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Synthesis and characterization of biodegradable TPP/genipin co-crosslinked chitosan gel beads

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Abstract

Novel chitosan gel beads were synthesized by a coupled ionic and chemical co-crosslinking mechanism. Tripolyphosphate (TPP) and a naturally occurring crosslinking reagent, genipin, which has been used in herbal medicine, were employed, respectively, as an ionic and a chemical crosslinkers to prepare the chitosan-based networks of gel beads. The competitive crosslinking of chitosan with ionic crosslinker (TPP) and chemical crosslinker (genipin) was characterized by FTIR, UV and EDAX spectroscopy (X-ray energy dispersion) spectroscopy. The variation of characteristic peak of genipin observed from UV spectroscopy and the characteristic peak of tripolyphosphate in crosslinked chitosan-based networks observed from FTIR spectroscopy suggests that the co-crosslinking mechanism is dependent on the pH of TPP/genipin co-crosslinker. The energy profiles of carbon and phosphorus estimated from confirms that chemical crosslinking dominates the co-crosslinking reaction at higher pH condition (pH 7.0 and 9.0) and ionic crosslinking dominates the co-crosslinking reaction at lower pH condition (pH 1.0, 3.0 and 5.0). The pH-dependent ionic/chemical co-crosslinking mechanism shows an obvious effect on the swelling property and enzymatic degradation behavior of prepared chitosan networks. These results reveal that the ionic/chemical co-crosslinked chitosan networks may be suitable for biomedical applications.

Keywords: Chitosan; Tripolyphosphate; Genipin

1. Introduction

Chitosan is a polysaccharide that is obtained by alkaline deacetylation of a naturally occurring abundant polysaccharide, chitin. It is biodegradable, biocompatible, non-immunogenic and non-carcinogenic, which makes it a suitable compound for biomedical applications, such as wound management [1–4], tissue engineering [5–7] and drug delivery vehicle [8–12].

Chitosan is stable in neutral condition due to the fact that the amine and hydroxyl groups on glucosamine unit can form strong inter- and intra-molecular hydrogen bond to crystallize. The crystal structure was broken down in acidic condition due to the electrostatic repulsion between protonated amine groups on chitosan. To preserve the stability of chitosan gel under GI (gastrointestinal) tract delivery or enzymatic degradation, its amine groups on polymeric chains have to be fixed by crosslinking. In the previous studies the preparation and application of cross-linked chitosan gels for drug delivery and biomedical purposes was reported. These chitosan gels were prepared by chemically crosslinked with glutaraldehyde, ethylene glycol diglycidyl ether (EGDE), and hexamethylenediisocyanate [13–16]. Many studies also reported the synthesis of chitosan-based derivatives via chemical modification [17–22].

Tripolyphosphate (TPP) is a non-toxic polyanion which can interact with chitosan via electrostatic forces to form ionic crosslinked networks [23]. TPP can be used for the preparation of chitosan beads and microspheres because of its quickly gelling ability [23]. Recently, we also reported the preparation of chitosan gel using a naturally occurring crosslinker—genipin [24–26]. Genipin was obtained from its parent compound traditionally used as a component of

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Chinese medicine, geniposide, which may be isolated from the fruits of Gardenia jasminoides Ellis [27,28]. In the previous study of Sung et al., it was found that genipin-crosslinked networks are significantly less cytotoxic than those crosslinked by glutaraldehyde and can be widely used for various biomedical applications [29–32]. The crosslinking of chitosan in TPP/genipin co-crosslinker may be competitive and complementary due to the fact that chitosan can be ionically and chemically crosslinked by TPP and genipin, respectively. The physicochemical properties such as chain fixation (crosslinking) and relaxation (swelling), and enzymatic degradability of these chitosan-based networks prepared under different pH conditions may be manipulated by the competitive ionic/chemical co-crosslinking reaction. This prompted us to evaluate the possibility of using such a combined ionic and chemical co-crosslinking method to prepare suitable chitosan-based networks for biomedical and drug delivery applications.

This paper aims to examine the effects of ionic and chemical crosslinking on the preparation of different chitosan-based networks, and also to evaluate which factor dominates the co-crosslinking reaction. The fixation of polymer chain, respectively, by ionic and chemical crosslinking varied with the change in pH value of TPP/genipin co-crosslinker, were studied by UV, FTIR spectroscopy and EDAX (X-ray energy dispersion) analysis to elucidate this pH-dependent co-crosslinking mechanism. The second aim was to examine the effect of ionic/chemical co-crosslinking reaction on swelling properties (chain relaxation) and enzymatic degradation (chain scission) of the crosslinked chitosan hydrogels. All the results investigated were used to evaluate the possibility of the ionic/chemical co-crosslinked chitosan hydrogels as potential materials for biomedical applications.

2. Experimental

2.1. Materials

Sodium tripolyphosphate (TPP) and lysozyme (48,000 unit/ml, extracted from egg white) were purchased from Sigma Chemical Co. (USA). Chitosan [degree of deacetylation (DD) = 85%] in the form of powder was of commercial grade, and genipin (obtained from geniposide) was kindly gifted by Challenge Bioproducts Co., Taiwan. All other reagents and solvents used were of reagent grade.

2.2. Preparation of crosslinked chitosan gel beads

Chitosan powder (3 g) was dispersed in 50 ml of water containing 0.5 wt% acetic acid. The mixture was mechanically stirred at 600 rpm for 3 h to prepare dissolved chitosan solution. The TPP/genipin co-crosslinker is an aqueous solution composed of ionic crosslinker (TPP) and chemical crosslinker (genipin). Firstly, the TPP powder was dissolved in deionized water to prepare 0.1 M TPP aqueous solutions. The pH values of TPP aqueous solutions were adjusted from pH 9.0 (original pH value) to pH 7.0, 5.0, 3.0 and 1.0, respectively. Afterwards, genipin was added into different pH values of TPP aqueous solutions to prepare the TPP/genipin co-crosslinker (0.1 M TPP/0.1 M genipin). The chitosan solution was directly dropped through a syringe needle into the solution of TPP/genipin co-crosslinkers, and the chitosan droplets were stood in the solution for 24 h to cross-link the gel beads. After crosslinking, the solidified gel beads were separated and washed thoroughly with deionized water under stirring for 2 days to remove residual ionic/chemical crosslinking agents (TPP and genipin), then dried in air for 24 h to collect the final products.

2.3. Characterization

The chitosan gel beads were prepared by co-crosslinked in different pH values of TPP/genipin aqueous solutions. The crosslinked chitosan derivatives were recorded as KBr pellets on a Perkin–Elmer 983 spectrometer. The co-crosslinking agents (genipin/TPP aqueous solutions) withdrew at predetermined time were diluted and spectrally analyzed by Hitach 250 UV–vis spectrophotometers (Japan) to examine the crosslinking mechanism at different pH conditions.

2.4. EDAX analysis

The energy profiles of carbon and phosphorus distributed in the co-crosslinked chitosan gel bead was analyzed by X-ray energy dispersion analysis (EDAX). The beads were cut by a razor and were adhered onto double-sided tape, sputter-coated with gold to about 500×10^{-8} cm thickness using an Hitachi coating unit IB-2 coater under a high vaccum, 0.1 Torr, high voltage, 1.2 kV and 50 mA. Cross-section of coated samples were examined by Hitachi S-2300 scanning electron microscopy with an attachment of EDAX analyzer (Delta Class Analyzer, Level I).

2.5. Determination of crosslinking degree

The degree of crosslinking degree could be determined by ninhydrin assay, was defined as the percentage of free amino groups in the crosslinked chitosan gel beads. In the ninhydrin assay, the gel beads was lyophilized for 24 h and then weighed. Subsequently, the lyophilized gel beads were heated with a ninhydrin solution for 20 min. After heating with ninhydrin, the optical absorbance of the solution was recorded with a spectrophometer (Model UV-150-02; Shimadzu Corp., Kyoto, Japan) using D-glucosamine at various known concentrations as standard. It is known that the amount of free amino groups in the test sample, after heating with ninhydrin, is proportional to the optical absorbance of the solution.

2.6. Swelling

The swelling capacities of co-crosslinked chitosan gel beads were determined by swelling the beads in pH 1.2 and 7.4 of medium at room temperature, respectively. A known weight (200 mg) of the chitosan beads was placed in the medium for the required period of time. The swollen beads were collected by filter and the wet weight of the beads were determined by first blotting the cross-linked beads with filter paper to remove adsorbed water on the surface, then weighed immediately on an electronic balance. The swelling ratio of co-crosslinked chitosan beads in the media were then calculated from the formula

$$E_{sw} = [(W_e - W_o)/W_o]$$

where E_{sw} is the swelling ratio of co-crosslinked chitosan beads at equilibrium. W_e denotes the weight of co-crosslinked chitosan beads at equilibrium swelling ratio and W_o is the initial weight of co-crosslinked chitosan beads.

Swelling reversibility of chitosan gel beads was studied by measuring the beads swelling in response to repeated changes in pH between 1.2 and 7.4. Each swelling experiment was repeated 3 times and the average value was taken as the percentage swelling value.

2.7. Enzymatic degradation

In vitro degradation of the chitosan-based networks prepared by crosslinking at different conditions were performed using lysozyme with an activity of 48,000 U/mg (solid form). The co-crosslinked chitosan gel beads were immersed in a 1000 U/ml lysozyme solution and incubated at 37 °C in pH 7.4 buffer solution for 12 weeks. After degradation, the cleaving β-glycosidic bonds of chitosan and the formation of oligomers containing *N*-glucosamine units resulted in increments in free amino group content in the incubation medium, which could be determined by ninhydrin assay. In the ninhydrin assay, the incubation medium were heated with ninhydrin for 20 min. After heating with ninhydrin, the optical absorbance of the solution at 570 nm was recorded with a Hitach 250 UV–vis spectrophotometer. It is known that the amount of free amino groups in the test sample, after heating with ninhydrin, is proportional to the optical absorbance of the solution. Digestion rates of the chitosan beads were examined by analyzing the increased *N*-glucosamine units in the incubation medium at pre-determined time.

3. Results and discussion

3.1. Properties of crosslinked chitosan beads

The TPP/genipin co-crosslinker at different pH conditions (pH 1.0, 3.0, 5.0, 7.0 and 9.0) were all optically clear, and were used in this study to prepare chitosan gel beads. After crosslinking, the co-crosslinkers were also kept clearly transparent; however, colors of the beads changed from white to dark blue with the variation of pH value of co-crosslinker from pH 1.0 to 9.0. The observation of color change reveals that the co-crosslinking mechanism may be pH-dependent. The beads prepared in higher pH value of co-crosslinker were brittle than the beads prepared at acidic condition. All chitosan gel beads prepared by the co-crosslinked method at different pH conditions were in good sphericity.

3.2. Characterization of crosslinking mechanism

Chitosan gel beads were obtained by the fixation of its amine groups with a co-crosslinking agent composed of TPP (ionic crosslinker) and genipin (chemical crosslinker). The negative charged TPP ions can react with positive charged chitosan via electrostatic attraction to form ionic crosslinked networks, and genipin reacts with chitosan via a covalent bonding to form chemical crosslinked networks [23,24]. The extent of chemical crosslinking could be examined by UV spectra analysis. It was found that genipin could be dissolved in TPP aqueous solution to form the ionic/chemical co-crosslinker, and displayed its characteristic absorption peak at 240 nm. Genipin reacted chemically with chitosan via a nucleophilic attack by amino group on the olefinic carbon atom at C-3 of deoxyloganin aglycone, followed by opening the dihydropyran ring and attacked by the secondary amine group on the resulting aldehyde group. In the solution of TPP/genipin co-crosslinker, the characteristic absorption peak at 240 nm decreased with the increase of reaction time due to the ring-opening of genipin via the crosslinking reaction. As shown in Fig. 1, the absorbance of characteristic peak at 240 nm decreased slowly at lower pH conditions (pH 1, 3 and 5 of TPP/genipin co-crosslinker) but quicker at neutral and basic conditions (pH 7.0 and 9.0 of TPP/genipin co-crosslinker), suggested that chemical crosslinking (crosslinked by genipin) of chitosan gel beads can be inhibited due to the presence of H^+ in the co-crosslinking process.

Fig. 2 shows the IR spectrum of chitosan gel beads crosslinked by TPP/genipin co-crosslinker at different pH conditions. The IR spectrum of chitosan showed peaks of assigned saccharide structure around 905 and 1153 cm^{-1} and a protonated amino characteristic peak at around 1570 cm^{-1}. There is a stronger absorption band at 1650 cm^{-1} of assigned amide groups. The appearance of characteristic peak at 1150 cm^{-1} assigned to P=O groups of TPP is the evidence of ionic crosslinking of chitosan. It is clearly found that the intensity of P=O absorbance at 1150 cm^{-1} of crosslinked chitosan gel beads increases with the decrease in pH values of the co-crosslinker; suggests that chitosan can bind with TPP ions more easily at lower pH conditions. These results from UV and FTIR analysis indicate that the reaction of ionic/chemical co-crosslinking

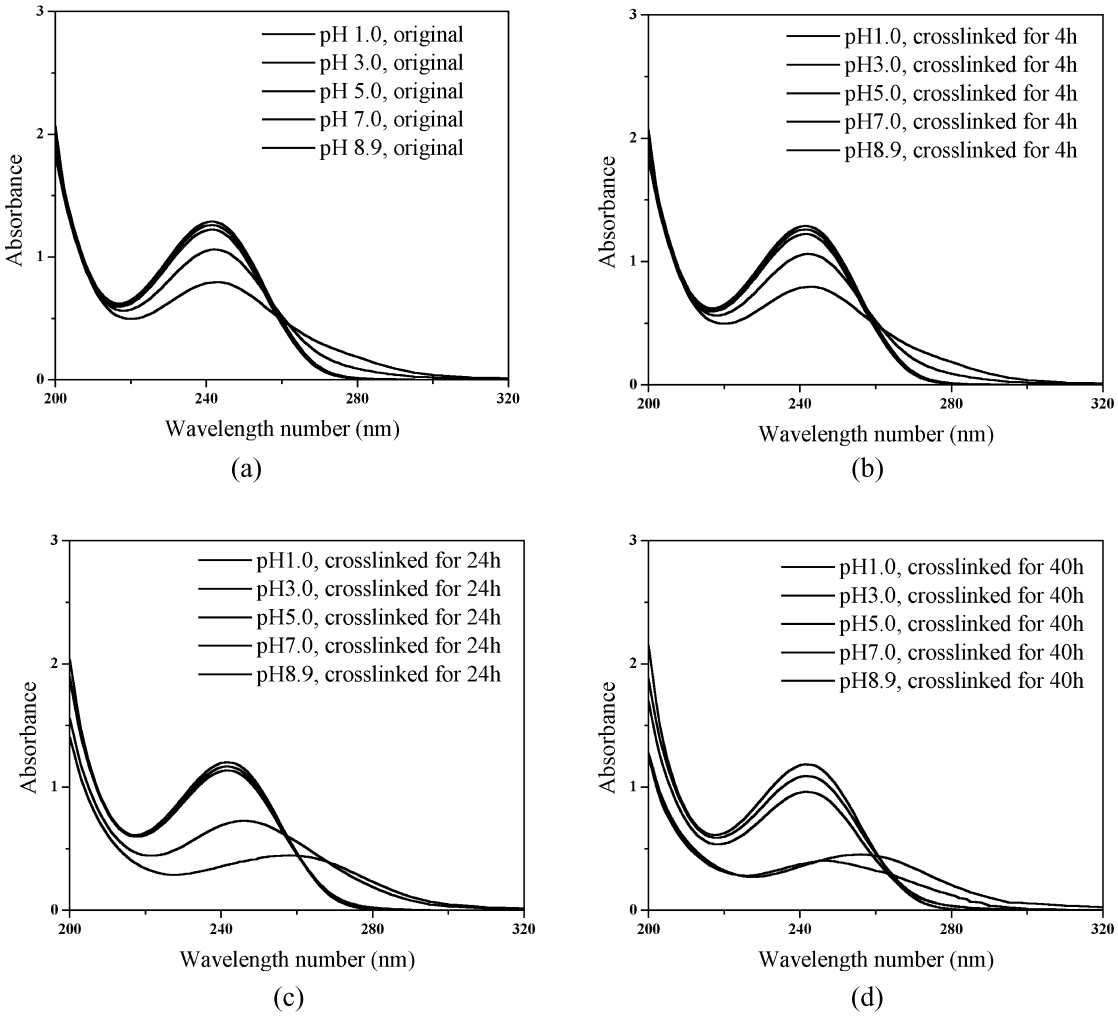


Fig. 1. Variation of UV spectra detected from TPP/genipin co-crosslinking agent after reacted with chitosan at different pH-conditions.

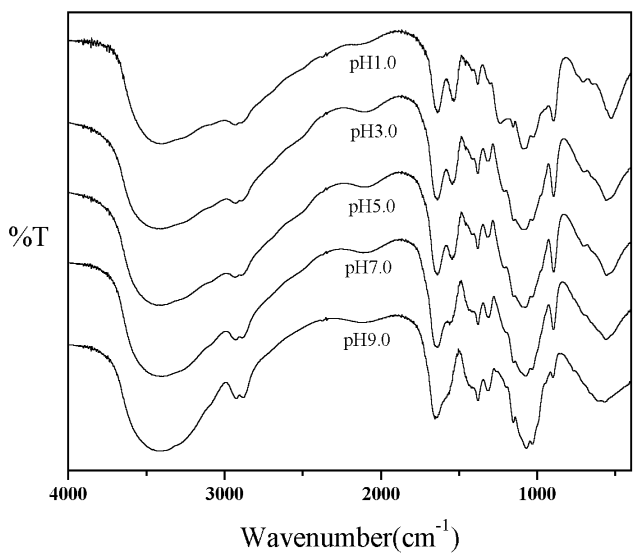
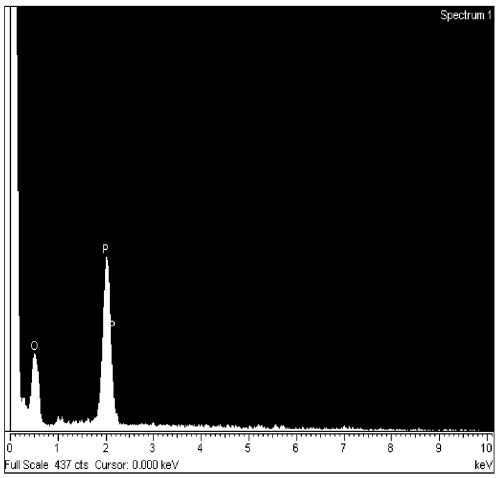


Fig. 2. FTIR spectra of TPP/genipin co-crosslinked chitosan networks prepared at different pH-conditions.

mechanism may be pH-dependent; however, the effect of pH on ionic and chemical crosslinking, respectively, are inversive.

3.3. EDAX studies

The study of UV and IR analysis reveal that the crosslinking of chitosan gel beads in TPP/genipin co-crosslinker are manipulated by ionic crosslinking with TPP ions and chemical crosslinking with genipin, and is dependent on pH value of the co-crosslinker. This hypothesis can be further confirmed by EDAX analysis to evaluate the extent of ionic and chemical crosslinking of chitosan gel beads, respectively. The profiles of carbon and phosphorus distributed on the cross-section of each chitosan bead are determined by EDAX to examine the relative ionic and chemical crosslinking tendency (Fig. 3). The energy profile is a significant index to examine the content of carbon and phosphorus in crosslinked chitosan gel beads, respectively. It is obvious that pH of TPP/genipin co-crosslinker is a major parameter which greatly influences the uptake of carbon and phosphorus to the crosslinked



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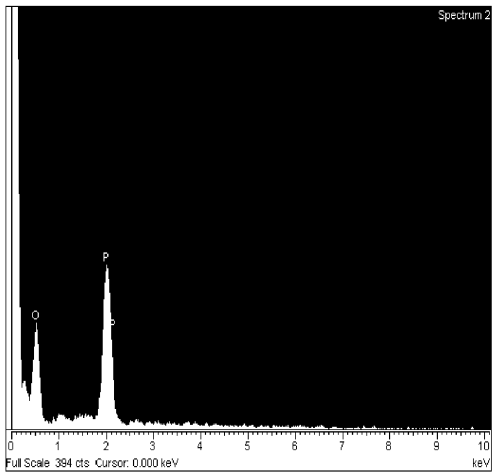

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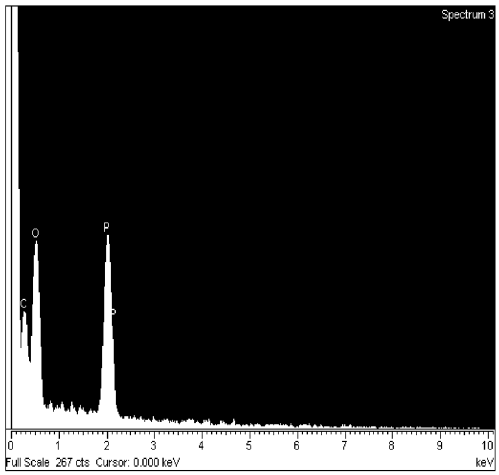

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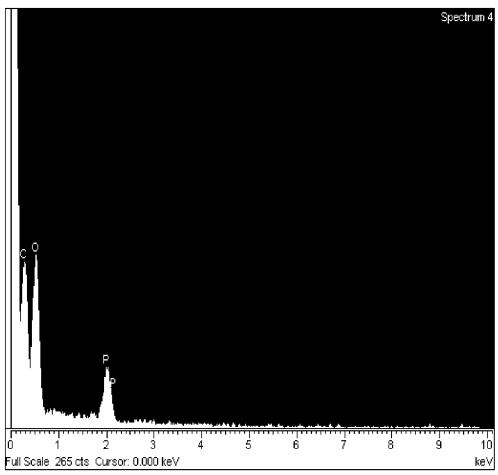

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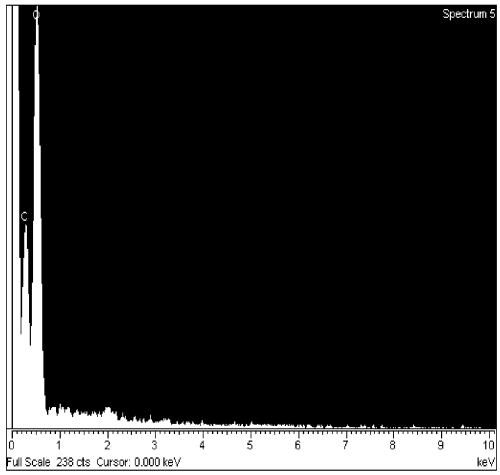


(5)

Fig. 3. EDAX analysis of energy profiles of TPP/genipin co-crosslinked chitosan networks: (1) pH 1.0 (2) pH 3.0 (3) pH 5.0 (4) pH 7.0 (5) pH 9.0.

chitosan beads. The degree of cross-linking and data of elemental analysis also demonstrates the same tendency (Table 1). At high pH condition, the competition between TPP and genipin causes a low uptake capacity of phosphorus and a high uptake capacity of carbon under crosslinking, suggests that amine groups on chitosan are majorly bound with genipin via chemical linkage. The contrasting condition for the uptake of carbon and phosphorus are observed from the chitosan gel bead prepared in low pH of TPP/genipin co-crosslinker, indicates the crosslinked chitosan networks prepared at lower pH condition are majorly bound with TPP ions via ionic linkage. These results suggest that the presence of hydrogen ion in TPP/genipin co-crosslinker significantly encourages the ionic crosslinking reaction but depresses the chemical crosslinking reaction.

In the original pH of TPP/genipin (pH 9.0, basic) co-crosslinker, OH^- and $P_3O_{10}^{5-}$ ions, and genipin coexisted in the aqueous solution. The NH_3^+ groups on chitosan are mostly deprotonated by OH^-, and partially ionic crosslinked by $P_3O_{10}^{5-}$ instantaneously. The free amino groups ($-NH_2$) on chitosan are chemically crosslinked with genipin more easily. On the contrary, H^+ and $P_3O_{10}^{5-}$, and genipin coexisted in the acidic TPP/genipin co-crosslinkers (pH 1.0, 3.0, and 5.0). Due to the reason that the rate of electrostatic attraction between $P_3O_{10}^{5-}$ and NH_3^+ group on protonated chitosan is very quick, and genipin cannot easily react with protonated amino groups on chitosan via nucleophilic attack. It may therefore be postulated that the ionic crosslinking of chitosan with TPP should be a dominated reaction for the co-crosslinking of chitosan gel beads in lower pH. Oppositely, the chemical crosslinking of chitosan with genipin is a dominated reaction for the co-crosslinking of chitosan gel beads in higher pH. The schematic co-crosslinking mechanism is shown in Fig. 4.

3.4. Swelling studies

To examine the possibility of biomedical application, the prepared chitosan gel beads is swollen in different pH aqueous solution to simulate the conditions of GI tract drug delivery. It is known that whether the macromolecular chains of a polymer are fixed by ionic crosslinking or chemical crosslinking, the swelling ability of the polymeric networks is reduced. Fig. 5 shows the equilibrium swelling behavior of the TPP/genipin co-crosslinked chitosan gel beads at different pH conditions (pH 1.2 and 7.4). An obvious increase in water uptake, together with pH varying from pH 7.4 to 1.2, was observed for the TPP/genipin co-crosslinked chitosan gel beads. Since the pK_a of chitosan is ~6.3, the hydrogen bonds was broken in acid, electrostatic repulsion would arise between protonated amine groups on chitosan macromolecule. It is worth noting that the chitosan gel beads prepared in more acidic TPP/genipin co-crosslinker have lower swelling ratio. Because the ionic crosslinking dominated co-crosslinking reaction is much quicker than its chemical crosslinking dominated counterpart, the chitosan gel beads prepared by crosslinking in acidic TPP/genipin co-crosslinker leads to the increase of entire crosslinking degree (containing less free NH_3^+ moieties). The increased entire crosslinking degree is responsible for the decreased swelling extent of chitosan gel beads.

Table 1
The degree of cross-linking and data of elemental analysis of TPP/genipin co-crosslinked gel beads

Type	C (%)	H (%)	N (%)	O (%)	P (%)	Crosslinking degree
pH 1	13.70	3.45	2.14	50.34	30.37	84%
pH 3	20.23	4.34	3.31	50.08	22.04	78%
pH 5	28.53	6.51	4.95	51.54	8.47	65%
pH 7	33.42	6.68	5.02	49.38	5.50	71%
pH 9	43.49	7.06	5.72	43.61	0.12	47%

To examine the dynamic swelling properties of prepared chitosan gel beads under medium jump from pH 1.2 to 7.4, water uptake of the crosslinked chitosan beads in different pH aqueous solution is investigated (Fig. 6). The results demonstrated that the beads changed their ability to absorb solution when the environmental pH is altered. The beads swell in simulated gastric juice (pH 1.2) and deswell in simulated intestinal fluid (pH 7.4). It seems that the response rate for swelling–deswelling of the chitosan gel beads prepared at different conditions are similar; however, the response amplitude for swelling–deswelling of the chitosan beads are quite different. Chitosan gel beads co-crosslinked at higher pH conditions have larger swelling–deswelling amplitude than the beads co-crosslinked at lower pH conditions. This is reasonable since the variation of response amplitude is majorly attributed to the difference of electrostatic repulsion arise from protonated amine groups (NH_3^+) in various crosslinked chitosan beads. At acidic crosslinking conditions, ionic crosslinking dominates the co-crosslinking mechanism. The increased fixation degree of free NH_2 moieties on the chitosan polymeric chain indeed depressed the electrostatic repulsion between adjacent protonated amino groups of the chitosan hydrogel beads, leading to the decrease of their swelling–deswelling amplitude.

Fig. 7 shows the morphology of various chitosan gel beads dried after repeated swelling–deswelling. It is clear to find that the chitosan gel beads prepared by crosslinking with lower pH of co-crosslinker (ionic crosslinking dominated) are intact; however, beads prepared by crosslinking with higher pH of co-crosslinker (chemical crosslinking dominated) are all cracked. This result can be attributed to the unequal swelling extent in the outer and inner regions of the chitosan gel beads prepared at basic conditions. As described in the previous studies [23], ionic crosslinking of chitosan gel beads with TPP at acidic condition resulted in homogeneously distributed phosphorus throughout whole chitosan gel beads. On the

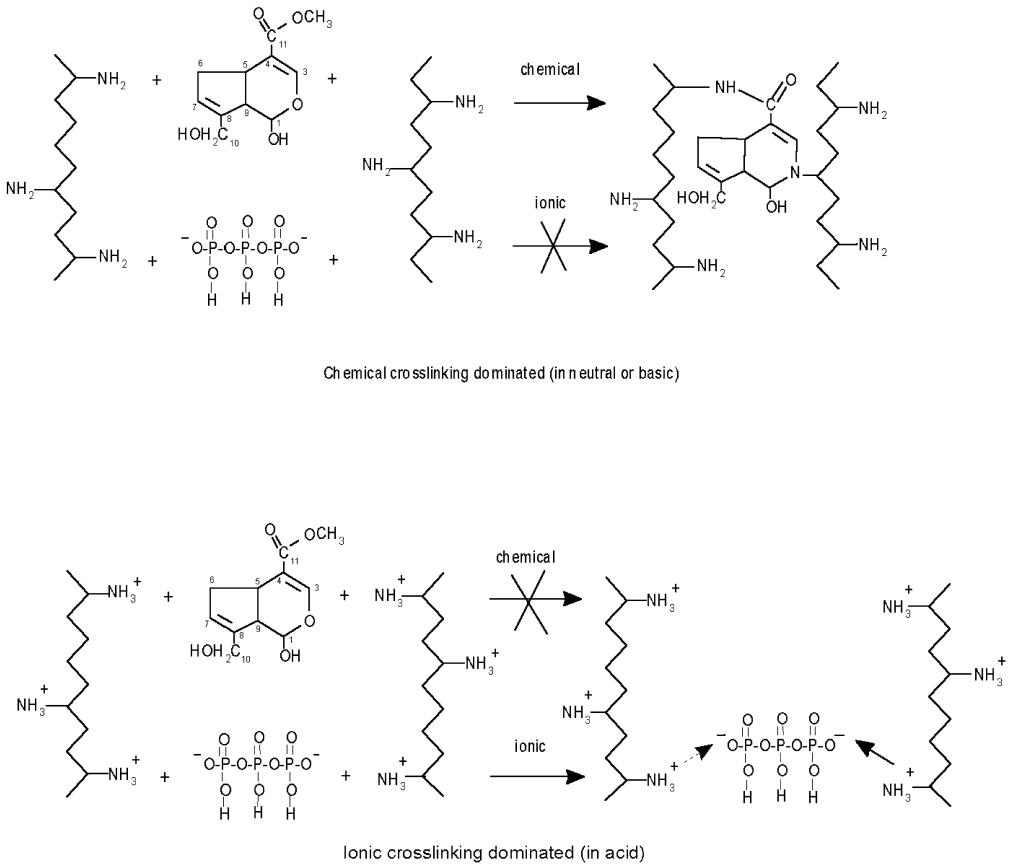


Fig. 4. Schematic TPP/genipin co-crosslinking mechanism for the preparation of chitosan networks.

contrary, the phosphorus of chitosan gel beads ionic-crosslinked at basic (or neutral) are locally distributed in outer region of the beads. Due to the reaction of ionic crosslinking is much slower than chemical crosslinking, the crosslinking of chitosan in basic TPP/genipin co-crosslinker will lead to the unequal swelling tendency in different region of co-crosslinked chitosan gel beads. Accordingly, the gel beads are cracked after repeated swelling–deswelling.

3.5. Enzymatic degradation of chitosan gel beads

Fig. 8 presents the degradation profile of the TPP/genipin crosslinked chitosan beads in terms of the increments in free

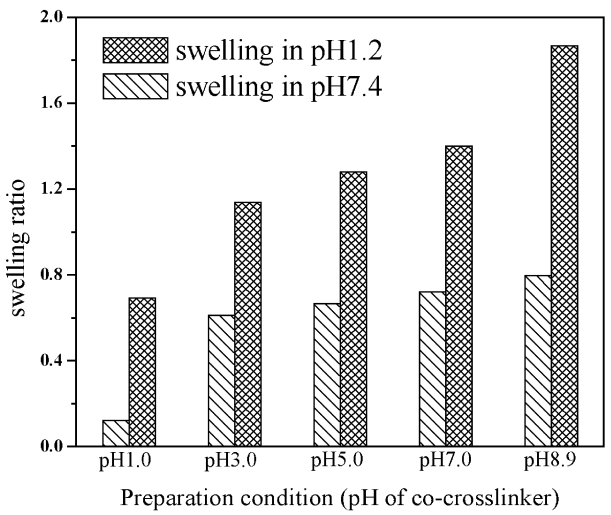


Fig. 5. Equilibrium swelling of the TPP/genipin crosslinked chitosan gel beads in pH 1.2 and pH 7.4 media.

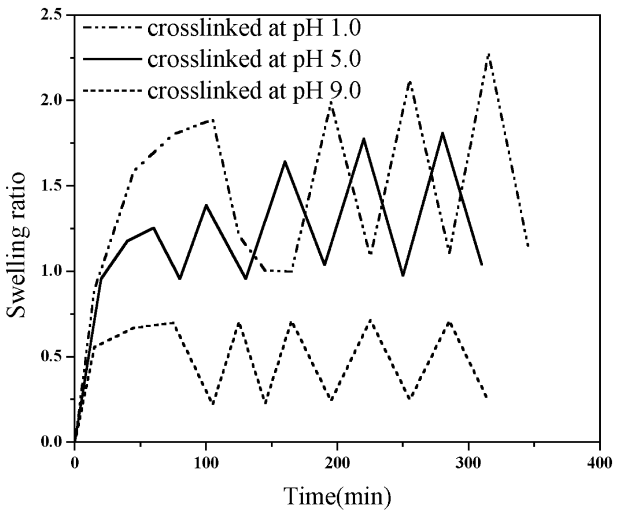


Fig. 6. Dynamic swelling–deswelling of the TPP/genipin crosslinked chitosan gel beads in simulated gastric juice and intestinal fluid.

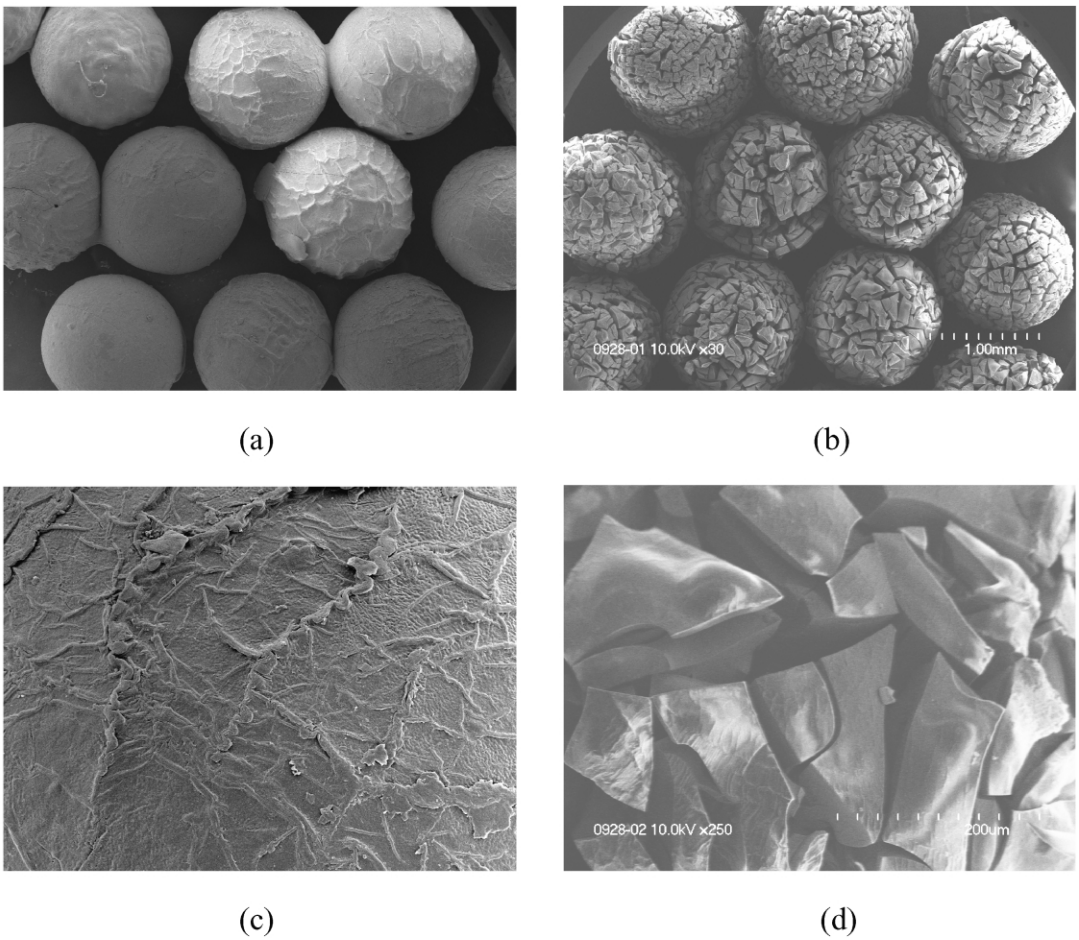


Fig. 7. Morphology of TPP/genipin crosslinked chitosan gel beads (crosslinked at pH 3.0 and pH 9.0) dried after repeated swelling–deswelling: (a) crosslinked at pH 3.0 (× 30) (b) crosslinked at pH 9.0 (× 30) (c) crosslinked at pH 3.0 (× 250) (d) crosslinked at pH 3.0 (× 250).

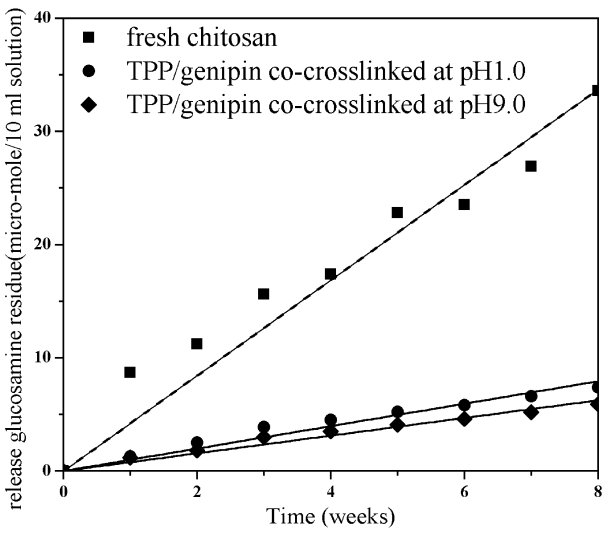
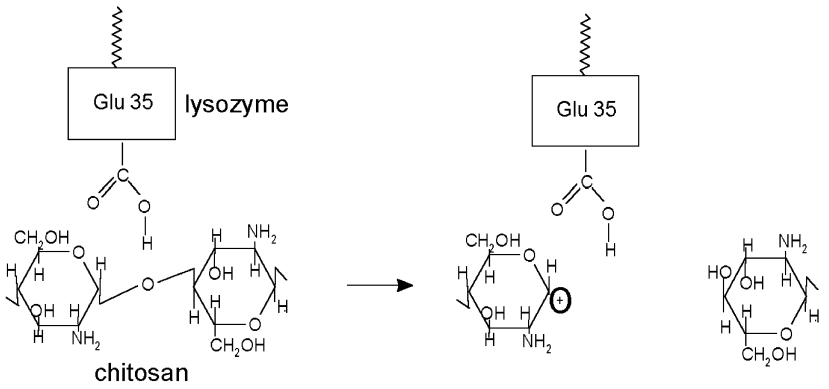


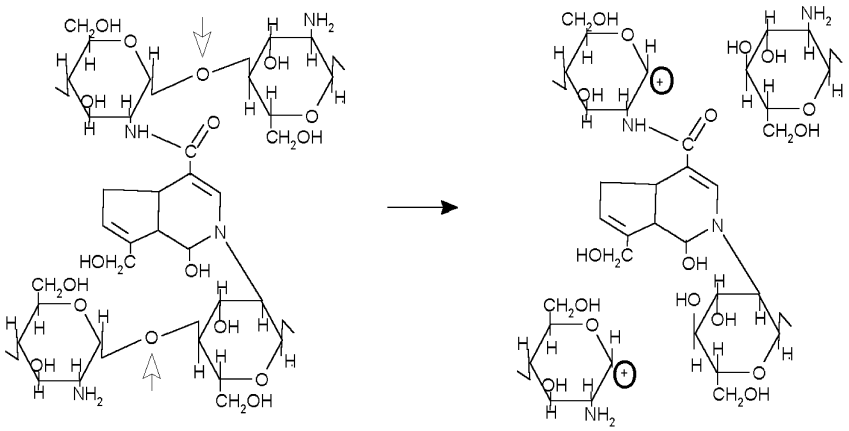
Fig. 8. Degradation profile of the TPP/genipin crosslinked chitosan beads in terms of the increments in free amino group content in the incubation medium vs. time: (■) fresh chitosan bead, (●) TPP/genipin co-crosslinked at pH 1.0, (◆) TPP/genipin co-crosslinked at pH 9.0.

amino group content within 8 weeks of lysozyme incubation. As shown in the figure, it was observed that the increment in free-amino-group content of medium incubated with fresh chitosan gel bead (without crosslinking) was significantly greater than that observed in the media incubated with the TPP/genipin co-crosslinked chitosan beads. The TPP/genipin co-crosslinked chitosan networks display a significant slower biodegradation rates than fresh chitosan gel beads. It was reported that the degradation of chitosan by lysozyme could be owing to the binding of *N*-acetylglucosamine residues to active site of lysozyme. Cleavage of the glucosidic linkage can occur from the interaction of alternate sites of lysozyme with the acetamide side chains of chitin or chitosan [33]. As shown in Fig. 9, it seemed that the cyclic covalent-crosslinked structure of the genipin–crosslinked chitosan network made its intermolecular crosslinks between adjacent chain denser than that in the linear ionic crosslinked structure of TPP–crosslinked chitosan network and fresh chitosan bead. According to the predicition, chitosan networks prepared by chemical crosslinking dominated reaction should be more stable than that prepared by ionic crosslinking dominated reaction under enzymatic digestion. Nevertheless, for the media



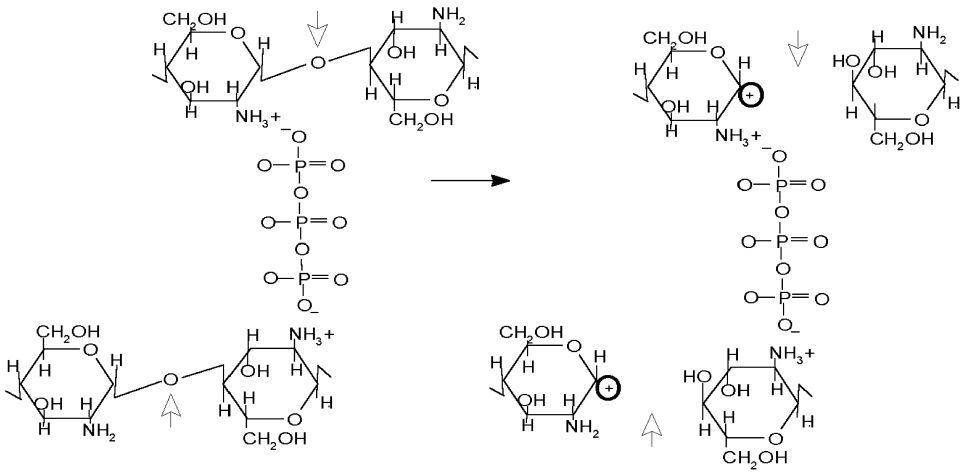
Degradation of chitosan by lysozyme

(a)



Degradation of genipin-crosslinked chitosan by lysozyme

(b)



Degradation of TPP-crosslinked chitosan by lysozyme

(c)

Fig. 9. Mechanism of enzymatic degradation of chitosan gel beads: (a) degradation of chitosan by lysozyme (b) degradation of genipin–crosslinked chitosan networks by lysozyme (c) degradation of TPP–crosslinked chitosan networks by lysozyme.

incubated with the co-crosslinked chitosan beads, the difference of increment in free-amino-group content for the medium incubated with various TPP/genipin crosslinked chitosan beads was not prominent. As described earlier, the ionic crosslinking dominated co-crosslinking reaction leads to the formation of higher crosslinking degree of chitosan networks than its chemical crosslinking dominated counterpart. Besides, the ionic TPP–chitosan networks are not easily decomposed in PBS solution. Being crosslinked in acidic TPP/genipin co-crosslinker, crosslinking degree of the co-crosslinked chitosan gel bead was increased as compensation for the decreased stability of its linear crosslinked structure, and therefor the chitosan gel beads prepared by ionic- and chemical-crosslinking dominated reaction demonstrates similar biodegradation rates under enzymatic digestion.

4. Conclusion

In this study, novel chitosan-based networks were prepared by curing in the TPP/genipin co-crosslinker. TPP and the herbal medicine, genipin, were used as an ionic and chemical crosslinking agent for the crosslinking of chitosan, respectively. UV, FTIR and EDAX analysis revealed that the co-crosslinking mechanism is pH-dependent. At lower pH condition, the co-crosslinking reaction is ionic crosslinking dominated; however, chemical crosslinking dominates the co-crosslinking reaction at neutral and basic conditions. It is interesting to find that the swelling property and biodegradability of prepared chitosan gel bead are significantly influenced by the pH-dependent co-crosslinking method. Preparation of chitosan gel beads via such an ionic/chemical co-crosslinking process may improve their usage for biomedical applications.

References

[1] Yeo JH, Lee KG, Kim HC, Oh YL, Kim AJ, Kim SY. Biol Pharm Bull 2000;23:1220.
[2] Stone CA, Wright H, Clarke T, Powell R, Devaraj VS. Br J Plast Surg 2000;53:601.
[3] Ueno H, Yamada H, Tanaka I, Kaba N, Matsuura M, Okumura M, Kadosawa T, Fujinaga T. Biomaterials 1999;20:1407.
[4] Mi FL, Shyu SS, Wu YB, Lee ST, Shyong JY, Huang RN. Biomaterials 2000;22:165.
[5] Chupa JM, Foster AM, Sumner SR, Madihally SV, Matthew HWT. Biomaterials 2000;21:2315.
[6] Suh JKF, Matthew HWT. Biomaterials 2000;21:2589.
[7] Ma J, Wang H, He B, Chen J. Biomaterials 2001;22:331.
[8] Tokumitsu H, Ichikawa H, Fukumori Y. Pharm Res 1999;16:1830.
[9] Seferian PG, Martinez ML. Vaccine 2001;19:661.
[10] Takeuchi H, Yasuji T, Yamamoto H, Kawashima Y. Pharm Res 2000; 17:94.
[11] Kofuji K, Ito T, Murata Y, Kawashima S. Chem Pharm Bull 2000;48: 579.
[12] Kato Y, Onishi H, Machida Y. J Controlled Release 2001;70:295.
[13] Nishioka Y, Kyotani S, Okamura M, Miyazaki M, Kazaki K, Ohnishi S, Yamamoto Y, Ito K. Chem Pharm Bull 1990;38:2871.
[14] Akbuga J, Bergisadi N. J Microencapsul 1996;13:161.
[15] Jameela SR, Misra A, Jayakrishnan A. J Biomater Sci Polym Ed 1994; 6:621.
[16] Jameela SR, Kumary YV, Lal AV, Jayakrishnan A. J Controlled Release 1998;52:17.
[17] Kurita K, Shimada K, Nishiyama Y, Shimojoh M, Nishimura SI. Macromolecules 1998;31:4764.
[18] Andrady AL, Xu PJ. Polym Sci, Part A: Polym Chem 1997;35:517.
[19] Wirsen XQA, Albertsson AC. J Polym 2000;41:4589.
[20] Cha SY, Lee JK, Lim BS, Lee TS, Park WH. J Polym Sci, Part A: Polym Chem 2001;39:880.
[21] Angelis AA, Capitani D, Crescenzi V. Macromolecules 1998;31: 1595.
[22] Peng T, Yao KD, Yuan C, Goosen MFA. J Polym Sci, Part A: Polym Chem 1994;32:591.
[23] Mi FL, Shyu SS, Lee ST, Wong TB. J Polym Sci, Part B: Polym Phys 1999;37:1551.
[24] Mi FL, Sung HW, Shyu SS. J Polym Sci, Part A: Polym Chem 2000; 38:2804.
[25] Mi FL, Sung HW. J Biomater Sci Polym Ed 1994;6:621.
[26] Mi FL, Sung HW. Biomaterials 1994;6:621.
[27] Tsai TH, Westly J, Lee TF, Chen CF. J Liq Chromatogr 1994;17: 2199.
[28] Fujikawa S, Yokota T, Koga K, Kumada J. J Biotechnol Lett 1987;9: 697.
[29] Tsai CC, Huang RN, Sung HW, Liang HC. J Biomed Mater Res 2000; 52:58.
[30] Sung HW, Chang Y, Chiu CT, Chen CN, Liang HC. J Biomed Mater Res 1999;47:116.
[31] Sung HW, Liang IL, Chen CN, Huang RN, Liang HC. J Biomed Mater Res 2001;55:538.
[32] Sung HW, Chang Y, Liang IL, Chang WH, Chen YC. J Biomed Mater Res 2000;52:77.
[33] Aiea S. Int J Biol Macromol 1992;14:225.