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Synthesis of dehydroabietic acid-modified chitosan and its drug release behavior

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ABSTRACT

A new type of chitosan derivative, dehydroabietic acid-modified chitosan (DAMC), was synthesized by the acylation reaction of chitosan with dehydroabietic acid chloride (DHAC) under microwave irradiation. The resulting product (DAMC) was characterized by FT-IR, UV, ^{1}H NMR, X-ray diffraction (XRD), scanning electron microscopy (SEM), thermal gravimetric analysis (TGA), and elemental analysis. The degree of substitution (DS) of DAMC was 16.5%. And chitosan and DAMC were used as carriers of fenoprofen calcium (FC), and their controlled release behavior in artificial intestinal juice was studied. The results showed that the controlled release of FC from the carrier of DAMC is better than that from original chitosan.

1. Introduction

Chitosan, a partially deacetylated derivative of chitin, is nontoxic and biocompatible and can be completely digested by the colonic bacteria.[1–5] And chitosan is also a good candidate for the development of novel gastrointestinal drug delivery systems. Chemical modification is usually utilized to improve the water solubility of chitosan and its derivatives, and it can introduce desired properties into chitosan and enlarge its potential application fields.[6–13] On the other hand, rosin and its derivatives have degradability, excellent solubility, biocompatibility, and lower polarity compared to chitosan.[14,15] In this research, a new type of chitosan derivative, dehydroabietic acid-modified chitosan (DAMC), was synthesized by the acylation reaction of chitosan with dehydroabietic acid chloride (DHAC) under microwave irradiation. The DHAC was obtained via the reaction of dehydroabietic acid (DHAA, the major component of disproportionated rosin) with $SOCl_2$. Also, chitosan and the product (DAMC) were used as carriers of fenoprofen calcium (FC), and their controlled release behavior in artificial intestinal juice was studied.

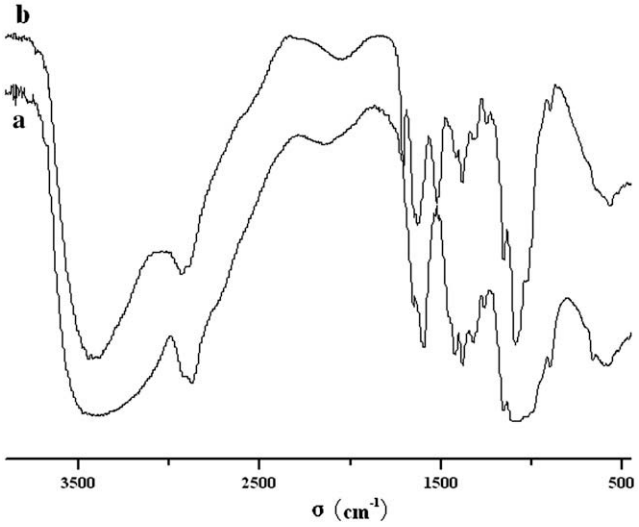

Figure. 1. FT-IR spectra of chitosan (a) and DAMC (b).

2. Characterization of DAMC

The FT-IR spectra of chitosan and DAMC are shown in Figure 1. Raw chitosan (Fig. 1a) shows signals of non-modified chitosan at 1599 cm^{-1}, 1426 cm^{-1}, 1380 cm^{-1}, and 1322 cm^{-1} attributed to N–H bending of the amino groups, C–N stretching coupled with N–H plane deformation, and symmetrical angular deformation of C–N stretching, respectively. Compared with the FT-IR spectrum of chitosan (a), DAMC (Fig. 1b) shows a new peak around 1710 cm^{-1} corresponded to C=O of the amide groups in DAMC, but the characteristic peak of C=O in an ester groups at

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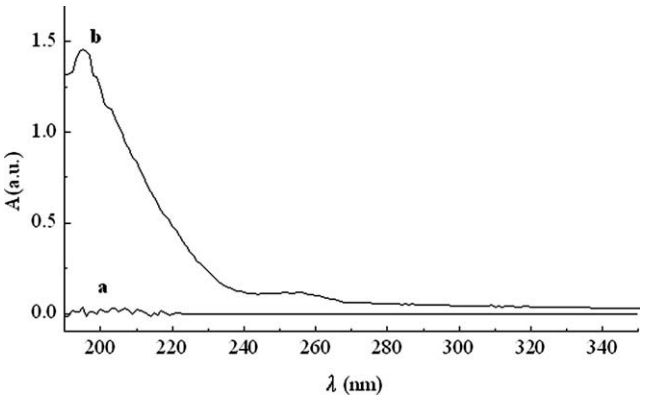

Figure. 2. UV spectra of chitosan (a) and DAMC (b).

1730 cm^{-1} was not observed.[16,17] And the absorption peak at 1599 cm^{-1} due to N–H bending of the amino groups disappeared. The above FT—IR data confirmed that the DMAC was synthesized as shown in Figure 8. According to elemental analysis and following formula DS = 7 × ($n_s - n_p$)/120, the degree of substitution (DS) of DAMC was 16.5%.

The UV spectra of chitosan and DAMC are shown in Figure 2. As can be seen, the UV spectrum of DAMC (Fig. 2b) shows an absorption band for a benzene ring. The λ_{max} value was at 195 nm for the absorption of the E1 and E2 bands arising from the π–π^* electronic transition in the benzene ring. The λ_{max} at 255 nm was attributed to the characteristic peak of the B band of the benzene system present in DAMC.

The ^{1}H NMR spectra of the raw chitosan in DCl/D_2O and DAMC in D_2O are shown in Figure 3. The spectrum of chitosan (Fig. 3a) shows a singlet peak at 3.0 ppm, and doublets peak at 3.5–3.9 ppm corresponded to the methine protons of D-glucosamine ring.[16,18] And the proton signal at around 2.6 ppm attributed to N–H peak of the amino groups. In the spectrum of DAMC (Fig. 3b), the peak at around 7.8 ppm is attributed to the protons of the benzene ring. And two new signals at 3.2 ppm and 1.90 ppm are assigned to the protons of carbons directly connected to the –NHCO– groups and C–H (–CH(CH_3)) of DHAA side groups, respectively.[19] These evidences obviously support that the amino groups of the raw chitosan were converted to amides.

Figure 4 shows the X-ray diffraction patterns of chitosan and DAMC. The pattern of chitosan (Fig. 4a) shows distinct crystalline peaks at 10°and 20°. Compared with chitosan, it can be found that the peak at 10° disappeared, and the characteristic peak at 20° weakened obviously in the pattern of DAMC (Fig. 4b). The decrease in crystallinity of DAMC was resulted from the destruction of the strong hydrogen bonds in the raw chitosan because of the

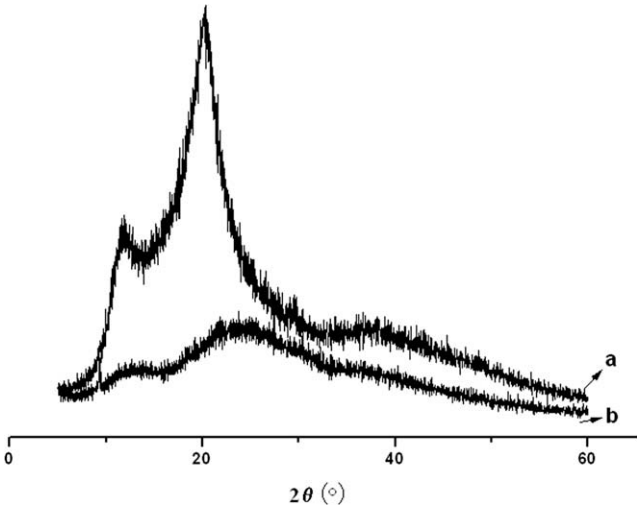

Figure. 4. X-ray diffraction patterns of chitosan (a) and DAMC (b).

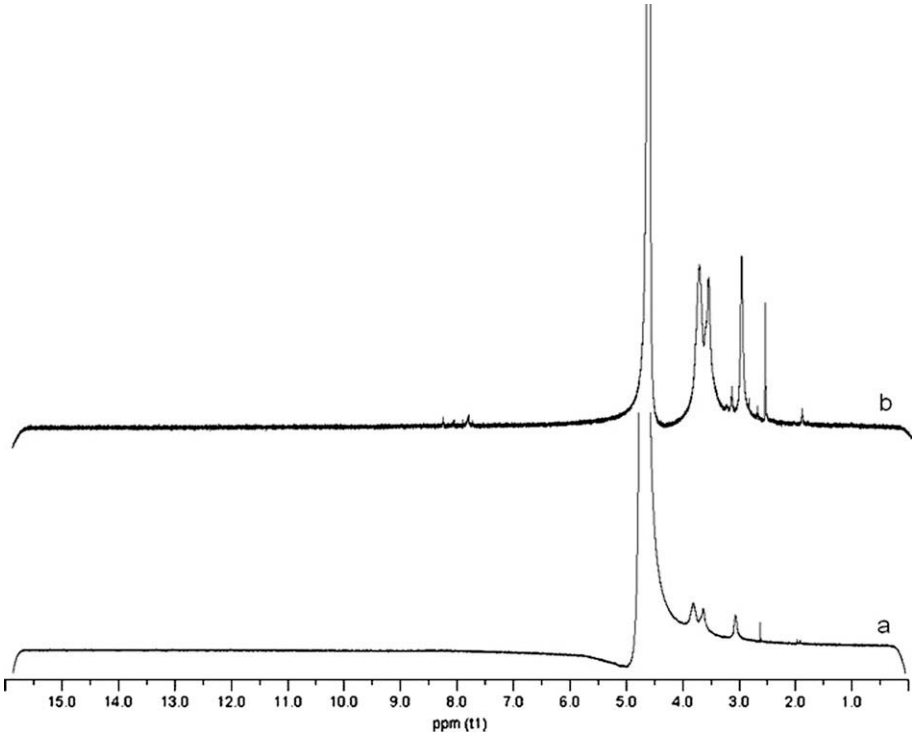

Figure. 3. ^{1}H NMR spectra of chitosan in D_2O/DCl (a) and DAMC in D_2O (b).

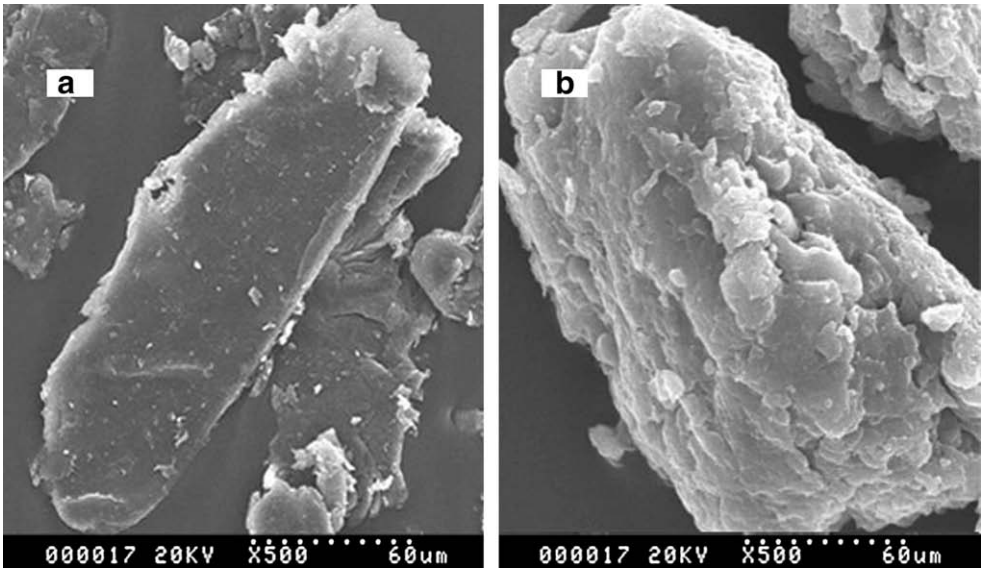

Figure. 5. SEM images of chitosan (a) and DAMC (b).

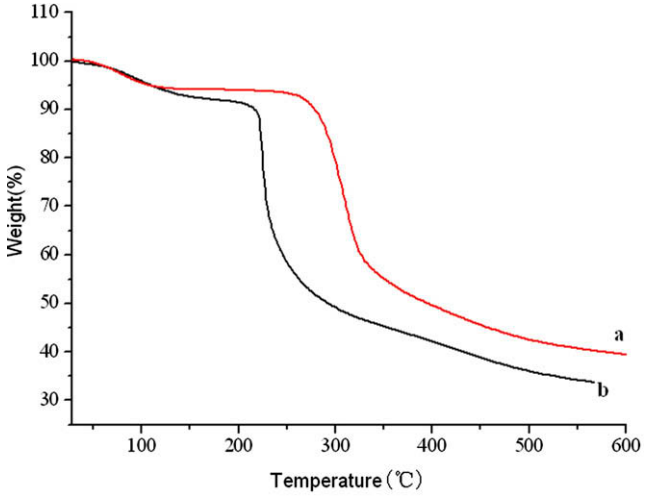

Figure. 6. TGA curvers of chitosan (a) and DAMC (b).

N-acylation reaction of the amino groups. The low crystallinity of DAMC indicates that it was more amorphous than chitosan.[20] Therefore, it can be concluded that the DHAA side groups were introduced into chitosan main chains.

The SEM images of chitosan and DAMC are shown in Figure 5. The SEM image of chitosan (Fig. 5a) shows larger clustered structure, because there are stronger interactions between chitosan molecules. Compared with chitosan, in the surface of DAMC (Fig. 5b), fluffy morphology and globular shapes with some irregularities are clearly observed. It may be attributed to the polar difference between chitosan and DAMC, and the destruction of the intermolecular hydrogen bonds and the crystalline regions of chitosan, which indicate intuitively that the DHAA side groups were introduced into chitosan main chains.

Figure 6 shows the results of TGA analysis of chitosan (a) and DAMC (b). As can be seen in Figure 6, two consecutive weight loss steps were observed in both chitosan and DAMC. No matter chitosan or DAMC the first weight loss occurred at temperatures lower than 100 °C corresponded to the evaporation of the water physically absorbed. The second weight loss occurred at temperature higher than 200 °C corresponded to the decomposition of chitosan and DAMC. Compared with the raw chitosan, the decomposition temperature of DAMC shifted toward low temperature. It indicates that the decomposition of the DHAA side group played a significant role in the decomposition of DAMC.

3. Drug release behavior

The rate of release of FC for two drug troche of chitosan and DAMC was determined by dialysis method against the artificial intestinal juice, and the results are shown in Figure 7. For the raw chitosan, the release of FC increased rapidly up to 40% within an hour, which may be attributable to the solubility of FC in water. Then, the rate of release increased slowly until 11 h, and the release kept in about 57%. However, the rate of release of DAMC increased gradually from 12% to 63% in the determination of time, which indicates that the controlled release of FC from DAMC is better than that from chitosan in artificial intestinal juice. The reason may be related to the fact that the DHAA side group surrounded FC and played an important part in the controlled release. It is

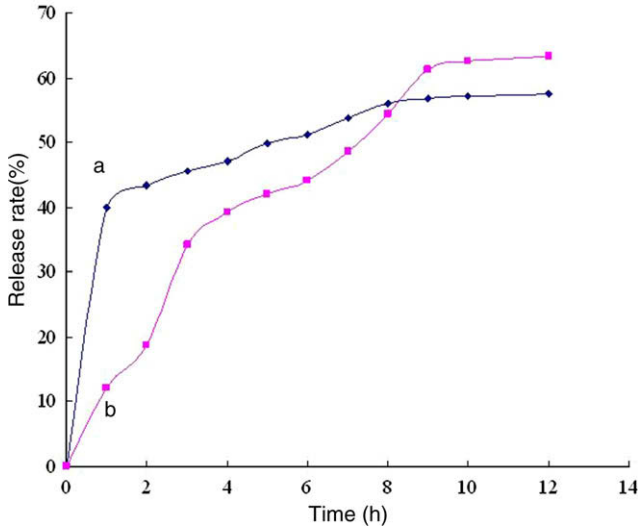

Figure. 7. The rate of release of drug troche of chitosan (a) and DAMC (b) in artificial intestinal juice.

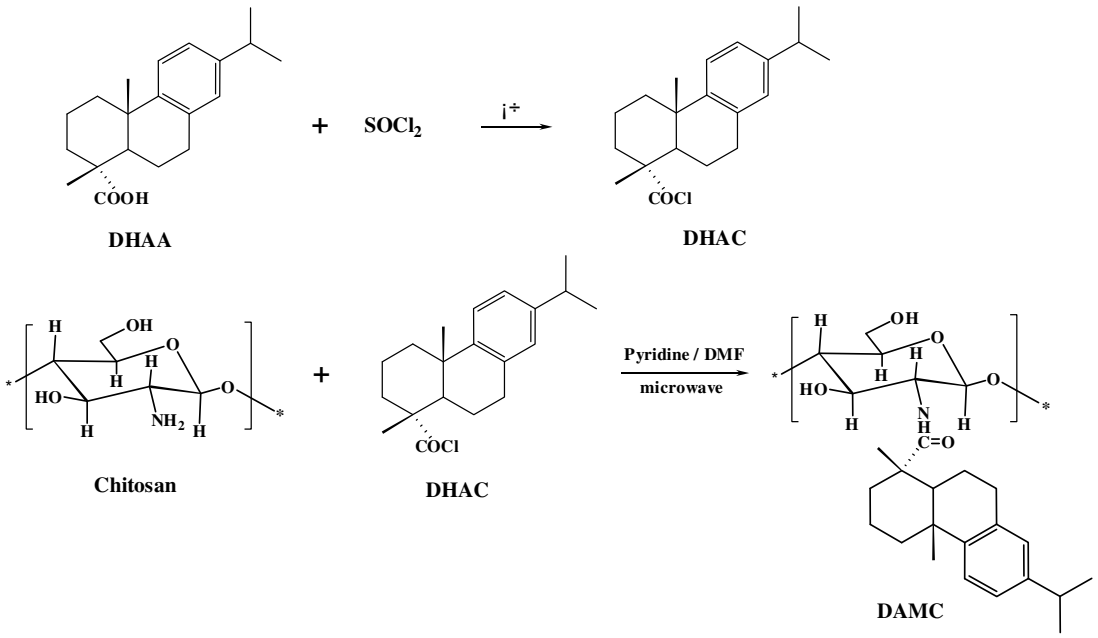

Figure. 8. Synthetic route of DAMC.

possible that a very slight coordination between the amide group and calcium ions may exist, that is, calcium ions act as a bridge between the carbonyl groups in DAMC and FC. On the other hand, there are hydrophobic bonds between the hydrophobic groups in DHAA and FC. All the above reasons lead to the controlled release of FC from DAMC.

4. Experimental

4.1. Materials and equipments

Chitosan was purchased from Zhejiang Jinqiao Biochemical Co., Ltd, China. The degree of deacetylation (DD) of chitosan was 0.95 as measured by elemental analysis.[21] Fenoprofen calcium was supplied by Jiangsu Nantong Chemical Co., Ltd, China. Dehydroabietic acid was separated from disproportionated rosin according to the previous work.[22] All other chemicals and reagents used were of analytical grade, and were used without further purification. A Xianghu microwave-induced synthesis/extraction apparatus (XH-100B), produced by Beijing Xianghu science and technology development Co., Ltd, China, was employed in the synthesis of DAMC.

4.2. Synthesis of DAMC

Synthesis of DAMC was carried out as the route shown in Figure 8. The intermediate dehydroabietic acid chloride (DHAC), was prepared as in the previous study.[23] The acylation reaction of chitosan with DHAC was performed in two steps, that is, the activation of chitosan and the acylation reaction under microwave irradiation. The procedure is as follows:[24] chitosan powder (0.5 g) was dissolved in 0.5% (w/v) of aq HOAc (50 mL). With stirring, 2% (w/v) of aq $NaHCO_3$ (50 mL) was added dropwise over a 10-min period to obtain transparent gel. The obtained gel was washed with water till a neutral condition was obtained, then soaked in pyridine for one day, and filtered to obtain the activated chitosan (9.3 g). Then, the activated chitosan was added to round flask and dissolved in DMF (20 mL), and DHAC (5.4 g) dissolved in pyridine (10 mL) was added dropwise to the flask in a few minutes. After stirring for half an hour, the flask was placed in a microwave reactor, setting reaction time (2.0 h), temperature (100 °C), and power (700 W). After reaction, a heterogeneous aggregation of the product was observed in the solution. The resultant mixture was poured into methanol (100 mL), and the precipitated product was filtered, extracted in Soxhlet's extractor with methanol for 8 h and dried under vacuum for 24 h. The degree of substitution (DS) of DAMC was calculated by $n_c = 6 \times [6 + 2(1 - DD)]/7$, $n_p = 6 \times [6 + 2(1 - DD) + 20DS]/7$ and $DS = 7 \times (n_s - n_p)/120$, where n_c and n_p are the ratios of carbon to nitrogen percentage of native chitosan and DAMC, respectively, DD is the degree of deacetylation of native chitosan.

4.3. Characterization

Fourier transform infrared (FT-IR) spectra of chitosan and DAMC were recorded in the range of 4000–400 cm^{-1} using a Nicolet Nexus 470 spectrometer (Nicolet Co., Ltd, USA) with KBr disks. The elemental analysis (C,N) was performed on a PE2400 II elemental analyzer (Perkin Elmer Instruments Co., Ltd, USA). ^1H NMR spectra were recorded on an ADVANCE AV 500 MHz NMR spectrometer (Bruker Co., Ltd, Switzerland), with DCl/D_2O or D_2O as the solvent. The X-ray diffraction (XRD) patterns and scanning electron micrograph (SEM) images of chitosan and DAMC were obtained by Rigaku D/max2500V (Rigaku Co., Ltd, Japan) and JEM1200-EX/S (JEOL Co., Ltd, Japan), respectively. The degradation process and thermal stability of chitosan and DAMC were investigated using a NETZSCH STA 409PC thermogravimetric analyzer (NETZSCH Instrument Co., Ltd, Germany). The TGA (thermal gravimetric analysis) measurements were carried out under an argon atmosphere at a heating rate of 10 °C/min from room temperature to 800 °C. All the UV spectra of the release medium were recorded on a UV–vis spectrophotometer (UV-1201 PCS, Unico (Shanghai) Instruments Co., Ltd, China).

4.4. Drug release of chitosan and DAMC

The artificial intestinal juice (Tris–HCl buffer solution, pH 7.2) was prepared as follows: in a close glass vessel, 500 mL of 0.1 M tris(hydroxymethyl) aminomethane solution and 447 mL of 0.1 M

hydrochloric acid solution were added, and stirred for equilibration.

4.4.1. Preparation of drug troche

Dried chitosans or DAMC powder sample (0.3000 g) and 0.0200 g of fenoprofen calcium (FC) were mixed evenly, and pressed to drug troche and weighted. Then, the contents of FC were calculated.

The standard curve of FC was drawn as follows: 0.500 g of FC was weighed accurately, and put in a beaker in which there was 500 mL of Tris–HCl buffer solution. Then, the beaker was put into a shaking bed (at 37 ± 1 °C, 180 rpm) for 24 h to make FC dissolve completely. The solution was transferred to 1000 mL volumetric flask, to obtain the 0.05 g/L solution, and 100 mL of 0.000 M, 0.005 M, 0.010 M, 0.015 M, 0.020 M, 0.025 M, 0.030 M, 0.035 M, 0.040 M, and 0.050 M FC solution was prepared, respectively. Their absorbency was determined by UV spectrophotometry at 271 nm, respectively, and the standard curve of FC was drawn out by the absorbency ($\boldsymbol{A}$) evolution with concentration ($\boldsymbol{C}$), that is, $\boldsymbol{A} = 6.2238\boldsymbol{C} + 0.0337$ ($\boldsymbol{R}^2 = 0.9983$).

The rate of release of FC for two drug troche of chitosan and DAMC was determined as follows: the drug troche and 10 mL of distilled water were added to dialysis tubing (molecular weight cut off 7000), and the dialysis tubing was immersed in 390 mL of Tris–HCl buffer solution. Then, the solution was put into a shaking bed (at 37 ± 1 °C, 180 rpm) for 12 h, and 3 mL of the solution was sampled at every one hour. Subsequently, the amount of FC released from the drug troche was evaluated by means of UV spectrophotometry at 271 nm and the standard curve of FC, and the rate of release was calculated.

Acknowledgments

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