

CRITICAL CONCENTRATIONS OF α -(1 $\rightarrow$ 3)-D-GLUCAN FROM
LENTINUS EDODES IN NaOH AQUEOUS SOLUTION*

Ping-yi Zhang, Li-na Zhang** and Shu-yao Cheng

Department of Chemistry, Wuhan University, Wuhan 430072, China

Abstract Critical concentrations of α -(1 $\rightarrow$ 3)-D-glucan L-FV-II from *Lentinus edodes* were studied by viscometry and fluorescence probe techniques. The dependence of the reduced viscosity on concentration of the glucan in 0.5 mol/L NaOH aqueous solutions with or without urea showed two turning points corresponding to the dynamic contact concentration c_s and the overlap concentration c^* of the polymer. The values of c_s and c^* were found to be $1 \times 10^{-3} \text{ g cm}^{-3}$ and $1.1 \times 10^{-2} \text{ g cm}^{-3}$, respectively, for L-FV-II in 0.5 mol/L NaOH aqueous solutions. The two critical concentrations of L-FV-II in 0.5 mol/L NaOH aqueous solutions were also found to be $1.2 \times 10^{-3} \text{ g cm}^{-3}$ for c_s and $9.2 \times 10^{-3} \text{ g cm}^{-3}$ for c^* from the concentration dependence of phenanthrene fluorescence intensities. The overlap concentration c^* of L-FV-II in 0.5 mol/L NaOH aqueous solutions was lower than that of polystyrene with same molecular weight in benzene, owing to the fact that polysaccharide tends to undergo aggregation caused by intermolecular hydrogen bonding. A normal viscosity behavior of L-FV-II in 0.5 mol/L urea/ 0.5 mol/L NaOH aqueous solutions can still be observed in an extremely low concentration range at 25°C.

Keywords α -(1 $\rightarrow$ 3)-D-Glucan, *Lentinus edodes*, Critical concentration, Viscometry, Fluorescence

INTRODUCTION

Macromolecular conformation is considered to be an important factor in determining the bioactivity of polysaccharide^[1]. As it is well known that the conformation of a polymer molecule is influenced by its components and solution concentration, polymer solutions can be divided into three concentration regimes. In extremely dilute solutions the polymer coils are separate and isolated from each other. With increasing concentration the coils begin to overlap and interpenetrate, and finally the coils are packed in more concentrated solution^[2]. In the development of scaling theory of polymer solutions, de Gennes suggested an overlap concentration c^* for coil contact in solution^[3]. The overlap concentration is assumed to be inversely proportional to an effective volume occupied by macromolecules in solution, and can be predicted with remarkable precision^[4]. On the other hand, the existence of a new concentration c_s , which is lower than c^* and divides dilute solution into extremely dilute and dilute solutions, was suggested^[5]. Qian *et al.*^[6] pointed out that when the concentration reaches a value c_s , the coil dimension begins to shrink due to repulsive force between the segments of neighboring coils in solution. The c_s value for polystyrene was identified as the slope change of concentration dependence of the excimer fluorescence intensities. Subsequently, the existence of c_s was confirmed by size-exclusion chromatography (SEC)^[7] and by dynamic light scattering^[8]. The c_s values have been found to be much smaller than the theoretical overlap concentration c^* , and they decrease with increasing molecular weight. Usually, the values of c_s are all in the concentration range of $10^{-4} \sim 10^{-3} \text{ g cm}^{-3}$ ^[2]. However, Simionescu *et al.*^[9] pointed out that c_s is a dynamic phenomenon and is hardly observed when using static measurements. When the concentration of polymer increases, the distance between coils decreases, and at some concentration c^* the coils

* This work was supported by the National Natural Science Foundation of China (No. 29374170) and the Research Grant Council of Hong Kong Government Earmarked Grant (CUHK 4161/99M).

** Corresponding author: Li-na Zhang (张俐娜), E-mail: lnzhang@public.wh.hb.cn

Received December 27, 1999

overlap and begin to entangle. Ultraviolet spectroscopy^[10], osmometry^[11], NMR^[12], SEC^[7] and dynamic light scattering^[8, 13] merely showed a change of slope of the concentration dependence around critical concentrations.

Polysaccharides have recently become attractive as a functional food and a source for the development of natural drugs. It is worth noting that the solution properties of polysaccharides are difficult to study because of their low water solubility caused by the strong intermolecular hydrogen bonding. In general, the addition of HAC, NaOH or Me₂SO is essential for the enhancement of water solubility^[1]. In our previous work^[14], α -(1 $\rightarrow$ 3)-*D*-glucan (L-FV-II) isolated from fruiting bodies of *Lentinus edodes* was investigated by using light scattering, membrane osmometry and size exclusion chromatography (SEC) in aqueous 0.5 mol/L urea/0.5 mol/L NaOH and DMSO/0.25 mol/L LiCl. NaOH and urea can break the hydrogen bonds of α -(1 $\rightarrow$ 3)-*D*-glucan in aqueous solution, and urea can destroy the intermolecular hydrogen bonds more easily than NaOH, leading to the enhancement of the water-solubility of polysaccharides. Interestingly, variations of polymer chain dimensions with the urea content in solutions were observed^[15]. It is well known that the molecular weight, solubility and conformation of polysaccharides in solution significantly affect their antitumor and immuno-modulator activity. Therefore, the accuracy of these measurements is important, as it is limited by the following two facts: the validity of the method for extrapolating those measured parameters to zero concentration, which are related to polymer conformation, and the tendency of cluster and aggregate formation of polysaccharides in solution^[16]. The concentration dependence of these parameters in extremely dilute and semi-dilute regimes is more complex than that of ordinary polymers. It has been reported^[17] that below the critical concentration c^* , the reduced viscosity η_{sp} was proportional to $c^{1.3}$, while at higher concentrations η_{sp} was proportional to $c^{5.1}$. Their exponent is higher than that usually observed for polymers interacting with each other purely by physical entanglement. Therefore, this prompted us to choose an appropriate solvent and to investigate the critical concentrations of the α -(1 $\rightarrow$ 3)-*D*-glucan in 0.5 mol/L NaOH aqueous solutions. This involves finding the dilute regime for reducing the uncertainty of viscometry and light scattering measurements and the semi-dilute regime for avoiding the aggregation of polymers.

EXPERIMENTAL

α -(1 $\rightarrow$ 3)-*D*-glucan L-FV-II used were previously isolated from fruiting bodies of *Lentinus edodes* by extraction with 5% NaOH/0.05% NaBH₄ aqueous solutions followed by precipitation with 1 mol/L acetic acid and washing with water and menthnlol for 5 times, respectively (yield: 3.2%)^[14]. The weight-average molecular weight (M_w) of L-FV-II was determined to be 5.21×10^5 by light scattering measurements of its 0.5 mol/L urea/0.5 mol/L NaOH aqueous solutions^[15]. All chemicals were of analytical grade.

Infrared (IR) spectra of L-FV-II were recorded using a Nicolet FT-IR spectrometer. Test specimens were prepared by the KBr-disk method.

Viscosities of L-FV-II solutions were carefully measured at $25 \pm 0.1^\circ\text{C}$ by using a modified capillary viscometer with a capillary inner diameter of 0.5 mm, which was a gift from the Institute of Industrial Science of Tokyo University. L-FV-II was dissolved in a mixture of 1.0 mol/L NaOH containing 0 mol/L, 0.4 mol/L, 1.0 mol/L, 1.6 mol/L and 2.0 mol/L urea, respectively for 20 min. The mixture was diluted with water to form the solutions of 0.5 mol/L NaOH, 0.2 mol/L urea/0.5 mol/L NaOH, 0.5 mol/L urea/0.5 mol/L NaOH, 0.8 mol/L urea/0.5 mol/L NaOH, and 1.0 mol/L urea/0.5 mol/L NaOH, respectively. In addition, L-FV-II was dissolved in aqueous solutions containing 20%~100% cadoxen (29 wt% aqueous solution of ethylenediamine saturated with CdO, which was prepared in our laboratory^[16]) in order to compare their viscosity behavior. To ensure the accuracy of the viscosity determination, the solutions were filtered with Millipore filters and each specimen was repeatedly measured for 5 to 7 times, then average values were calculated. The viscometer was thoroughly washed with distilled water and acetone, then dried in vacuum for 1 h.

The fluorescence spectra were recorded on a Shimadzu RF-5301 fluorescence spectrophotometer at 25°C with a quartz cell (1 $\times$ 1 cm cross-section) equipped with a 150-W xenon lamp and dual monochromator. L-FV-II was dissolved in 0.5 mol/L NaOH, and then phenanthrene (Phe) methanol stock solution was added as a fluorescence probe. The fluorescence probe concentration was adjusted to 2.5×10^{-6} mol/L. N₂ gas was purged

into each medium for 10 min with stirring to inhibit fluorescence quenching by oxygen in the 0.5 mol/L NaOH aqueous solutions. The slit openings for excitation and emission were both set at 3.0 nm. The excitation and emission wavelengths were 251 and 364 nm, respectively. I_0 and I denote the fluorescence emission intensity of the solution in the absence and presence of polysaccharide, respectively. An Rf-530X-PC software package was used for data treatment.

RESULTS AND DISCUSSION

Intermolecular Hydrogen Bonding

The IR spectra for pullulan, L-FV-II and L-FV-II film, which was prepared by coating a solution of L-FV-II in DMSO/0.25 mol/L LiCl on KBr disk surface, are shown in Fig. 1. L-FV-II and L-FV-II film have the character of α -(1 $\rightarrow$ 3)-D-glucan^[14]. The —OH stretching vibration band of L-FV-II broadened and shifted to a lower wave-number (at 3300 cm^{-1}) as compared with pullulan and L-FV-II film, suggesting the existence of strong intermolecular hydrogen bonds in L-FV-II. Because DMSO can break the inter- and intra-molecular hydrogen bonds of polysaccharides^[18], the hydrogen bonds in the L-FV-II film were significantly weakened, so its spectrum was quite similar to that of the water-soluble pullulan. The water-insolubility of L-FV-II is attributed to the strong interaction of interchain hydrogen bonding. However it dissolves very easily in a 1.0 mol/L urea/1.0 mol/L NaOH aqueous solution, owing to the breaking of interchain hydrogen bonds.

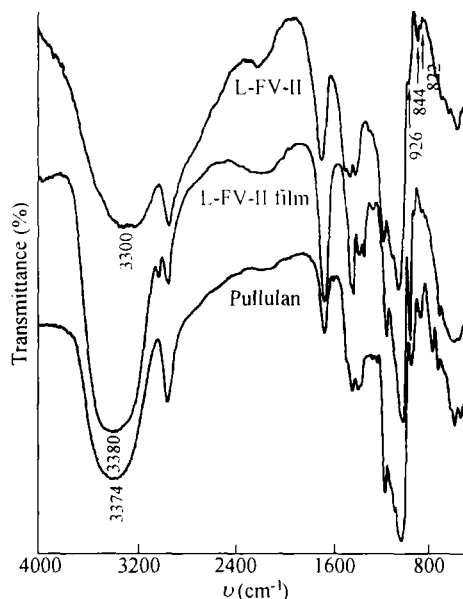


Fig. 1 Infrared spectra for pullulan, L-FV-II, and L-FV-II film

Viscosity Behavior at Critical Concentrations

Figure 2 shows the η_{sp}/c - c plots of the glucan L-FV-II in 0.5 mol/L NaOH aqueous solution at 25°C containing 0.0 mol/L, 0.2 mol/L, 0.5 mol/L, 0.8 mol/L, and 1.0 mol/L urea, respectively. The outstanding feature is that in extremely dilute solutions (at polymer concentrations below $1 \times 10^{-3} \text{ g cm}^{-3}$), η_{sp}/c - c curves deviated from linearity, except for the 0.5 mol/L urea/0.5 mol/L NaOH solution. It can be explained that the adsorption of polymers on the capillary gives rise to the up-bending of η_{sp}/c - c curves at extremely dilute concentration. While the down-bending of η_{sp}/c - c curves are caused by the conversion of capillary flow from a viscous model to a slip model^[19], due to the strong adsorption of excess urea on the glass surface by forming hydrogen bonds in the 0.8 mol/L urea/0.5 mol/L NaOH and 1.0 mol/L urea/0.5 mol/L NaOH aqueous solutions. From the turning points in Fig. 2, the dynamic contact concentration c_s was estimated to be in the range of 0.6 to $1 \times 10^{-3} \text{ g cm}^{-3}$ for L-FV-II in 0.5 mol/L NaOH aqueous solutions containing 1~0 mol/L urea.

It is noticeable that the η_{sp}/c - c curve for 0.5 mol/L urea/0.5 mol/L NaOH aqueous solution is a straight line. In addition, the η_{sp}/c - c line in 0.5 mol/L urea/0.5 mol/L NaOH was at the top among the others (see Figs. 2 and 3), indicating that the chains are more extended in this solvent. Therefore, 0.5 mol/L urea/0.5 mol/L NaOH is a suitable solvent for L-FV-II to extrapolate η_{sp}/c to zero concentration.

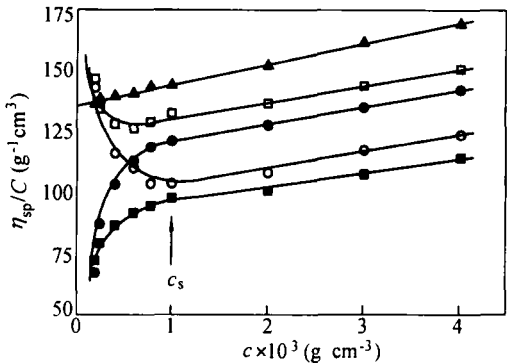


Fig. 2 η_{sp}/c - c curves of L-FV-II in 0.5 mol/L NaOH aqueous solutions of different urea content at 25°C in the polymer concentration range of $2 \times 10^{-4} \sim 4 \times 10^{-3} \text{ g cm}^{-3}$
○ 0 mol/L urea, □ 0.2 mol/L urea, ▲ 0.5 mol/L urea, ● 0.8 mol/L urea, ■ 1.0 mol/L urea

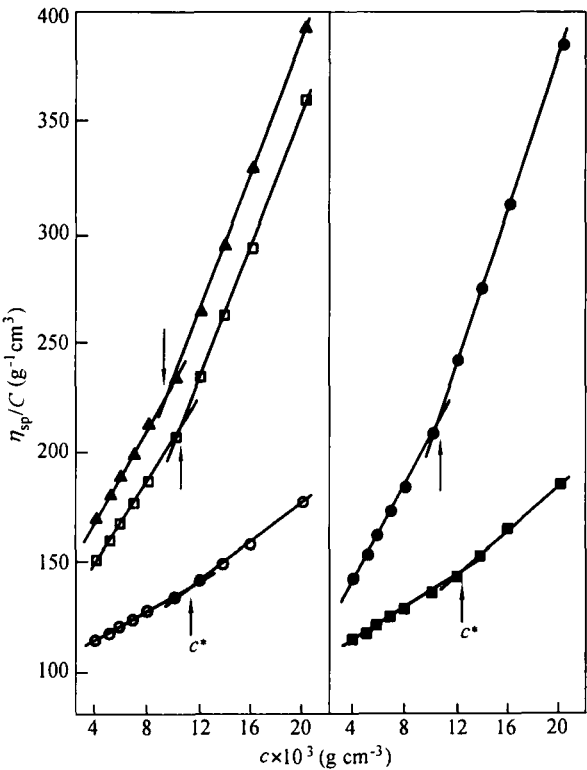


Fig. 3 η_{sp}/c - c curve of L-FV-II in 0.5 mol/L NaOH aqueous solutions of different urea contents in the polymer concentration range of $4 \times 10^{-3} \sim 2 \times 10^{-2} \text{ g cm}^{-3}$ at 25°C
○ 0 mol/L urea, □ 0.2 mol/L urea, ▲ 0.5 mol/L urea, ● 0.8 mol/L urea, ■ 1.0 mol/L urea

In Fig. 3, a change of slope of η_{sp}/c - c plots of L-FV-II going from dilute solution to semi-dilute solution occurred around a critical concentration. The crossover point corresponded to the overlap concentration c^* of polymers. Figure 4 shows the dependences of overlap concentration c^* and $[\eta]$ for L-FV-II in 0.5 mol/L NaOH aqueous solutions containing urea on urea concentration from 0 to 1.0 mol/L. The c^* value in 0.5 mol/L NaOH aqueous solution containing 0.5 mol/L urea is significantly lower than others, indicating that the polymer solubility is more strong in this mixed solution. The $[\eta]$ value at 0.5 mol/L urea content is significantly greater than that of the other urea concentration, indicating that molecular chain are more extended in this mixture, which is consistent with the result of urea effect on L-FV-II molecular conformation^[15]. The c^* values of L-FV-II in 0.5 mol/L NaOH aqueous solution containing urea lay in the range from 0.9 to $1.2 \times 10^{-2} \text{ g cm}^{-3}$.

Meanwhile, the η_{sp}/c - c curves of the L-FV-II aqueous solution containing cadoxen from 20% to 100% at 25°C are shown in Figs. 5 and 6. According to the above mentioned method, the values of c_s and c^* were also estimated to be from 0.9 to $1.5 \times 10^{-3} \text{ g cm}^{-3}$ and 8 to $9 \times 10^{-3} \text{ g cm}^{-3}$, respectively. Figure 7 shows the cadoxen dependence of c^* of L-FV-II in the aqueous solutions. It indicates that c^* increases slowly with increasing cadoxen concentration, suggesting that the clusters were broken by cadoxen, and resulting in the enhancement of the overlap concentration.

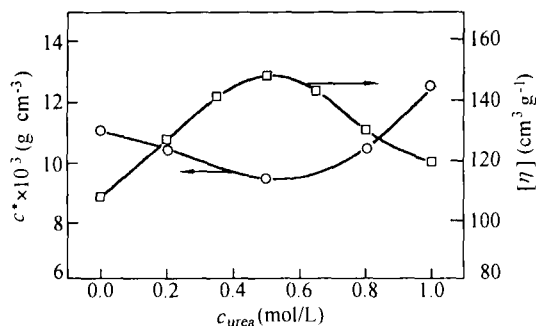


Fig. 4 Dependence of c^* (open circle) and $[\eta]$ (open square) for L-FV-II in 0.5 mol/L NaOH aqueous solutions on the urea concentration at 25°C

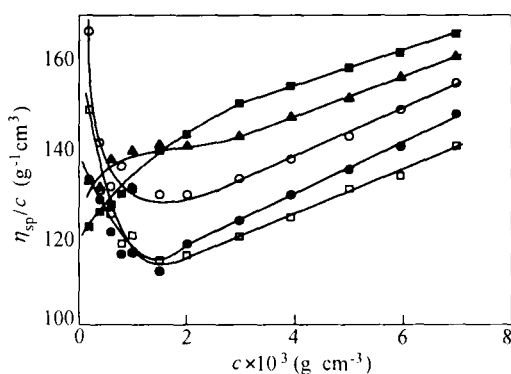


Fig. 5 η_{sp}/c - c curves of L-FV-II in cadoxen solvent systems at 25°C in the polymer concentration range of $2 \times 10^{-4} \sim 7 \times 10^{-3} \text{ g cm}^{-3}$
○ 20% cad, □ 40% cad, ▲ 60% cad, ● 80% cad, ■ 100% cad

Fluorescence Evidence of Critical Concentration

The fluorescence intensity of probes was sensitive to their microenvironment in polymer solution^[11], and the concentration dependence of the excimer fluorescence intensities was used to evaluate the critical concentrations of polystyrene in solutions^[6]. Phenanthrene used as probe in polysaccharide solutions was identified with physical interaction, to be hydrophobic in nature^[20]. The fluorescence intensity of phenanthrene decreases with increasing polymer concentration from 0 to $1.52 \times 10^{-2} \text{ g cm}^{-3}$. When the glucan concentration increases, the chance of contact and overlap of the polysaccharide chains is increased, favoring the formation of clusters and aggregates in the polysaccharide solution^[11]. Figure 8 illustrates the double logarithmic plots of Phe fluorescence emission intensity I/I_0 versus glucan concentration c_{glu} of L-FV-II in 0.5 mol/L NaOH aqueous solutions. Two changes of slope denoted as c_s and c^* were obviously found at $1.2 \times 10^{-3} \text{ g cm}^{-3}$ and $9.2 \times 10^{-3} \text{ g cm}^{-3}$, respectively. These results are in agreement with the values obtained from viscosity measurements.

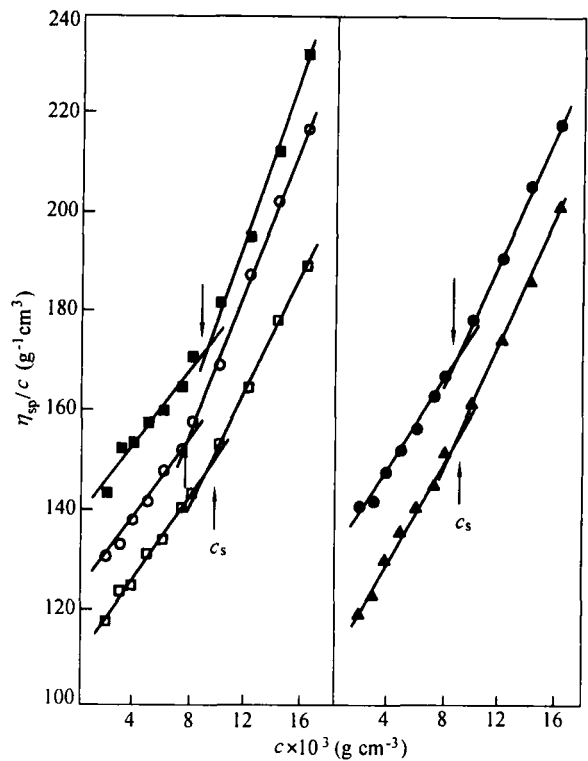


Fig. 6 η_{sp}/c - c curves of L-FV-II in aqueous solutions of different cadoxen contents at 25°C in the polymer concentration range of $2 \times 10^{-3} \sim 1.6 \times 10^{-2} \text{ g cm}^{-3}$
○ 20% cad, □ 40% cad, ▲ 60% cad, ● 80% cad, ■ 100% cad

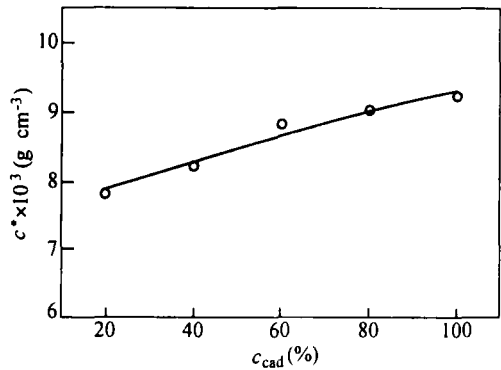


Fig. 7 Cadoxen concentration c_{ad} dependence of c^* of L-FV-II in aqueous solutions

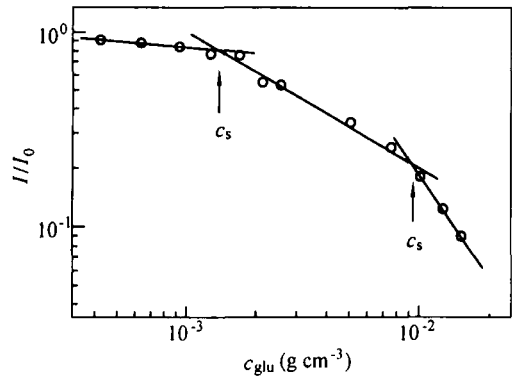


Fig. 8 Double logarithmic plot of fluorescence emission intensity (I/I_0) versus polymer concentration (c_{glu}) for $2.5 \times 10^{-6} \text{ mol/L Phe}/0.5 \text{ mol/L NaOH}$ aqueous solution of L-FV-II

CONCLUSIONS

Strong intermolecular hydrogen bonding exists in L-FV-II, an α -(1 $\rightarrow$ 3)-*D*-glucan. The c_s and c^* for the glucan in 0.5 mol/L NaOH aqueous solutions were satisfactorily estimated from the polymer concentration dependence of induced viscosity and fluorescence emission intensity. The values of c_s and c^* for L-FV-II in the 0.5 mol/L NaOH aqueous solutions were found to be approximately 1×10^{-3} and $1.1 \times 10^{-2} \text{ g cm}^{-3}$ by viscometry, and 1.2×10^{-3}

and $9.2 \times 10^{-3} \text{ g cm}^{-3}$ by the fluorescence method, respectively. The overlap concentration c^* of glucan on going from the dilute solution regime to the semi-dilute solution regime was lower than that of polystyrene ($M_w = 4.1 \times 10^5$) in benzene, which has $c^* \cong 1.5 \times 10^{-2} \text{ g cm}^{-3}$ [21]. This suggests that the polysaccharide in aqueous solutions tends to undergo aggregation caused by interchain hydrogen bonding and thus favors the overlap of polymer chains. Moreover, 0.5 mol/L urea/0.5 mol/L NaOH is a good solvent for this glucan at 25°C, where a normal viscosity behavior of the glucan solution still exists at extremely dilute concentration, and permits the linear extrapolation of η_{sp} to zero concentration in order to get the $[\eta]$ value.

REFERENCES

- 1 Young, S.H. and Jacobs, R.R., *Carbohydr. Res.*, 1998, 310: 91
- 2 Liu, M., Cheng, R. and Qian, R., *J. Polymer. Sci., Part B. Polymer Phys.*, 1995, 33: 1731
- 3 de Gennes, P.G., "Scaling Concepts in Polymer Physics", Cornell University Press., Ithaca, NY, 1979
- 4 Ying, Q. and Chu, B., *Macromolecules*, 1987, 20: 362
- 5 Dondos, A., Skondras, P., Pierri, E. and Benoit, H., *Makromol Chem* 1983, 184: 2153
- 6 Qian, R., Cao, D., Cheng, S. and Bai, F., *Chinese Sci. (Series B)*, 1983, 12: 1080
- 7 Cheng, R. and Yan, X., *J. Appl. Polym. Sci., Appl. Polym. Symposium*, 1991, 48: 123
- 8 Wu, C., *J. Polym. Sci., Part B. Polym. Phys.*, 1994, 32: 1503
- 9 Bercea, M., Ioan, C., Ioan, S., Simionescu, B.C. and Simionescu, C.I., *Prog. Polym. Sci*, 1999, 24: 379
- 10 Destor, C., Langevin, D. and Rondelez, F., *J. Polym. Sci.: Polym. Lett. Ed.*, 1978, 16: 229
- 11 Cotton, J.P., Farnoux, B., Jannink, G. and Strazielle, C., *J. Polym. Sci. Symposium*, 1973, 42: 81
- 12 Meyer, C.T. and Cohen-Addad, J.P., Boileau, S., *Polymer*, 1978, 19: 1107
- 13 Takada, A., Nishimura, M., Koike, A. and Nemoto, N., *Macromolecules*, 1998, 31: 436
- 14 Zhang, P., Zhang, L. and Cheng, S., *Biosci. Biotechnol. Biochem.*, 1999, 63(7): 1197
- 15 Zhang, P., Zhang, L. and Cheng, S., *Carbohydr. Res.*, 2000 (in press)
- 16 Zhang, L., Ding, Q., Zhang, P., Zhu, R. and Zhou, Y., *Carbohydr. Res.*, 1997, 303: 193
- 17 Robinson, G., Ross-Murphy, S.B. and Morris, E.R., *Carbohydr. Res.*, 1982, 107: 17
- 18 Zhang, L. and Yang, L., *Biopolymers*, 1995, 36: 695
- 19 Yang, Y., Yan, X. and Cheng, R., *J. Macromol. Sci., Physics*, 1999, B38: 237
- 20 Barkay, T., Navon-Venezia, S., Ron, E.Z. and Rosenberg, E., *Applied and Environmental Microbiology*, 1999, 65: 2697
- 21 Pierri, E. and Dondos, A., *Eur. Polym. J.*, 1987, 23(5): 347