Synthesis and Characterization of Carboxymethyl Chitosan Containing Functional Ultraviolet Absorber Substituent

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ABSTRACT: A series of carboxymethyl chitosan (CMC) with 2.4-dihydoxybenzophenone (UV-0) substituent were synthesized by the Mannich reaction of CMC hydrochloride and UV-0 in the solvent of methanol and water. The different molar degree of substitution (MS) was achieved by changing the UV-0 content. The effects of MS on ultraviolet absorbability, crystallinity, moisture-absorption and -retention property, and photostability were investigated respectively. The obtained products were characterized by means of Fourier transform infrared spectra (FTIR), proton nuclear magnetic resonance spectroscopy (^{1}H NMR), X-ray diffraction, and UV spectrophotometer. It was found that the grafted products were water-soluble, and the increased MS values could enhance the moisture-retention property and photostability, while decreased the crystallinity and moisture-absorption property. The ultraviolet absorption peaks became stronger by introducing UV-0 into the CMC backbones. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 106: 4098–4103, 2007

Key words: graft copolymers; UV–vis spectroscopy; water-soluble polymers

INTRODUCTION

In recent years, there has been a great need for ultraviolet (UV) absorber in cosmetic sunscreen in order to avoid all kinds of UV-induced skin damages such as photoallergies, skin wrinkles, sunburn, or even skin cancer.[1,2]

It is well known that sun radiations ranging from 280 to 400 nm, subdivided into UV-B (280–315 nm) and UV-A (315–400 nm), are noxious to human skin.[3] Some compounds derived from cinnamic acid, 4-aminobenzoic acid, and benzylidencamphor were used for the sun-protecting formulations for the protection from the UV-B radiations. However, their photostabilities were not satisfying. Benzophenone derivatives have a very good photostability, and can act both as UV-B filters with absorption at about 290 nm, and as UV-A filters with absorption at about 325 nm.[4] Therefore, benzophenone derivatives have been well developed as effective UV absorber and some of them are now commercially available. Besides good photostability and UV-absorbability, the cosmetic sunscreen compositions should, however, comply with a number of other properties such as low penetration into the skin, low content of residual monomers, good biocompatibility, and enhancement of moisture.[5,6] To meet these requirements, preparation of suitable graft polymers with UV-absorber substituent could be a solution.

Chitosan (CS) is a polymer of D-glucosamine and N-acetylglucosamine derived from chitin. It has prospective applications in pharmaceutical material owing to its bioantifungal activity, good biocompatibility, and low toxicity.[7] Recently there has been a growing interest in chemical modification of chitosan to improve its water solubility and widen its applications.[8–10] Among various methods, graft polymerization is most attractive because it is a useful technique for modifying the chemical and physical properties of natural polymers. Chitosan bears two types of reactive groups that can be grafted, including the free amino group on deacetylated units and the hydroxyl groups on the C_3 and C_6 carbons on deacetylated units. Thus, grafting of chitosan allows the formation of functional derivatives by covalent binding of a molecule onto chitosan backbone.[11] Some researches have been carried out on the graft polymerization of chitosan in view of preparing polysaccharide-based advanced materials with unique bioactivities and thus widening their applications in biomedicine fields such as controlled drug delivery, antiseptic, and tissue engineering.[7,12–15] Analogously, chitosan can also be used in cosmetic fields by introducing functional UV-absorber as the graft. From this point of view, our current research is aimed at exploring the possibility of developing chitosan-based UV-absorber with better biocompatibility as well as still having UV-absorbability.

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In this study, multiple-derivatized chitosan (CMC-g-UV-0) with various degrees of substitution were prepared by graft polymerization of carboxymethyl chitosan hydrochloride and 2.4-dihydoxybenzophenone (UV-0). Then, the compositions and properties of the obtained polymers were investigated in detail.

EXPERIMENTAL

Materials

CS (off white powder; moisture content: 5%; deacetylation degrees: 84%; $M_w = 3 \times 10^4$) was supplied by Xianju Co. (Zhengjiang, China), purified by extracting with methanol, and dried in vacuum under reduced pressure of about 10 torr for several hours before use. 2.4-Dihydoxybenzophenone (UV-0) was provided by Huayang Co. (Hubei, China). Chloroacetic acid (CA), isopropanol, methanol, ethanol, and formaldehyde were purchased from Lianbang Chemical Reagent Co. (Shenyang, China). All the solvents were purified by distillation over calcium hydride before use. The other reagents were used as received.

Synthesis of carboxymethyl chitosan

Carboxymethyl chitosan (CMC) was prepared according to the literature[16]; concretely, 10 g of chitosan dissolved in 200 mL of isopropanol was placed into a 500 mL flask. After 1 h of stirring at 50°C, 20 g of 50% sodium hydroxide solution was added, and the reactive system was alkalized at this temperature for 1 h. Then a solution of 15 g of CA in 20 mL of isopropanol was added dropwise, and the mixture was reacted at 60°C for 4 h. The resulting product was then removed from the reaction flask, dissolved in distilled water, precipitated in a 10-fold amount of cold methanol, and collected on a filter. The obtained follow product was purified by reprecipitation from water/methanol and then dried in vacuum at 40°C under reduced pressure of about 10 torr for 12 h.

^{1}H NMR (200 MHz, D$_2$O): δ = 2.01 (—NHCOCH$_3$, H); 2.97–3.13 (C$_2$, H); 3.30–3.72 (C$_3$–C$_5$, H; —CH$_2$OH, H); 4.06 (C$_1$, H); 4.60 (D$_2$O); 4.92 (—CH$_2$COO$^-$, H).

Synthesis of carboxymethyl chitosan hydrochloride

10 g of CMC and 100 mL of 20 wt % hydrochloric acid were added into a three-necked flask, stirred for 2 h at 65°C. Then, 50 mL of ethanol was added. After 2 h of reflux, the offwhite precipitate was filtered at room temperature, washed with ethanol, and dried at 40°C in vacuum under reduced pressure of about 10 torr for 12 h.

FTIR (KBr, cm^{-1}): ν = 3427 w (—NH$_2$, —OH), 2925 w (—CH), 1740 vs. (—COOH), 1641–1563 w (imide group), 1070 s (C—O).

^{1}H NMR (200 MHz, D$_2$O): δ = 3.18–3.74 (C$_2$–C$_5$, H; —CH$_2$OH, H); 4.28 (C$_1$, H); 4.61 (D$_2$O); 4.89 (—CH$_2$COO$^-$, H).

Graft polymerization

A series of graft polymer were synthesized by the Mannich reaction of carboxymethyl chitosan hydrochloride (CMCHC) with UV-0 in the solvent of methanol and water. Typical procedures are as follows: CMCHC was treated with formaldehyde in water at 65°C for 1 h. To the reaction mixture, a solution of UV-0 in methanol and water (1 : 1) was added. After stirring at 65°C for 30 h, the N-selectively modified product was collected by precipitation using acetone (1000 mL). The yellow precipitate was collected by filtration, washed with acetone, and dried in vacuum under reduced pressure of about 10 torr at room temperature for 24 h. The different molar degree of substitution (MS) was achieved by changing the UV-0 content. The feed ratios and the corresponding MS of the products are listed in detail in Table I.

Characterization

The Fourier transform infrared (FTIR) spectra (in KBr pellets) were recorded with a Nicolet 20DXB FTIR spectrophotometer. ^{1}H NMR spectra were measured on a Bruker Unity 400 NMR spectrometer at room temperature, with D$_2$O as solvent and TMS as internal reference. The UV spectra were obtained on a 756MC spectrophotometer. Wide angle X-ray diffraction experiments were performed using a XD-3A diffractometer at room temperature with a Cu Kα radiation.

TABLE I
Feed Ratios and Ultraviolet Absorptions of CMC-g-UV-0 with Different MS Values

CMC-g-UV-0 no.	CMCHC : UV-0 (mol/mol)	MS	λ_{max} (nm)	A_{max} (I)
1	1 : 5.0	0.26	338	0.970
2	1 : 3.5	0.24	336	0.779
3	1 : 2.5	0.20	336	0.664
4	1 : 1.5	0.18	338	0.618
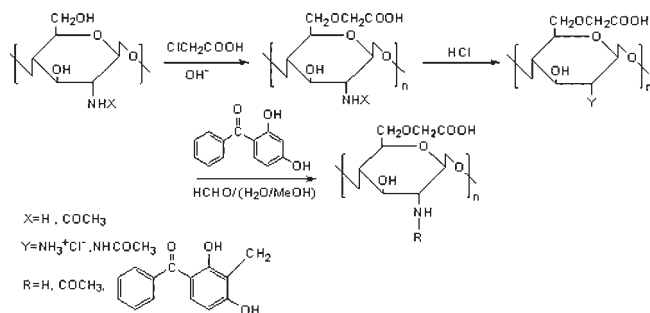
Scheme 1 The synthesis of CMC-*g*-UV-0.

Determination of the MS

^{1}H NMR method is the most effective technique concurrently to determine the substitution site of chitosan derivatives.[17] In the ^{1}H NMR spectrum, d(H) in UV-0 and C_2(H) in CMC were both isolated, and the integral values of them were signed as *A* and *B*, respectively. The MS was thus determined by the equation (MS = *A*/*B*).

Moisture absorption and retention test

The moisture-absorption and -retention ability of the samples were determined according to the method reported by Sun et al.[18] Prior to the moisture-absorption testing, the samples were dried over P_2O_5 in vacuum under reduced pressure of about 10 torr at room temperature for 24 h. The water-absorption ability was evaluated by the percentage of weight increase of dry sample (R_a):

$$R_a(\%) = 100 \times (W_n - W_0)/W_0 \tag{1}$$

W_0 and W_n are the weights of sample before and after putting in the saturated $(NH_4)_2SO_4$ desiccator (81% relative humidity) and the saturated K_2CO_3 desiccator (43% relative humidity) at 20°C for 24 h.

In the moisture-retention test, wet samples were prepared by adding 10% of water to dried samples. The moisture-retention ability was evaluated by the percentage of residual water of wet sample (R_h):

$$R_h(\%) = 100 \times (H_n - H_0) \tag{2}$$

H_0 and H_n are the weights of water in the sample before and after putting in the saturated K_2CO_3 desiccator (43% relative humidity) and the silica gel at 20°C for 24 h.

RESULTS AND DISCUSSION

Synthesis and compositions

A significant effort was made in this study to synthesize a series of graft polymers CMC-*g*-UV-0 with

different MS by the Mannich reaction. The synthesis route of monomers and polymers is shown in Scheme 1.

The compositions of the products were established by FTIR and ^{1}H NMR spectrums. The FTIR spectrum of chitosan [Fig. 1(b)] showed a broad-OH stretch absorption band between 3404 and 3100 cm^{-1} and the aliphatic C—H stretch between 2990 and 2850 cm^{-1}. The absorption peaks between 1200 and 1020 cm^{-1} represented the free primary amino group (—NH_2) at C_2 position. The peak at 1647 cm^{-1} represented acetylated amino group of chitin, which indicated that the sample was not fully deacetylated. The peak at 1384 cm^{-1} represented the —C—O stretch of primary alcoholic group (—CH_2—OH). In the FTIR spectrum of CMC [Fig. 1(a)], the strong peaks at 1598 and 1406 cm^{-1} could be assigned to the asymmetry and symmetry stretch vibration of COO$^-$, respectively. The results also indicated that the carboxymethyl substitution occurred at the C_6 position of chitosan.

The FTIR and ^{1}H NMR spectrums of graft polymers with different MS were almost identical and differed only in relative signal intensities; therefore, we chose CMC-*g*-UV-0/MS = 0.26 as a typical example. In the FTIR spectrum of CMC-*g*-UV-0/MS = 0.26 [Fig. 1(c)], the absorption peak at 1618 cm^{-1} could be assigned to the carbonyl stretch of secondary amides (amide I band), and that at 1500 cm^{-1} could be assigned to the N—H bending vibration of the amide II band. In addition, the broad peak between 2887 and 2950 cm^{-1}, ascribed to the aliphatic chain (—CH_2) and UV-0-CH_2-CMC (—CH_2), were wider in Figure 1(c), compared with the corresponding peaks in Figure 1(a,b). The absorption peak at 1444 cm^{-1} could be assigned to C—H of UV-0-CH_2-CMC. The absorption peak at 1741 cm^{-1} could be represented the (—COOH) stretch vibra-
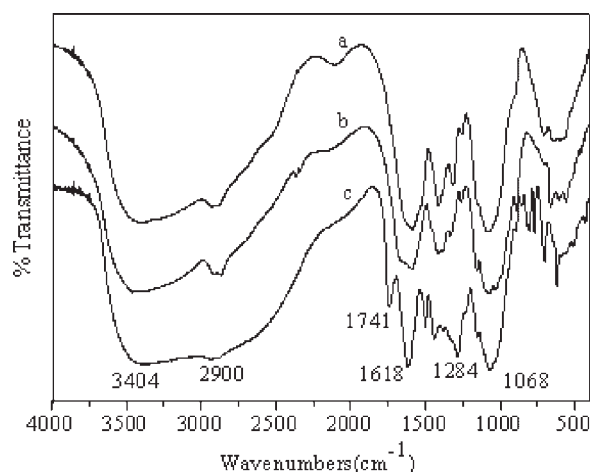

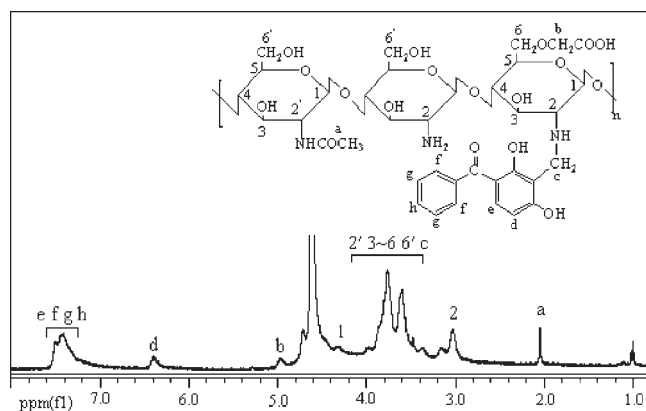**Figure 1** FTIR spectra of (a) CMC, (b) CS, and (c) CMC-*g*-UV-0/MS = 0.26.

Figure 2 ^{1}H NMR spectra of CMC-*g*-UV-0/MS = 0.26 in D$_2$O.

tions of carbonyl groups. Another absorption peaks at 1618 and 1068 cm^{-1} could be represented the stretch vibrations of C=O, and C—O of UV-0 group, respectively. The absorption peak at 1600 and 858 cm^{-1} could be assigned to the skeleton of benzene ring.[19–22] Figure 2 shows the ^{1}H NMR spectrum of CMC-*g*-UV-0/MS = 0.26. The multiplet signals at 3.2–4.2 ppm were originated from C$_3$—C$_6$ (H) and c (H) of methylene. The signal around at 4.61 ppm was ascribed to D$_2$O. The peak at 6.4 ppm was d (H) of UV-0. The multiplet signals at 7.3–7.5 ppm were regarded as the e–h (H) of UV-0. Accordingly, the results confirmed that UV-0 was successfully grafted into CMC.

UV spectra

The ultraviolet patterns of UV-0 and the grafted products with different MS are shown in Figure 3(a,b), respectively. It could be found that UV-0 displayed characteristic ultraviolet absorption peaks at 242, 289, and 326 nm, while the product of CMC without UV-0 substituent could not exhibit any absorption peaks ranging from 220 to 400 nm. When the UV-absorber was introduced into the CMC molecular chain as a graft, the ultraviolet absorption peaks were shifted to 246, 296, and 338 nm, exhibiting that the grafted products possessed UV-absorbability attributed to UV-0, and shifted to infrared. The maximal absorption peak (λ_{max}) and the intensity of peak (A_{max}) are listed in Table I. It was noticed that the ultraviolet absorption peaks became stronger with the increased MS of the grafted products. Furthermore, the better ultraviolet absorbability of the grafted products could also be demonstrated by the broad-spectra from 280 to 400 nm in Figure 3(b), which was attributed to the presence of hydrogen bonds, formed with the chelated reaction among the hydroxyl groups, the carboxyl group in UV-0, and the 2-N group in CMC. The grafted products

existed in the form of macromolecules, which might twist with each other, and thus the chelate ring formed.

Solubility

The solubilities of CMC-g-UV-0 with different MS were measured in the common solvents at 25°C at a concentration of 5 g/L. The results are listed in Table II. It was found that the solubilities of the products were hardly changed in the homogeneous solvents. All the products were well soluble in water, but insoluble in some of organic solvents. Furthermore, the solubilities of the products in isopropanol, DMSO, and DMF were enhanced with the UV-0 graft.

XRD patterns

The XRD patterns of CS, CMC, and CMC-g-UV-0/MS = 0.24 are shown in Figure 4. The sharp diffraction peaks at 15.22°, 20.94°, and 24.83° in Figure 4(a) were indicative of the presence of crystallites in CS.
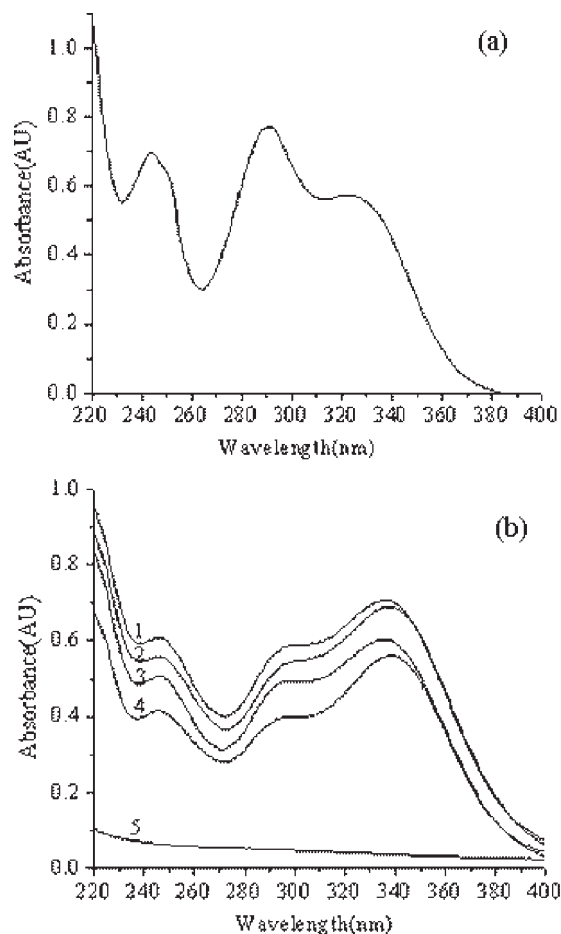

Figure 3 UV spectra of (a) UV-0 and (b) CMC-g-UV-0. 1, MS = 0.26; 2, MS = 0.24; 3, MS = 0.20; 4, MS = 0.18; 5, MS = 0.

TABLE II
Solubility of CMC-g-UV-0 in Various Solvents

Solvents	CMC-g-UV-0/MS				
	0	0.18	0.20	0.24	0.26
Ethanol	Δ	Δ	Δ	Δ	Δ
Isopropanol	Δ	○	○	○	○
Acetone	×	×	×	×	×
Ethyl acetate	×	×	×	×	×
Ethyl ether	×	×	×	×	×
Dichloromethane	×	×	×	×	×
THF	×	×	×	×	×
DMSO	Δ	○	○	○	○
DMF	Δ	○	○	○	○
Toluene	×	×	×	×	×
Pyridine	Δ	Δ	Δ	Δ	Δ
Water	○	○	○	○	○

O: soluble, ○: partly soluble, Δ: swollen, ×: insoluble.

However, for the diffraction patterns of CMC and CMC-g-UV-0/MS = 0.24 in Figure 4(b,c) respectively, the sharp peaks disappeared, but two broadened diffuse peaks at $10.78°$ and $21.23°$ emerged. The probable reason might be that an introduction of carboxymethyl groups destroyed the regularity of the molecule chains of chitosan, resulting in the reduction of crystallinity. Furthermore, compared with the diffraction patterns of CMC, the diffuse peaks of the grafted product were relatively flatter, as a result of the destroyed regularity and integrity of CMC molecule chains by introduction of the UV-0 graft.

Moisture absorption and retention property

The moisture-absorption and -retention ability of CMC-g-UV-0 depended on the MS values. As shown in Table III, with an increase in MS, the retention ability of the grafted products increased gradually;
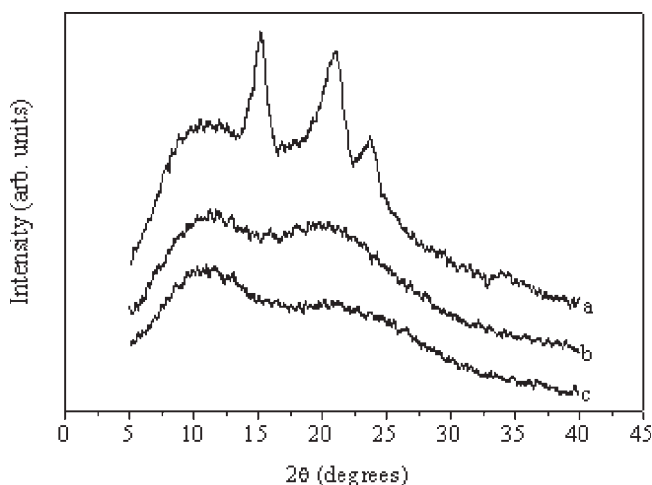

Figure 4 XRD patterns of (a) CS, (b) CMC, and (c) CMC-g-UV-0/MS = 0.24.

TABLE III
Moisture-Absorption and -Retention Abilities of
CMC-g-UV-0 with Different MS Values

CMC-g-UV-0 no.	MS	R_a/% (dry sample)		R_h/% (wet sample)	
		RH = 43%	RH = 81%	RH = 43%	Silica gel
1	0.00	19.78	34.95	84.31	24.86
2	0.18	17.10	32.86	85.08	9.52
3	0.20	15.62	29.58	92.56	10.89
4	0.24	10.66	26.89	106.91	11.57
5	0.26	8.05	22.09	128.92	11.83

RH, relative humidity.

however, the moisture-absorption slightly decreased. The probable reason was that with increasing UV-0 groups, the intermolecular hydrogen bonds in the chelate ring, formed with the chelated reaction among the hydroxyl groups, the carboxyl group in UV-0 and the 2-N group in CMC, increased gradually, which helped to bond large amounts of water and retain them in a spacious network. Therefore, the products with UV-0 graft have potential applications as moisture-retention ingredient in cosmetics. Furthermore, as an oil soluble group, UV-0 substituted for hydrogen of amide, which weakened the moisture-absorption of the products. In silica gel, the moisture-retention ability of the grafted products also increased with increasing in MS, but was weaker than that of CMC. The results indicated that the grafted products had incompetent moisture-retention ability in low RH atmosphere.

Photostability

The photostability was estimated by residue in UV absorption spectra in detail as follows: 60 ppm grafted products in water and 10 ppm UV-0 in 95% ethanol were exposed in sun-irradiation for 2 weeks, and then measured by UV spectra. The residue was thus obtained by the ratio of the absorption peaks (200–400 nm) between after and before sun-exposure.[23] The evaluations of the photostability are listed in Table IV. It was found that the graft polymers had better photostability than the monomer UV-0, probably due to the presence of intermolecular hydrogen bonds, resulting in enhancing the intermolecular interactions.

CONCLUSION

A series of CMC-g-UV-0 with different MS were synthesized by graft polymerization of CMCHC and UV-0. The effects of MS on ultraviolet absorbility, crystallinity, moisture-absorption and -retention property, and photostability were investigated respectively. The introduction of UV-0 was confirmed by FTIR

TABLE IV
The Evaluation of Phtotostability of CMC-*g*-UV-0 and UV-0

Products	CMC-*g*-UV-0				UV-0
	MS = 0.26	MS = 0.24	MS = 0.20	MS = 0.18	
Residue (UV-absorption)	96.6	98.2	96.4	99.2	90.9
Photostability	□	□	□	□	○
Color change	No	No	No	No	No

□, Residue ≥ 95%; ○, Residue < 95%.

and ^{1}H NMR spectra. The obtained products were well soluble in water. With the increasing MS values, the UV-absorbability, moisture-retention property, and photostability increased, while crystallinity decreased, owing to the presence of intermolecular hydrogen bonds in chelate ring and the reduction in molecular chain regularity, respectively. The broad UV spectra from 280 to 400 nm and excellent moisture-retention properties make the grafted products have potential applications as sunscreen ingredient in cosmetics.

Reference

1. Afaq, F.; Mukhtar, H. J Photoch Photobiol B 2001, 63, 61.
2. Schulz, J.; Hohenberg, H.; Pflucker, F.; Gärtner, E.; Will, T.; Pfeiffer, S.; Wepf, R.; Wendel, V.; Gers-Barlag, H.; Wittern, K. P. Adv Drug Deliver Rev 2002, 54, S157.
3. Czajkowski, W.; Paluszkiewicz, J.; Stolarski, R.; Kazmierska, M.; Grzesiak, E. Dyes Pigments 2006, 71, 224.
4. Raspanti, G.; Malpede, A. U.S. Patent 5,776,439 (1998).
5. Keller, H. G. U.S. Patent 5,869,099 (1999).
6. Chaudhuri, R. K.; Bower, D. B. U.S. Patent 5,736,128 (1998).
7. Chen, S. C.; Wu, Y. C.; Mi, F. L; Lin, Y. H.; Yu, L. C.; Sung, H. W. J Control Release 2004, 96, 285.
8. Sashiwa, H.; Shigemasa, Y. Carbohydr Polym 1999, 39, 127.
9. Terada, N.; Morimoto, M.; Saimoto, H.; Okamoto, Y.; Minami, S.; Shigemasa, Y. Chem Lett 1999, 28, 1285.
10. Heras, A.; Rodriguez, M. N.; Ramos, V. M. Carbohydr Polym 2001, 44, 1.
11. Joshi, J. M.; Sinha, V. K. Carbohydr Polym 2007, 67, 427.
12. Hu, Y.; Du, Y.; Yang, J.; Kennedy, J. F.; Wang, X.; Wang, L. Carbohydr Polym 2007, 67, 66.
13. Guo, Z.; Xing, R.; Liu, S.; Yu, H.; Wang, P.; Li, C.; Li, P. Bioorg Med Chem Lett 2005, 15, 4600.
14. Shi, X.; Du, Y.; Yang, J.; Zhang, B.; Sun, L. J Appl Polym Sci 2006, 100, 4689.
15. Qian, F.; Cui, F.; Ding, J.; Tang, C.; Yin C. Biomacromolecules 2006, 7, 2722.
16. Liu, X. F.; Guan, Y. L.; Yang, D. Z.; Li, Z.; Yao, K. D. J Appl Polym Sci, 2001, 79, 1324.
17. Chen, L.; Du, Y.; Wu, H.; Xiao, L. J Appl Polym Sci, 2002, 83, 1237.
18. Sun, L.; Du, Y.; Yang, J.; Shi, X.; Li, J.; Wang, X.; Kennedy, J. F. Carbohydr Polym 2006, 66, 168.
19. Omura, Y.; Taruno, Y.; Irisa, Y.; Morimoto, M.; Saimoto, H.; Shigemasa, Y. Tetrahedron Lett 2001, 42, 7273.
20. Chen, X. G.; Park, H. J. Carbohydr Polym. 2003, 53, 355.
21. Zhang, L.; Guo, J.; Zhou, J.; Yang, G.; Du, Y. J Appl Polym Sci 2000, 77, 610.
22. Zhao, X.; Kato, K.; Fukumoto, Y.; Nakamae, K. Int J Adhes Adhes 2001, 21, 227.
23. Masaru, S.; Eijiro, H. Jpn Patent 371,265 (2002).