Articles

Saccharide Effect on the Lower Critical Solution Temperature of Poly(organophosphazenes) with Methoxy-poly(ethylene glycol) and Amino Acid Esters as Side Groups

Sang Beom Lee, Youn Soo Sohn,[‡] and Soo-Chang Song[*]

Division of Life Science, Korea Institute of Science and Technology, Seoul 130-650, Korea
[‡]Department of Chemistry, Ewha Womans University, Seoul 133-791, Korea
Received February 27, 2003

The lower critical solution temperature (LCST) of thermosensitive poly(organophosphazenes) with methoxy-poly(ethylene glycol) (MPEG) and amino acid esters as side groups was studied as a function of saccharide concentration in aqueous solutions of mono-, di-, and polysaccharides. Most of the saccharides decreased the LCST of the polymers, and the LCST decrease was more prominently observed by saccharides containing a galactose ring, such as D-galactose, D-galactosamine and D-lactose, and also the polysaccharide, 1-6-linked D-dextran effectively decreased the LCST of the polymers. Such an effect was discussed in terms of intramolecular hydrogen bonding of saccharides in polymer aqueous solution. The saccharide effect was found to be almost independent on the kinds of the amino acid esters and MPEG length of the polymers. Such a result implies that the polymer-saccharide interaction in aqueous solution is clearly influenced by the structure of saccharides rather than by that of the polymers. The acid saccharides such as D-glucuronic and D-lactobionic acid increased the LCST, which seems to be due to their pH effect.

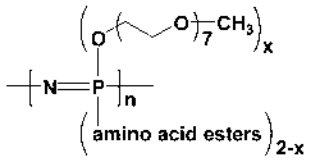
Key Words : Polyphosphazene, LCST, Saccharide effect, Intramolecular hydrogen bonding

Introduction

Thermosensitive polymers exhibit a reversible phase transition phenomenon with temperature change, which arise from the balance of hydrophilicity and hydrophobicity of the polymers. The main mechanism of the phase transition in the polymer solution is a drastic change of interactions between water molecules and the hydrophilic region of the polymer through hydrogen bonding as well as hydrophobic interactions between the polymer molecules. In general, the phase transition of the thermosensitive polymers is known to be affected by additives such as salts,[1,2] surfactants,[3,4] saccharides,[5,6] and solvents.[7,8] These additives mainly influence the interaction between the polymer and water molecules. These solution effects can provide an important information on the interactions of the polymers-solvent-additives.

The biological systems are composed of very complex components such as proteins, salts, saccharides, nucleic acids, and so on. In general, thermosensitive polymers have shown to be sensitive to pH,[9] salts and solvents. Therefore, the LCST of the polymers is responsive to many species in biological systems, and changed "salting-in" (LCST increase) or "salting-out" (LCST decrease). Such LCST properties depending on various additives[1-8] in aqueous solutions have been extensively studied for the well-known thermosensitive polymers, poly(*N*-isopropylacrylamide) (PNIPAM), poly(*N*-vinylacetamide), poly(ethylene oxide), poly(vinyl alcohol), poly(vinyl methyl ether), poly(vinylpyrrolidone), and ethylene oxide-propylene oxide block copolymer. However, in spite of high potential for application to biomedical materials,[10,11] the third component effects on the LCST of polyphosphazenes have been reported only in a few papers.[7] Furthermore, saccharide effect on the LCST of polyphosphazenes has not been reported previously.

In particular, polyphosphazenes with amino acid ester substituents are known to be biocompatible and biodegradable.[12,13] In our previous work, it was reported that the poly(organophosphazenes) bearing methoxy-poly(ethylene glycol) (MPEG) and amino acid esters as side groups are not only biodegradable but also thermosensitive, showing a wide range of LCST depending on compositions and kinds of the side groups.[14] We have also studied the salt effect on these polymers in aqueous solutions.[15]



In this study, saccharide effects on the LCST of poly(organophosphazenes) were investigated in detail employing several structurally different saccharides.

Experimental Section

Polymers. Among the poly(organophosphazenes) with methoxy-poly(ethylene glycol) and amino acid esters as side groups previously synthesized.[14,15] the following copolymers were used: $[NP(MPEG350)_{1.42}(GlyEt)_{0.58}]_n$ (**1**), $[NP(MPEG350)_{0.99}(GlyEt)_{1.01}]_n$ (**2**), $[NP(MPEG350)_{0.58}(GlyEt)_{1.42}]_n$ (**3**), $[NP(MPEG350)_{1.03}(GlyMe)_{0.97}]_n$ (**4**), $[NP(MPEG350)_{1.00}(GlyBz)_{1.00}]_n$ (**5**), $[NP(MPEG750)_{1.09}(GlyEt)_{0.91}]_n$ (**6**), $[NP(MPEG350)_{1.00}(AlaEt)_{1.00}]_n$ (**7**), $[NP(MPEG350)_{0.97}(MalEt_2)_{1.03}]_n$ (**8**), $[NP(MPEG350)_{1.01}(AspEt_2)_{0.99}]_n$ (**9**), and $[NP(MPEG350)_{1.03}(\beta\text{-AlaEt})_{0.97}]_n$ (**10**).

Materials. Guaranteed reagent grade D-glucose, D-galactose, D-glucosamine hydrochloride, D-galactosamine hydrochloride, D-mannosamine hydrochloride, D-lactose, D-maltose, D-cellobiose, D-gluguronic acid, D-lactobionic acid, and dextran from Aldrich and D-mannose, D-glucitol, D-mannitol, and D-sucrose from Acros were used without further purification.

Measurements of LCST. The LCST temperature of the aqueous solution of the polymer (5%) containing different kinds and concentrations of saccharides (0-1.0 M) was detected visually in a closed glass tube and the temperature was controlled by immersion of the glass tube in an oil bath. The LCST was identified as the temperature at which the solution became turbid.

Results and Discussion

The effect of each saccharide on the LCST of poly(organophosphazenes) was studied as a function of saccharide concentration. In this study, six structurally different types of saccharides, monosaccharides, disaccharides, polysaccharides, amino saccharides, acidic saccharides and alditols shown in Figure 1 were employed to examine the saccharide effect on the LCST of polyphosphazenes in aqueous solutions.

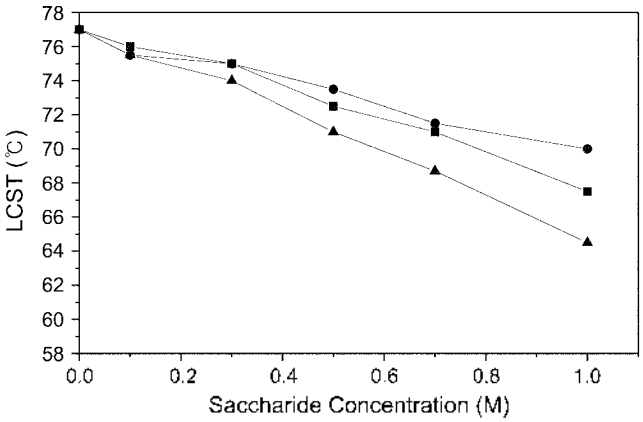


Figure 2. The LCST Change of polymer **2** in aqueous solution depending on the concentration of mannose (●), glucose (■), and galactose (▲).

Figure 2 shows the monosaccharide effect on the LCST of polymer **2**, $[NP(MPEG350)_{0.99}(GlyEt)_{1.01}]_n$. The LCST was significantly affected by both concentration and structure of saccharides. All the monosaccharides decreased the LCST of polymer **2**, and the LCST decreased remarkably with increasing concentration of saccharides. Such a phenomenon was known to be a general trend in the saccharide effect on the LCST of thermosensitive polymers.[5,6] A general explan-

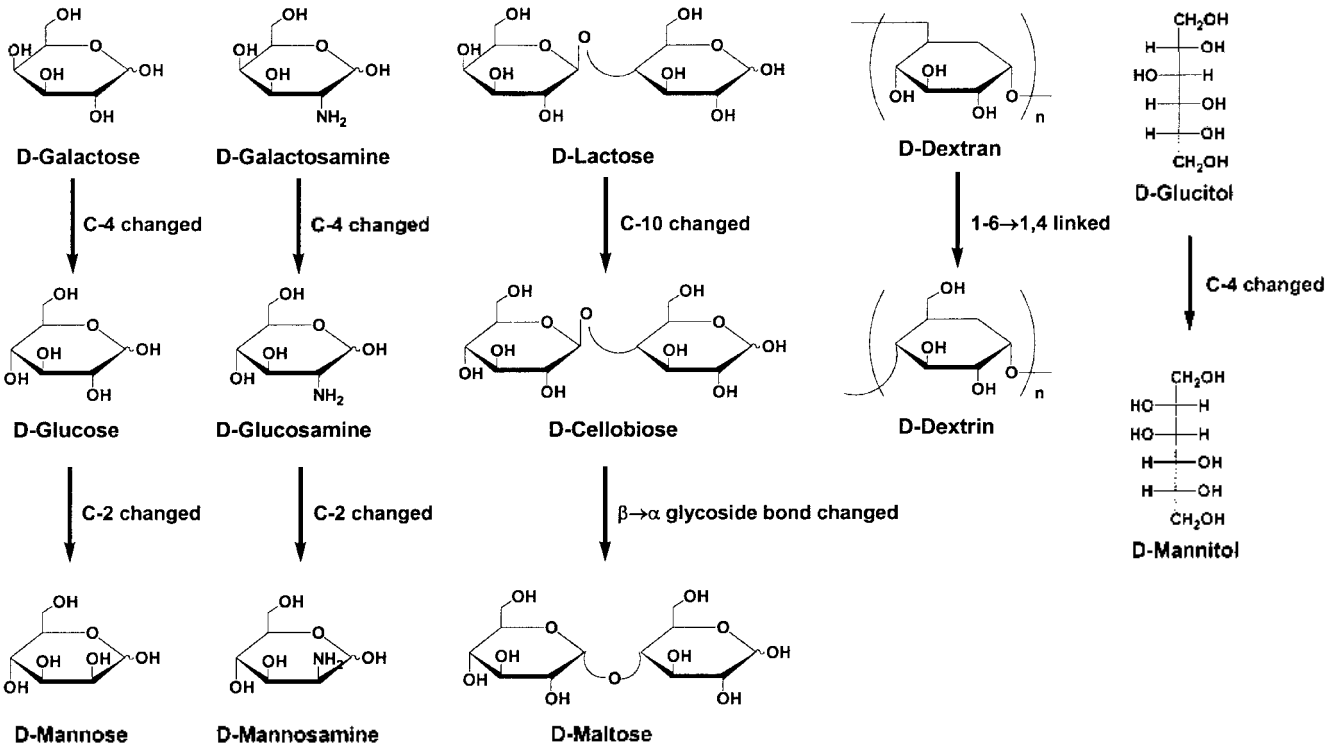


Figure 1. Saccharide structures and their relationships.

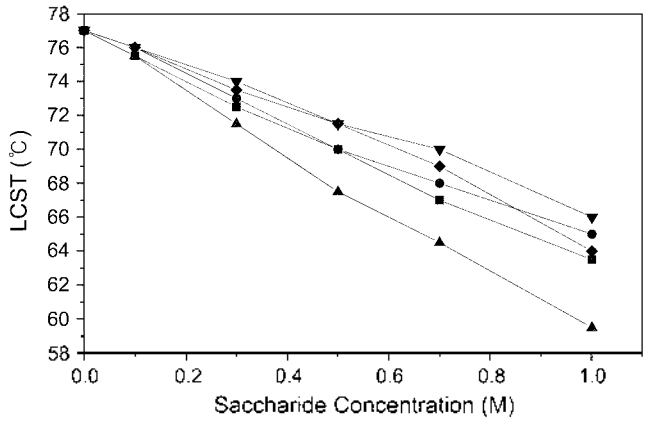


Figure 3. The LCST Change of polymer **2** in aqueous solution depending on the concentration of mannosamine hydrochloride (●), glucosamine hydrochloride (■), galactosamine hydrochloride (▲), mannitol (▼), and glucitol (◆).

ation for the LCST decrease by addition of saccharides is that the saccharide molecules disrupt the hydrogen-bonding between polymer and water molecules in solution. Thus, the higher saccharide concentration results in larger LCST decreasing effect. Interestingly, the degree of LCST decrease for polymer **2** was found to be dependent on the structure of saccharides: the LCST decreasing effect of galactose was more effective than that of glucose, and glucose was more effective than mannose. The ΔLCST ($T_{1.0M}$-T_{0M}), the change of LCST by addition of 1.0 M galactose, glucose, and mannose, was -12.0, -9.5, and -7.0 °C, respectively. A similar trend was also observed in Figure 3 showing the effect of amino saccharides and acyclic alditols on the LCST of polymer **2**. As the saccharide concentration in the polymer solution increased, the LCST decreased in a similar way to the monosaccharides shown in Figure 2. However, the amino saccharides have shown more prominent concentration effect on the LCST of polymer **2** compared with their corresponding monosaccharides.

Alditols are acyclic or polyhydric alcohols that have a similar configuration to cyclic monosaccharides: glucitol and mannitol are prepared by reduction of glucose and mannose, respectively. These alditols have shown nearly the same effect on the LCST as monosaccharides as shown in Figure 3. Thus the saccharide effect on the LCST of the polymer increases in the order of mannose > glucose > galactose derivatives: the more effective LCST decrease was observed for saccharides with the galactose ring, and the results were summarized in Table 2. As seen in the table saccharides such as glucuronic acid and lactobionic acid, increased the LCST of the polymer solutions. The LCST of the pH sensitive polymers bearing weakly basic amino side group are known to be mostly increased at lower pH,[16,17] but the LCST of the polymers with weakly acidic side groups decreases at lower pH.[18,19] Thus the present pH effect may be understood by assuming that the polymer becomes hydrophilic by ionization of the secondary amine in the amino acid ester side group at lower pH. In other words, it is suggested that the LCST increasing effect due to acidity of these acidic saccharide systems is superior to their LCST decreasing effect through water structure making.

It is known that low molecular weight saccharides are strong water structure makers.[6,20-22] The stabilization of water structure by addition of saccharides may cause the interaction through hydrogen bonding between water and polymer chain to decrease in an aqueous polymer solution, which may enhance the hydrophobic interaction between polymer chains. Therefore, it is reasonable that the LCST of the present polymer solutions decreases with increasing saccharide concentration. It should also be pointed out that the effect of the saccharides on the LCST were dependent on their structures. Each group of monosaccharides, amino saccharides and alditols has the same numbers of hydroxyls, carbons, and oxygens in their molecular structure as shown in Figure 1, that is, the molecules in each group is either stereo or positional isomers with different hydroxyl posi-

Table 1. Characteristics of Poly(organophosphazenes)

polymer	formula	LCST (°C)[a]	M_w ($\times 10^{-4}$)[b]	ΔLCST (°C) ($T_{1.0M}$-T_{0M})[c]
1	$[NP(MPEG350)_{1.42}(GlyEt)_{0.58}]_n$	93.2	4.73	-9.5
2	$[NP(MPEG350)_{0.99}(GlyEt)_{1.01}]_n$	77.5	3.84	-9.5
3	$[NP(MPEG350)_{0.58}(GlyEt)_{1.42}]_n$	64.5	1.77	-6.5
4	$[NP(MPEG350)_{1.03}(GlyMe)_{0.97}]_n$	88.5	3.08	-8.5
5	$[NP(MPEG350)_{1.00}(GlyBz)_{1.00}]_n$	49.5	2.13	-7.5
6	$[NP(MPEG750)_{1.09}(GlyEt)_{0.91}]_n$	98.5	4.14	-9.5
7	$[NP(MPEG350)_{1.00}(AlaEt)_{1.00}]_n$	67.0	3.58	-8.0
8	$[NP(MPEG350)_{0.97}(MalEt_2)_{1.03}]_n$	65.5	2.24	-7.5
9	$[NP(MPEG350)_{1.01}(AspEt_2)_{0.99}]_n$	60.2	4.40	-8.5
10	$[NP(MPEG350)_{1.03}(\beta\text{-}AlaEt)_{0.97}]_n$	70.3	2.18	-8.5

[a,b]Data from ref. 14 and 15. [c]The LCST change by addition of 1.0 M glucose.

Table 2. Saccharide Dependence of LCST for Polymer **2**

saccharide	ΔLCST (°C) ($T_{1.0M}$-T_{0M})
D-Galactose	-12.0
D-Glucose	-9.5
D-Mannose	-7.0
D-Galactosamine hydrochloride	-17.5
D-Glucosamine hydrochloride	-13.5
D-Mannosamine hydrochloride	-12.0
D-Lactose	-24.0
D-Maltose	-17.0
D-Cellobiose	-16.5
D-Sucrose	-12.0
D-Glucitol	-13.0
D-Mannitol	-11.0
D-Glucuronic acid	+[a]
D-Lactobionic acid	+[b]
D-Dextran	-23.0
D-Dextrin	-12.5

[a]Cloud point was not observed throughout from 0.1 M to 1.0 M. [b]Cloud point was 99.5 °C at 0.1 M, but not observed beyond 0.1 M up to 1.0 M.

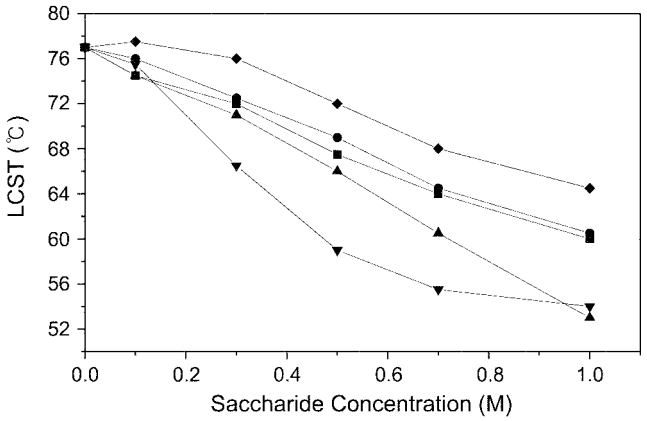


Figure 4. The LCST Change of polymer **2** in aqueous solution depending on the concentration of cellobiose (●), maltose (■), lactose (▲), dextran (▼), and dextrin (◆).

tions: the D-galactose differs from the D-glucose only in the configuration of the hydroxyl group at C-4, and therefore, is intrinsically equivalent to D-glucose. However, in D-galactose, the hydroxyl group at C-4 forms an intramolecular hydrogen bond with the ring oxygen[23,24] and thus is less easily available than the hydroxyl group at C-4 of the D-glucose to interact with water molecules. Generally, the intramolecular hydrogen bonding makes most of the saccharides hydrophobic.[25] From the result of such intramolecular hydrogen bonding, the galactose derivatives will play as a stronger water structure maker and consequently increase the hydrophobic interactions between polymers, thereby, resulting in decrease their LCST. Kawasaki *et al.*[26] reported the saccharide-induced volume phase transition of the poly(*N*-isopropylacrylamide) (NIPA) gel. In that study, it was also found that the LCST of NIPA gel was more decreased by D-galactose than by D-glucose.

To confirm the tentative explanations for the saccharide effect on the LCST of polyphoaphazene, we studied how the LCST of poly(organophosphazene) solutions was changed when 1-4-linked disaccharides was added. Figure 4 shows the di- and polysaccharide effects on the LCST of polymer **2** solution. The disaccharides have one glucose ring in common but a different C-10 hydroxyl configuration or1-4-glycosidic bond. If the hydroxyl group of C-10 of lactose moves to axial position, then lactose become cellobiose and if the β glycosidic linkage of cellobiose is changed to a linkage, the cellobiose becomes maltose. By using these isomers, we were able to examine the galactose ring and glycosidic bond effects of saccharide on the LCST of the polymer. The ΔLCSTs ($T_{1.0M}$-T_{0M}) of polymer **2** by lactose, maltose, and cellobiose are -24.0, -17.0, and -16.5 °C, respectively. This result indicates that disaccharides composed of two monosaccharide rings are two times more efficient in LCST decrease than monosaccharides, and the disaccharides containing galactose ring decrease more effectively the LCST. Furthermore, the LCST was shown to be independent on the kinds of glycosidic linkages. Hydroxyl group of C-10 in lactose is equatorial, and those of cellobiose and maltose are axial. That is, the hydroxyl group of C-4 in monosaccharide and C-10 in disaccharide seems to play an important role in the saccharide effect. The galactose ring in D-galactose or D-galactosamine and D-lactose increases intramolecular hydrogen bonding. Thus galactose derivatives may play a stronger water structure maker.

Dextran and dextrin are representative polysaccharides having glycosidic bonds of α-1,6 and α-1,4 glycosidic linkages, respectively. The dextran with the α-1,6 glycosidic linkage was more effective in decreasing the LCST of polyphosphazene than dextrin of the α-1,4 linkage. Such a result means that dextran has stronger intramolecular hydrogen bonding than dextrin: dextran has the hydroxyl at C-4, but dextrin does not, and thus dextran is more hydrophobic than dextrin. This result shows that C-4 hydroxyl of saccharides play an important role in hydrogen bonding with water molecules. Sjoberg *et al.*[5] reported the saccharide effect on the LCST of the homopolymer of poly(ehylene glycol) (PEG). The 1-6-linked isomaltose have also shown to interact more favorably with the PEG segment in an aqueous solution than 1-4-linked maltose and cellobiose.

Table 1 shows the glucose effect on the LCST of the polymers with different amino acid esters, composition of side groups, and MPEG length. The ΔLCST was almost independent on the kinds of amino acids and esters. The ΔLCST of polymer **6** bearing MPEG 750 by addition of glucose was same as that of polymer **2** bearing MPEG 350. Also polymer **3** with 0.58 : 1.42 mole ratio of MPEG : GlyEt showed only a slight LCST change compared with polymers **1** and **2**. Consequently, the structure and composition of the side groups of the polymers exhibited negligible effects on the ΔLCST obtained by the addition of saccharides. In contrast, inorganic and organic salts[15] showed sensitive effects on the ΔLCST of the present polymers depending on their structures. From such results, it may be concluded that the saccharide effect on the LCST of the thermosensitive poly(organophosphazenes) is affected by saccharide structure rather than by the polymer structure.

Conclusion

The LCST of the thermosensitive poly(organophosphazenes) was affected by the molecular structure of saccharides. Most of the saccharides, except for acid saccharides, decreased the LCST of the polymers: the saccharides having galactose ring and 1-6-linked glycosidic bond decreased more significantly the LCST of the polymers. This result was interpreted as an intramolecular hydrogen bonding ability of the saccharide molecule: the more intramolecular hydrogen bonding of saccharide is populated, the more remarkably the LCST of the polymers is decreased. These saccharide effects on the LCST of thermosensitive polymers may be an important information for designing glucose sensitive drug release systems for diabetes using their thermosensitive properties.

Acknowledgement. This research was financially sup-

ported by the Ministry of the Science and Technology in Korea.

References

1. Park, T. G.; Hoffman, A. S. *Macromolecules* **1993**, *26*, 5045.
2. Saito, S. *J. Polym. Sci. Part A-1* **1969**, *7*, 1789.
3. Kokufuta, E.; Zhang, Y. Q.; Tanaka, T.; Mamada, A. *Macromolecules* **1993**, *26*, 1053.
4. Isogai, N.; Gong, J. P.; Osada, Y. *Macromolecules* **1996**, *29*, 6803.
5. Sjoberg, A.; Karlstrom, G.; Tjerneld, F. *Macromolecules* **1989**, *22*, 4512.
6. Kim, Y. H.; Kwon, I. C.; Bae, Y. H.; Kim, S. W. *Macromolecules* **1995**, *28*, 939.
7. Pandya, K.; Lad, K.; Bahadur, P. J. *M. S. Pure Appl. Chem. A1* **1993**, *30*, 1.
8. Yang, J.; Guo, R.; Friberg, S. *J. Disp. Sci. Technol.* **1995**, *16*, 249.
9. Crommen, J. H. L.; Schacht, E. H.; Mense, E. H. G. *Biomaterials* **1992**, *13*, 601.
10. Allcock, H. R.; Dudley, G. K. *Macromolecules* **1996**, *29*, 1313.
11. Tanigami, T.; Ono, T.; Suda, N.; Sakamaki, Y.; Yamaura, K.; Matsuzawa, S. *Macromolecules* **1989**, *22*, 1397.
12. Goedemoed, J. H.; Mense, E. G. H.; Groot, D. D.; Claessen, A. M. E.; Scheper, R. J. *J. Control. Rel.* **1989**, *170*, 245.
13. Crommen, J. H. L.; Schacht, E. H.; Mense, E. H. G. *Biomaterials* **1992**, *13*, 511.
14. Song, S.-C.; Lee, S. B.; Jin, J.-I.; Sohn, Y. S. *Macromolecules* **1999**, *32*, 2188.
15. Lee, S. B.; Song, S.-C.; Jin, J.-I.; Sohn, Y. S. *Macromolecules* **1999**, *32*, 7820.
16. Siegel, R. A.; Firestone, B. A. *Macromolecules* **1988**, *21*, 3254.
17. Feil, H.; Bae, Y. H.; Feijen, J.; Kim, S. W. *Macromolecules* **1992**, *25*, 5528.
18. Kou, J. H.; Amidon, G. L.; Lee, P. I. *Pharm. Res.* **1988**, *5*, 592.
19. Dong, L.-C.; Hoffman, A. S. *J. Control. Rel.* **1991**, *15*, 141.
20. Robinson, R. A.; Stokes, R. H. *J. Phys. Chem.* **1961**, *65*, 1954.
21. Herskovits, T. T.; Kelly, T. M. *J. Phys. Chem.* **1973**, *77*, 381.
22. Gustafsson, A.; Wennerstrom, H.; Tjerneld, F. *Fluid Phase Equilibria* **1986**, *29*, 365.
23. Aspinall, G. O. *The Polysaccharides*; Academic Press: New York, U.S.A., 1982; Vol. 1, Chapter 5.
24. Birch, G. G.; Dziedzic, S. Z.; Shallenberger, R. S.; Lindley, M. G. *J. Pharm. Sci.* **1981**, *70*, 277.
25. Hudson, C. S.; Yanovsky, E. *J. Am. Chem. Soc.* **1917**, *39*, 1013.
26. Kawasaki, H.; Sasaki, S.; Maeda, H.; Mihara, S.; Tokita, M.; Komai, T. *J. Phys. Chem.* **1996**, *100*, 16282.