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Preparation of *N*-Succinyl-chitosan and Their Physical-Chemical Properties as a Novel Excipient

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The aim of this work is to prepare *N*-succinyl-chitosan (Suc-Chi) and measure physical-chemical properties for Suc-Chi as excipients. Suc-Chi were prepared via ring-opening reactions with succinic anhydride in Dimethyl Sulfoxide system. The physical-chemical properties of Suc-Chi, such as the degree of substitution (DS), solubility, isoelectric point (pI), glass transition temperature (Tg), partition coefficient (P_{app}) and zata potential were detected respectively in order to evaluate their possibility as drug carriers. We obtained Suc-Chi DAC-90 (DS=0.33) and the data of physical-chemical properties for the product. The knowledges of physical-chemical properties for Suc-Chi are valuable for basic or applied purposes in biomedical and pharmaceutical sciences stabilization.

Key words——*N*-succinyl-chitosan; preparation; physical-chemical properties; excipients

INTRODUCTION

Chitin and chitosan derivatives have been investigated as polymeric drug carriers for the optimization of drug delivery in the pharmaceutical field because of their biocompatibility and biodegradability.[1—5] Generally, chitosan dissolves aqueous acidic medium below pH 6.5, it precipitates above this pH by the addition of alkali solution. The application of chitosan was limited for using as a drug carrier owing to the insolubility at neutral or high pH region. To improve the soluble property of chitosan, generally, partial *N*-acetylation or chemical modification is required.

Suc-Chi was obtained by introduction of succinyl groups into chitosan *N*-terminal of the glucosamine units. Polyion complexes may be formed between the $-NH_3^+$ and $-COO^-$ groups in the succinyl chitosan molecule. The chemical structure and synthesis route of the *N*-succinyl chitosan are shown in Scheme 1. Suc-Chi displays good water soluble property at various pHs.[6] Suc-Chi was initially developed as wound dressing materials,[7] it is currently also applied as cosmetic materials (Moistfine liquid®).[8] New wound dressings composed of Suc-Chi and gelatin were also developed.[9] In addition, water-soluble chitin and chitosan including Suc-Chi were applied for a patent as a treatment of arthritis in Japan.[10] Suc-Chi has unique characteristics *in vitro* and *in vivo* such as biocompatibility, low toxicity and long-term retention in the body. Suc-Chi is valuable for the drug carrier to readily prepare its conjugates with various drugs to avoid vexatious complications in cancer chemotherapy,[11—16] due to having $-NH_2$ and $-COOH$ groups in its structure.

In the work we report the preparation and the physical-chemical properties of water soluble *N*-succinyl-chitosan as excipients and drug carriers.

MATERIALS AND METHODS

Materials Chitosan (90% deacetylated chitin, DAC-90, MW 3.4×10^5) was obtained from Qi-dao Shengyang Co. (Qi-dao, China). Succinic anhydride and dimethyl sulfoxide were purchased from Shenyang Pharmaceutical Co. (Shen-yang, China). All other chemicals were obtained commercially as reagent-grade products.

Methods

Synthesis of *N*-Succinyl-chitosan DAC-90 (1.0 g) was dissolved in dimethyl sulfoxide (20 ml). Prescribed succinic anhydride (1.0 g) was added to the diluted solution and stirred at 60°C. After standing for 24 h, the pH of the mixture was adjusted to 5.0 with 5% w/v aq. NaOH to give a precipitate. The

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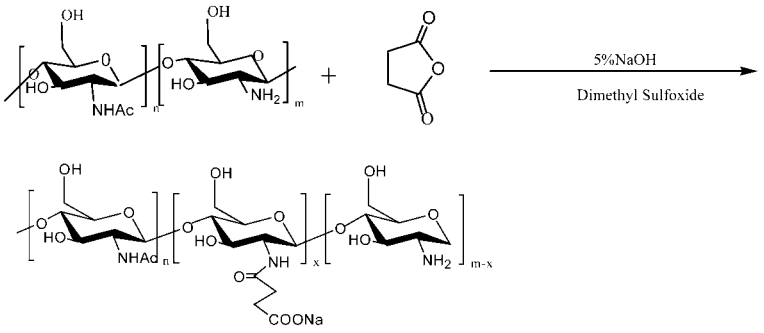

Scheme 1. Synthesis Route of *N*-Succinyl-chitosan

precipitate was collected by filtration and dispersed in 50 ml of H_2O. The pH of the dispersion was adjusted to 10—12 with 5% w/v aq. NaOH to give a pale yellow solution. The solution was dialyzed using dialysis membrane (molecular weight cut off, 12000—14000; Viskase Sales Co.) at room temperature for 2—3 days and lyophilized. The lyophilized samples were recovered. The synthesis route is shown in Scheme 1.

Analysis The products were characterized by IR, ^1H and ^{13}C NMR spectra. IR spectra were obtained with a Shimadzu FTIR-8900 spectrophotometer (KBr pellets) in the 4000—400 cm^{-1} range. ^1H and ^{13}C NMR spectra were recorded for solutions in D_2O on JEOL JMN-GX270 spectrometer, using sodium 3-(trimethylsilyl) propanesulfonate as an internal standard.

Degree of Substitution (DS) Determined by ^1H NMR; DS was determined from the area ratio of substituted group protons and methyl proton of *N*-acetyl group (0.36 H); data in parentheses were determined from the area ratio of substituted group protons to H-3, H-4, H-5, H-6 protons of the hexosamine residue and H-2 proton of Nacylated hexosamine residue.

Solubility Solid sample (200 mg) was placed in a glass stoppered flask containing 20 ml of distilled water. The pH of the solution was adjusted with 0.5 % (w/v) aqueous HCl and NaOH. The samples were placed on a shaker, agitated at 28℃ until equilibrium was achieved (24 h) and the aliquots were filtered through 0.22 μm nylon disc filter. The filtered samples were diluted suitably and assayed spectrophotometrically at 210 nm.

Isoelectric Point (pI) Suc-Chi has acid groups carboxy (pK_1) and the amount of unsubstituted basic amino groups (pK_2). Thus, the pI of Suc-Chi in water solution is

$$pK = pK_1 + pK_2/2$$

Suc-Chi was dissolved in acetic acid, added sodium hydroxide (NaOH) to the solutions. The pH and the volumes of solutions adding to every time were recorded. A curve of pH versus V was given. To find out the two changes of PH on curve, they are the values of pK_1 and pK_2. Using above equation, we obtained the pK.

Differential Scanning Calorimetry (DSC) The DSC thermograms were recorded using DSC-7 Differential Scanning Calorimeter (Perkin-Elmer Co., New York, USA) Approximately 2 mg of samples were heated from 30 to 400℃ at a scanning rate of 10℃/min under a stream of nitrogeo.

Partition Coefficient (P_{app}) The Partition coefficients of Suc-Chi at different pH value at 25℃ were independently measured by the shake-flask method.[17] The aqueous phase (PBS): *n*-octanol ratio employed was kept the same (100) unless otherwise stated. Samples before and after partition were quantified by using UV–9200 spectrophotometer ZRS-4 (Shimadzu, JAN). The partition coefficient was calculated by the following equation:

$$\mathbf{P = C - Cw/Cw}$$

where **C** and **Cw** represent the UV–vis absorption value of the compound at the aqueous phase before and after partition, respectively,

Zata Potential (ξ) Delsa 440SX Zeta Potential Analyze (BECKMAN COULTER) was utilized for surface zeta potential analysis of Suc-Chi at different pH value. The curve of zeta potential versus system pH value was given.

RESULTS AND DISCUSSION

We obtained Suc-Chi DAC-90 (DS=0.33, Yield 98 %). The product was characterized by IR, ^1H NMR and ^{13}C NMR spectra.

IR (KBr) cm^{-1}: 3423, 3082, 2940, 2874, 1655, 1574, 1404, 1026, 1070. ^1H NMR (D_2O) δ: 2.06 (0.36 H, s, $-NH(CO)CH_3$), 2.40 (0.63 H, br s, $-NH(CO)-CH_2-$), 2.49 (0.62 H, br s, $-CH_2-COONa$), 2.76 (0.54 H, br m, H-2 of GlcN), 3.52—4.0 (br m, H-2 of *N*-acylated GlcN and H-3, H-4, H-5, H-6 of monosaccharide residue); DS= (0.63+0.68)/4=0.33; ^{13}C NMR δ: 24.8 ($-NH(CO)CH_3$), 35.2, 35.5 ($-CH_2-CH_2-$), 57.8—59.1 (C-2), 62.7 (C-6), 74.8—81.0 (C-3, C-4, C-5), 103.7 (C-1), 178.9 ($-NH(CO)-CH_2-CH_2-$), 183.7 ($-COONa$).

The spectral characteristics of the compounds are identical to the already reported results.[18] The product was obtained by ring-opening reaction, though the DS values in our cases were somewhat low, the desired DS value of products could be obtained by use of excess amount of anhydride (6—15 equiv) and controlling reaction time for the preparation of Suc-Chi DAC-90. Furthermore, the molecular weight of Suc-Chi was inexpensively reduced using hydrochloric acid.[19]

Figure 1 shows the solubility of Suc-Chi DAC-90 (DS=0.33) in water of various pH. DAC-90, which is the starting material in study, dissolved only in the acidic region below pH 6.5. Suc-Chi DAC-90 (DS=0.33) showed the solubility even in the basic region above pH 7—8. As the derivative (DS=0.33) has both amino and carboxy group, the solubility in acidic region would be caused by the protonation of amino group (changed from $-NH_2$ to $-NH_3^+$). The solubility in alkaline region would also be caused by the change of carboxy group to carboxylate ion (from $-COOH$ to $-COO^-$). The insolubility between pH 4.5—6.8 would be owing to the isoelectric point which exists equimolar of $-NH_3^+$ and $-COO^-$ groups in the molecule. The DS value is the average value in each polysaccharide. Therefore, the insoluble pH region of sample was widely distributed (pH 4.5—6.8).

Suc-Chi DAC-90 (DS=0.33) has both amino and carboxy group. The isoelectric point which exists equimolar of $-NH_3^+$ and $-COO^-$ groups in the molecule can be calculated according to the literature.[20] Thus we see that the pI of Suc-Chi DAC-90 (DS=0.33) is 5.26. Whereas DAC-90 has not the property.

Suc-Chi DAC-90 DSC measurements were performed to investigate changes in the physical state of the compounds. The curve of Suc-Chi DAC-90 -DSC was given as follows (Fig. 2). From the Curve, we concluded a glass-to-liquid transition-onset temperature Tg at about 304.16°C, by theoretical analysis. The Tg is higher than that of DAC-90.

We obtained the average values P_{app} in this work for Suc-Chi DAC-90 (DS=0.33). Figure 3 shows the relationship of the partition coefficient and pH values. At pH=13.37 the P_{app} is 0.05, the P_{app} increases as the pH decreases until at pH=5.87 the P_{app} is 0.16, which is its maximum P_{app}. Below pH=5.87, as the graph shows, the P_{app} decreases. The facts of above are due to the derivative with a property as polyampholyte. The octanol/water partition coefficient (P_{app}) is the ratio of a compound's concentration in octanol to its concentration in water when the phases are at equilibrium. The partition coefficient of solutes between octanol and water, is extensively used in biomedical sciences as a descriptor of the lipophilicity–hydrophilicity properties of different compounds. And also the partition coefficient has been widely used in calculating numerous physical proper-

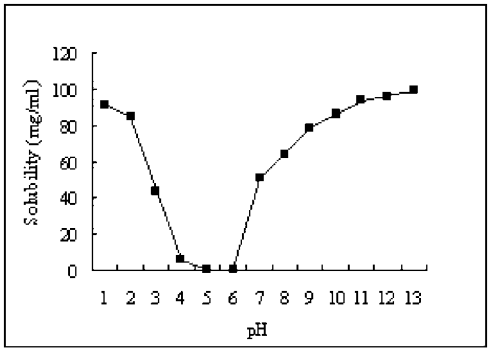

Fig. 1. Solubility of Suc-Chi DAC-90 (DS=0.33) in Water of Various pHs

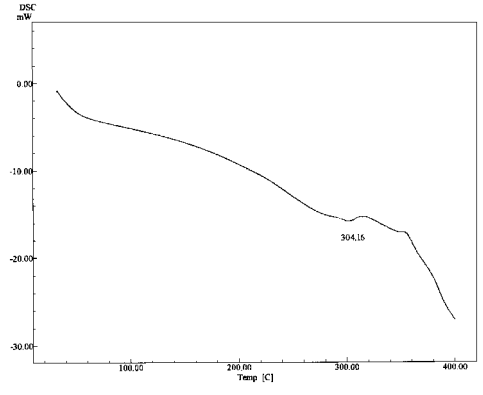

Fig. 2. DSC Pattern of Suc-Chi DAC-90 (DS=0.33)

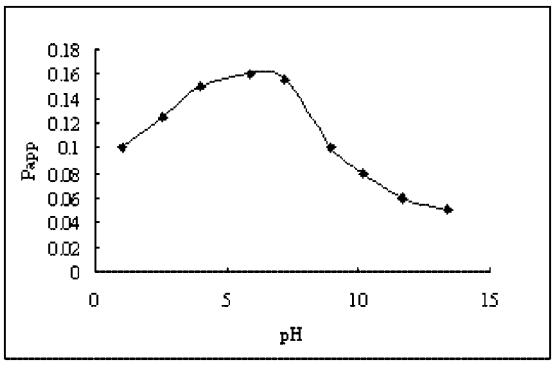

Fig. 3. Relationship of the Partition Coefficient and pH Values

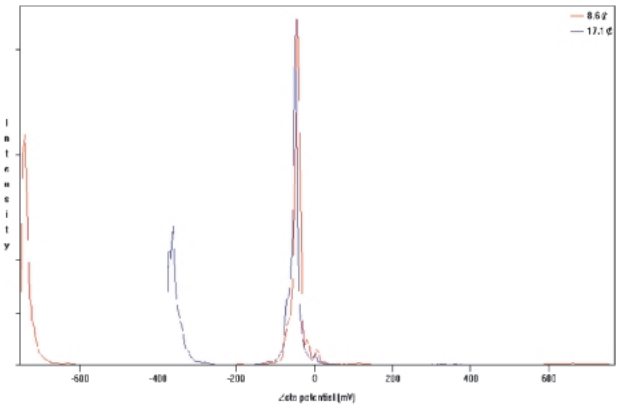

Fig. 4. Zata Potential (ξ) Pattern of Suc-Chi DAC-90 (DS=0.33)

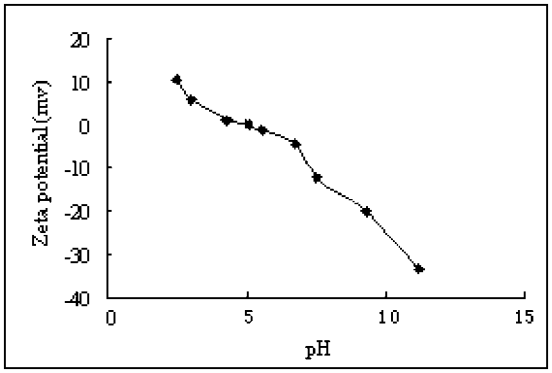

Fig. 5. Curves of Zeta Potential versus System pH Value of Suc-Chi DAC-90 (DS=0.33)

ties such as membrane transport and water solubility.

The zeta potential measurements reveal that Suc-Chi DAC-90 exhibits a net positive charge of the surface below a pH value of approximately 4 (Figs. 4 and 5). The zeta potential versus system pH value are quite different, especially in alkaline environments. Adjusting the pH of the system can change positive and negative zeta potential. Generally, DAC-90 only exhibits positive zeta potential in the acidic solution. Measurements of zeta potentials provides a straightforward way of ascertaining adsorption mechanisms and conditions in systems.

Chitosan is one of the most abundant polysaccharides in nature, second only to cellulose. Suc-Chi can be readily prepared from chitosan. Ordinary chitosan can be dissolved in acidic water but not in alkaline, whereas Suc-Chi exhibits the opposite behaviour. Suc-Chi can function well as a drug carrier due to long systemic retention, low toxicity and accumulation in the tumour tissue. It is valuable for the drug carrier to readily prepare its conjugates with various drugs to avoid vexatious complications and the conjugates with various anticancer drugs showed good antitumour activities against various tumours. In the study, the knowledges of physical-chemical properties for Suc-Chi are valuable for basic or applied purposes in biomedical and pharmaceutical sciences stabilization.

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