# Preparation and Characteristics of a Water-Soluble Chitosan–Heparin Complex

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**ABSTRACT:** To improve the wound-healing ability of chitosan, heparin, known to be effective in wound healing, was complexed with water-soluble chitosan (WSC) by chemical reaction. The chemical structure of the water-soluble chitosan–heparin (CH) complex was analyzed, and CH complex formation was confirmed with an FTIR spectrometer. The mechanical and thermal properties of the CH complex were measured by a tensile tester and thermal analyzers (DSC and TGA). Within the heparin content up to ≈470 IU/g in the aqueous CH complex solution, the intrinsic viscosity and tensile strength of the water-soluble CH complex gradually increased, but thermal stability slightly decreased by introducing the heparin into the WSC. When the heparin content was greater than these values (470 IU/g), the water-insoluble CH complex, which is supposed to have a multisubstituted or crosslinked structure, precipitated in the aqueous water-soluble CH complex solution. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 87: 1784–1789, 2003

**Key words:** water-soluble chitosan–heparin complex; intrinsic viscosity; tensile strength; thermal property

## INTRODUCTION

Chitin is the second most abundant biopolymer in nature, found in the shell of crustaceans, the cuticles of insects, and the cell walls of fungi.[1] It is composed of poly(*N*-acetylglucosamine) units bound by β-1,4-glycosidic bonds.[2,3] Chitosan may be obtained from chitin by deacetylation using a strong alkaline solution. Methods for this process are recorded elsewhere.[4]

Previous studies have demonstrated the nontoxic effect of chitosan in animals, and many investigations have been performed to explore its potential use in pharmaceutical and medical applications. Through these earlier studies it has been shown that chitin and chitosan have useful biological properties such as biocompatibility, biodegradability, hemostatic activity, and wound-healing ability. An extensive study has been carried out of the uses of chitosan in biomedical applications, such as an absorbable suture, a drug carrier, an antitumor agent, a hemostatic agent, and a wound-healing agent.[5] The physiochemical interactions with the most important components in living matter have appeared to be essential for interpretation of the biological responses induced by the presence of chitosan.[6–9]

Chitosan has polycationic properties that allow interaction with polyanions, leading to the formation of insoluble polyelectrolyte hydrogels. These hydrogels can be prepared as microsphered, films, particles, beads, and sponges, depending on the desired use. However, the applications of these chitin, chitosan, and polyelectrolyte hydrogels have been limited by their low solubility in most common solvents. It is known that the solvents for chitin and chitosan are usually concentrated acids (HCl, $CH_3COOH$) and amide–LiCl mixtures (*N*,*N*-dimethylacetamide–LiCl and *N*-methyl-2-pyrrolidone–LiCl).[10] But these solvents come accompanied by various problems, such as the difficulty of removing the solvents and their toxicity.

However, water-soluble chitin or chitosan has many advantages in biomedical applications.[11–14] Therefore, much attention has been paid to preparing water-soluble chitosan (WSC) that is also soluble in body fluid.[15–18]

In the mean time, many researchers have investigated how to improve the cell affinity and biological activity of chitosan by using functional proteins.[19,20]

Among many proteins, heparin found in the granules of mast cells exerts anticoagulant activity by binding and activating the plasma protein antithrombin.[21] Heparin stimulates the proliferation of some cell types[22,23] and inhibits the growth of others.[24,25] It also modulates several phases of wound healing.[26–28] In clinical practice heparin must be applied in a manner that ensures therapeutic concentrations in the wound-healing area over a prolonged period of time; one way to do this is to immobilize the heparin molecules by

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binding them to a biodegradable carrier such as chitosan.[29]

Thus far, investigations of possible uses of chitosan modified using heparin, such as in wound healing, have been done.[29,30] But to elicit the full potential of chitosan, basic studies are needed.

The main disadvantage of using the chitosan complex in various applications is its insolubility in water or other common solvents, which not only reduces its healing effect but also makes processing or molding difficult. Hence, in this study a water-soluble CH complex was prepared by reacting both WSC and heparin. Its structure and physical (mechanical and thermal) properties were investigated using different weight ratios of WSC and heparin.

## EXPERIMENTAL

### Materials and Reagents

Water-soluble chitosan ($M_w = 2.0 \times 10^5$) was supplied by Ja Kwang RNC Tech. Co. Ltd. (Seoul, Korea). Solid-type deaminated heparin (100 unit/mg) and liquid-type heparin sodium (5000 unit/mL) were supplied by Aldrich Chemical Co. (St. Louis, MO) and Korea Green Cross Co. (Seoul, Korea), respectively.

### Preparation of water-soluble chitosan–heparin complex

Water-soluble CH complex was prepared by reacting both WSC and heparin in aqueous media. WSC (0.5 g) and distilled water (8–10 mL) were charged into a four-necked flask equipped with a stirrer, reflux condenser, and $N_2$ inlet, and then the mixture was stirred over 1 h until well dissolved. Separately, two kinds of heparin solutions were prepared: a solution of deaminated heparin (0.01–0.08 g) dissolved in distilled water (1 mL) and a commercial solution (0.25–2.00 mL).

The heparin solution was slowly added into the flask containing the WSC solution while stirring (200 rpm). The reaction mixture was stirred at 40°C for 1 h and then glass-filtered to remove water-insoluble product. Finally, the water-soluble CH complex was obtained in white powder by lyophilization at −40°C for 24 h.

### Preparation of water-soluble chitosan–heparin complex film

Water-soluble CH complex (0.5 g) was dissolved in distilled water (15 mL) by stirring for 1 h at room temperature, and then undissolved complex was removed by glass filter. The complex solution was degassed under vacuum and cast on a clean glass plate. The film was obtained by drying the casted solution in an air oven at 40°C for 24 h.

### Structural, mechanical, and thermal characterization

Fourier transform infrared (FTIR) spectra were obtained from KBr pellets of the WSC, heparin, and water-soluble CH complex with a FTIR spectrometer (Perkin-Elmer Spectrum GX, Beaconsfield, U.K.)

The tensile strength and elongation of the WSC and water-soluble CH complex film were measured using an Instron Universal Machine (Model 4465; Canton, MA) under these conditions: sample size of 50 mm × 5 mm, crosshead speed of 50 mm/min, and distance of grip of 30 mm. All samples were preconditioned at 30°C and 65% relative humidity for 24 h prior to testing. The measurements were repeated more than 3 times to obtain an average for each sample.

Thermal characteristics of the WSC, heparin, and water-soluble CH complex were measured using both a differential scanning calorimeter (DSC; Seiko 6100, Chiba, Japan) in the temperature range of 30°C–250°C at a heating rate of 10°C/min and a thermal gravimetric analyzer (TGA; Mettler TG 50, Greifense, Switzerland) in the temperature range of 20–800°C at a heating rate of 10°C/min.

### Intrinsic viscosity

The intrinsic viscosity ($\eta$) of the WSC and water-soluble CH complex with a change of heparin content was measured in distilled water at 30°C using an Ubbelohde viscometer. The sample concentration in distilled water was in the range of 0.0035–0.0414 g/dL.

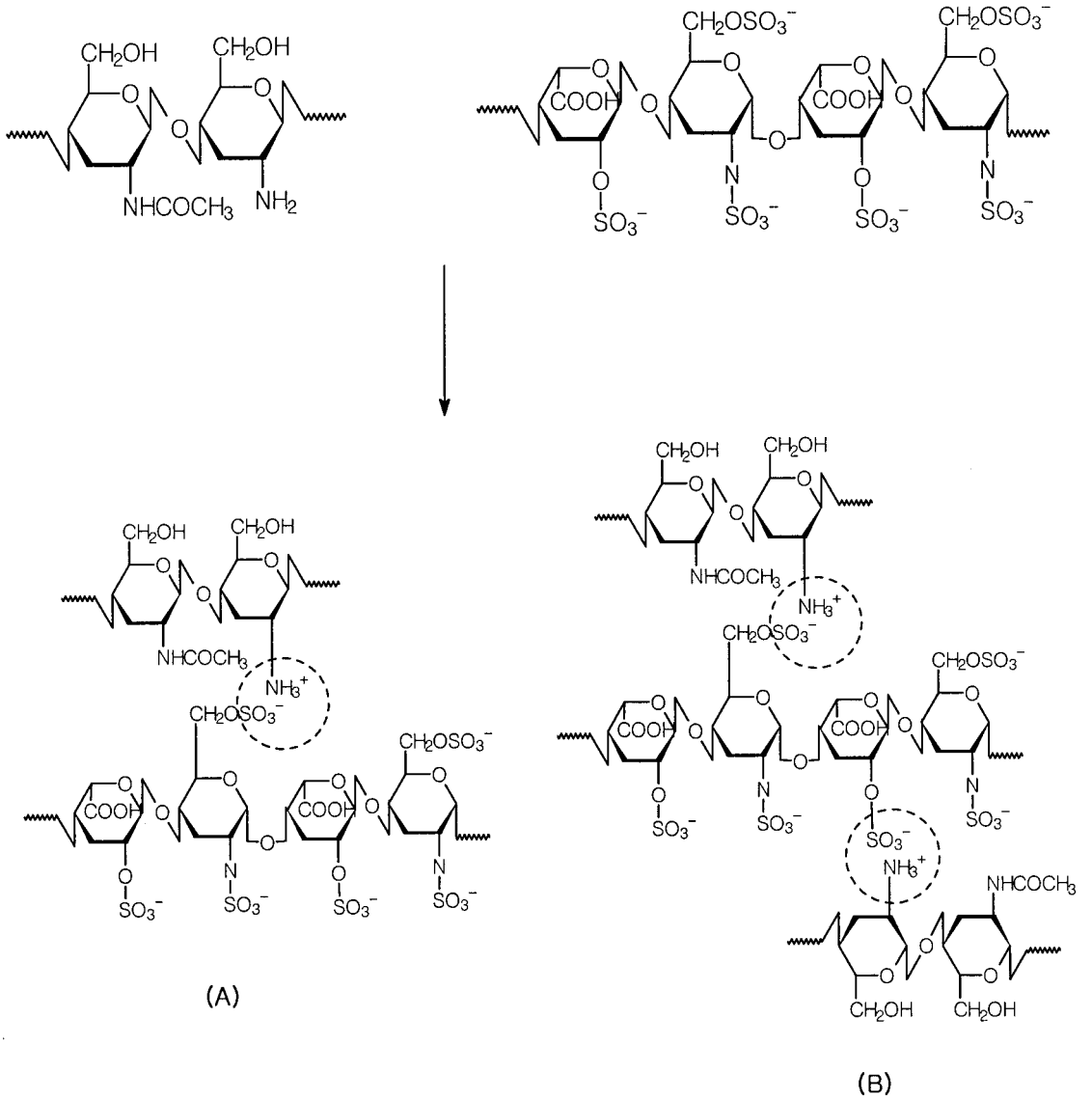
## RESULTS AND DISCUSSION

### Preparation of water-soluble chitosan–heparin complex

The reaction for water-soluble CH complex preparation is briefly shown in Scheme I. Composition ratio, heparin units, and reaction yield under various conditions are shown in Table I.

When the heparin content was less than 470 IU/g, the reaction yield for the water-soluble CH complex based on the initial amount of WSC and heparin was 91%–98%, indicating that most of the heparin complexed with the WSC, possibly in a mono-substituted structure [Scheme I(A)]. However, the yield for the water-soluble CH complex decreased prominently to 61%–71% when the heparin content was raised. It was assumed that at the higher heparin content (625 IU/g), water-insoluble CH complex also might be formed in a multisubstituted or crosslinked structure [Scheme I(B)], which was subsequently separated from the aqueous CH complex solution.

The FTIR spectra of the WSC, heparin, and water-soluble CH complex (sample 4) are shown in Figure 1.




**Scheme I**

The FTIR spectrum of the WSC showed a strong peak around 2500–3000 $cm^{-1}$, ascribed to the stretching of the —$NH_2$ group with overlapping strong hydroxyl peak around 3000–3700 $cm^{-1}$. By the time the complex between WSC and heparin had formed, the broad peak at 2500–3000 $cm^{-1}$ (marked by arrow) had decreased substantially, which was presumed to be attributed to the formation of a complex on this —$NH_2$ group. At the same time, by the time CH complex had formed, the peak at 1680 $cm^{-1}$, ascribed to the carboxylic group of heparin, had increased slightly more than that of WSC. For samples 1–3 and 5–10 in Table I, the FTIR spectra were similar to that of sample 4 (data not shown).

## Intrinsic viscosity

By introducing heparin in WSC, the intrinsic viscosity ($\eta$) was changed (Fig. 2). As shown in Figure 2, although the intrinsic viscosity of WSC was about 8.54 dL/g, that of water-soluble CH complex gradually increased from 9.78 to 10.28 dL/g as the heparin content was raised from 95.2 to 476.1 IU/g. But when the heparin content was greater than these values, intrinsic viscosity was almost constant regardless of heparin content. For heparin content less than 476.1 IU/g, the CH complex was supposed to form only in the mono-substituted structure [scheme I(A)], and thus the intrinsic viscosity gradually increased. But over the range of heparin content, CH complex might become insoluble in water not only in the mono-substituted structure but also in multi- or crosslinked structures [scheme I(B)].

## Tensile strength

Tensile strength and elongation of WSC and water-soluble CH complex films with different heparin con-

**TABLE I**
**Composition Ratio, Heparin Unit, and Yield of Water-Soluble Chitosan–Heparin Complex in Various Reaction Conditions[a]**

| Sample no. | WSC (g) | Heparin | | Distilled water (mL) | Heparin units (IU/g) | Yield (%)[b] |
|---|---|---|---|---|---|---|
| | | Solid (g) | Liquid (mL) | | | |
| 1 | 0.5 | 0.01 | · | 10 | 95.2 | ≈98 |
| 2 | | 0.02 | · | 10 | 190.4 | ≈97 |
| 3 | | 0.03 | · | 10 | 285.7 | ≈98 |
| 4 | | 0.05 | · | 10 | 476.1 | ≈95 |
| 5 | | 0.07 | · | 10 | 761.9 | ≈71 |
| 6 | | · | 0.25 | 9.75 | 116.3 | ≈92 |
| 7 | | · | 0.50 | 9.50 | 227.3 | ≈92 |
| 8 | | · | 1.00 | 9.00 | 434.8 | ≈91 |
| 9 | | · | 1.50 | 8.50 | 625.0 | ≈70 |
| 10 | | · | 2.00 | 8.00 | 810.0 | ≈61 |

[a] Reaction was carried out at — Temperature: 30°C; stirring speed: 200 rpm.
[b] Weight of water-soluble CH complex/(weight of WSC + weight of heparin).

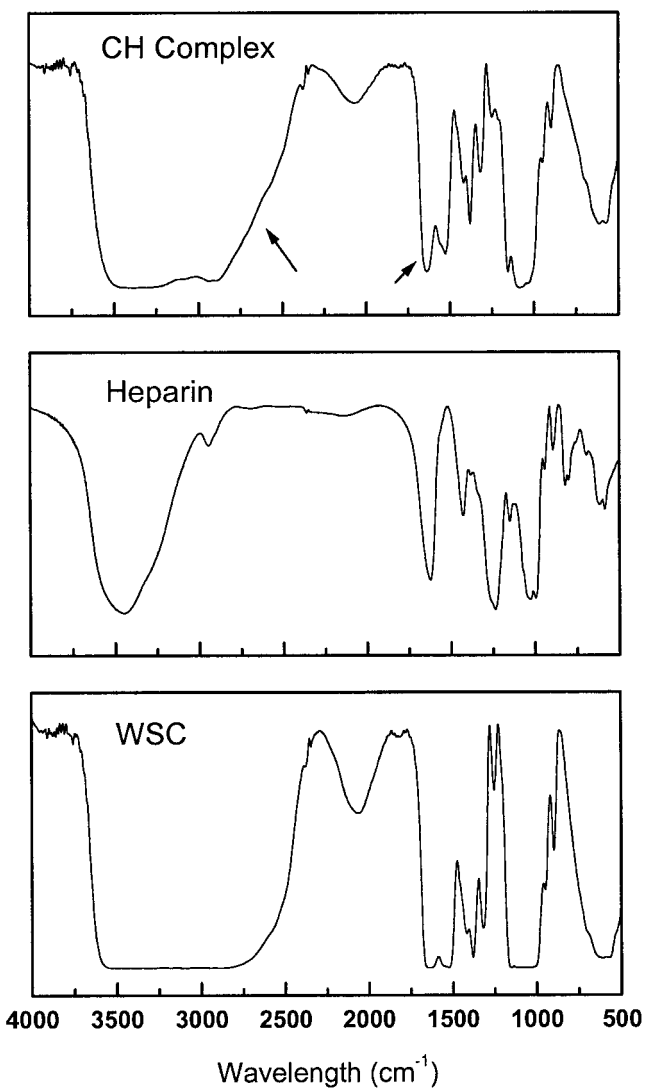



**Figure 1** FTIR spectra of WSC, heparin, and water-soluble CH complex.

tent are shown in Figure 3. The tensile strength and elongation of WSC film were about 69 $N/mm^2$ and 16%, respectively. By introducing heparin into WSC, the tensile strength gradually increased from 69 to 82 $N/mm^2$, with the increase of heparin content in the range of 95.2–476.1 IU/g. But when the heparin content was greater than these values, tensile strength was not changed much, regardless of the amount of heparin. It was supposed to result from the different reactions, as mentioned in the intrinsic viscosity results.

The elongation of water-soluble CH complex film was almost constant, about 13%–15%, regardless of heparin content.

## Thermal properties

With the introduction of heparin into WSC, the thermal characteristics also changed, as shown in Figures 4 and 5. The DSC thermograms for WSC and heparin (Aldrich Chemical Co.) showed one major exothermic sharp peak due to thermal degradation at the peak temperatures of 175°C–185°C and 230°C, respectively. By introducing heparin into WSC, the exothermic degradation peak for water-soluble CH complex (sample 4) shifted to a temperature range that was slightly higher (about 183°C–188°C) than that of WSC. The thermogram of the water-soluble CH complex (sample 8) prepared in a different condition with heparin (Green Cross Co.) was the same as that of sample 4 (data not shown).

As shown in Figure 5, the thermal degradation of the WSC and heparin started at approximately 183°C and 210°C ($T_d$), respectively, in accordance with the temperature region in the DSC results (Fig. 4). The degradation of WSC progressed gradually within a broad temperature range (183–320°C), whereas that of heparin progressed abruptly in a narrow temperature range (210–310°C), according to the TGA curves. And

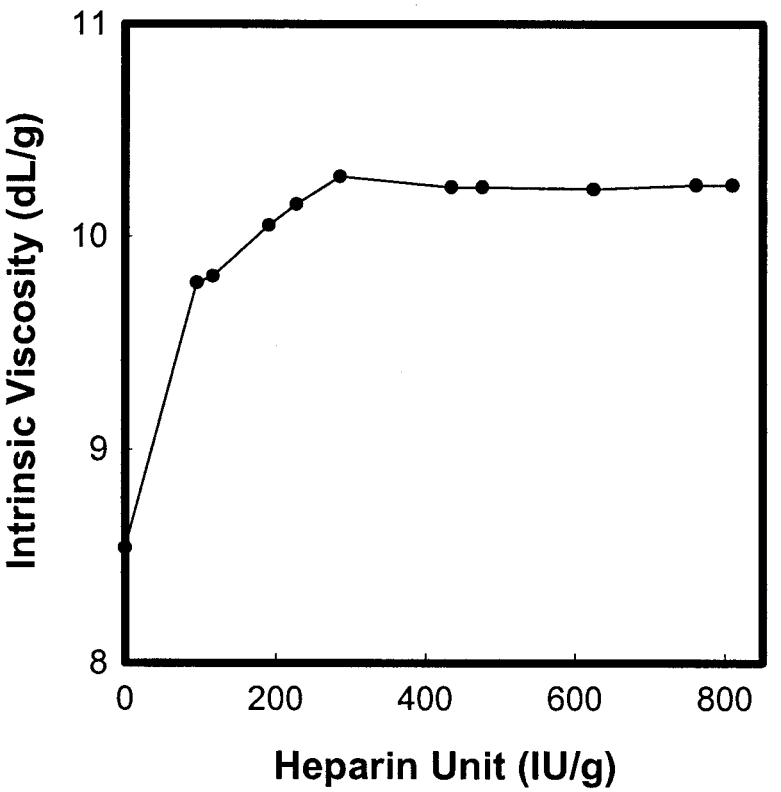



**Figure 2** Intrinsic viscosity of water-soluble CH complex with change of heparin content.

the thermal stability of heparin was less than that of WSC. By introducing heparin into WSC, the $T_d$ of the water-soluble CH complex (sample 4), which was where the degradation started, was shifted to a slightly higher temperature, at approximately 186°C, but thermal stability was slightly decreased compared with that of WSC. The TGA curve of the water-soluble CH complex (sample 8) prepared with heparin (Green Cross Co.) was similar to that of sample 4 (data not shown).

## CONCLUSIONS

The water-soluble CH complex could be prepared by reacting both WSC and heparin in an aqueous solution. By introducing heparin into WSC, the tensile strength and intrinsic viscosity increased, but thermal stability decreased slightly. Under our experimental conditions, the complex was soluble in water in which heparin content was less than about 476 IU/g. Although the CH complex prepared with ordinary chi-

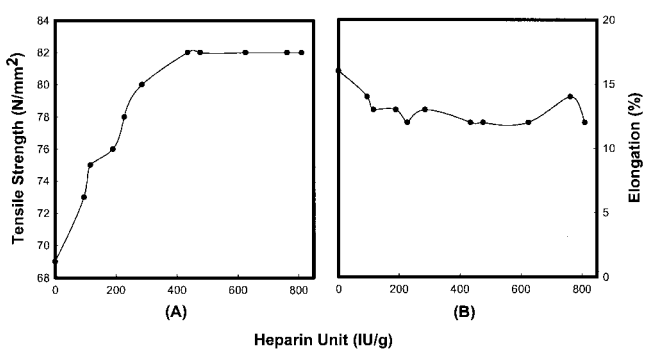



**Figure 3** (A) Tensile strength and (B) elongation of water-soluble CH complex with change of heparin content.

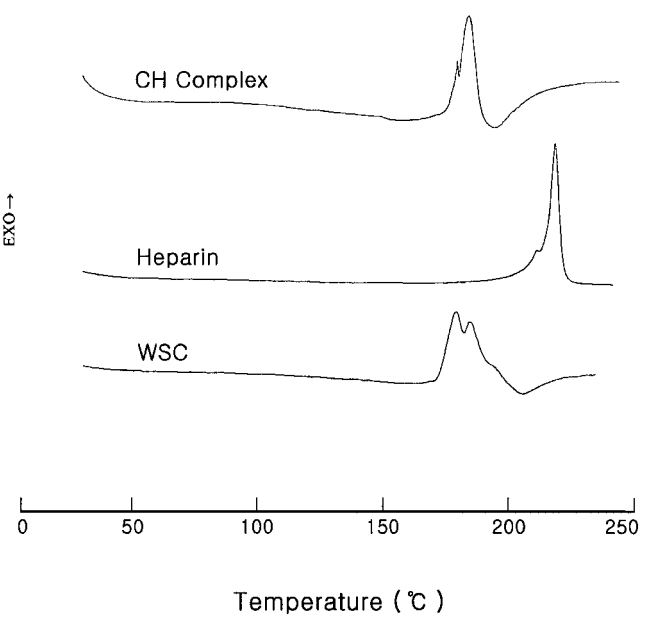



**Figure 4** DSC thermograms of WSC, heparin, and water-soluble CH complex with change of heparin content.

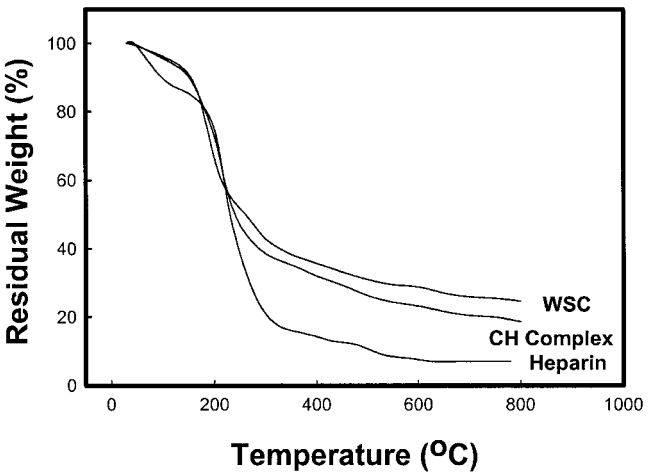



**Figure 5** TGA thermograms of WSC, heparin, and water-soluble CH complex with change of heparin content.

tosan (only soluble in acid media) is insoluble in water, the water-soluble CH complex prepared in this study had excellent solubility in water. These results suggest that water-soluble CH complexes may be useful as bioactive, bioabsorbable, and biocompatible materials in the pharmaceutical and medical fields. A future article will report in detail about this CH complex's cell affinity and wound-healing ability.

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