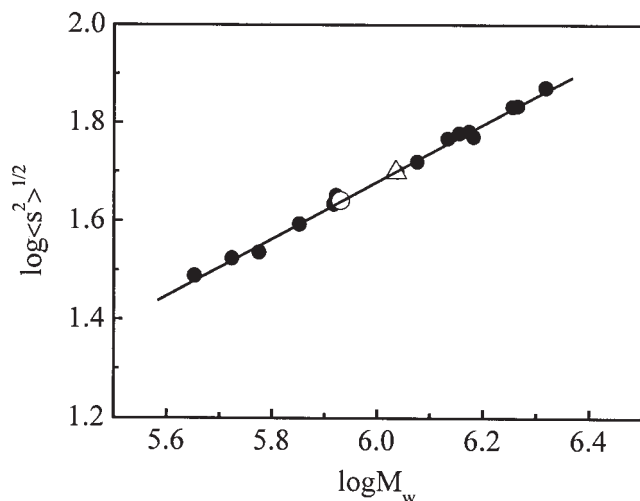


Figure 5 The dependence of the radius of gyration ($\langle s^2 \rangle^{1/2}$) on the weight-average molecular weight (M_w) for (●) A gum fractions fractionated in DMSO in 0.20M LiCl/DMSO, and A-5 (○) in 0.20M urea/0.25M NaOH and (△) in 0.20M LiCl/DMSO at 25°C.

on the order of $10^{-4} \text{ mol g}^{-2} \text{ cm}^3$, which is close to that in 0.20M LiCl/DMSO⁷ and similar to those of general polymers in good solvents. This suggests that the aggregation was prohibited as a result of intermolecular interactions between the A-5 chains and the solvent. The relationship of M_w and $\langle s^2 \rangle^{1/2}$ is often used to characterize the conformation of polymers in dilute solution.^{17,19–21} Figure 5 shows the dependence of $\langle s^2 \rangle^{1/2}$ on M_w for the A gum fractions in 0.20M LiCl/DMSO,¹⁷ and A-5 in 0.20M urea/0.25M NaOH aqueous solution and in 0.20M LiCl/DMSO at 25°C. Obviously, the data points of A-5 in 0.20M urea/0.25M NaOH and 0.20M LiCl/DMSO fell on the line of those of A gum fractions in 0.20M LiCl/DMSO, further confirming that the polysaccharide in 0.20M urea/0.25M NaOH aqueous solution exists as single chains like that in 0.20M LiCl/DMSO. It has been reported that A gum fractions exist as semiflexible chains in 0.20M LiCl/DMSO.^{17,18} Thus, it can be deduced that A-5 also exists as semiflexible chains in 0.20M urea/0.25M NaOH aqueous solution.

CONCLUSIONS

A new safe solvent, 0.20M urea/0.25M NaOH aqueous solution, for A gum was found by viscometry and

light scattering measurements. The solution of A gum fraction A-5 in this solvent was relatively stable, and it can be used as a good solvent to study the chain conformation of polysaccharides in aqueous solution in place of cadoxen and organic solvent (LiCl/DMSO). The values of M_w , $\langle s^2 \rangle^{1/2}$, and $[\eta]$ for A-5 in this solvent are consistent with those in 0.20M LiCl/DMSO, suggesting that A-5 was dissolved into single chains in 0.20M urea/0.25M NaOH aqueous solution. The conformation parameters M_w and $\langle s^2 \rangle^{1/2}$ for the samples in 0.20M urea/0.25M NaOH aqueous solution and in 0.20M LiCl/DMSO suggests that the chains of A-5 exist as semiflexible chains in 0.20M urea/0.25M NaOH aqueous solution.

We thank the National Natural Science Foundation of China for financial support.

References

- Murphy, R. M. *Curr Opin Biotechnol* 1997, 8, 25.
- Burchard, W. *Adv Polym Sci* 1983, 48, 4.
- Vanneste, K.; Sloodmaekers, D.; Reynaers, H. *Food Hydrocolloids* 1996, 10, 99.
- Ma, C.; Sun, L.; Bloomfield, V. A. *Biochemistry* 1995, 34, 3521.
- Fishman, D. M.; Patterson, G. D. *Biopolymers* 1996, 38, 535.
- Berth, G.; Dautzenberg, H.; Rother, G. *Carbohydr Polym* 1994, 25, 177.
- Berth, G.; Dautzenberg, H.; Rother, G. *Carbohydr Polym* 1994, 25, 187.
- Berth, G.; Dautzenberg, H.; Rother, G. *Carbohydr Polym* 1994, 25, 197.
- Muller, A.; Pretus, H. A.; McNamee, R. B.; Jones, E. L.; Browder, I. W.; Williams, D. L. *J Chromatogr B Biomed Appl* 1995, 666, 283.
- Rodgers, K. K.; Bu, Z.; Fleming, K. G.; Schatz, D. G.; Engelman, D. M.; Coleman, J. E. *J Mol Biol* 1996, 260, 70.
- Zhou, J.; Zhang, L. *Polym J* 2000, 32, 866.
- Zhang, P.; Zhang, L.; Cheng, S. *Carbohydr Res* 2000, 327, 4.
- (a) Tanaka, S. *Jpn Kokai Tokyo Koho* 89-13360, March 6, 1989, p 8; (b) Tanaka, S. *Jpn Kokai Tokyo Koho* 89-206971, August 21, 1989, p 3.
- Zhang, L.; Xu, X.; Jiang, G.; Iijima, H.; Tsuchiya, H. *Polym J* 1999, 31(20), 150.
- Xu, X.; Zhang, L. *J Polym Sci Part B: Polym Phys* 2000, 38, 2644.
- Xu, X.; Zhang, L. *J Polym Sci Part B: Polym Phys* 2002, 40, 2269.
- Xu, X.; Zhang, L.; Nakamura, Y.; Norisuye, T. *Polym Bull* 2002, 48, 491.
- Xu, X.; Zhang, L.; Nakamura, Y.; Norisuye, T. *Biopolymers* 2002, 65, 387.
- Wittgren, B.; Borgstrom, J.; Piculell, L. *Biopolymers* 1998, 45, 85.
- Mendichi, R.; Giamona, G.; Cavallaro, G.; Shieron, A. G. *Polymer* 2000, 41, 8649.
- Majdoub, H.; Roudesli, S.; Picton, L.; Lecert, D.; Muller, G.; Grisel, M. *Carbohydr Polym* 2001, 46, 69.