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NOVEL HOLLOW MICROBEADS BASED ON HYDROXYPROPYL CHITOSAN BY W/O EMULSION CROSS-LINKING METHOD

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Abstract

A simple method to prepare hollow microbeads composed of hydroxypropyl chitosan (HPCH) using W/O emulsion method was described. For this purpose, HPCH was synthesized by chitosan and propylene oxide under alkaline catalyst. FT-IR and solid state CP/MAS ^{13}C-NMR confirmed the incorporation of hydroxypropyl moieties on chitosan. The degree of substitution(DS) of HPCH was around 3.5 as detected by elemental analysis. Wide angle X-ray diffraction (WAXD) pattern of HPCH showed lower crystallinity than that of chitosan. The physical properties such as solubility, hydrophilic character, and thermal stability of the HPCH were significantly changed as compared with those of chitosan. HPCH had improved solubilities in several organic solvents, compared with chitosan. After cross-linking HPCH with epichlorohydrin in water-in-oil (W/O) emulsion system, microbeads were obtained. Scanning electron microscope picture reveals that they are hollow microbeads which have an asymmetric shell structure with nonporous inner skin layer and porous outer surface layer. The mean diameter of the hollow microbeads was 180 μm with shell thickness of 1.5 μm.

Introduction

Chitosan [poly (1→4)-2-amino-2-deoxy-β-D-glucan], prepared by alkaline N-deacetylation of chitin [poly (1→4)-2-acetamide-2-deoxy-β-D-glucan], is a potentially useful material owing to its good biocompatibility and biodegradability [1]. The industrial use of the chitosan has been steadily increasing since 1970s

[2]. The major applications of chitosan were centered on sludge dewatering, food processing, and metal ion chelation [3]. The present trend, however, is toward producing high value products, such as cosmetics, drug carriers, feed additives, semipermeable membranes, and pharmaceutics [4-5].

Advantages of this polymer include easy availability, low cost, high biocompatibility and ease of chemical modification. Chitosan has many free amino groups which are open to alkylation along with the hydroxy groups in itself. Many papers have recently been published concerning utilization of chitosan as functionalized polysaccharides [1-5]. It has now been found that HPCH having hydroxypropyl substituents both on some of the hydroxyl oxygen atoms and on some of nitrogen atoms of the glucosamine units can be prepared, and that such substituted chitosans, designated as hydroxypropyl chitosan, are water soluble, in acidic as well as in alkaline media [6]. Although HPCH has been exploited for its potential in the biomedical field, the development and application studies on the hollow microbeads is a relatively virgin area. Until now, there is no reports about the hollow microbeads based on HPCH. In the present study, we attempted to develope a novel and simple method for the preparation of HPCH hollow microbeads by W/O emulsion method. We report here the preparation of HPCH hollow microbeads using epichlorohydrin as crosslinking agent.

Experimental

Materials and instrument

Chitin was extracted from the shells of crabs according to the method of Hackman [7]. N-deacetylated chitin, chitosan, was prepared from chitin following the method proposed by Mima *et al.* [8]. The chitosan has been found to have M_n = 6.8 x 10^5 [9], 94% of deacetylation [10]. Propylene oxide was purchased from Jassen and stored at 4℃ before use. Epichlorohydrin and dimethoxyethane were purchased from Aldrich. Other reagents are all of special reagent grade. Autoclave was Parr model 4561 with SUS 316.

Preparation of HPCH

HPCH was synthesized from chitosan and propylene oxide by using alkaline catalyst. The synthesis procedure was as follows: 1.1g (7.8 mmol) of chitosan, which was crushed by roll-mill, was placed in an autoclave together with 20 ml of 0.25*N* NaOH. After stirring for overnight, 20 ml of dimethoxyethane and 54g (93.6 mmol) of propylene oxide were introduced into an autoclave. The mixture was heated for 24 hr at 90℃. After finishing the reaction, the residual propylene oxide was vented out by N_2 stripping. After dilution with 200 ml of water, the solution was adjusted to pH 7.0 by adding 2*N* HCl and concentrated in a rotary evaporator, and then precipitated with 10 times volume of acetone. The resulting

precipitate was washed with acetone and hot distilled water (85~90℃). Finally, the product was dissolved in small amount of water and freeze dried. 3.3g of hydroxypropyl chitosan was obtained, degree of substitution : 3.5 hydroxypropyl/glucosamine.

Characterization

FT-IR spectra were obtained by FTS-40 system of Bio-Rad Laboratories. Solid state CP/MAS ^{13}C-NMR spectra were obtained by Bruker MSL 200 spectrometer operating at room temperature. Thermogravimetry analysis (TGA) was carried out with a Perkin-Elmer Series-7 in the range of 70~610℃ at a heating rate of 10℃/min under nitrogen atmosphere. To compare the crystalline character of chitosan and HPCH, WAXD patterns were measured by the reflection method with nickel-filtered Cu-Kα radiation using a Rigaku Geigerflex D/MAX IIA operated at 30kV, 15mA in the 2θ scanning mode between 4° to 30° , scanning speed of 2° /min. Electron microscope analysis was performed using JEOL JSM-35 scanning electron microscope (SEM). The samples were coated with gold using a Fine Coat Ion Spatter (JEOL JFC-1100) for 30 min, under a vacuum of 0.1 torr and at a voltage of 1.2 kV and 10 mA. Elemental analysis was performed by CHNS-932 Elemental Analyzer of Leco Corporation. Degree of substition was estimated in proportion to the relative contents of CHN in chitosan and in HPCH.

Preparation of hollow microbeads

10g of HPCH was dissolved in distilled water to give 3% solution. The resulting solution was degassed under vacuum. 50g of HPCH solution was mixed with 50g of toluene, 0.3g of sorbitan monooleate (Span 80). Thus formed W/O emulsion was mechanically stirred at 400 rpm for 1 hr to stabilize the emulsion. 1.25 ml of 40% NaOH was added dropwisely into the emulsion. After mixing the emulsion for 20 min, 7 ml of epichlorohydrin was added by dropwise. The reaction temperature was slowly raised to 60℃. The reaction was continued for 8 hr. After the microbeads were formed, they were filtered, washed with 1% Triton X-100, and washed with ethanol. Finally, after washing with acetone, the microbeads were dried in vacuum under P_2O_5. Particle size distribution of the microbeads was measured by using a nest of U.S. standard sieves. Apparent density was determined by the graduated cylinder method [11].

Results and discussion

Characterization

The alkaline-catalyzed reaction of propylene oxide with chitosan is considered to be S_N2 type reaction, in which the nucleophile provides the driving force for

ring-opening at the less substituted carbon. The site and degree of substitution were determined by solid state CP/MAS ^{13}C-NMR and elemental analyses. Fig. 1 shows the solid state CP/MAS ^{13}C-NMR spectra for chitin, chitosan, and HPCH, respectively. From the comparision of spectra (b) and (c), a peak at 19 ppm in (c) is assigned to be a CH_3 peak in the hydroxypropyl unit in HPCH. A broad peak detected at around 66 ppm is caused by the CH_2 and CH unit in the hydroxypropyl unit. Since the signal of the carbon attached to the hydroxyl group in glucose is known to shift to a lower magnetic field (8~10 ppm) when blocked with methyl group [12-13], the signals of 60 ppm and 73 ppm are assigned to those for the unsubstituted C_6 and C_3, as shown in spectra (b). It is clearly evident that the peak intensity of the C_6 position (60 ppm) is reduced and a new peak is observed at 69 ppm due to the substitution. There was no clear indication of a chemical shift change due to the substituted C_3 at 73 ppm. Consequently, the hydroxypropylation site on the chitosan was assumed to be the C_6 as well as the C_2 position. The degree of substitution (DS) of HPCH was around 3.5 as confirmed by elemental analyses, as shown in Table 1.

The IR spectrum of the HPCH exhibits absorptions at 1375 cm^{-1}. The sharp band at 1375 cm^{-1} has been assigned to the CH_3 symmetrical deformation mode. The peaks at 1100 cm^{-1} are assigned to the sum of C-O stretching vibration in the ether bond of hydroxypropyl substituents and chitosan.

Thermal properties

Polysaccharide, like all polyhydroxylated linear polymers, do not have a melting temperature, although usually at high temperatures, they have a degradation point (Td). The thermal behavior of HPCH was examined by TGA analysis. A typical trace for HPCH is shown in Fig. 3. The first weight losses observed in chitosan and HPCH correspond to the loss of adsorbed water. HPCH showed an onset of weight loss at 270℃. At 390℃, HPCH had lost approximately 80% of its weight. Chitosan showed significant weight losses at 360~420℃. Furthermore, it can be seen that the Td at 50% weight loss (350℃) for HPCH is lower than the Td for chitosan (465℃). This indicates that the HPCH is thermally less stable than chitosan.

Solubilities

Table 2 lists the solubilities of chitosan and HPCH in various organic and aqueous solvents. As seen in Table 2, HPCH is soluble in water as well as most of organic solvents. Physical properties such as solubility and hydrophilic character of HPCH were significantly changed as compared with chitosan. This could be taken as an additional proof of substitution by propylene oxide.

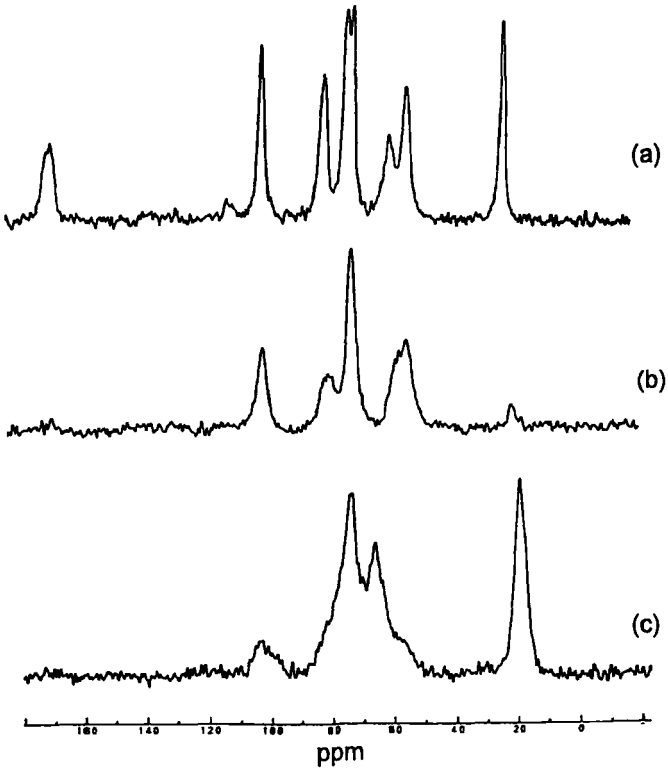


Fig. 1. Solid state CP/MAS ^{13}C-NMR spectra of (a) chitin, (b) chitosan, and (c) hydroxypropyl chitosan.

Table 1. Elemental analysis of chitosan and HPCH

Sample	C(%)	H(%)	N(%)	DS
Chitosan	44.88	7.20	8.26	0
HPCH	53.46	8.88	3.80	3.5

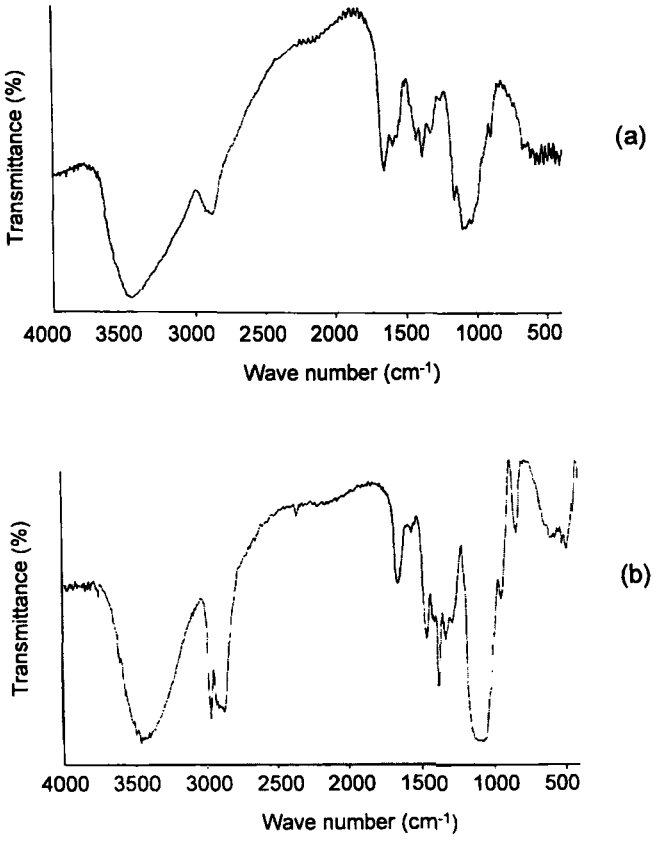


Fig. 2. FT-IR spectra of (a) chitosan and (b) HPCH

The solubilities of HPCH compared to chitosan were tested in water, acetic acid, DMF, NMP, 1*N* NaOH, acetone and alcohols. It was found that the HPCH remained soluble in water, acetic acid, DMF, NMP and 1*N* NaOH. Chitosan is insoluble in water, alkalis, alcohol, and other organic solvents, but only soluble in diluted acids. HPCH showed much higher solubility than chitosan and was soluble in polar organic solvents such as DMF, NMP and acetic acid. By converting the primary amines to hydroxypropyl groups, chitosan became soluble in wide pH range and in some organic solvents. This may be due to the inherent structural disruption of the molecular packing in the lattice by the introduction of bulky side groups to chitosan.

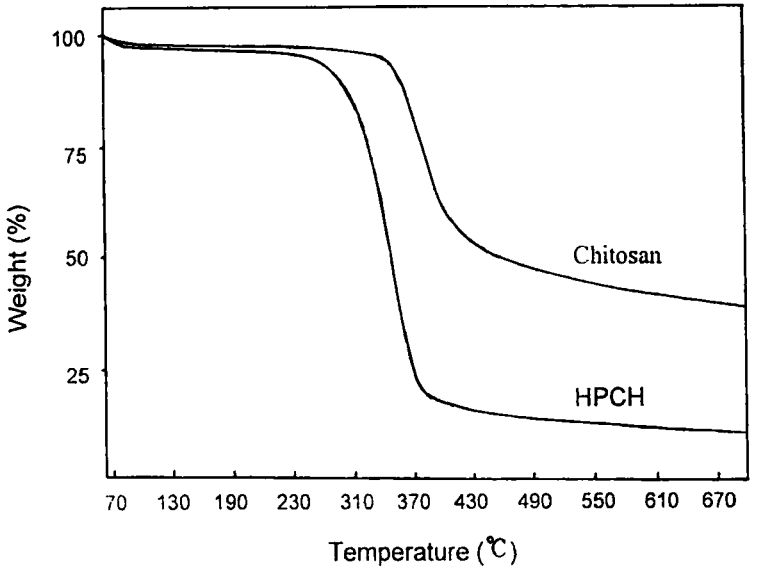


Fig. 3. TGA thermodiagrams of chitosan and HPCH

Table 2. Solubility of chitosan and HPCH

solvent	chitosan	HPCH
water	x	o
acetic acid	o	o
1N-NaOH	x	o
DMF	x	o
DMSO	x	o
NMP	x	o
MC	x	Δ
ethanol	x	Δ
iso-PrOH	x	x
acetone	x	x

(o: soluble, Δ: swelling, x: insoluble)

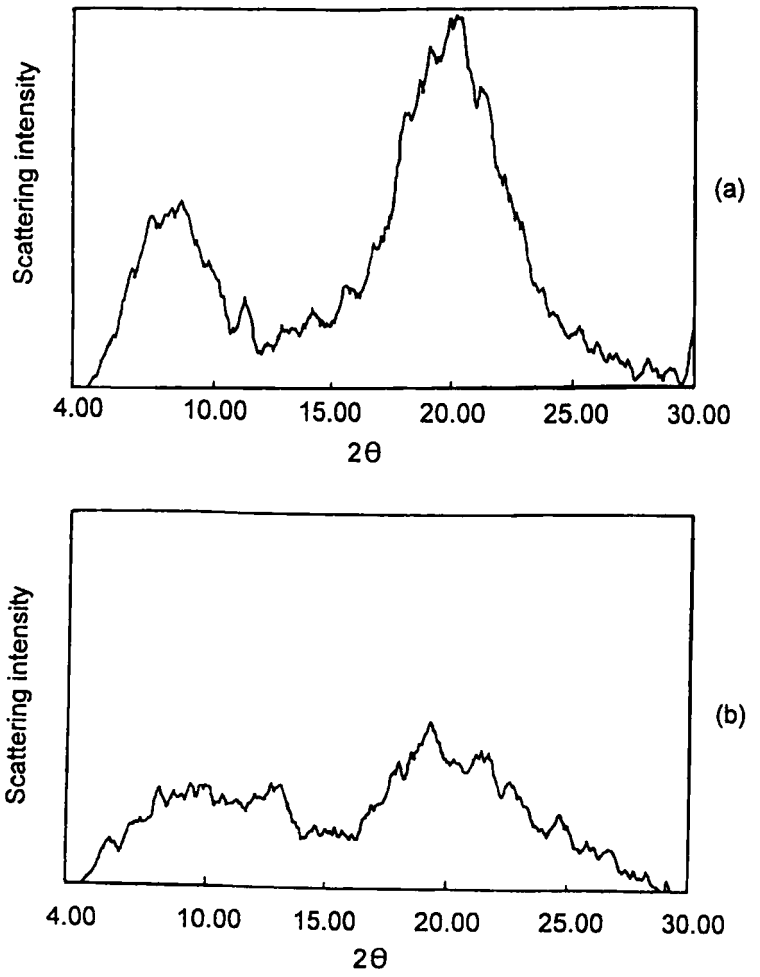
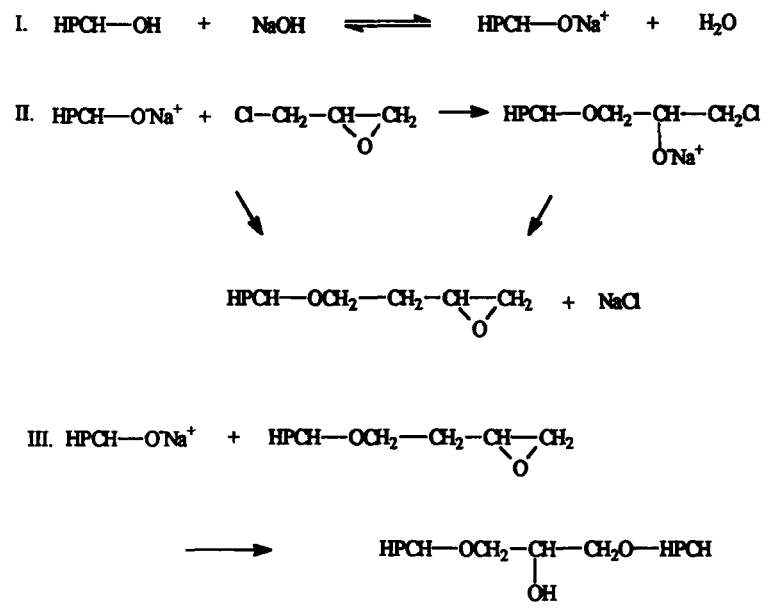


Fig. 4. X-ray diffraction pattern of (a) chitosan and (b) HPCH



Scheme 1. Proposed mechanism of HPCH crosslinking reaction with epichlorohydrin under basic condition

X-ray analysis

The crystallinity of HPCH was examined qualitatively by X-ray diffraction experiments. Fig. 4 shows the X-ray diffration patterns of HPCH and chitosan. They showed that the relative intensity of crystalline peak became smaller for HPCH. The peaks of HPCH were somewhat broader than those of chitosan, indicating lower crystallinity or less ordered structure. The crystallinity was decreased markedly with the incorporation of hydroxypropyl group. As the crystallinity is decreased for HPCH, it can be easily expected that HPCH be soluble in many organic solvents. It can be said that a decrease in hydrogen bonding force resulting from substituted bulky hydroxypropyl moiety affects the decrease of crystallinity.

The mechanism of cross-linking reaction

HPCH microbeads were manufactured from W/O emulsion crosslinking of HPCH with epichlorohydrin under alkaline condition. The crosslinking process theoretically

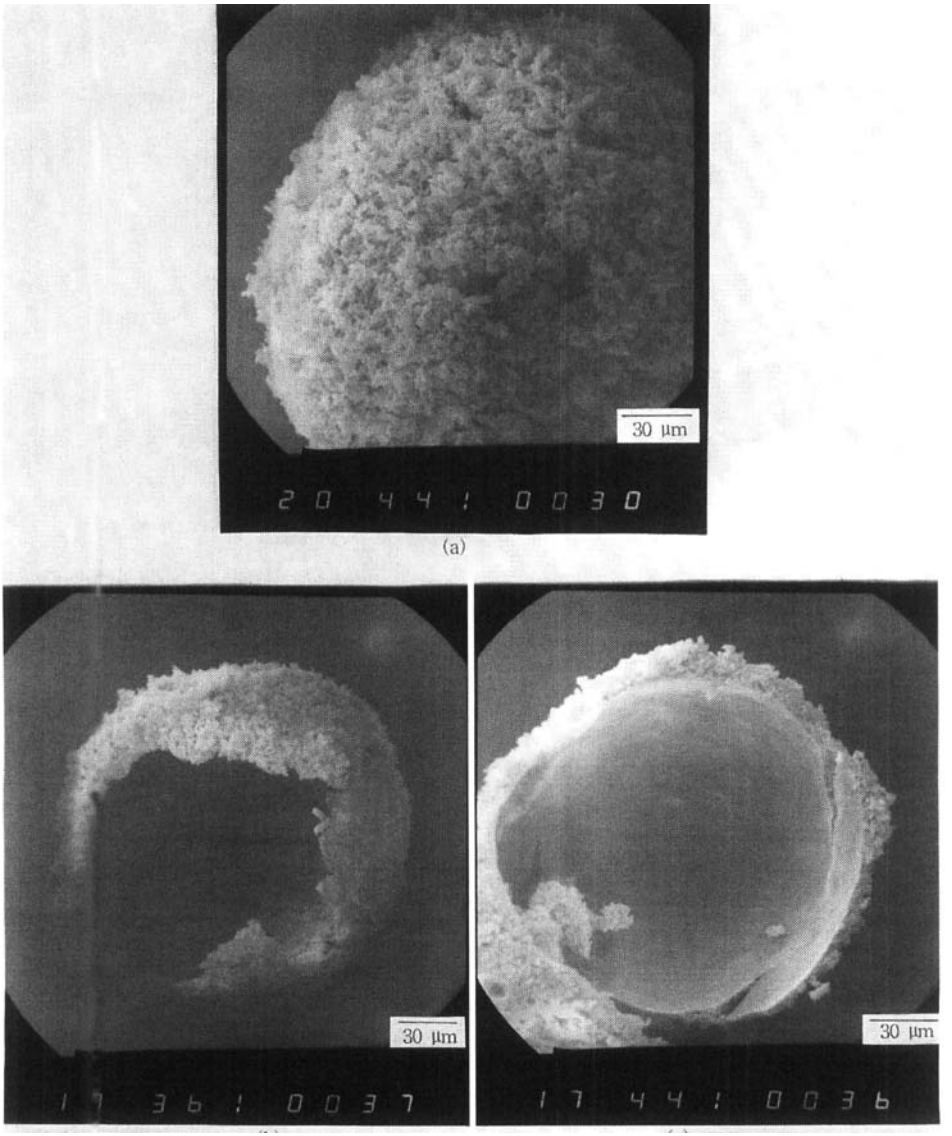


Fig. 5 SEM photographs of HPCH hollow microbeads. (a) outer surface morphology, (b) surface of fractured bead, (c) inner structure of fractured bead

contains three steps as shown in Scheme 1. Epichlorohydrin acts as a bifunctional molecule toward hydroxyl groups in basic medium. The epoxide group reacts readily with the hydroxyl group in basic medium. As the results, HPCH microbeads were formed by crosslinking with epichlorohydrin in W/O emulsion.

Morphology of HPCH hollow microbeads

The external and internal morphologies of HPCH microbeads were analysed by scanning electron microscope (SEM). Fig. 5 shows the microbeads prepared from W/O emulsion of HPCH which was cross-linked with epichlorohydrin. It can be seen in Fig. 5 that the HPCH microbeads exhibited an asymmetrically porous shell layer and hollow internal structures. The inner surface structure revealed the smooth and nonporous skin layer. However, the outer surface morphology revealed the rough and porous structure. The epichlorohydrin used as crosslinking agent has somewhat hydrophobic property, compared to the conventional crosslinking agent such as glutaraldehyde. As the results, the crosslinking reaction started from outside of the emulsion droplets and the hollow microbeads with smooth inner skin layer and outer porous sand rough structure was formed. The mean diameter of the hollow microbeads was 180 μm with shell layer of 1.5 μm. Apparent density of the hollow microbeads was 81 mg/ml. Application studies of the HPCH hollow microbeads as polymer supports for the enzyme immobilization and the chromatographic separation are under way.

Conclusion

HPCH was prepared from chitosan by reacting it with propylene oxide. Solid state CP/MAS ^{13}C-NMR spectra for HPCH confirmed the incorporation of hydroxypropyl moiety. The degree of substitution was around 3.5 as detected by elemental analysis. WAXD patterns of chitosan and HPCH showed that an incorporation of hydroxypropyl unit in chitosan contributed to reducing the crystallinity and enhancing the solubility in organic solvents. Hollow microbeads of HPCH which have an asymmetric shell structure was prepared by W/O emulsion crosslinking method using epichlorohydrin.

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