Paper D22

Study on synthesis and antibacterial property of polyamino chitosan derivates

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Abstract: We use propylene epoxide to hydroxylate chitosan, so that to obtain water-soluble hydroxypropyl chitosan (CS-H). Through Michael additive reaction with methyl acrylate (MA), we obtain N-ethyl carboxyl hydroxypropyl chitosan methyl ester CS-HC. We use diethylenetriamine to amination react with esters CS-HC to obtain water-soluble hydroxypropyl chitosan .Through IR and ^{1}HNMR to study its material structure and properties .We choose escherichia coli as our test strain to study its antibacterial property .The result shows that the products have high antibacterial property.

Key Words: Polyamino, Chitosan derivate, Sythesis, Antibacterial property

1 Introduction

Chitosan have properties of moisture absorption, retentiveness, antibacterial property, biocompatible and other chemical physical properties and biological activity, so it is widely used in medication, foodstuff, environment protection and chemical sectors. Chitosan is fine functional natural green fabric finishing agents, after finishing, the fabric have good antibacterial and finishing deodorization properties, it's comfortable when wearing, chitosan also can modify the coloring property of fabric. But the relative mass is too big, the structure is density, so it can't dissolve in water, only dissolve in acid, which greatly limit its application area. Chitosan have large amount of amino groups, hydroxyl groups, and pyranoid ring and other activity group, we can induce special group to change its molecule structure, finally change its physical chemical properties, give chitosan new functions, expanding its application area.

This paper use chitosan as main material, we use a novel chemical method to modify chitosan to obtain arborizate polyamino chitosan derivates. Then study on the antibacterial property of the modified products.

2 Experimental

2.1 Materials and Equipments

Chitosan, industry products, Zhejiang Aoxing-biology technology limited company; Protein peptone, beef extract, agar, Escherichia coli, Beijing Aoboxing biotechnology limited company; Fourier Transform Infrared Spectroscopy,Type: VECTOR-22, Germany BRUKER company; NMR Spectrometer, type: ADVAIII 400MHz,produce country: Germany-Switzerland Brock company; Water Insulation Electrothermal Constant Temperature Incubator, type: PYX-DHS, Shanghai Yujin medical treatment instrument factory.

2.2 The synthesis principle of CS-HCA

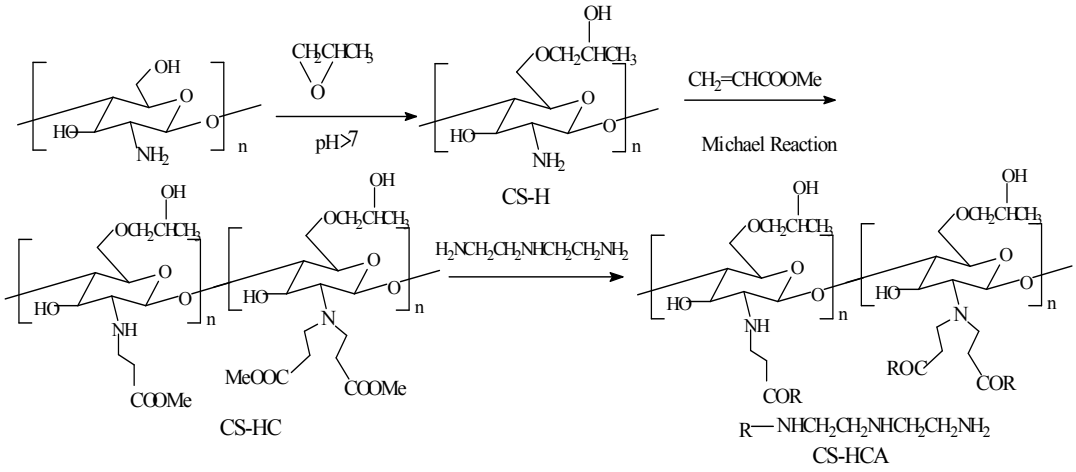


Fig1 Synthesis routes of CS-HCA

2.3 The synthesis method of CS-HCA

2.3.1 The synthesis method of CS-H

Weigh 2.5g chitosan into 50ml small beaker, add 5ml 33% NaOH solution , when chitosan sewlling sufficiently, put it into refrigerator overnight ,thawing the next day, put it into the 3 necks boiling flask with reflux device, Add 50ml isopropanol, stirring 30min. Then add 0.25g 10% mass concentration tetramethylammonium hydroxide solution, then add 20g propylene oxide when stirring, stirring for another 1h, rising temperature to 50℃ with refluxing reaction for 6h, cooling, filtration, washing, drying to obtain white or light yellow powdery products.

2.3.2 The synthesis method of CS-HC

Put 2.0g CS-H and 50ml 30% mass concentration ethanol solution into 3-necks boiling

flask, add 5g methyl acrylate (MA) slowly, react for 32h under 50□, remove redundant MA and water through rotating evaporimeter, use mass amount of acetone to precipitate, filtration, washing, drying to obtain white CS-HC.

2.3.3 The synthesis method of CS-HCA

Weigh 1g CS-HC into 3 necks boiling flask, add 40ml anhydrous methanol and 3g diethylenetriamine, stirring 2d under 40□, precipitation, filtration, washing, drying to obtain final product which is CS-HCA.

2.4 Structure characterization

Dry CS, CS-H, CS-HC, CS-HCA, use KBr squash method, detect through Fourier Ttransform Infrared Spectroscopy; use deuteroxide as deuterated reagent, detect the H-NMR of CS-H, CS-HC, CS-HCA through ADVAIII 400MHz NMR Spectrometer

2.5 Determination of characteristic viscosity η, substituted degree

We use ubbelohde to determine the characteristic viscosity; use the same method as literature [6] to determine substituted degree

2.6 antibacterial property experiments

Use the same method as literature [7] to determine antibacterial property.

3 Results and Discussion

3.1The determine of the product property

3.1.1 Qualitative analysision of CS-H

Add CS-H into iodoform reagent, if there are light yellow precipitation, the product have the $-CH_2CH(OH)CH_3$ structure, the raw material CS doesn't have primary alcohol ,which prove that CS successfully modify to CS-H.

3.1.2 Testing physical properties of products

We can conclude that the characteristic viscosity of CS-H is much small than CS from figure 1, because the reaction is under alkalinity condition, the molecule chain will degradate which result in the decrease of viscosity. The deacetylation degree of CS-H is 6% less when compared to CS, This is probably the hydroxylate reaction between propylene epoxide and CS are mainly happen in C_6-O,few of them happen in —NH_2.

Through substituted degree of CS-H and deacetylation degree of CS we can conclude the largest productivity of CS-H under the most appropriate conditions is as follows:

$$P = \frac{2.2394 / (161\times 0.9 + 58\times 0.52)}{2.5 / 161\times 0.9} = 74.1\%$$

Fig 1 Testing physical properties of products

product	η/ml·g-1	solubility/g(H_2O 100ml)-1	D.D/%	DS
CS	580	--	91.2%	--
CS-H	97.2	6.88	85%	0.52
CS-HC	210.8	4.231	--	--
CS-HCA	131.7	7.345	--	--

The characteristic viscosity η of CS-HC is 210.8 ml·g-1which is much bigger than that of CS-H 97.2 $ml \cdot g^{-1}$ this is probably because introduce ethyl carbomethoxy which increase the relative molecular mass, the viscosity is increase; with the increase of viscosity, the water-solubility is not good when compared to CS-H, result in the water –solubility of CS-HC is smaller than CS.

The characteristic viscosity η of CS-HCA is 131.7 $ml \cdot g^{-1}$, smaller than that of CS-HC 210.8 $ml \cdot g^{-1}$,this is probably because the synthesis of CS-HCA is react under amine, which will result in rupturing of glucosidic bond in CS molecular chain, the viscosity decrease. The decrease of viscosity and the increase of hydrophilicity groups make the water solubility of CS-HCA higher than CS-HC.

3.2 The Characterization of products structure

1. IR analysis: IR spectrums of chitosan CS, chitosan modification product CS-H, CS-HC, CS-HCA are showed in Figure 2.

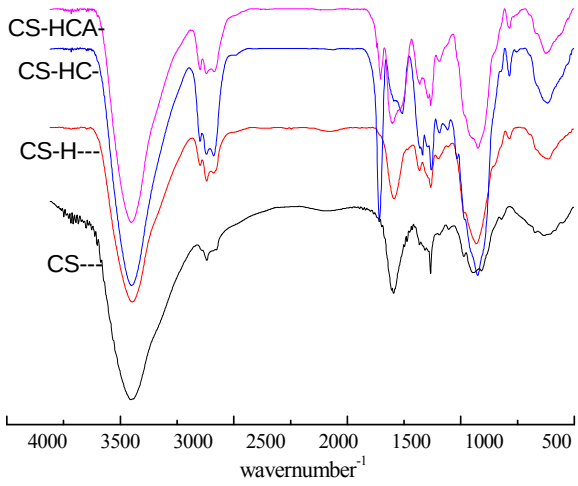


Fig.2 IR spectra of CS and Productions

Through IR spectrum we can see that CS-H save the CS's -OH stretching vibration peak under 3440cm^{-1} and N-H stretching vibration peak; 2969 cm^{-1}, 2870 cm^{-1} and 2923 c m^{-1} appear $-CH_3$ and $-CH_2$ stretching vibration peak, 1461 cm^{-1} and 1380 cm^{-1}appear $-CH_3$ and

-CH_2 bending vibration peak; because there are new C-O and C-O-C bonds form, the C-O upon –OH adsorption peak of CS displace to 1070 cm^{-1},the intensity of the peak is stronger apparently, primary -OH in 1033 cm^{-1} disappear, which indicate that the hydroxylate mainly react in C_6-OH.

We can see from the IR spectrum of CS-HC, The appearance of ester C=O bond stretching vibration peak under 1736 $cm^{-1}$and the intensity of adsorption is relatively big, the new peak around 1260 cm^{-1}, 1202 cm^{-1} are the C-O-C of ester antisymmetric and symmetric bending vibration peak; the distortion absorption peak of N-H around 1640 cm^{-1} red shift to 1580 cm^{-1}; the peak around 2969 cm^{-1}, 2923 cm^{-1}, 2870 cm^{-1}, 1460 cm^{-1}, 1380 cm^{-1} are contraction and bending vibration peak of -CH_3, -CH_2, the peak have different degrees of increase, these features indicate that MA are induced into CS-H successfully.

According to the spectrum of CS-HCA, the stretching vibration peak of C=O on the ester around 1736 cm^{-1} decrease, the bending vibration peak of C-O-C on the ester around 1260 cm^{-1}, 1202 cm^{-1} have disappeared, which indicate that most ester are replaced and substituted. the distortion absorption peak of N-H around 1640 cm^{-1} enhance, which indicate that the amount of amide bonds increase.

2. ^{1}HNMR analysis: the ^{1}HNMR spectrum of the modified chitosan CS-H, CS-HC, CS-HCA are showed in figure 3-13, figure 3-14, figure 3-15.

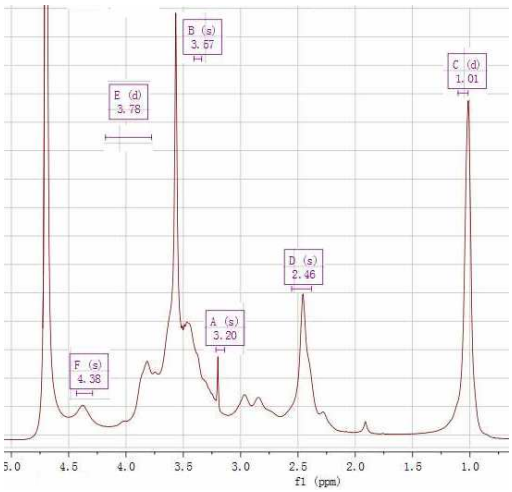


Fig 3 Typical ^{1}HNMR spectra of CS-H

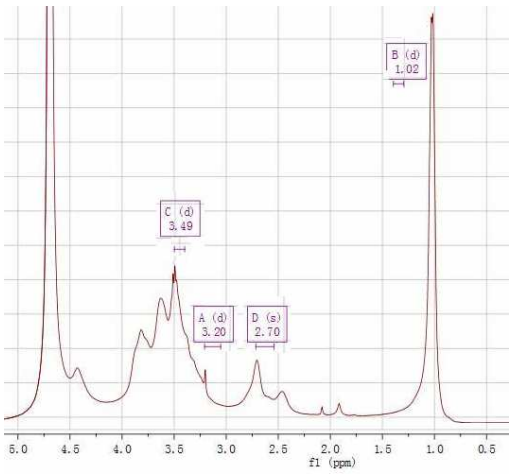


Fig 4 Typical ^{1}HNMR spectra of CS-HC

We can conclude from figure 2 that the product CS-H modified from CS appear obvious proton peak chemical shift of -CH_3 around δ1.0ppm (d), the proton peak chemical shift of the methylene on -$OCH_2CH(OH)CH_3$ structure appear around proton peak chemical shift, which indicate that CS have been replaced by hydroxypropyl; the peak around δ3.2ppm is the proton peak of the C_2 on the pyranose ring. The peak around δ3.5~4.0ppm is the proton peak of hydroxypropyl and C_3~C_6,which indicate that the substitute reaction of hydroxypropyl mainly on hydroxyl groups, especially on C_6-OH, because there aren't intramolecular hydrogen bond between C_6-OH and adjacent repeating unit ,that reactivity is bigger than C_3-OH.

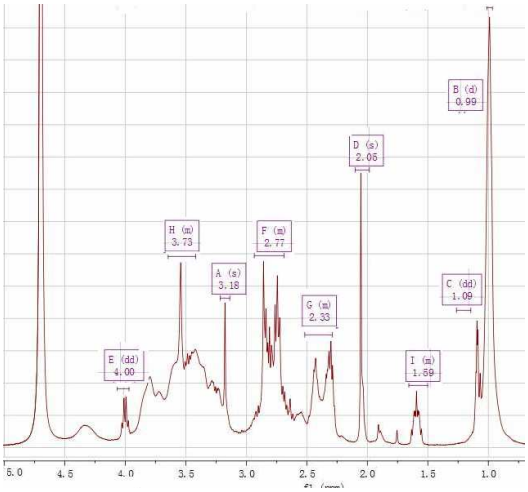


Fig 5 Typical ^{1}HNMR spectra of CS-HCA

We can conclude from figure3 that the product CS-HC modified from CS-H appear obvious chemical shift around δ3.57ppm (s), which is proton peak chemical shift of the methyl on the -$COOCH_3$ group, the peak around δ2.2~3.0ppm is the proton peak of hydroxypropyl on -$NHCH_2CH_2COOCH_3$ of the pyranose ring C_2 position. The proton peak around δ1.0ppm (d)is the chemical shift of -CH3, the proton peak around δ3.2ppm is the pyranose ring C_2 position, the proton peak around δ3.5~4.0ppm is the methylene on C_3~C_6, -$CH_2CH(OH)CH_3$.

We can conclude from figure 3-15 that the chemical structure of product CS-HCA modified from CS-HC appears great change. According to picture 4, the peak around δ2.0ppm is the proton adsorption peak of the methylene adjacent to C=O on the amide group, the peak around δ2.5~2.5ppm is the proton adsorption peak of the methylene adjacent to N on the amide group, the peak around δ2.6~2.9ppm is the proton adsorption peak of the methylene adjacent to amino group or imino group, the peak around δ1.0ppm (d)is proton peak chemical shift of -CH_3, δ3.2ppm is the proton peak of the C_2 on the pyranose ring. the peak around δ3.73ppm (s) is proton adsorption peak of the methyl on the -$COOCH_3$ group, the proton peak around δ3.5~4.0ppm is the methylene on C_3~C_6, -$CH_2CH(OH)CH_3$.

3.3 The result of antibacterial experimental

(1) The selection of strain

The four typical strain have been study in fabric and leather products are as follows: the resistance of Staphylococcus aurous is the strongest pathogenic bacteria among the non spore bacteria,it can represent gram-positive bacteria, Escherichia coli is widely distribution, it can represent gram-negative bacteria; Bacillus subtilis can form spore easily, strong resistance, the experiments results show that this strain can survive through heating under 100ºC for 5min, it can represent spore bacteria; Candida albicans is an usual pathogenic fungus in Human Skin mucosa ,it can represent fungus. The four strains are the most usually distribution category in nature and human skin mucosa, it can essentially reflect the antibacterial property of faric and leather products

For shoe leather, Staphylococcus aurous and Escherichia coli are the main antibacterial object, we choose Escherichia coli as test strain.

(2) Antibacterial function

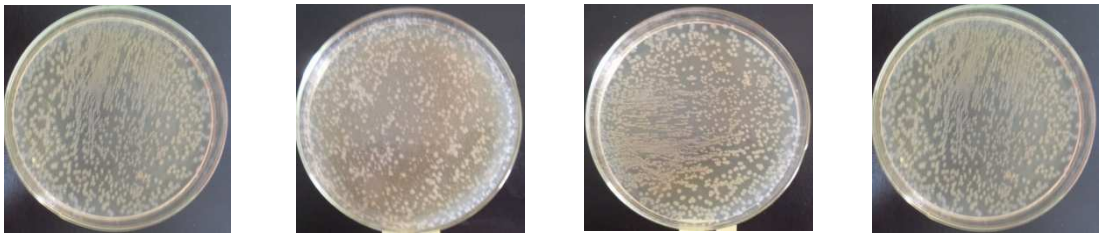
Put chitosan into acetic acid solutions to obtain 5% mass concentration chitosan solution, adjust pH to 6,then use acetic acid sodium acetate buffer solution (pH=6) to dilute part 5% chitosan solution to 1% and 0.5% chitosan solution; use distilled water (pH=7)and acetic acid sodium acetate buffer solution (pH=6) to dissolute CS-H, CS-HC and CS-HCA, then compound 5%, 1%, 0.5% mass concentrate solution, mix with agar-solidified medium accord 1:9 (volume ratio) to reach 0.5%, 0.1%, 0.05% mass concentration solution, then start antibacterial experiments, the results are showed in figure 2, photo 3

Tab 2 Antibacterial effect of products

Antibacterial ratio%	distilled water (pH=7)			acetic acid sodium acetate buffer solution (pH=6)		
	0.5%	0.1%	0.05%	0.5%	0.1%	0.05%
CS	--	--	--	100%	99%	98%
CS-H	0%	0%	0%	50%	85%	98%
CS-HC	0%	0%	0%	90%	95%	98%
CS-HCA	0%	0%	0%	75%	86%	99%

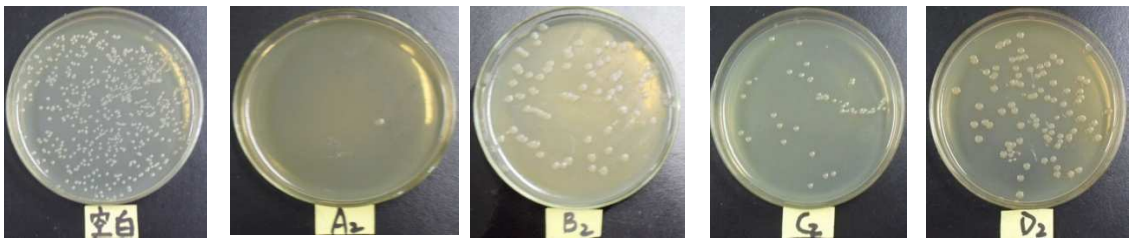
According to Figure 2 and photo 4,the product not only have antibacterial property,but also help the growth of bacteria, when use acetic acid sodium acetate buffer solution (pH=6) as solvent, they all show good antibacterial properties, because CS and their synthetic products are not protonated under neutral condition, so they don't have antibacterial properties, when use acetic acid as solvent, the amino-groups on CS and their synthetic products are protonated. The synthetic products aren't show good antibacterial property according to the picture, because when use acetic acid sodium acetate buffer solution (pH=6) to dissolve CS-H, CS-HC and CS-HCA, the amino-groups on CS-H, CS-HC and CS-HCA are not protonated under high mass concentration totally, the unprotonated CS-H, CS-HC and CS-HCA are nutrition.

Only the protonated CS-H, CS-HC and CS-HCA have antibacterial property when use CS-H, CS-HC and CS-HCA as antibacterial material, we should the protonated the amino-group first, then control the concentration when use.

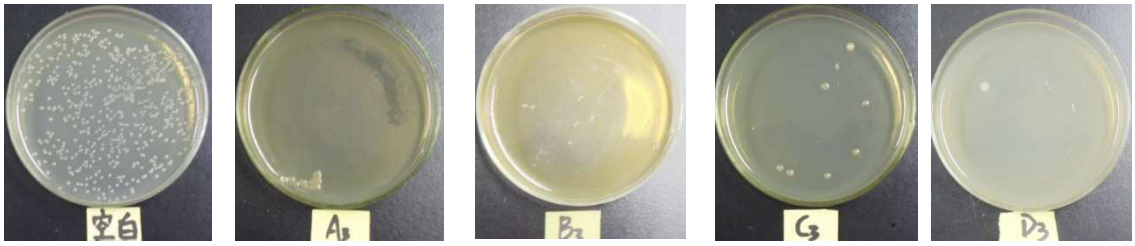


pH=7 空白 B: CS-H(0.5%) C: CS-HC(0.5%) D: CS-HCA(0.5%)

pH=6 空白 A_1 : CS-H(0.5%) B_1 : CS-H(0.5%) C_1 : CS-HC(0.5%) D_1 : CS-HCA(0.5%)



pH=6 空白 A_2 : CS-H(0.1%) B_2 : CS-H(0.1%) C_2: CS-HC(0.1%) D_2 : CS-HCA(0.1%)



pH=6 空白 A_3 : CS-H(0.05%) B_3 : CS-H(0.05%) C_1 : CS-HC(0.05%) D_1 : CS-HCA(0.05%)

Fig.6 Results of antibacterial experiment

4 Conclusions

1. We use propylene epoxide to hydroxylate chitosan under alkaline condition in order to obtain hydroxylate product CS-H, through Michael Addition react with methyl acrylate(MA), we obtaine CS-HC, We use diethylenetriamine to amination react esters with CS-HC to obtaine water-solubility, highly branched hydroxypropyl chitosan CS-HCA.
2. CS, CS-HC and CS-HCA have antibacterial property under neutral condition , have high antibacterial property under acidic condition.

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