Polyelectrolye Complex Formation Between Alginate and Chitosan as a Function of pH

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Received 9 January 2002; accepted 11 July 2002

ABSTRACT: Chitosan and alginate are two polyelectrolytes that can be used as thickening agents in the food industry, in drug-release systems in pharmaceutical applications as biomaterials in wound healing, and cell culture applications, or as ion exchange material for the removal of heavy metal ions from industrial wastewaters. These two polysaccharides can also be used together to form a polyelectrolyte complex, especially to encapsulate proteins, cells, and enzymes. Although there are many applications of these polyions, few publications explain the interaction between their functional groups. This is mostly because of the difficulty of following ionic interaction in an interface of macromolecules, especially since they alter much with the reaction conditions such as pH. The present study reveals the interaction between chitosan and alginate at different pH values by means of a particular method for Fourier transform infrared (FTIR) studies. A previously reported disagreement between the yield of the complexes in weight and density of the interacting functional groups is explained through this method. The obtained results are supported with the morphological studies of the polyelectrolyte beads prepared at different pH values. Freeze-dried beads of both alginate and chitosan-coated alginate beads could be viewed after hexamethyl disilazane (HMDS) treatment. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 88: 346–351, 2003

Key words: polyelectrolyte complex; chitosan, alginate; Fourier transform infrared; scanning electron microscopy

INTRODUCTION

Polyelectrolytes are macromolecules carrying a relatively large number of functional groups that either are charged, or under suitable conditions, can become charged. The molecules may constitute either polycations or polyanions, since the net charge of these macromolecules depends on the functional groups, which may be either positively or negatively charged, or both. Chitosan and alginate are well known polyelectrolytes that can be used as thickening agents in the food industry, in drug-release systems in pharmaceutical applications,[1–4] as biomaterials in wound healing[5] and cell culture applications,[6] or as an ion exchange material for the removal of heavy metal ions from industrial waste waters.[7–10] These two polysaccharides can also be used together to form a polyelectrolyte complex, especially to encapsulate proteins, cells and enzymes.[11–14] Polyelectrolyte complexes can be obtained as precipitates when cationic polymers are mixed with anionic polymers in aqueous solutions. Mixing of solutions containing polymeric acids and polymeric bases can lead to mutual precipitation, even in extremely dilute solutions. A coulombic force is believed[6,13–15] to be the primary binding force for the formation of these complexes, the interactions taking place primarily between ionizable groups bearing opposite charges. Secondary bonding forces, like hydrogen bonding or covalent bonding, may also be important in the formation.

Recently, the use of natural polymers for encapsulation of drugs, proteins, and viable cells has received much attention because of their biocompatibility. In some applications, the polymer matrix has been coated with another polymer to control the release of encapsulated material.[3,4,13–17] The success of the coatings is attributed in large part to the coulombic interactions between these polymers, which are polyelectrolytes. Chitosan, alginate, carboxymethylcellulose, λ-carrageenan, and dextran sulfate are the most extensively studied polysaccharides used in the formation of polyelectrolyte complexes. Some synthetic polyelectrolytes, like poly(L-lysine) and polyacrylates, have been used to make complexes with these polysaccharides.

The effect of pH on complex formation between amine groups of chitosan and carboxyl groups of alginate is the focus of the present study. The pH has a strong influence on polyelectrolyte functional groups, and hence on both the yield and the permeability of the membranes formed from these complexes.

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Journal of Applied Polymer Science, Vol. 88, 346–351 (2003)
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Polyelectrolyte complexes of chitosan with sodium dextransulfate, prepared at high and low pH values, were studied by Fukuda and Kikuchi,[15] who suggested that the complexes prepared at different pH values were different in molecular structure. The polyelectrolyte complexes in the low-pH series were found to be appreciably different from those in the high-pH series, in such properties as solubility, color, reaction with toluidine, and thrombus formation.

Fukuda and Kikuchi[16] also studied the effects of several parameters on the chemical reaction between sodium carboxymethyl cellulose (CMS) and chitosan leading to the formation of polyelectrolyte complexes. Being the salt of a weak acid, carboxymethylcellulose has functional groups of —CH_2COO^- in its binding sites, interacting with chitosan through its —NH_3^+ groups. The Fourier transform infrared (FTIR) absorption bands around 1520 and 1740 cm^{-1}, attributed to NH_3 and COOH groups respectively,[16] were observed for the complex, whereas no such absorption was seen for the simple mixture of polyions. These results suggest that the —NH_3^+ groups in chitosan participate in binding to carboxymethylcellulose, probably through their —COO^- groups. The sodium and chlorine contents in the complexes were distinctly smaller than those found in carboxymethylcellulose and chitosan separately. The yield (by weight) of the complex prepared at pH 2.5 was found to be higher than that of the complex prepared at pH 5.0. Elemental analysis showed that the nitrogen content of the complex is not influenced by the mixing order, or by the molar ratio of N/Na in the reaction mixture. The samples prepared at high pH values were more soluble. The nitrogen, sodium, and chlorine contents in the complex prepared at higher pH was found to be greater, although the low-pH complexes gave the higher yield.

Chitosan–alginate complex systems have been studied[12–14] in the form of beads, used generally as a controlled-release system for high-molecular-weight proteins or drugs. Huguet et al.[13] reported in 1994 that the release of encapsulated hemoglobin (M_w: 60,000), during storage of chitosan-coated alginate beads in water, depended on the conditions of their formation and particularly on the chitosan molecular weight and the pH at which the beads were prepared. The best retention during bead formation was obtained with beads prepared at pH 5.4. The best retention during storage in water was obtained with beads prepared at pH 2. A suggested explanation for this was the presence of a greater concentration of —COOH functional groups in the interface when the beads were formed at pH 2. These functional groups do not interact with chitosan, and some kind of loop formation was suggested[13] to occur on the alginate surface, resulting in a thicker and less dense membrane. In contrast, when the procedure was carried out at pH 5.4, the alginate chains would keep the greater part of their ionized carboxyl groups. The greater number of chitosan–alginate ionic linkages would result in a denser or higher yield membrane formation at pH 5.4 than at pH 2.

There appears to be a disagreement between the data for the yield and for the permeability of polyelectrolyte complexes prepared at different pH values. The yield of the complexes obtained at high pH values must be higher according to the permeability results of Huguet et al.[13] Fukuda and Kukichi,[16] however, showed that the yield of the solid polyelectrolyte complex obtained from sodium carboxymethyl cellulose and chitosan is higher when prepared at pH 2.5 than at pH 5. This disagreement was also pointed out by Huguet et al.[13]

A lower-molecular-weight enzyme, carbonic anhyrdase (M_w: 30,000), was encapsulated in chitosan–alginate beads in our previous studies.[11,12] The release properties of the enzyme from beads prepared at different pH values exhibited the same trend that was observed by Huguet et al.[13] In the present study, this apparent disagreement was both demonstrated and explained for the first time, based on an experiment to compare the yield performance of the chitosan–alginate system with the permeability of the chitosan–alginate beads. The effect of pH on bead formation was studied not only in the chitosan–alginate system but also in the alginate matrix itself. The effect of pH on the morphology of the alginate matrix and the presence of chitosan coating on the alginate surface was viewed by scanning electron microscopy (SEM).

FTIR spectroscopy has been used to explain the interaction between the functional groups of the oppositely charged polyions. In earlier studies, the complexes obtained from solutions at different pH values have been compared[15–17] with simple physical mixtures of the individual polyions. There was an underlying assumption that only complexes were precipitated from mixed solutions. Spectra from pure polyions precipitated separately from solutions at different pH values were not examined, and the possibility that one of the polyions (in our case alginate and chitosan) could coprecipitate with the complex, depending on the pH range, was previously neglected. In the present study, the interaction between chitosan and alginate was explained for the first time by means of the FTIR method. Instead of a mixture of powdered chitosan and alginate (as the form purchased from vendor), self-precipitated forms of these two polysaccharides were compared separately with the complexes obtained at different pH values. The method used in these studies also makes it possible to explain the apparent disagreement between data in the literature.

EXPERIMENTAL METHOD

Chitosan–alginate polyelectrolyte complexes were formed at different pH values from 0.05 wt % of alginate solution (Fluka lot no. 71238) mixed with 0.05 wt % of chitosan (Sigma lot no. 50K0180) solution at room temperature. Dilute solutions were used to prevent gel formation. The solutions were diluted from 2 wt % aqueous alginate solution and 2 wt % chitosan solution, which was prepared in 1 vol % acetic acid, in deionized water. The pH of the reaction medium was adjusted with either HCl or NaOH solutions. The complex precipitates were separated from the supernatant solution by mild centrifuging, then washed, freeze dried, and weighed. Precipitation was observed in the alginate solution at pH values less than 3.6, and in the chitosan solution at pH values higher than 6.5. FTIR (laser analytical model: RFX-40, WA, USA) was used to show the interactions between the functional groups of alginate and chitosan. Experimental spectra of solid samples were obtained with KBr pellets prepared with 3:100 "product-to-KBr" ratio.

Alginate and chitosan-coated alginate beads were prepared as described previously.[11,12] Morphological and microstructural features of the beads were investigated on freeze-dried samples dehydrated in a Mitsubishi moisture meter (model CA-02 Refrigeration for Science, Inc., freeze drier). Prior to the drying process the beads were washed in hexamethyl disilazane (HMDS) solution for 1 min, and placed in a humidified chamber on a watch glass. HMDS was used to stabilize the beads under the beam. Dried samples were cut in half and viewed in a JEOL model JSM-6100 scanning electron microscope.

RESULTS

Chitosan–alginate polyelectrolyte complexes were formed at various pH values at room temperature. Dilute solutions were used to prevent gel formation. Pure chitosan precipitated at pH values higher than 6, while alginate precipitated at pH values lower than 3.6. Yield of the precipitates was determined at pH 2 and pH 9 for pure alginate and chitosan respectively. Centrifuged precipitates from the solutions were freeze dried and weighed for yield calculations. Table I shows the yield of the complexes, as well as the yields from the individual chitosan and alginate solutions, obtained at different pH values. FTIR spectra of the samples listed in Table I are shown in Figure 1.

TABLE I
Yield of the Complexes and the Independent Precipitates Obtained from the Dilute Solutions of Chitosan and/or Alginate at Different pH Values

Sample	Chitosan solution (mL)	Alginate solution (mL)	pH	Yield (g)
C1	8	4	2	0.0027
C2	8	4	3.6	0.0019
C3	8	4	4.6	0.0015
C4	8	4	6	0.0013
C5	8	4	9	0.0026
Alginate	0	12	2	0.0017
Chitosan	12	0	9	0.0050

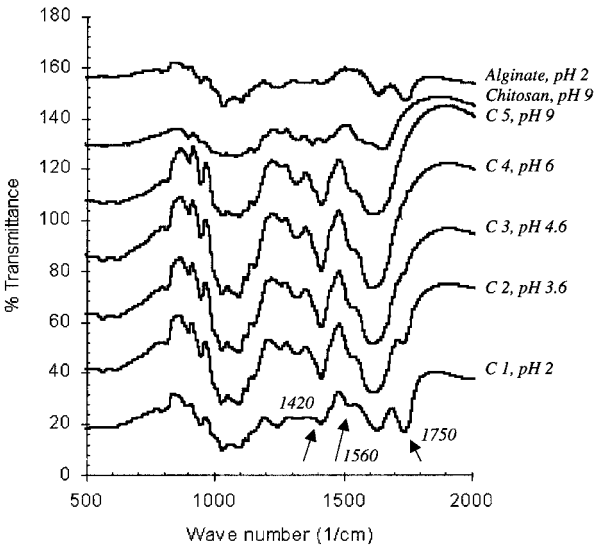

Figure 1 FTIR spectra of the chitosan–alginate polyelectrolyte complexes compared to chitosan and alginate precipitates.

A new peak at around 1420 cm^{-1} can be seen for all complexes (C5–C1) in Figure 1. Intensity of this peak increases from the complex C1 (pH 2) to C4 (pH 6) and decreases a little for C5 (pH 9). This peak is attributed to the $—NH_3^+$ groups of chitosan interacting with the $—COO^-$ groups of alginate. A strong peak at 1750 cm^{-1} is seen in C1 (pH 2) and C2 (pH 3.6) complexes, and in pure alginate precipitate. Intensity of this peak is also smaller for the C2 (pH 3.6) complex than for the C1(pH 2) complex on the separate alginate precipitate. This peak is explained by nonionized —COOH groups of alginate at low pH values. The peak seen for all complexes at 1560 cm^{-1}, and as a shoulder for the pure chitosan precipitate, is explained in terms of the unreacted NH_3 groups of chitosan.

The effect of the preparation pH on the structure of alginate beads is seen in Figures 2 and 3 for beads prepared at pH 2 and pH 5, respectively. The presence of the chitosan coating and the morphology of the inner alginate matrix of chitosan–alginate beads are seen in Figure 4.

DISCUSSION

Contrary to the general method, which is to compare the FTIR spectra of the complexes with those from a corresponding physical mixture of the reagents, pure chitosan and alginate respectively, self-precipitated

from solutions, were compared with the complexes in the present study. Chitosan precipitation was observed above ~ pH 6 and alginate precipitation was observed below ~ pH 3.6. The observed pH values causing precipitation correlated well with the published pK values of these polyions.[1,2] The alginate chains are composed of mannuronic and guluronic acid units whose pK values are published as 3.38 and 3.65, respectively.[1] The chitosan chain pK is known to be around 6.3.[2] Given therefore that there would not be any chitosan precipitated below pH 6, except in complex form (C1–C3 in Figure 1), the newly formed amine peak at 1420 cm^{-1} can be attributed to the amine groups of chitosan reacting with the carboxyl groups of alginate. This is more obvious when the FTIR spectrum for the C5 (pH 9) complex is compared with that of the chitosan precipitate obtained at pH 9. The peak corresponding to carboxyl ions that precipitate in the complex formation, on the other hand, is not clearly distinguishable in the spectra for the complexes, perhaps due to the presence of a shoulder around 1650–1700 cm^{-1} in all complexes. Observation of the strong peak at 1750 cm^{-1} only for the C1 (pH 2) and C2 (pH 3.6) complexes, and for pure alginate precipitate, reveals the presence of unionized carboxylic acid groups in the alginate structure. Intensity of this peak is also smaller for the C2 (pH 3.6) complex than for the C1 (pH 2) complex or the separate alginate precipitate. The decrease in intensity of the peak at 1420 cm^{-1} at lower precipitation pH similarly may indicate the declining density of polyelectrolyte formation at lower pH. In our earlier studies,[11] Kjeldahl nitrogen analysis was performed on the beads and showed that the nitrogen content of the chitosan–alginate beads, which is directly related with the chitosan coating on the alginate surface, also declines with decreasing pH. These results demonstrate that the yield of the complex formation must be higher when the complex is prepared at pH 5 than at pH 2, and this result is related to the available ionized carboxylic acid groups on the alginate surface.

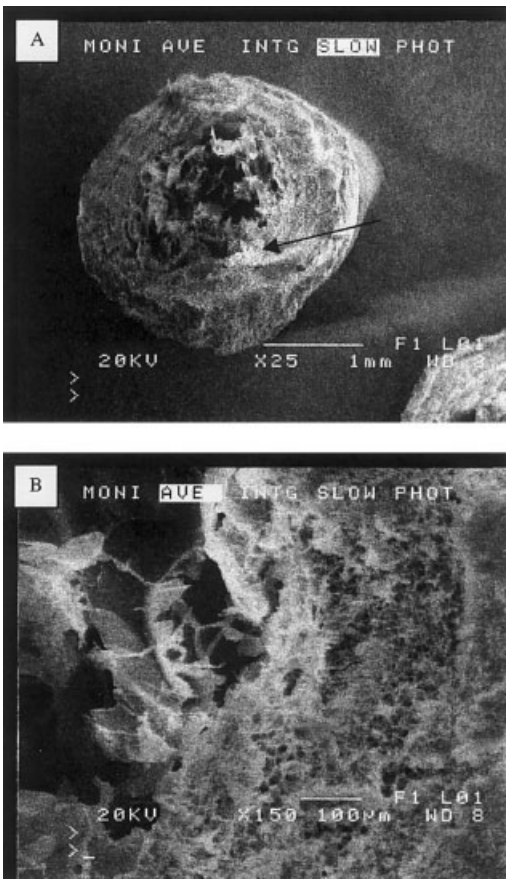

Figure 2 (A) Cross-sectional view of an alginate bead prepared at pH 2. (B) Cross-sectional view of an alginate bead prepared at pH 2 at higher magnification (an arrow in A indicates the area).

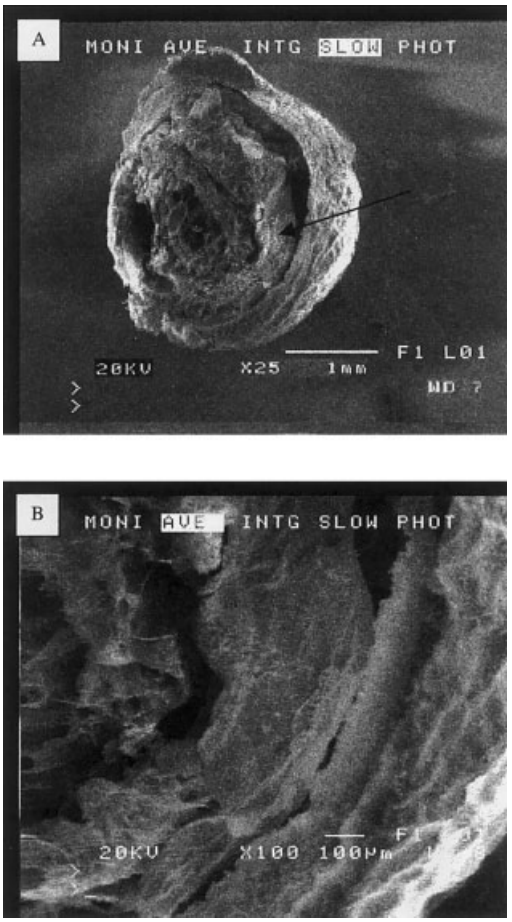

Figure 3 (A) Cross-sectional view of an alginate bead prepared at pH 5. (B) Cross-sectional view of an alginate bead prepared at pH 5 at higher magnification (an arrow in A indicates the area).

The change in structure of alginate beads when they are prepared at pH 2 is shown in Figure 2. Compared to typical alginate beads prepared at pH 5 (Figure 3),

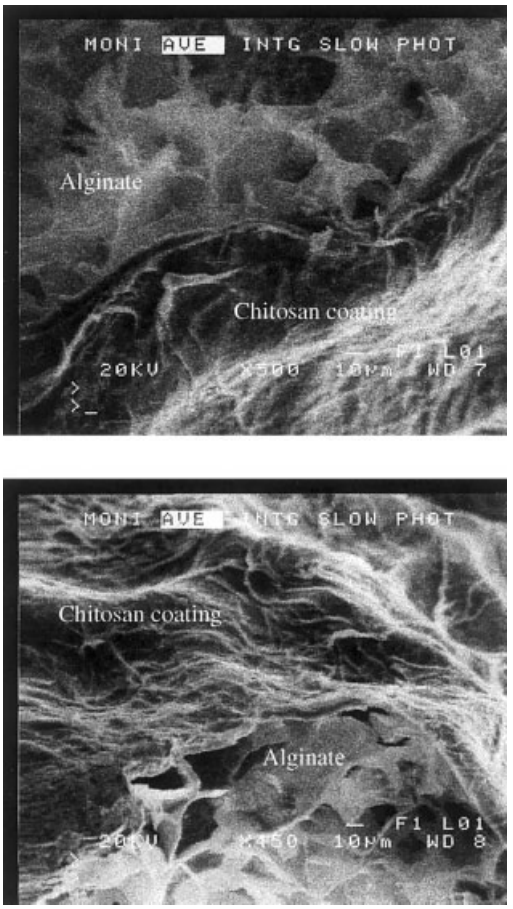

Figure 4 Cross-sectional views of a half-cut chitosan–alginate bead prepared at pH 5.

the bead seen in Figure 2 has a denser and less open alginate structure to the surface. This morphology may also indicate the presence of loop kind-containing alginate network not only on the surface of alginate beads available for coating, as suggested by Huguet et al.,[13] but also in the matrix. Chitosan-coated alginate beads were viewed more easily and at higher magnification because the coating helped to maintain the structure during the drying process. The smooth structure of the coated beads and the layered formation of the alginate matrix inside the beads can be seen in Figure 4. Presence of the coating can also be seen clearly at the edges of the cut beads.

The yield of the chitosan–alginate polyelectrolyte complexes appears to decrease with increasing pH up to 6 from C1 (pH2) complex to C4 (pH6) complex, as seen in Table I. These results support the yield results obtained for carboxymethylcellulose-chitosan complexes published by Fukuda and Kikuchi.[16] Further increase in the reaction pH, however, raises the yield to higher values due to self-precipitation of chitosan, as seen in Table I, sample C5. Thus the relatively high yield at pH 2 can be attributed to self-precipitation of alginate in the reaction medium together with the complex. The earlier elemental analysis results[15,18] that showed a decrease in the content of additional ions incorporated from the reaction solution for the complexes formed at lower pH, which also show higher yield in weight but have lower density of functional groups available for complex formation, can also now be explained by the self-precipitation of alginate in the medium at lower pH (in addition to complex formation).

The formation of complexes between alginate and chitosan at pH values below 3.6 or above 6.5 raises an interesting question. The pK values imply that there should be no —COO^- groups in alginate below pH 3.6, and no —NH_3^+ groups above pH 6.3. If this were the case, however, complex formation could not occur, and yet it does. The complex actually forms so fast that, immediately after dilute solutions of chitosan and alginate are mixed, precipitate or gel formation occurs.

CONCLUSION

The interactions between the polyanion alginate matrix and the polycation chitosan coating are explained by FTIR analysis of the resulting polyelectrolyte complexes. The method used in these studies also explains a discrepancy existing in the literature, between yield studies and the permeability of polyelectrolyte membranes prepared at different pH values.

Nitrogen analysis performed on the beads, which is directly related with the chitosan coating on the alginate surface, demonstrates that the yield of the complex formation must be higher when the complex is prepared at pH 5 than at pH 2, and this result is related to the available ionized carboxylic acid groups on the alginate surface. The presence of the coating and the morphology of the alginate matrix prepared at different pH values are demonstrated with SEM pictures.

References

1. Haug, A. Norwegian Institute of Seaweed Research, Report No. 30, 1964, p 85.
2. Yalpani, M.; Hall, L. D. Macromolecules 1984, 17, 272.
3. Kawashima, Y.; Handa, T.; Kasai, A.; Takanaka, H.; Lin, S. Y.; Anda, Y. J Pharm Sci 1985, 74, 264.
4. Wheatley, M. A.; Chang, M.; Park, E.; Langer, R. J Appl Polym Sci 1991, 43, 2123.
5. Kim, H.; Lee, H.; Oh, J.; Shin, B.; Oh, C.; Park, R.; Yang, K.; Cho, C. J Biomater Sci Polym Add 1999, 10, 543.
6. King, G. A.; Daugulis, A. J.; Faulkner, P.; Goosen, M. F. A. Biotechnol Progress 1987, 3, 231.
7. Kuhn, S. P.; Pfister, R. M. Appl Microbiol Biotechnol 1989, 31, 613.
8. Crist, R. H.; Martin, J. R.; Carr, D.; Watson, J. R.; Clarke, H. J.; Crist, D. R. Environ Sci Technol 1994, 28, 1859.

9. Nestle, N.; Kimmich, R. Appl Biochem Biotechnol 1996, 56, 9.
10. Gregor, J. E.; Fenton, E.; Brokenshire, G.; Brink, P. V. D.; O'Sulvian, B. Water Res 1996, 30, 1319.
11. Simsek-Ege, F. A.; Bond, G. M.; Stringer, J. In Environmental Challenges and Greenhouse Gas Control for Fossil Fuel Utilization in the 21st Century; Moroto Valer, M. M., Soong, Y., Song, C., Eds.; Kluwer Academic/Plenum: New York, in press.
12. Simsek-Ege, F. A.; Bond, G. M.; Stringer, J. World Resource Rev 2001, 13, 74.
13. Huguet, M. L.; Groboillot, A.; Neufeld, R. J.; Poncelet, D.; Dellacherie, E. Polym Sci 1994, 51, 1427.
14. Kokufuta, E.; Shimuzi, N.; Tanaka, H.; Nakamura, I. Biotechnol Bioeng 1988, 32, 756.
15. Fukuda, H.; Kikuchi, Y. Macromol Chem 1977, 178, 2895.
16. Fukuda, H.; Kukuchi, Y. Macromol Chem 1979, 180, 1631.
17. Takashi, T.; Takayama, K.; Machida, Y.; Nagai, T. Int J Pharm 1990, 61, 35.
18. Huguet, M. L.; Neufeld, R. J.; Dellacahherie, E. Process Biochem 1996, 31, 347.