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Faculty of Chemical and Biopharmaceutical Technologies
Department of Industrial Pharmacy

Master's thesis
on the topic

STUDY OF THE CONTROLLED RELEASE OF POORLY WATER-SOLUBLE API IN SYSTEMS WITH NATURAL POLYMERIC MATERIALS

Completed: student of the group MPhch-20
of the speciality 226 Pharmacy, industrial pharmacy
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ASSIGNMENTS
FOR THE MASTER'S THESIS

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2. Scientific supervisor Volodymyr Bessarabov, Doctor of Engineering Sciences, Assoc. Prof.

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6. Consultants of the master's thesis sections

Section	Surname, initials and positionof the consultant	Signature	
		the task was issued	the task accepted
Section 1	Volodymyr Bessarabov, Doctor of Engineering Sciences, Assoc. Prof.		
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Execution schedule

№	The name of the stages of the master's thesis	Terms of performance of stages	Note on performance
1	Introduction	20.09 –27.09.2021	Wei Tang
2	Section 1. Physico-chemical properties of chitosan and methods of its modification. Literature review.	28.09. – 11.10.2021	Wei Tang
3	Section 2. Synthesis, characterization, physicochemical properties and application of tetradecyl carboxylic acid modified O-carboxymethyl chitosan	12.10 – 25.10.2021	Wei Tang
4	Section 3. Loading and releasing of curcumin from TCA-m-CMCh aggregates	26.10 - 08.11.2021	Wei Tang
5	Conclusions	09.11.-15.11.2021	Wei Tang
6	Draw up a master's thesis (*final version*)	16.11.-04.12.2021	Wei Tang
7	Submission of master's thesis to the department for review	06.12.-14.12.2021	Wei Tang
8	Checking the master's thesis for signs of plagiarism	14.12-18.12.2021	Wei Tang
9	Submission of master's thesis to the master's department to check the implementation of the appendix to the individual curriculum	16.12-18.12.2021	Wei Tang
10	Submission of master's thesis for approval by the head of the department	18.12-21.12.2021	Wei Tang

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SUMMARY

Wei Tang. Study of the controlled release of poorly water-soluble API in systems with natural polymeric materials. – Manuscript.

Master's thesis on the speciality 226 Pharmacy, Industrial Pharmacy. - Kyiv National University of Technology and Design, Kyiv, 2021.

In this paper, Tetradecyl carboxylic acid was used to modified O-carboxylmethyl chitosan, and synthesizing tetradecyl carboxylic acid-modified-O-carboxylmethyl chitosan (TCA-m-CMCh). The chemical structures of TCA-m-CMCh were characterized by FTIR, 1H NMR and XRD methods. Properties of TCA-m-CMCh including thermal properties, antibacterial activity, cytotoxicity, aggregation behavior, self-aggregate particle size and particle size distribution were studied. In solution of 1.0 g/L, TCA-m-CMCh could form aggregate with average particle size of 221.8 nm and Zeta potential of -23.6 mV. TCA-m-CMCh showed moderate antibacterial activity against E. coli and S. aureus, and non-toxicity. The curcumin could be released continuously from TCA-m-CMCh aggregates for 600 min, and the accumulated concentration was up to 0.1 g/L. The results indicated that TCA-m-CMCh is one of potential curcumin carriers.

Keywords: *tetradecyl carboxylic acid-modified-O-carboxylmethyl chitosan (TCA-m-CMCh), physicochemical property, drug loading, releasing*

АНОТАЦІЯ

Вей Тан. Дослідження контрольованого вивільнення малорозчинних у воді АФІ в системах з природними полімерними матеріалами. – Рукопис.

Дипломна магістерська робота за спеціальністю 226 Фармація, промислова фармація. – Київський національний університет технологій та дизайну, Київ, 2021 рік.

У цій роботі тетрадецилкарбонова кислота була використана для модифікації О-карбоксиметилхітозану та синтезу О-карбоксиметилхітозану, модифікованого тетрадецилкарбоновою кислотою (TCA-m-CMCh). Хімічні структури TCA-m-CMCh охарактеризовано методами FTIR, ^{1}H ЯМР та XRD. Досліджено властивості TCA-m-CMCh, включаючи теплові властивості, антибактеріальну активність, цитотоксичність, агрегаційну поведінку, розмір часток самоагрегату та розподіл частинок за розміром. У розчині 1,0 г/л TCA-m-CMCh може утворювати агрегат із середнім розміром частинок 221,8 нм і дзета-потенціалом -23,6 мВ. TCA-m-CMCh показав помірну антибактеріальну активність щодо E. coli та S. aureus та нетоксичність. Куркумін міг безперервно вивільнятися з агрегатів TCA-m-CMCh протягом 600 хв, а накопичена концентрація становила до 0,1 г/л. Результати показали, що TCA-m-CMCh є одним із потенційних носіїв куркуміну.

Ключові слова: *модифікований тетрадецилкарбоновою кислотою-О-карбоксиметилхітозан (ТСА-м-КМХ), фізико-хімічні властивості, навантаження, вивільнення*

List of abbreviations

TCA-m-CMCh-tetradecyl carboxylic acid-modified-O-carboxyl methyl chitosan

DD- degree of deacetylation

HMCS- hydrophobically modified chitosan

OD- oxidized dextran

OSA-CS- Octenyl succinic anhydride acylated modified chitosan

PAZO- prepared azo polymer

CMCh- O-carboxymethyl chitosan

CLE- curcumin loading

CEE- encapsulation efficiency

CONTENTS

INTRODUCTION

The purpose of the study: Through chemical modification, a kind of hydrophobic group, octadecyl carboxylic acid groups, was grafted into the carboxymethyl chitosan molecules, endowing the chitosan with hydrophobicity. Meanwhile, the carboxylic group and unreacted -NH_2 groups in the chitosan molecules have made the chitosan derivative pH response. The prepared chitosan derivatives with double responsivity of hydrophobic and pH have self-aggregation behavior in aqueous solution and can load oil-soluble drugs. The double responsivities of NOCAOCMC make them have the property of fixed-point release.

The main work is composed of three sections.

(1) A review of the structure, modification of chitosan, properties of chitosan and hydrophobically modified chitosan, and utilization of chitosan derivatives.

(2) Synthesis of TCA-m-CMCh. Tetradecyl carboxylic acid was used to modified O-carboxylmethyl chitosan, and synthesizing tetradecyl carboxylic acid-modified-O-carboxylmethyl chitosan (TCA-m-CMCh). The chemical structures of TCA-m-CMCh were characterized by FTIR, ^{1}H NMR and XRD methods. Properties of TCA-m-CMCh including thermal properties, antibacterial sactivity, cytotoxicity, aggregation behavior, self-aggregate particle size and particle size distribution were studied.

(3) Curcumin loading and releasing behavior. The curcumin loading and encapsulation efficiency in TCA-m-CMCh aggregates were 13.3% and 65.7%,

respectively. The curcumin could be released continuously from TCA-m-CMCh aggregates for 600 min, and the accumulated concentration was up to 0.1 g/L.

Research objective: To synthesize an amphiphilic chitosan for drug delivery.

Task: To synthesis TCA-m-CMCh chitosan derivative, to study their properties and application basis.

The active pharmaceutical ingredient is curcumin.

Scientific Novelty: an amphiphilic chitosan derivative was synthesized using O-CMCh as raw material, and it was used to encapsulate and release curcumin.

Methodology: Tetradecyl carboxylic acid was used to modified O-carboxylmethyl chitosan, and synthesizing tetradecyl carboxylic acid-modified-O-carboxylmethyl chitosan (TCA-m-CMCh). The chemical structures of TCA-m-CMCh were characterized by FTIR, ^{1}H NMR and XRD methods. Properties of TCA-m-CMCh including thermal properties, antibacterial sactivity, cytotoxicity, aggregation behavior, self-aggregate particle size and particle size distribution, active pharmaceutical ingredient loading and releasing behavior were studied.

SECTION 1 PHYSICO-CHEMICAL PROPERTIES OF CHITOSAN AND METHODS OF ITS MODIFICATION. LITERATURE REVIEW

1. 1 Chitosan

Chitosan, also known as deacetylated chitin, is the product of chitin after the removal of acetyl groups from the molecule under alkaline conditions[1], and is the second most abundant biopolysaccharide in nature and the only alkaline polysaccharide. The chemical name of chitosan is poly(glucosamine) (1-4)-2-amin-B-D-glucose. There are a large number of hydrogen bonds in chitosan molecules, which make the chitosan molecule a tight crystal structure and insoluble in water and alkaline solutions. It can only be dissolved in dilute acid solutions such as hydrochloric acid, acetic acid, lactic acid, benzoic acid and formic acid [2]. Chitosan has excellent properties such as biodegradability, cell affinity and biocompatibility[3], and has important potential applications in many fields such as pharmaceutical, food, chemical, cosmetic, water treatment, metal extraction and recovery biochemical and biomedical engineering. Li et al[4] found that chitosan with a relative molecular weight of 3 kDa and degree of deacetylation (DD) had the strongest hygroscopicity and moisturizing properties, and the moisturizing rate was close to that of the control propanetriol. Chitosan films prepared from chitosan/acetate solution was transparent films and with uniform texture. More importantly, these films could delay the color change and prevent the loss of nutritional quality of fat-rich fruits and vegetables while the film-forming solution

was coated on the fruits and vegetables due to oxidative rancidity. Meanwhile, chitosan films reduce the respiratory intensity of fruits and vegetables, reduce the damage caused by free radicals, maintain the firmness of fruits and vegetables, and maintain a high dry matter content of fruits and vegetables [5-7]. However, the water insolubility of chitosan limits its wide application in many fields, chemical modifications have been attempted to increase its solubility, giving chitosan derivatives more functions, and expand its application range.

1.2 Modification of chitosan and properties of the modified chitosan

Chitosan molecules have three functional groups with strong reactivity such as the primary hydroxyl group at the C6 position, the secondary hydroxyl group at the C3 position, and the primary amino group at the C2 position, which can be modified or modified by functionalization to improve its water solubility and to give chitosan various functional properties. Common methods of chitosan modification include acylation, carboxylation, etherification, alkylation, quaternization, cross-linking, copolymerization modification, etc. [8].

1.2.1Acylation modification

Acylation modification is one of the earliest methods of chitosan modification, and acylation reagents are generally used such as acid anhydride and chloride. The result of acylation modification is to destroy intermolecular interaction among

chitosan, reducing the crystallinity and increasing the solubility. Han et al[9] used acetic anhydride and phthalic anhydride to increase the solubility of chitosan by acylation on the primary amino group of chitosan using ethanol as solvent, and found that half of the amino groups were acylated. The acylated chitosan accesses hydrophilic groups while its crystal structure is disrupted, so it can dissolve well in water. Qi et al[10] modified chitosan by N-acylation using maleic anhydride and found that the yield of the modified product decreased with increasing the degree of N-acylation modification, however, the water solubility was improved with increasing the content of maleic acid group. Chen et al[11] acylated chitosan with succinic anhydride at 50 ºC and 4 h of reaction and obtained N-succinyl chitosan with the best water solubility of 9.72 g/L. Yu et al[12] prepared benzoyl chloride modified chitosan with good water solubility by using benzoyl chloride to esterify chitosan in the pH range of 3.5-7.5, and the modified chitosan had good bacterial inhibitory properties in the pH range of 3.5-7.5. The optimum conditions for this reaction were 6:1 molar ratio of benzoyl chloride/chitosan repeating units, temperature of 0 ºC and 3 h. Cok et al[13] prepared N-isopropyl chitosan with both pH and thermal responsiveness. The results showed that the introduction of isopropyl increased the solubility of chitosan and improved the flexibility of the polysaccharide backbone. The results of cell growth experiments showed that N-isopropyl chitosan had good biocompatibility.

The acylation reaction can also be carried out on -OH to produce O-acylated chitosan. Since the reactivity of -OH is weaker than that of -NH2, the O-acylation

reaction of chitosan requires the use of benzaldehyde to protect the reactive -NH2, and the protecting group is removed after the completion of the O-acylation reaction. Otherwise, the acylation reaction is highly like to occur on C2-NH2 and C6-OH at the same time. Wang et al[14] generated N,N,N-trimethyl-O-hexanoyl chitosan with good water solubility by Eschweiler-Clarke reaction followed by dropwise acetyl chloride. David et al[15] acylated chitosan with chloroacetic anhydride, which underwent acylation on C2-NH2 and C6-OH simultaneously with N- and O-chloroacetylation rates of 0.32 and 0.15, respectively. The N-phthaloyl chitosan derivatives were more active than N,O-phthaloylated chitosan derivatives and the reaction of fully deacetylated chitosan with biphenyldicarboxylic anhydride yielded N-phthaloyl chitosan with high solubility in polar solvents[16].

1.2.2 Carboxyl modification

Carboxymethyl is a hydrophilic group, and the water solubility of carboxymethyl-modified chitosan is greatly improved[17]. Carboxymethyl chitosan with substitution degree greater than 0.6 is easily soluble in water, and the higher the substitution degree, the better its water solubility. In general, N, O-carboxymethyl chitosan is soluble at all pH conditions, except for the pH range of 2-6, where it is insoluble in water. The solubility of O-carboxymethyl chitosan is closely related to the preparation conditions. Carboxymethyl chitosan prepared by the interaction of chitosan and chloroacetic acid at 0-20 ºC is soluble in water in all pH ranges. In

contrast, carboxymethyl chitosan prepared after reaction temperature higher than 20 ºC showed insoluble zone at pH of about 7[18]. Carboxylic acids containing aldehyde groups could react with chitosan directly, so that the aldehyde group reacts with the amino group of chitosan in a schiff-base reaction and finally N-carboxymethylated chitosan is obtained by reduction with NaBH4[19]. Carboxymethylation can also take place on the hydroxyl group. Wang et al[20] used chitosan as a raw material and produced N,N,N-trimethyl chitosan using formaldehyde formic acid and iodomethane firstly, and then carboxylated with chloroacetic acid at the C6 position to produce C6-O-carboxy-N-quaternary chitosan. This chitosan derivative has significantly improved water solubility and antibacterial properties due to the weakened intramolecular hydrogen bonds containing quaternary ammonium ions. The carboxymethylation reaction can react on both N and O. Anitha et al[21] prepared O-carboxymethyl chitosan and N,O-carboxymethyl chitosan using sodium tripolyphosphate and calcium chloride as ionic cross-linking agents and evaluated the cytotoxicity of nanoparticles by thiophene blue colorimetric method. They also studied the antibacterial activity of nanoparticles of both chitosan derivatives against Staphylococcus aureus by the minimum inhibitory concentration method. The results showed that the nanoparticles of the two chitosan derivatives showed little toxicity to breast cancer cells, and the antibacterial activity proved that the antibacterial activity of O-carboxymethyl chitosan and N,O-carboxymethyl chitosan nanoparticles was stronger than that of chitosan nanoparticles, with N,O-carboxymethyl chitosan nanoparticles showing the best antibacterial activity.

1.2.3 Quaternized modification

Quaternized chitosan not only has large solubility but also antimicrobial activity, which has become one of the most popular chitosan derivatives. The use of epoxy quaternary ammonium salt modified chitosan has the advantages of chitosan, but also increased the quaternary ammonium salt positive electricity and bactericidal and bacteriostatic properties. Liu et al[22] grafted 2,3-epoxypropyl trimethyl ammonium chloride to the amino group at the C2 position of chitosan to obtain water-soluble chitosan quaternary ammonium salts. The quaternary ammonium modified chitosan not only increased solubility but also significantly improved antibacterial activity against Staphylococcus aureus compared with chitosan. Liu et al[23] studied the antibacterial activity of iodinated N-trimethyl chitosan quaternary ammonium salt against Gram-positive and Gram-negative bacteria, and the experiments showed that the antibacterial activity of quaternary ammonium modified chitosan was more significant against Gram-positive bacteria. When the molar ratio of 2, 3-epoxypropyltrimethylammonium chloride to chitosan was 3:1, the highest substitution of chitosan quaternary ammonium salt was produced by the reaction at 80 ºC for 3 h. The introduced quaternary ammonium group disrupted the crystal structure of chitosan, which resulted in good water solubility in the pH range of 3.0-11.0, and the hygroscopicity, water retention and antibacterial properties of the quaternary ammonium chitosan film were significantly improved[24, 25].

Both the amino group at the C2 position and the hydroxyl group at the C6

position have strong reactivity and are competitive in the reaction process. If the modification take place on the hydroxyl group, the amino group at the C2 position can be protected by the quaternization reaction first, and then the C6 hydroxyl group modified chitosan can be made by the C6 hydroxyl group reaction. Che et al[26] used chitosan of different molecular weights as raw materials, first synthesized N,N-dimethyl chitosan with formaldehyde and formic acid, then alkylated tertiary amine with iodomethane to make N,N,N-trimethyl chitosan quaternary ammonium salt, and finally introduced cyanogen trimer on its hydroxyl group to synthesize O-cyanogen trimer-N,N,N-trimethyl chitosan quaternary ammonium salt, and the principle is shown in Figure 1.1. O-cyanogen trimer-N,N,N-trimethyl chitosan -trimethyl chitosan quaternary ammonium salt was superior to N,N,N-trimethyl chitosan quaternary ammonium salt in acid dyes for dyeing wool fabrics.

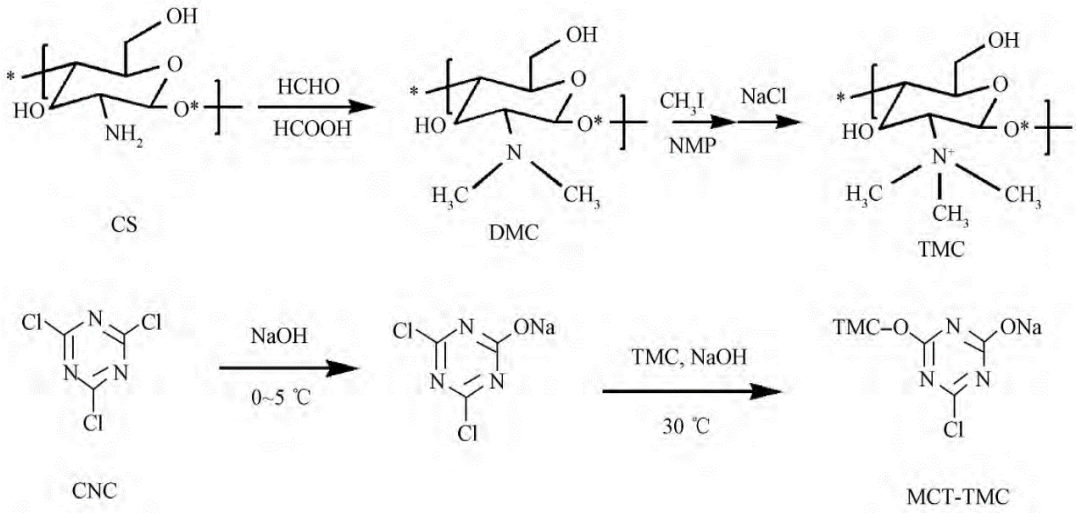

Figure 1.1 Synthetic route of O-trichloro-N,N,N-trimethyl chitosan quaternary ammonium salt.

Zhang et al[27] used chitosan as a raw material and formed ether bonds by reacting the C6-hydroxyl group with the etherizing agent 2,3-

epoxypropyltrimethylammonium chloride and then deacetylated to obtain N-modified chitosan, the principle is shown in Figure 1.2. Both methods use functional group protection strategies to introduce groups on the C6-hydroxyl group of chitosan.

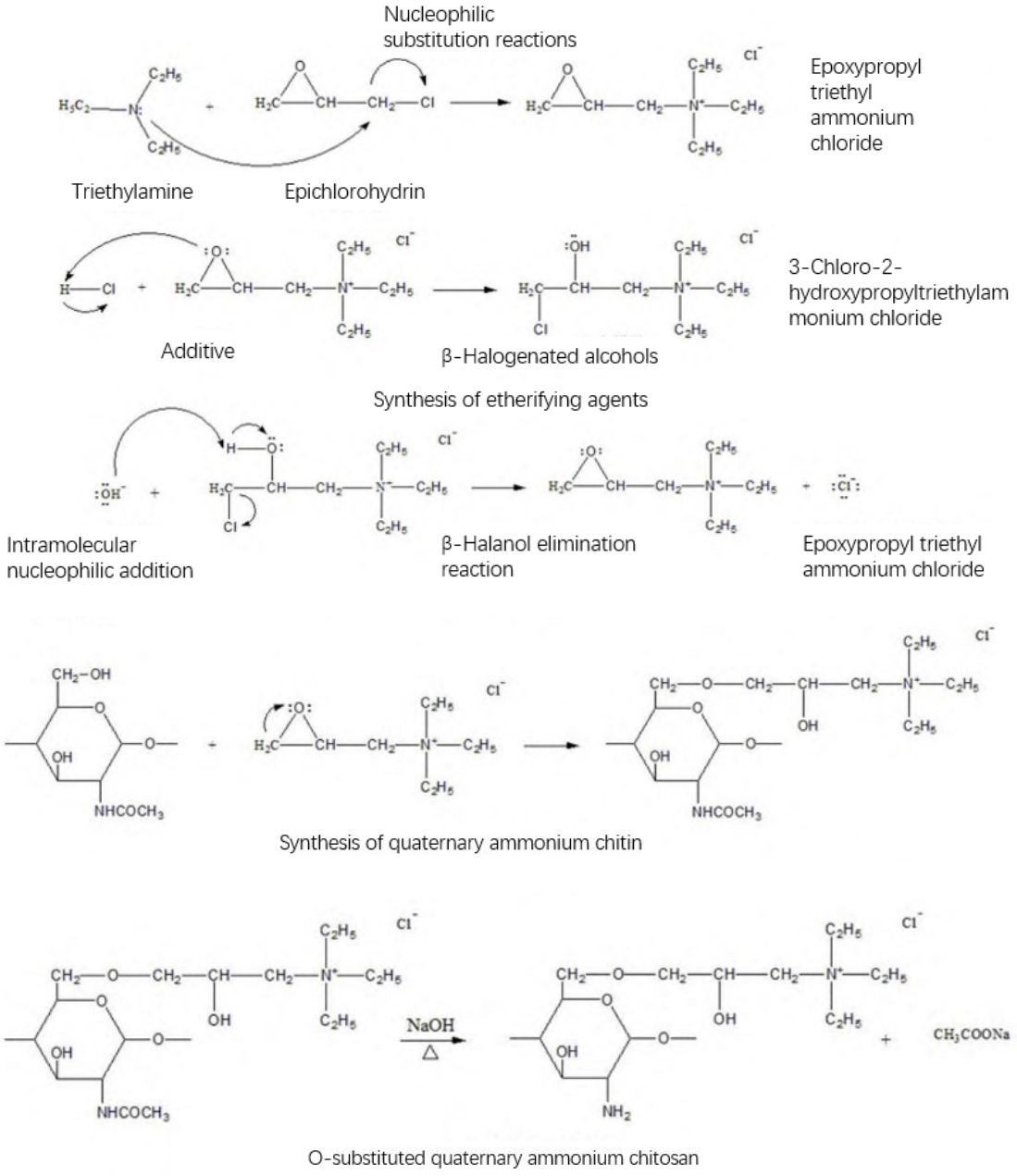

Figure 1.2 Principle of preparation of O-substituted quaternary ammonium chitosan.

1.2.4 Hydroxylation modification

The modification of chitosan by introducing hydroxyl groups at the C6-OH position through etherification reactions has also received considerable attention. Xu et al[28] introduced the hydrophilic group, hydroxyethyl group through the reaction at C6-OH of chitosan and produced hydroxyethyl chitosan with 65% substitution of C6-hydroxyethyl ether. Wang et al[29] prepared dihydroxy propyl chitosan with better water solubility by using glycidol as etherizing agent at 60 ºC and 8 h reaction.

1.2.5 Alkylation modification

Chitosan molecules have several hydroxyl and amino groups with typical hydrophilic properties, and the modified chitosan molecules were changed into amphiphilic chitosan by accessing alkyl groups in their molecules. Compared with chitosan, amphiphilic chitosan has stronger bacteriostatic properties and lower effective bactericidal concentration. The alkylation modification reaction can occur at two reaction sites, C2-NH2 and C6-OH.

N-alkylation is usually a product of the reaction of halocarbons with chitosan C2-NH2. Wang et al[30] modified chitosan with ethyl, n-butyl, n-octyl and cetyl haloalkyl hydrocarbons in isopropyl alcohol/sodium hydroxide solution, respectively, and showed that ethyl, n-butyl and n-octyl modified chitosan had good water solubility and good anticoagulant properties. Cetyl modified chitosan had good

amphiphilic and antibacterial properties[31]. Hydrophobic modification of chitosan was carried out using aluminum monostearate and dehydrothermal treatment. In 2% w/v lactic acid, aluminum monostearate dissociated stearate ions and amidated with dehydrothermally treated chitosan C2-NH2 to produce alkylated modified chitosan.

The nucleophilic addition and re-elimination of amino groups and aldehydes and ketones within the chitosan molecule yielded cephaline, which can be used not only to protect the primary amino group but also to reduce with sodium borohydride to produce N-alkylated chitosan (e.g., Figure 1.3) [32, 33]. Tang et al. [34] prepared N-ethyl chitosan and N-hexyl chitosan with substitution degrees of 81.8% and 61.5%, respectively, by the reaction mechanism in which the amino group reacts with the aldehyde group to form cephaline (-CH=N- structure), which is then reduced to methylene and secondary amines in the presence of sodium borohydride. The contact angle of the two hydrophobically modified chitosan was significantly larger than that of the chitosan, and the hydrophobicity increased significantly with the increase of alkyl substitution.

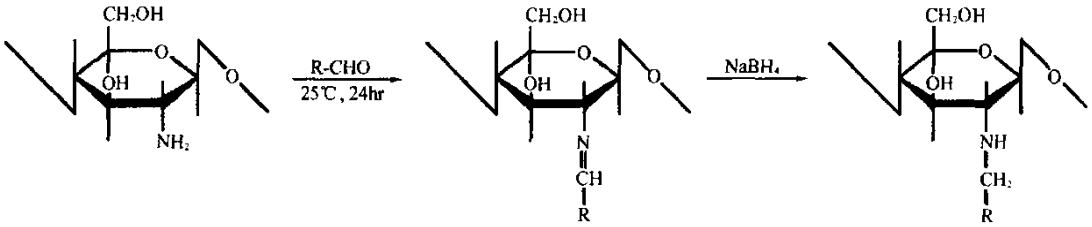

Figure 1.3 Schematic diagram of the synthetic route of N-alkyl chitosan

The preparation of hydrophobic chitosan by Schiff base method is complicated, using many reagents, generating a large amount of foam during the reaction, and the

aldehyde itself has a strong irritating odor, which is not easily removed from the product and has a high risk factor to the organism and the surrounding environment. The reaction of long-chain organic acids or anhydrides with chitosan is also a method to prepare amphiphilic chitosan. Wen et al [35] used the reaction between chitosan and lauric anhydride to produce N-alkyl-substituted chitosan in one step, and the reaction equation is shown in Figure 1.4. When the reaction temperature was 55 ºC, the highest degree of substitution of 10.77% was obtained for the modified chitosan with lauric anhydride, and the lowest characteristic viscosity of chitosan solution was obtained. The higher the degree of substitution of lauric acid in the modified chitosan, the lower the solubility of the modified chitosan. The contact angle between modified chitosan and water was 104.46º, which was much larger than that between chitosan and water (67.26 º), indicating that the access of lauryl alkane chain significantly improved the hydrophobicity of chitosan. The lauric anhydride modified chitosan has significant hemostatic effect and is expected to be developed as a new hemostatic material.

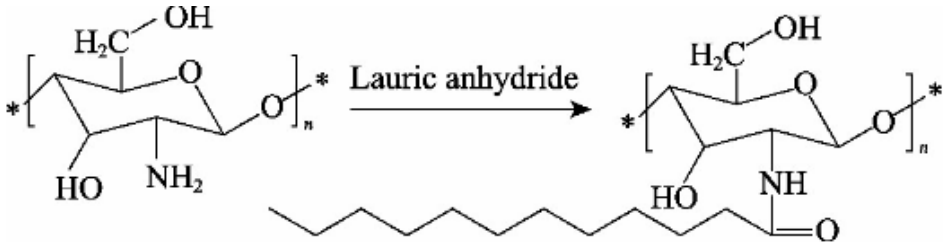

Figure 1.4 Schematic diagram of the reaction of amphiphilic chitosan prepared by C2-NH_2 substitution of organic acid/anhydride

Jothimani et al[36] prepared N-(thiophene-2-acetyl) chitosan (Figure 5) and the spectroscopic results confirmed the structure of the modified chitosan, and the highest N-substitution was obtained by elemental analysis as 0.96. Under the action of ultrafine grinding and sonication, N-(thiophene-2-acetyl) chitosan was transformed into particles with an average particle size of 83.1-95.54 nm, and the larger the degree of substitution, the larger the particle size. The thermal stability of N-(thiophene-2-acetyl) chitosan nanoparticles was affected by the crystallinity of chitosan and particle size. The higher the crystallinity of chitosan or the larger the particle size, the more stable the nanoparticles were.

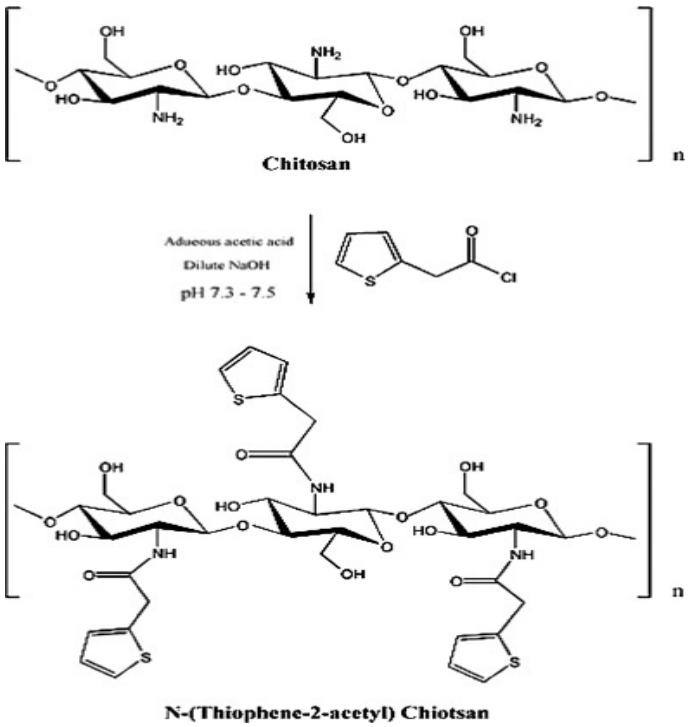

Figure 1.5 Synthesis of N-(thiophene-2-acetyl) chitosan

1.3 Application of modified chitosan

1.3.1 Application of hydrophilic modified chitosan

Hydrophilic modified chitosan is widely used in food, medicine, leather, chemical process and other fields because of its good water solubility. The unique biological properties of chitosan and its derivatives make it widely used in food additives, preservatives, clinging agents, cling films, and beverage clarifiers [37]. Compared with traditional preservatives and preservatives, hydrophilic modified chitosan not only has less dosage and more obvious preservation effect, but also is safe and non-polluting. Fan et al [38] added 2% of water-soluble chitosan as clarifying agent in fruit juice, and compared with the juice without clarifying agent, the added juice could reach more than 85% light transmittance and better color, and chitosan is non-toxic and harmless and does not affect the nutrients in the juice. Chitosan has good effect in inhibiting bacteria, and the hydrophilic modified chitosan can better inhibit the invasion and growth of bacteria and microorganisms, meanwhile, its good moisturizing and antioxidant properties can extend the shelf life of food. The good film-forming property of chitosan can be used to make food packaging film with antibacterial property, which can be used as preservation agent in food storage process. The film can effectively prevent the invasion of microorganisms and reduce the decay rate of fruits and vegetables.

In addition, hydrophilically modified chitosan has good antibacterial, biocompatible and biodegradable properties due to its good reactive functionality and

physiological activity, as well as its ability to promote soft tissue repair, wound healing, and guide bone and cartilage regeneration [39]. N,N,N-trimethyl chitosan quaternary ammonium salt is also widely used in biomedical and pharmaceutical applications. Worawan et al [40] and Worawan et al [41] investigated the induction of immune responses to mouse ovalbumin by chitosan and N,N,N-trimethyl chitosan quaternary as an immune aid in a nasal mucosal drug delivery system. The results showed that N,N,N-trimethyl chitosan quaternary was also effective in a delivery system for the delivery of antibodies for therapeutic procedures in the lung. Fiddes et al [42] prepared microgel capsules with microchannels of certain morphology by coating with chitosan quaternary, and explored the hydrodynamic issues related to them. The capsules were formed by wrapping alginate gels with positive and negative charges respectively with 2-hydroxypropyl-3-trimethyl ammonium chitosan, and then allowed to flow through the microchannel pores to observe the effect of the modified chitosan wrapped with methyl chloride, the interaction between the microcapsules and the surface of the channel walls, and the flow rate through the pores. The results showed that by regulating the electrostatic interaction between the positive charge of 2-hydroxypropyl-3-trimethylammonium chitosan and the negative charge of the microchannel wall, and by controlling the flow rate, microcapsules of different diameters could be obtained, which had good controllability. Liu et al [43] found that the quaternary ammonium salt of sulfhydrylated chitosan has good encapsulation ability for DNA when used as a gene carrier, and also has the ability to release for reduction-responsive genes.

Quaternary ammonium-modified chitosan coated on the surface of leather fibers can effectively adsorb the surrounding negatively charged bacteria and inhibit their proliferation, which is a good performance antimicrobial agent for leather[44]. The results of Xu et al[45] showed that carboxymethyl chitosan can be used as a dyeing aid in the tanning process, and it helped to deepen the color of the product and improved the dyeing rate, evenness, and dry and wet rubbing properties.

Hydrophilically modified chitosan has good water solubility and heat resistance, and has a strong adsorption capacity for heavy metal ions [46]. It can rapidly adsorb metal ions such as arsenic, chromium, mercury, cadmium, lead and copper present in industrial wastewater, providing an excellent solution to the problem of heavy metal water pollution. Fan et al [47] found that the flocculation effect of carboxymethyl chitosan with a carboxylation degree of 1.43 and a dosage of 8 mg/L on seawater, the efficiency of COD removal reached 55%, and the turbidity removal rate reached 94% to 95%. The printing and dyeing wastewater has complex composition, high chromaticity, high odor, high concentration of organic matter, and difficult to biodegrade. And the unique structure of modified chitosan makes it a great advantage in treating printing and dyeing wastewater [48].

Chitosan and its derivatives have similar structure to cellulose and excellent properties such as flocculation, bacterial inhibition and biodegradation, so they can be used as paper reinforcing agents, retention and filtration aids, flocculants and surface sizing agents. Hydrophilic modified chitosan good film-forming properties, stability can form intermolecular hydrogen bonds with the fibers in the paper and tight

bonding, so the hydrophilic modified derivatives of chitosan can be used in the paper industry as paper viscosity enhancers, in addition, hydroxypropyl modified chitosan as a wet sizing aid can significantly enhance the retention rate of cationic petroleum resin gum. It helps the retention of calcium carbonate filler in neutral sizing, while hydroxypropyl chitosan additives also contribute to the retention of sizing agent under high shear conditions and form more stable flocs, which will help the actual production operation of paper machines[49]. N-carboxymethyl chitosan has excellent moisturizing properties and stability, which prevents skin allergies. Hydroxypropyl trimethyl ammonium chloride chitosan has good hygroscopic and moisturizing properties, and has the antibacterial and antimicrobial properties of quaternary ammonium salts, which can be used as an additive in creams.

1.3.2 Application of hydrophobically modified chitosan

Hydrophobically modified chitosan is widely used in the medical field, Meghan et al [50] found that based on hydrophobically modified chitosan (HMCS), an amphiphilic derivative of the biopolymer chitosan, injectable expanded foam successfully induced decomposition of thrombus after 6 weeks of application with minimal inflammation and adhesion state in the lesion cavity, and HMCS had faster biodegradation compared to chitosan. Huang et al[51] found that alkylated chitosan with degrees of substitution (DS) of 7%, 16%, 26%, and 40% had better in vitro hemostatic effects, and that DS of 16% alkylated chitosan having the best in vitro

hemostatic effect and 40% substitution alkylated chitosan having the best in vivo hemostatic effect. Du et al [52] prepared hydrophobically modified chitosan (HMCS)/oxidized dextran (OD) hydrogels. Their antibacterial activity on Pseudomonas aeruginosa and Staphylococcus aureus reached 96.4% and 95.0% respectively [53]. Based on the synergistic effects of electrostatic and hydrophobic interactions and the adhesive properties of the hydrogels, the HMCS (1.0 wt%)/OD hydrogels had a better in vivo hemostatic effect and could effectively bind to tissues, which was beneficial to promote wound repair. Deoxycholic acid modified chitosan (DCS-CS) autopolymer can self-aggregate into nanoparticles with particle size of 280-310 nm and charge of 26 mV, and the synergistic effect with hyaluronic acid (HA) can achieve a high encapsulation rate of 56% for adriamycin. The drug delivery system was exposed to a phosphate buffer solution at 37 ºC, pH=7.4, and adriamycin could maintain slow release without bursting effect [54]. Octenyl succinic anhydride acylated modified chitosan (OSA-CS) self-aggregates into negatively charged aggregates in aqueous solution, had good biocompatibility, a large and pH-responsive loading rate for curcumin, facilitates the extraction efficiency of the drug by specific cells, and could better exploit the anti-inflammatory and antioxidant capacity of the drug [55]. Amphiphilic chitosan obtained from carboxymethyl chitosan modified by 2,3-epoxypropyl n-butyl and isooctyl ethers also has self-aggregation behavior and pH responsiveness, and the electronegativity of the aggregates increases from -40 to 20 mV as the solution pH decreases from 9.18 to 1.0. Controlled release of curcumin can be achieved by the aggregates [56].

Chitosan is an important new food packaging material because of its biocompatibility, high antimicrobial activity, degradability, non-toxicity and excellent film-forming ability. Fernández et al [57] prepared azo polymer (PAZO) modified chitosan by solvent casting method with azo polymer. The hydrophobic modified chitosan can form films with a thickness of about 25 μm, which had weak water absorption properties and strong tensile strength. The film of N-2-hydroxypropyl-3-trimethyl-O-carboxymethyl chitosan and carboxymethyl cellulose system inherits the biocompatibility of chitosan and cellulose, with smooth and flat surface, transmittance greater than 90%, maximum tensile strength greater than 5 MPa, elongation at break up to 120%, and significant inhibition of Escherichia coli and Staphylococcus aureus. More importantly, the film could effectively prolong the shelf life of fresh pork [58].

Hydrophobically modified chitosan is also used as a water treatment agent. To enhance the binding ability to antibiotics, hydrophobic segments were introduced on hydrophilic chitosan polymers to enhance the hydrophobic association between flocculants and antibiotic molecules. Yang et al [59] prepared three hydrophobic chitosan flocculants by grafting hydrophobic branches of different lengths onto hydrophilic chitosan. The results showed that the hydrophobic chitosan flocculants removed up to 80%-90% of antibiotics and the possibility of negative environmental impact from residual flocculants. Dodecanal modified chitosan sponge prepared by Vo et al [60] had a porous structure filled with smaller pore channels and the maximum adsorption capacity of methyl orange was 168 mg/g, which was 60%

higher than that of chitosan sponge. The adsorption capacity of the dodecanal modified chitosan sponge was two times higher than that of the chitosan sponge when used repeatedly. Bidgoli et al [61] used a simple homogeneous esterification reaction to access fatty acid esters with five carbon atoms in the hydroxyl and amino groups of chitosan to produce oil-soluble chitosan esters. Subsequently, the oil-soluble chitosan esters were further cross-linked to produce ultra-low density aerogels for use as oil absorbents. The results showed that the aerogel has very good oil-water selectivity and high adsorption capacity, and does not produce secondary water pollution, which is an environmentally friendly adsorbent.

Hydrophobic chitosan has so many advantages, it can be used to load, transfer and release of oil soluble drugs. Based on this, this article will to modify chitosan by a long-chain carboxylic acid, preparing hydrophobic chitosan derivatives and characterize their molecular structures, study their physical and chemical properties, explore their application properties on packaging, transferring and releasing active ingredients of curcumin.

Conclusion of Section 1

This section has reviewed chitosan modification and the properties of modified chitosan by reading and studying the literature related to natural polymer-based chitosan. There are various manipulations for the modification of chitosan. For example, acylation, carboxylation, sulfation, hydroxylation, quaternization, and other modification methods. As well as the mechanism of the influence of reaction conditions on the structural characteristics and properties of modified chitosan, the applications of modified chitosan are also introduced, and the prospect of chitosan modification and the application of modified chitosan is made.

SECTION 2 SYNTHESIS, CHARACTERIZATION, PHYSICO-CHEMICAL PROPERTIES AND APPLICATION OF TETRADECYL CARBOXYLIC ACID MODIFIED O-CARBOXYMETHYL CHITOSAN

2.1 Introduction

Chitosan, the second most abundant polysaccharide in the world, was introduced into the pharmaceutical field in the early 1990s and has gained significant attention [44]. Chitosan-based nanoscale drug carriers can enhance the therapeutic value of drugs by improving their bioavailability, solubility and retention time, and benefit patients due to lower costs and reduced toxicity [62]. Therefore, a large number of nanoscale drug delivery technologies have been explored regarding chitosan, including polyelectrolyte complexes (PECs), hydrogels, sponges, emulsions, self-aggregating micelles, and vesicles [63].

Carboxymethyl chitosan is pH sensitive and carboxyl group has natural affinity to cells. Amphiphilic chitosan can be prepared by modifying carboxymethyl chitosan to make it amphiphilic, and to achieve the purpose of drug delivery.

In this paper, n-decanoic acid was used to modify O-carboxymethyl chitosan and prepare tetradecyl carboxylic acid modified O-carboxymethyl chitosan (TCA-m-CMCh). The structure of TCA-m-CMCh was characterized by FTIR, 1H NMR, 13C NMR methods, as well as the degree of substitution (DS). The physicochemical properties including thermal properties, aggregation behavior particle size and

particle size distribution in aqueous solution, potential of the aggregates, antimicrobial activity and toxicity were investigated. The inclusion and release properties of TCA-m-CMCh to curcumin were also studied.

2.2 Synthesis of TCA-m-CMCh

2.2.1 Reagents

1-Allyl-3-methylimidazolium chloride (purity ≥99.5 Lanzhou Institute of Physical Chemistry), carboxymethyl chitosan (DD=80%, viscosity 80 (mPa.s) Maclean's Biochemical Co., Ltd.), tetradecyl carboxylic acid (AR, Aladdin Reagent Company), acetone (AR, Aladdin Reagent Company), ethanol (AR, Aladdin Reagent Company), were used in the experiments.

2.2.2 Experimental instruments

Nuclear magnetic resonance spectrometer (Bruker Advance II 400, Switzerland), Elemental analyzer (Elementar, Germany), Fourier infrared spectrometer (Nicolet iS10, Thermo Scientific Co.), thermal gravimetric analyzer (Q600, TA, USA); scanning electron microscope (JSM6700F, JEOL, Japan), transmission electron microscope (tecnai12 Phillip Instruments, Netherlands), static tensiometer (Jinan Sentai Machinery Co., Ltd.), X-ray diffraction (D8-ADVANCE Bruker AXS,

Germany), nanoparticle size potential analyzer (ZS90 Malvern, UK), dynamic mechanics analyzer (Q800 Malvern, UK), fluorescence spectrophotometer (F-4600 TA, USA) were used in the experiments.

2.2.3 Synthesis method

0.2g of O-carboxymethyl chitosan (CMCh) was dissolved in 20ml of deionized water. Dissolve 0.18g of tetradecyl carboxylic acid in 20ml of anhydrous ethanol in equimolar amount, add EDC (1.5 times the molar amount of tetradecyl carboxylic acid) after dissolving, stir for 30min and then add NHS (1.5 times the molar amount of tetradecyl carboxylic acid) and continue to activate for 30min. heat the dissolved O-CMC in an oil bath to 80 ºC, then add the activated solution of tetradecyl carboxylic acid after the reaction was completed. The solution was cooled to room temperature, and then the reaction was stirred at room temperature for 16 h. The solution was washed with ethanol and the crude product was obtained by centrifugation. The refined product was obtained by centrifugation and dialyzed for 48 h. The final product was lyophilized. The synthesis procedure is shown in Figure 2.1.

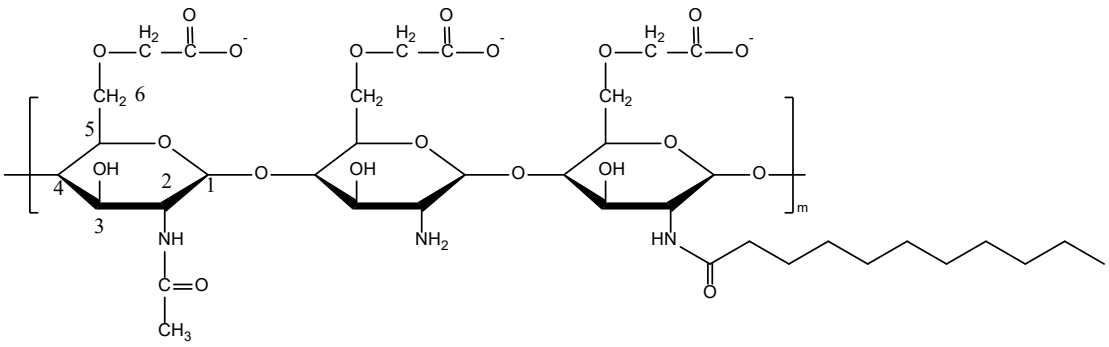

Figure 2.1 Synthesis procedure of TCA-m-CMCh.

2.2.4 Characterization

The molecular structure of TCA-m-CMCh was characterized by FTIR (Nicolet iS10, Thermo Scientific Co.), NMR (1H NMR and 13C NMR, Bruker Advance II 400 spectrometer, Bruker, Switzerland, using D2O as solvent), automatic elemental analyzer (Elementar UNICUBE, Germany) and XRD (AXS D8-ADVANCE X-ray diffractometer, Bruker, Germany). The thermal properties and microstructure of TCA-m-CMCh were investigated by TGA (SDT Q600 simultaneous thermal analyzer, TA Instruments, USA) and DMA (DMA Q800 instrument, TA Instruments, USA). The aggregation behavior, aggregate size and aggregate size distribution of TCA-m-CMCh were studied by fluorescence spectrophotometer (Hitachi F-4600, Japan), laser particle size analyzer (Zetasizer Nano ZS90, Malvern, England) and transmission electron microscope (Tecnai-12, PHilip Apparatus Co.). Cytotoxicity and antibacterial activity of TCA-m-CMCh were detected.

The TCA-m-CMCh aggregates were characterized TEM images.

2.3 Results and Discussion

2.3.1 Structure of TCA-m-CMCh

The FTIR spectra of TCA-m-CMCh shows characteristic absorption peaks at 2935 cm^{-1} (v-CH_2) and 2868 cm^{-1} (v-CH_3), which are the asymmetric and symmetric C-H stretching modes of –CH_2 groups of TCA (Figure 2.2). The characteristic

absorption peak of secylamine appears at 1648 cm^{-1} in the CMCh spectrum and was red-shifted to 1620 cm^{-1} in the TCA-m-CMCh FTIR spectra due to the decrease in intermolecular/intermolecular interactions. These results demonstrate that the N-substitution reaction and the synthesis of TCA-m-CMCh synthesis.

^{1}H NMR spectrum of TCA-m-CMCh was different from the 1H NMR spectra of CMCh (Figure 2.2(B)). The chemical shifts at 0.8 and 1.2 ppm were assigned to CH3 and CH2 of the TCA, confirming the formation of TCA-m-CMCh. The DS of TCA on CMCh was calculated from the integrate of 1H NMR was 15.3%.

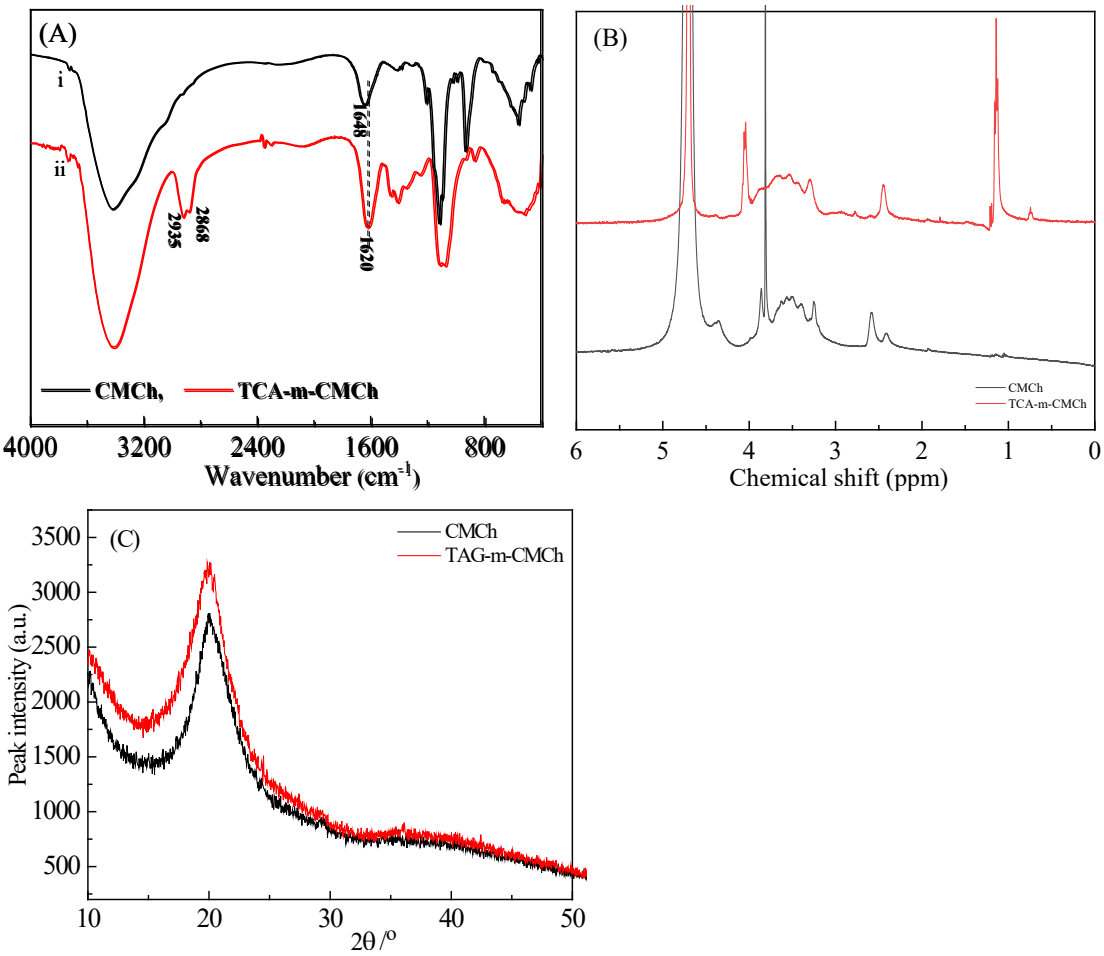

Figure 2.2 FTIR spectra (A), 1H NMR (B) and XRD (C) spectra of TCA-m-CMCh.

The XRD spectrum of CMCh showed a typical diffraction peak at 2θ = 20° (Figure 2.2C), which is produced by the synergistic effect of the (101) and (002) planes. The diffraction peak of TCA-m-CMCh was widened. This suggested that the CMCh crystallinity decreases due to the introduction of hydrophobic side chains and the disruption of inter- and intramolecular hydrogen bonds.

2.3.2 Thermal properties

The thermal stability of CMCh and TCA-m-CMCh was evaluated by TGA, which curves were shown in Figure 2.3 and the detailed data are listed in Table 1.

The TGA curves showed two significant weight losses, the first one took place in the range of room temperature to ca. 100 ºC, corresponding to the evaporation of cabbed water. The weight loss of TCA-m-CMCh (3.5%) was smaller than that of CMCh (8.8%), which might be due to the enhanced hydrophobic properties of TCA-m-CMCh and the disruption of inter- and intramolecular hydrogen bonds.

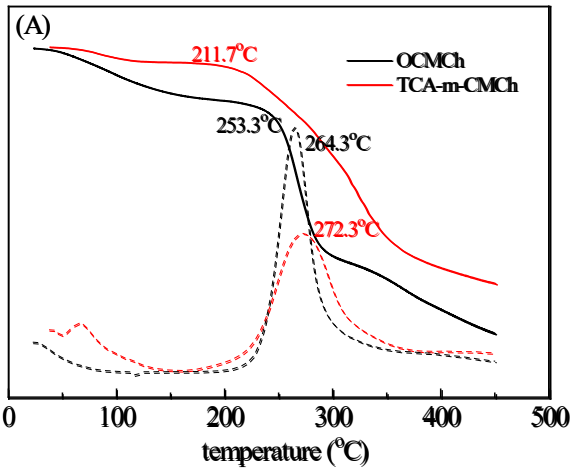

Figure 2.4 TGA curves of CMCh and TCA-m-CMCh.

The second weight loss occurred between 200 ºC and 350 ºC and corresponds to the breakdown of the molecular backbone of TCA-m-CMCh corresponding to the breakdown of the molecular backbones. The initial decomposition temperature was lower than CMCh (253.3ºC), however, the maximum decomposition temperature (T_{max}) was higher than CMCh. This indicated that the thermal stability of TCA-m-CMCh was improved by introducing TCA chain.

Table 2.1. Thermal degradation data of CMCh, TCA-m-CMCh.

Sample	ti,1 (ºC)	w1 (%)	ti,2 (ºC)	tmax (ºC)	w2 (%)	Tg,DMA (ºC)
CMCh	35.0	8.8	253.3	264.3	41.6	48.7
TCA-m-CMCh	57.8	3.5	211.7	272.3	41.9	58.5

2.3.3 Aggregation behavior of TCA-m-CMCh

The aggregation behavior of TCA-m-CMCh in aqueous solution was determined by steady-state fluorescence spectrophotometry. Pyrene, an oil-soluble compound and its fluorescence is very sensitive to the microenvironment, was used as a probe. Pyrene has five characteristic emission peaks in the range of 370-400 nm, and peak 1 and peak 3 are sensitive to the polarity of the microenvironment. Their intensity ratio (I1/I3) decreases sharply when the aggregates are formed. Therefore, the inflection point before and after the formation of aggregates is defined as the critical aggregation concentration (cac).

The plots of fluorescence intensity vs. TCA-m-CMCh concentration in the solution with pH 9.18 is shown in Figure 2.5, from which it could be seen that the cac is ca. 0.2 g/L. It is due to the strong hydrophobic intermolecular interactions. At pH 9.18, there is a strong electrostatic repulsion between TCA-m-CMCh molecules due to the presence of –COO-, therefore, its cac value is larger than those chitosan derivatives at lower pH solutions.

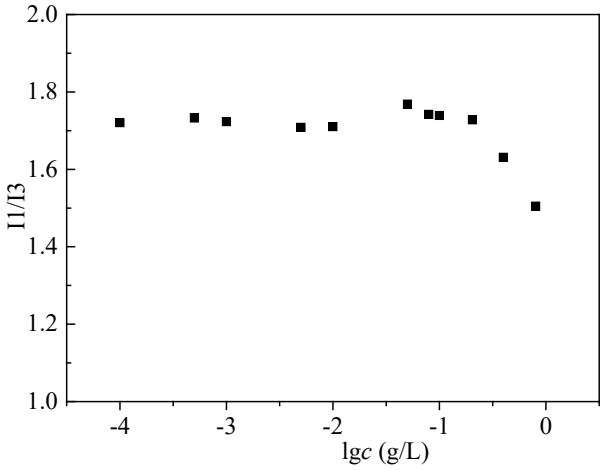

Figure 2.5 Plots of I1/I3 vs. TCA-m-CMCh concentration.

2.3.5 Diameter, Zeta potential and TEM images of TCA-m-CMCh aggregate

The average diameter of TCA-m-CMCh with 1.0 g/L is 221.8 nm and the particle distribution index (PDI) is 0.492, and the Zeta potential of TCA-m-CMCh aggregates are -23.6 mV at pH 9.18, indicating the adsorption of the -COO group on the surface of the aggregates.

The morphology and diameter of the aggregates were also studied by TEM

(Figures 2.6), which showed that all aggregates were spherical in shape and their size distribution was 23-40 nm. The size of the aggregates was much smaller than that determined by the dynamic particle size analyzer. It is generally believed that the smaller size of the aggregates in TEM images is caused by the dry state of the aggregates. In the TEM images, we were fortunately to find a large aggregate composed of small aggregates. That is, there are two reasons for the large diameter of the aggregates: one is the state of the sample, and the other is the small aggregation of the aggregates. All data from dynamic particle size analyzer, Zate potential and TEM images confirm the formation of aggregates, TCA-m-CMCh a potential nanocarrier for water-insoluble drugs.

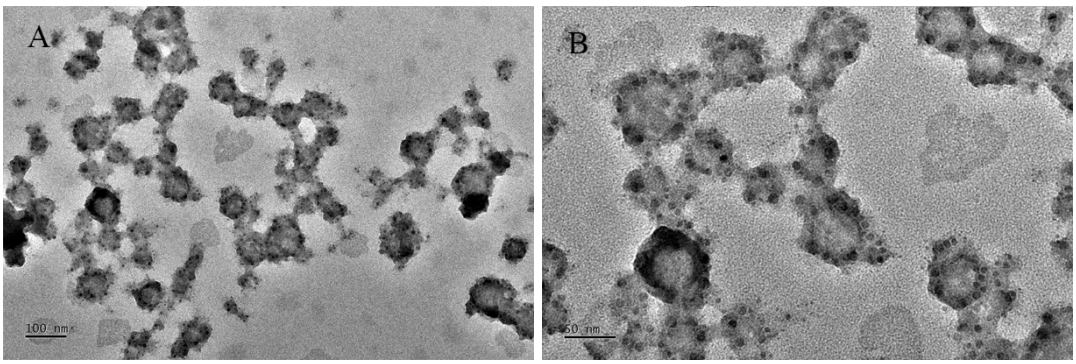

Figure 2.6 TEM images of TCA-m-CMCh aggregate.

2.3.6 In-vitro cytotoxicity

The in-vitro cytotoxicity of both TCA-m-CMCh and CMCh at 1.0 g/L were evaluated by MTT assay against endothelial cells (Figure 2.7). Compared to the growth of cells CMCh culture medium, the concentration of the cells in TCA-m-CMCh culture medium decreased with increasing concentration. The growth of cells was more than 90% compared to that of naïve sample, which means that the TCA-m-CMCh was still non-cytotoxicity.

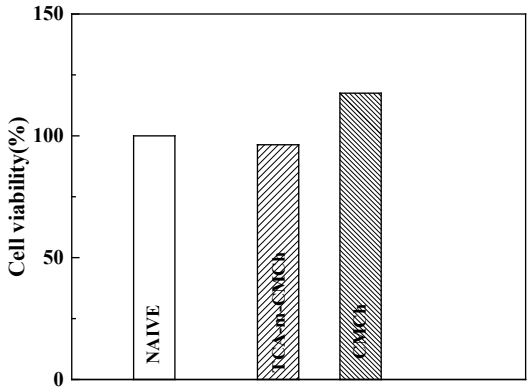

Figure 2.7 In-vitro cytotoxicity of TCA-m-CMCh.

2.3.7 Antibacterial activity

TCA-m-CMCh showed good antibacterial activity against E. coli and S. aureus while its concentration was larger than 0.5 g/L, as shown in Figure 2.8. The diameters of the inhibition circle zone were 0.6 cm (1), 1.5 cm (2) and 1.45 cm (3) for E. coli, respectively. And those for S. aureus were 0.65 cm (1), 1.25 (2) and 1.35 cm (3), respectively. That is, the larger the concentration TCA-m-CMCh, the stronger the antibacterial activity.

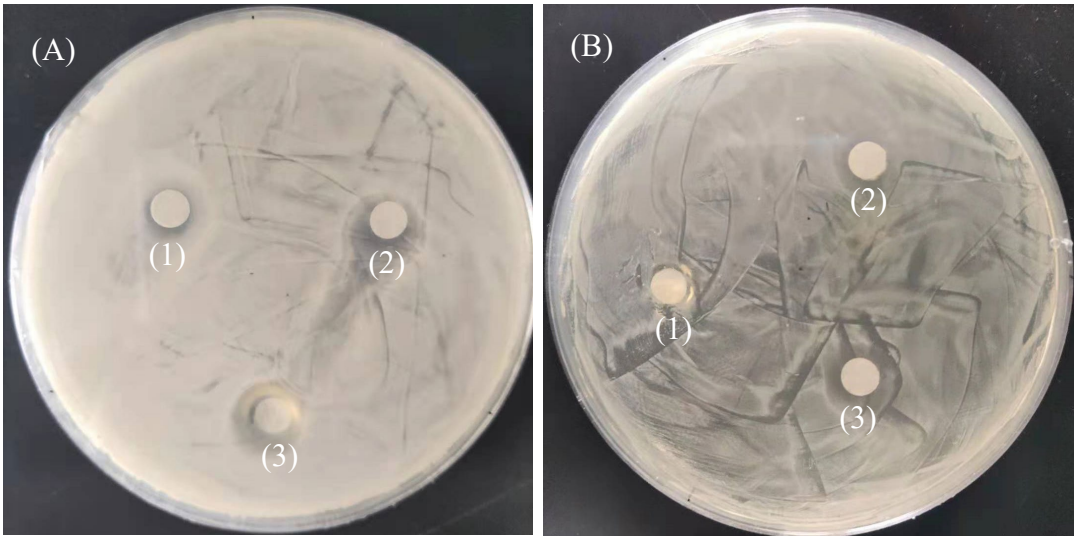

Figure 2.8 Antibacterial properties of TCA-m-CMCh against E. coli (A) and S. aureus (B). Concentrations of TCA-m-CMCh were 0.1 g/L (1), 0.5 g/L (2) and 1.0 g/L (3), respectively.

It is generally believed that hydrophobicity and intermolecular forces are the key factors affecting the antibacterial activity. Negatively charged TCA-m-CMCh interacted with positively charged S. aureus, the hydrophobic chains of TCA-m-CMCh form a barrier layer that prevents S. aureus from accessing nutrients. The TCA groups interacted with E. coil by hydrophobic interaction, and inhibited their growth. From the results, both the hydrophobicity of TCA-m-CMCh and electrostatic interactions are considered to be the key factors.

Conclusions of Section 2

TCA-m-CMCh was synthesized using 1-allyl-3-methyl imidazole chloride (AmimCl) as a solvent. The molecular structure was characterized by FTIR, NMR and XRD methods. The degree of substitution was calculated from the integrated spectrum of NMR, which was 15.3%. TGA results suggested that the thermal stability of TCA-m-CMCh was increased due to the introduction of TCA groups. Also, ascribed to the introduction of TCA, the TCA-m-CMCh showed strong amphiphilicity and could form self-aggregates in aqueous solutions with diameter ranged from 23 to 40 nm (1.0 g/L), which were negative charged. TCA-m-CMCh showed moderate antibacterial activity against E. coli and S. aureus, and non-toxicity, which could be used as water-insoluble plant active ingredients.

SECTION 3 LOADING AND RELEASING OF CURCUMIN FROM TCA-M-CMCH AGGREGATES

3.1 Introduction

Curcumin is a kind of diketone compound that extracted from the rhizome of some plants in ginger and Araceae. Its chemical formula is $C_{21}H_{20}O_6$. Turmeric contains about 3% ~ 6% curcumin, which is a rare pigment with diketone structure in the plant kingdom. Curcumin is orange yellow crystalline powder, taste slightly bitter, mainly used in food production for coloration of intestinal products, canned products, sauces and other products. Curcumin has anti-inflammatory, antioxidant, lipid-regulating, antiviral, anti-infection, anti-tumor, anti-coagulant, anti-liver fibrosis, anti-atherosclerosis and a wide range of pharmacological activities, and has the advantages of low toxicity and small adverse reactions. Curcumin is insoluble in water and ether, soluble in ethanol, propylene glycol, soluble in glacial acetic acid and alkali solutions, which limits its wide application.

Curcumin is commonly used by chemical modification to increase its water solubility, or by wrapping it in aggregates formed by amphiphilic substances. Chitosan (CS) is a naturally occurring basic polysaccharide with $-NH_2$ groups that are easily protonated to carry a positive charge. The $-NH_2$ moiety has mucoadhesive properties and has the potential to instantly open tightly attached epithelial cells. The good properties of chitosan along with its biocompatibility and biodegradability make it the most promising drug carriers.

Hydrophobically modified chitosan not only endods chitosan with amphiphilic properties, but also inherits the advantages of non-toxicity, biodegradability and biocompatibility of chitosan. The carboxyl and amino groups of carboxymethyl chitosan are pH responsive, so the hydrophobic modified carboxymethyl chitosan is an optional substance to encapsulate curcumin.

In this section, the TCA-m-CMCh aggregates were used as carriers of curcumin, and the curcumin loading (CLE) and encapsulation efficiency (CEE) were studied. The curcumin loaded aggregates were characterized by fluorescence confocal microscopy and TEM.

3.2 Experimental sections

3.2.1 Reagents

Curcumin (AR, Aladdin Reagent Company) and TCA-m-CMCh (synthesized ourselves) were used in the experiments.

3.2.2 Experimental instruments

Fluorescence confocal microscopy (Axio Scope. A1, Zeiss) were used in the experiments, except for the instruments used in Section 2.

3.2.3 Curcumin encapsulation and release

Encapsulation of curcumin was performed by dialysis by first dissolving 0.05 g of curcumin in 20 mL of DMSO and stirring in a dark room at room temperature for 6 h. Subsequently, 20 ml of 10 g/L of TCA-m-CMCh solution was added dropwise. The TCA-m-CMCh / curcumin / water mixture was then stirred for an additional 12 h

at room temperature, followed by dialysis (molecular weight cutoff = 14 kDa) for 72 h. The curcumin-loaded TCA-m-CMCh aggregates were lyophilized. The curcumin loading (CLE) and encapsulation efficiency (CEE) were calculated according to the following equations.

$$Load\ factor = \frac{Weight\ of\ curcumin\ in\ the\ aggregate}{Weight\ of\ aggregates\ containing\ curcumin} \times\ 100\% \quad (2\text{-}1)$$

$$Encapsulation\ rate = \frac{\text{Weight of curcumin in the aggregate}}{\text{Initial weight of curcumin}} \times\ 100\% \quad (2\text{-}2)$$

The release of curcumin from TCA-m-CMCh aggregates at 25 and 40°C was also investigated. 0.1 g of curcumin-loaded HBCC aggregates were dissolved in 100 mL of water. Every 10 min, 2.0 mL of the solution was removed and was mixed with the same amount of DMSO to dissolve the DMSO-soluble curcumin, and its UV absorption spectrum was measured at 435 nm. The pH of TCA-m-CMCh solution was 9.18. Prior to the UV absorption experiments, a standard working curve of curcumin in DMSO was plotted.

3.3 Results and Discussion

3.3.1 Properties of curcumin-loaded TCA-m-CMCh aggregates

To investigate the potential use of TCA-m-CMCh aggregates as water-insoluble plant active ingredient carriers, the loading and in vitro release behavior of curcumin was studied at pH 9.18. The average hydrodynamic diameter of TCA-m-CMCh aggregates (1.0 g/L) loaded with curcumin was 253 nm with PDI of 0.341 (Figure 3.1 (A)). The average diameter of curcumin-loaded TCA-m-CMCh aggregates obtained

from TEM images was 36.8 nm (Figure 3.1 (B)). The both diameters were larger than the aggregates without curcumin. This confirms the presence of curcumin in the TCA-m-CMCh aggregates. The fluorescence confocal morphology confirmed the existence of curcumin in TCA-m-CMCh aggregates (the blue arrow in Figure 3.1 (C)). Moreover, the particle size is large enough to be captured by macrophages.

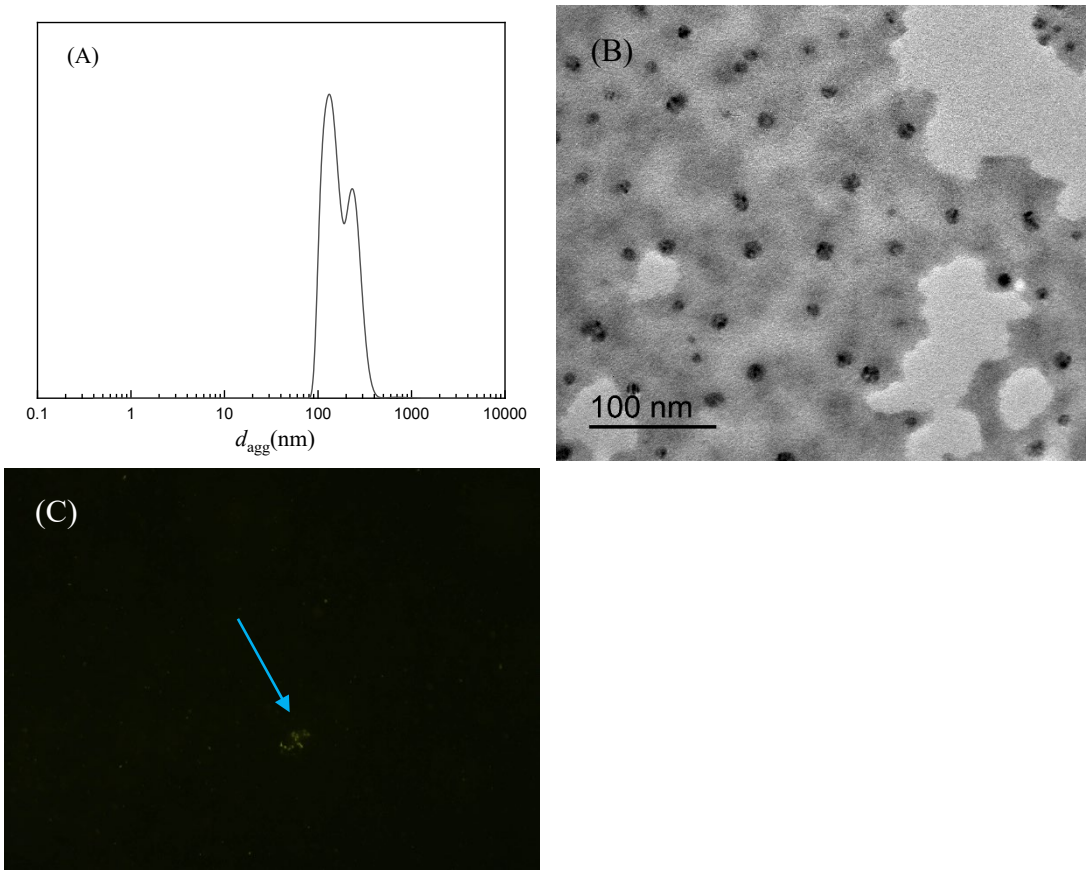

Figure 3.1 Hydrodynamic diameter (A), TEM morphology (B) and fluorescence confocal morphology (C) of curcumin-loaded TCA-m-CMCh aggregates.

The Zeta potential of curcumin loaded TCA-m-CMCh aggregates was -17.3 mV, which increased slightly compared to that of TCA-m-CMCh aggregates, indicating that the curcumin-loaded TCA-m-CMCh aggregates were more suitable for curcumin release.

The curcumin loading and encapsulation efficiency were 13.3% and 65.7%, respectively.

3.3.2 Release of curcumin from TCA-m-CMCh aggregates

The release efficiency of curcumin from TCA-m-CMCh aggregates were studied at room temperature. Before the determination of the released curcumin from TCA-m-CMCh, the working curve was plotted by measuring the relationship between UV intensity and concentration of curcumin in DMSO solution. The equation of working curve is $y=0.1436 \cdot x+0.0135$ ($R^2=0.9982$).

The released concentration of curcumin from TCA-m-CMCh aggregates with time is shown in Figure 3.2. It showed that the curcumin could be released continuously from TCA-m-CMCh aggregates for 600 min, and the accumulated concentration was up to 0.1 g/L. The results indicated that TCA-m-CMCh is one of potential curcumin carriers.

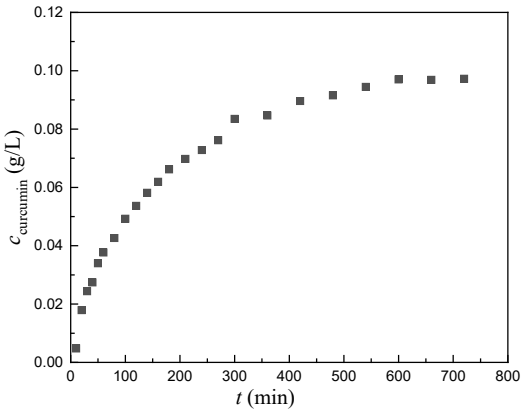

Figure 3.2 Plots of accumulated curcumin concentration released from TCA-m-CMCh aggregates vs. releasing time at room temperature.

In actual life, when oral drugs pass through oral cavity, stomach and digestive systems, the pH of each section is different, which will also affect the release time and efficiency of TCA-m-CMCh for the active ingredients of oil-soluble drugs. Due to the limited time, the influence of these factors on TCA-m-CMCh-encapsulated curcumin has not been studied yet. The work will be continued.

Conclusions to section 3

The TCA-m-CMCh aggregates could load curcumin by self-aggregation behavior and hydrophobic interaction. The increase of aggregate diameter, proved by both TCA-m-CMCh aggregates and TEM images, confirmed the loading of curcumin in TCA-m-CMCh aggregates, as well as the fluorescence confocal morphology. The curcumin loading and encapsulation efficiency in TCA-m-CMCh aggregates were 13.3% and 65.7%, respectively.

The curcumin could be released continuously from TCA-m-CMCh aggregates for 600 min, and the accumulated concentration was up to 0.1 g/L. The results indicated that TCA-m-CMCh is one of potential curcumin carriers.

CONCLUSIONS

TCA-m-CMCh was prepared by grafting TCA in CMCh using an ionic liquid AmimCl as solvent. Both FTIR spectra and NMR spectra proved that the substitution reaction of TCA on CMCh and the substitution degree of TCA was 15.3% by integral calculation of NMR spectra. The thermal stability of TCA is increased, and the concentration of TCA in aqueous solution is 0.2g/L. In solution of 1.0 g/L, TCA-m-CMCh could form aggregate with average particle size of 221.8 nm and Zeta potential of -23.6 mV. TCA-m-CMCh showed moderate antibacterial activity against E. coli and S. aureus, and non-toxicity.

The curcumin could be released continuously from TCA-m-CMCh aggregates for 600 min, and the accumulated concentration was up to 0.1 g/L. The results indicated that TCA-m-CMCh is one of potential curcumin carriers.

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