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Novel method for preparation of β-cyclodextrin/grafted chitosan and it's application

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Abstract

A novel technique for preparation of β-cyclodextrin-grafted chitosan was carried out by reacting β-cyclodextrin citrate (β-CD citrate) with chitosan. β-Cyclodextrin citrate was synthesis by esterifying β-cyclodextrin (β-CD) with citric acid (CA) in presence or absence of sodium hypophosphite as a catalyst in a semidry process. Different factors affecting preparation of β-CD citrates were studied to obtain β-CD citrate with high carboxyl content, such factors include reaction temperature, citric acid concentration, material to liquor ratio and duration. β-Cyclodextrin/grafted chitosan was prepared by coupling β-CD citrate with chitosan dissolved in different formic acid solutions having different concentrations. The reacting ingredients were subjected to various reaction conditions to attain the optimum condition. β-Cyclodextrin/grafted chitosan were evaluated by measuring the nitrogen content of both chitosan and grafted chitosan. Chitosan and β-cyclodextrin/grafted chitosan, having different molecular weights, were evaluated as antimicrobial agents for different microorganisms such as, *Bacillus Megaterium, Pseudomonas Fragi, Bacillus Cereus Staphylococcus Aureus, Escherichia Ecoli* and *Aeromonas hydra.*

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides. They are produced by enzymatic degradation of starch and were first obtained by Villies 1891. There are three main types of cyclodextrin,α, β and γ of six, seven and eight cyclic maltose, respectively (Kobayashi et al., 1981; Cox et al., 1984; Bender and Komiyama, 1978; Deratani and Poepping, 1995; Seo et al., 1987; Mizobuchi et al., 1980). The cyclodextrin consists of tours like macrocyclic ring. All hydroxyl groups are located at the top and bottom of the tours. Thus the hydrophobic cavity of cyclodextrins is capable of including a variety of hydrophobic compounds via host-guest complexation (Takashima et al., 2004). This property has been extensively exploited in the past to change physiocopharmacetical properties of lipophilic drugs such as water solubility, bioavailabilty, improved stability and effectiveness (Sortino et al., 2001).

Many attempts to utilize CD and CD derivatives in textile applications were carried out in the last decade (Szejtli, & Jozsef 2003; Buschmann, Knittel, & Schollmeyer., 2001). This was brought about by the recognition that the inclusion complex formation capability of CD can be applied to different area of applications such as deodorant, aroma, antimicrobial, insect repellent, mite repellent finishes that have recently become popular and in treating effluents.

Chitin is one of the most abundant naturally occurring polysaccharide next to cellulose. Chitin consists mainly of β-(1–4)-2-acetamido-2-deoxy-D-glucose units. Despite much recent research into its utilization, its tightly intermolecular hydrogen bonding and poor solubility to common organic solvents have so far prevented widespread utilization of chitin (George, 1992). Chitosan is N-deacetylated form of chitin that is obtained by alkaline treatment of chitin (50% of aqueous NaOH) at high temperature. Chitosan and its derivatives have become useful polysaccharides in the biomedical area because of its biocompatible, biodegradable, and non-toxic properties (Lee et al., 1997).

The antimicrobial and antifungal activities of chitosan and chitosan derivatives (Devlieghere, Vermeulen, & Debevere, 2004; Lim, Sang-Hoon; Hudson, &Samuel, 2004a,b) have been described, since chitosan inhibits the growth of a wide variety of bacteria and fungi. Moreover, chitosan has several advantages over other types of disinfectants, that is, it possesses a higher antibacterial activity, a broader spectra of activity, a higher killing rate, and lower toxicity toward mammalian cells.

Many attempts were carried out to prepare cyclodextrin-grafted chitosan in the literature. Zhang et al. prepared two new adsorbents by reacting of β-CD and sulfonated β-CD with

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epoxy-activated chitosan and chitosan, respectively (Zhang, Wang, & Yi, 2004). Wu Wen-Teng et al. immobilized β-CD to chitosan beads by crosslinking with1, 6-hexamethylene diisocyanate (Chiu, Chung, Giridhar, & WuChiu, 2004). Michel Morcellet studied the reaction of cyclodextrin monochlorotriazinyl derivative with chitosan (Martel et al., 2001), and Farusaki et al. (Furusaki et al., 1996) coupled carboxymethyl derivative of β-CD to chitosan.

In our pervious work, β-CD/grafted chitosan was prepared by graft copolymerization of β-CD itaconate onto chitosan using ceric ammonium nitrate as a redox initiation system (Gaffar et al., 2004). Cyclodextrin itaconate has been prepared by esterifying β-CD with itaconic acid using a semidry process. As continuity, efforts are made in this work to prepare cyclodextrin/grafted chitosan with high CD-content meanwhile it is easily and completely soluble in organic acid solutions. This was achieved by reacting of β-CD with citric acid at different reaction conditions to prepare β-CD citrate, as a reactive CD, of high carboxyl content. β-CD citrate was allowed to react with chitosan of three different molecular weights at various reaction conditions.

2. Experimental

2.1. Materials

β-Cyclodextrin was kindly supplied by Cerestar Co. (USA). Chitosan, having three levels of molecular weight, e.g. 50,000; 30,000; and 1500 was purchased Korea Chitosan Co., Ltd and other suppliers. Citric acid (CA), sodium hypophosphite monohydrate (SHP), formic acid, and sodium hydroxide were laboratory grade chemicals.

Bacillus megaterium 744 were obtained in lyophilized form, from Northen Regional Research Center, Illionion (USA). While *Pseudomonas* fragi NRRL B-727 was obtained from National Center for Agricultural Utilization Research (USA).

Bacillus cereus ATCC 11778 was obtained from American type culture collection (ATCC) (USA). While strain *Staphylococcus aureus* (No. 315) was obtained from US Food Drug Administration (FAD) Microbiology Lab (USA).

Tryptone Soya Broth and Tryptone Soya media were obtained from Oxoid Ltd, UK

2.2. Synthesis of β-cyclodextrin citrate

β-Cyclodextrin citrate (β-CD citrate) was prepared using a semidry reaction method by mixing of 2 g of β-CD with definite amount of water containing different citric acid concentrations (1–4 mole/1 g CD) in presence and absence of SHP. The reaction mixture was allowed to react in a circulating air oven at different reaction temperatures (80–140 °C) for specific times. The cured samples were purified by washing with isopropanol using a soxhlet for 6 h in order to remove unreacted components as well as any soluble fragments or by-products, followed by drying at 60 °C for 24 h. After drying, the β-CD citrate was kept over P_2O_5 for at least 48 h before analysis.

2.3. Preparation of β-cyclodextrin/grafted chitosan

Linking of β-CD citrate onto chitosan was taken place by reacting the pendant free carboxyl groups of β-CD citrate with the amino groups of chitosan. A definite volume of water containing different β-CD citrate concentrations was introduced into a solution containing chitosan dissolved in different formic acid concentrations (0–0.4 ml/1 g chitosan). The reaction mixture was then magnetically stirred and heated at different reaction temperatures (80–140 °C) for 3 h using different material-to-liquor ratios (1:10–1:25). At the end of the reaction, the products were precipitated by adding 100 ml of NaOH solution (0.2 N). The samples were thoroughly washed with distilled water till neutral (pH 7) to ensure the removal of unreacted β-cyclodextrin citrate. Finally, the samples were washed with acetone and oven dried at 60 °C for 24 h.

2.4. Antibacterial spectrum

Tryptone soya agar and broth were used for estimation the antibacterial spectrum of chitosan and cyclodextin/grafted chitosan, having three different molecular weights, on several spoilage and pathogen strains namely *B. cereus B. megaterium, Pseudomonas fragi, S. aureus, Aeromonas Hydra, and Escherichia coli*

Chitosan and cyclodextin/grafted chitosan samples of different concentrations (12.5–100 mg) were added prior pouring. All treatments were incubated at 37 °C for 24 h. All incubated media were visually observed to find out whether the strain had grown or not. Besides, the minimum inhibition concentration (MIC) of each strain was determined.

2.5. Testing and analysis

The carboxyl content of β-CD citrate was determined using acid base titration method according to a reported method (Yang & Wang, 2000).

The grafting efficiency of β-CD citrate onto chitosan was calculated mathematically via determining the nitrogen % of chitosan before and after modification according to Kjeldal method (Vogel, 1966).

The minimal inhibition concentration (MIC) of bacteriocin was qualitatively determined for several bacterial strains according to the reported method (Davidson & Parish, 1989).

3. Results and discussion

3.1. Tentative mechanism

Esterification of polysaccharides (e.g. cellulose (El-Tahlawy, 1999; Voncina & Le Marechal, 2005), starch (Xueju & Qiang, 2004)) can be prepared by the reaction of polycarboxylic acid with hydroxyl groups of these polymers in the presence of alkaline or amphoteric catalyst under curing conditions via reactive cyclic anhydride intermediate mechanism.

Preparation of β-CD citrate was carried out by the reaction of β-CD with citric acid, in presence and absence of SHP, via the

formation of a reactive cyclic anhydride intermediate by the dehydration of two adjacent carboxyl groups (Lim, Sang-Hoon, Hudson, & Samuel 1997) under curing conditions. Since the primary hydroxyl groups of β-CD are the most basic, they are more favorable for esterification than secondary one.

The mechanism of esterification reaction would be accompanied by several possibilities as in Scheme 1. The dominant of which may be as follows:

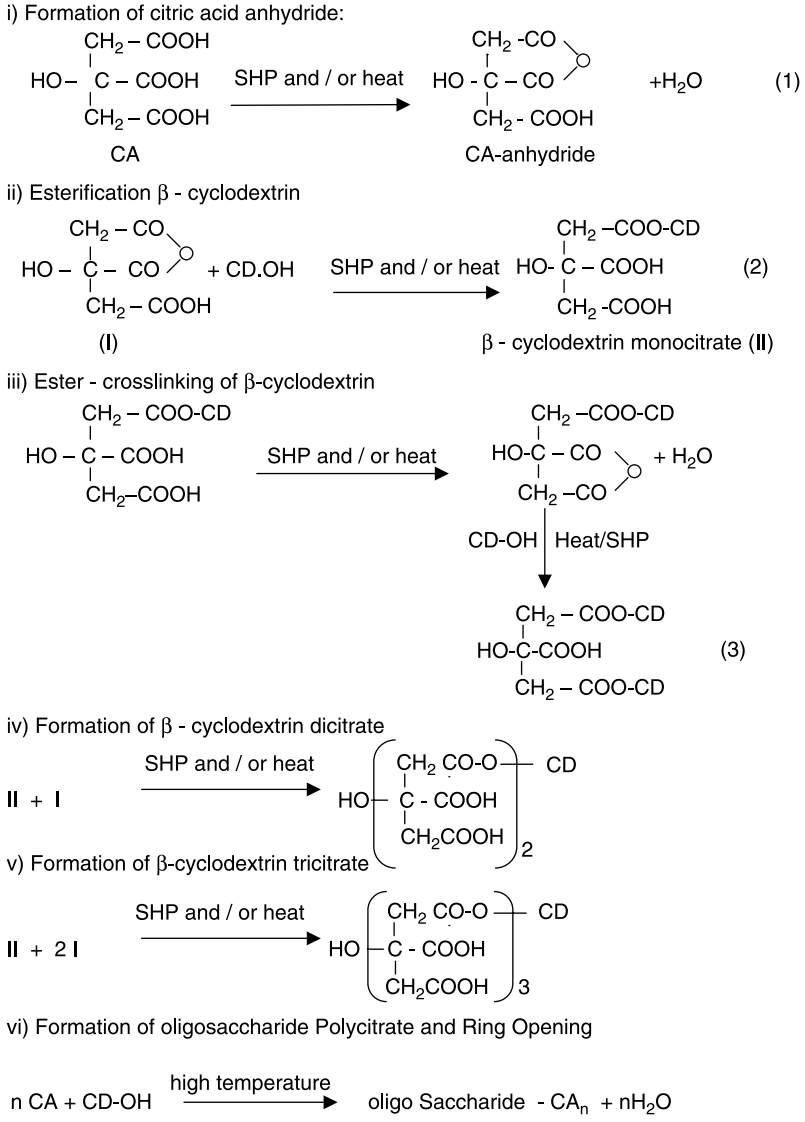
FTIR- spectra of β-CD citrate shows strong peak at 1730 cm^{-1}. This peak indicates the formation of carboxylic ester of cyclosextrin citrate esters.

4. Preparation of β-cyclodextrin citrate

Heating of citric acid is accompanied by formation of anhydride, when β-CD is present in the reaction mixture, the anhydride can react with β-CD to form β-CD citrate adduct. Major factors affecting esterification of CD with CA in presence of/or absence of SHP as a catalyst were studied. Given below are the results obtained along with their appropriate discussions (Fig. 1).

4.1. Effect of sodium hypophosphite concentration

Fig. 2 shows the effect of sodium hypophosphite concentration (SHP) (0–0.5 mole/mole CA) as a catalyst versus the carboxyl content of β-CD citrate, when β-CD was treated with citric acid at different reaction temperatures (80–140 °C) using material to liquor ratio 1:0.6. It is clear from the data that, increasing SHP concentration from 0 to 0.5 mole/mole CA is accompanied by increase in the carboxyl content of β-CD citrate, such increase in the carboxyl content could be attributed to the enhancement in the esterification efficiency of CA to react with β-CD even at low temperature (80–100 °C). The carboxyl content of β-CD citrate prepared by esterifying of β-CD with CA in absence of SHP gave us a promising results, since the formed β-CD citrate is easier in the purification step. The data (Fig. 2) indicates also that the reaction temperature



Scheme 1. The reaction possibilities of citric acid with cyclodextrins.

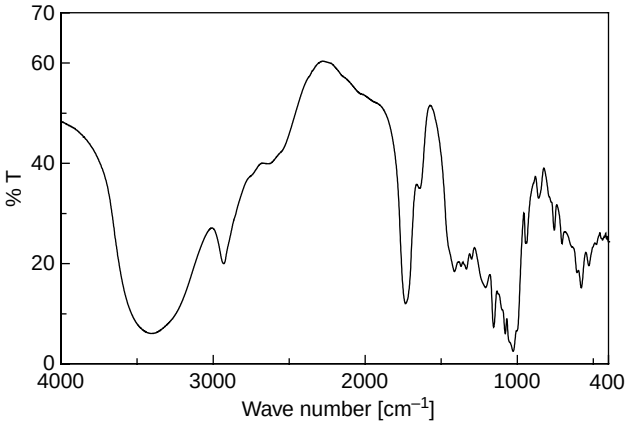


Fig. 1. Representation of the FTIR spectra of β-CD citrate.

plays an important role during the esterification process. It is clear from the data that, regardless of SHP concentration used, increasing the reaction temperature from 80 to 120 °C is accompanied by a significant increase in the carboxyl content of β-CD citrate. Further increase in the reaction temperature beyond 120 °C is accompanied by marginal increase in the carboxyl content. This behavior could be attributed to the favorable effect of raising the reaction temperature (1) dehydration of CA resulting in the formation of the reactive anhydride intermediate, and (2) esterification process as a whole. Since our target is preparation of β-CD citrate with high carboxyl content without affecting the CD-ring. It could be concluded that 100 °C is the optimum reaction temperature for preparation of β-CD citrate.

4.2. Effect of reaction duration

The relation between the reaction duration of esterification reaction and the carboxyl content of β-CD citrate at three different reaction temperatures (80, 100, 120 °C) is shown in Fig. 3. The esterification reaction was carried out using CA, 2 mole/1 mole CD and material to liquor ratio 1:0.6. It is clear from the data that, (a) the carboxyl content of β-CD citrate is increasing gradually till reaches the maximum of the reaction

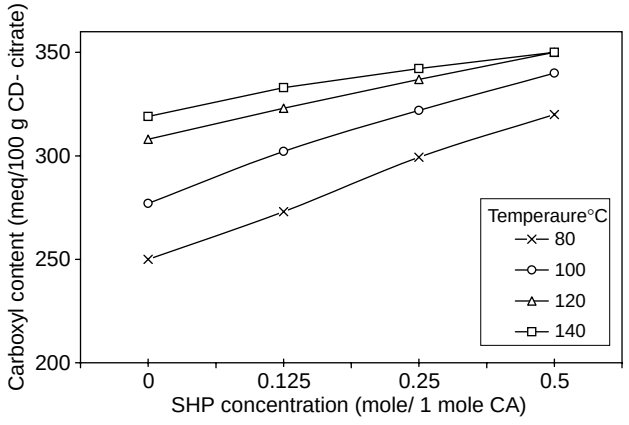


Fig. 2. Effect of SHP concentration on the the carboxyl content of CD citrate at different reaction temperature [CA], 2 mole/1 mole CD; M/L—ratio 1:0.6.

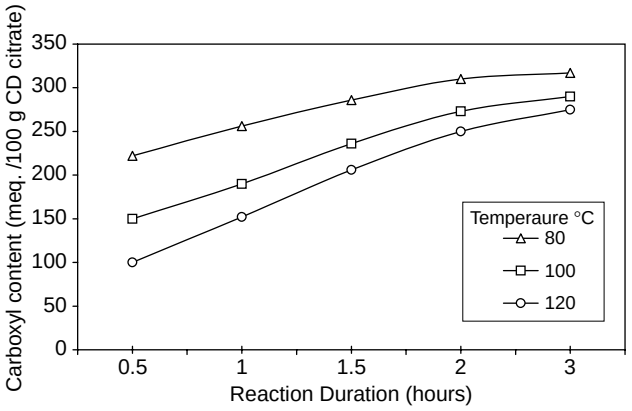


Fig. 3. Effect of reaction duration on the the carboxyl content of CD citrate at different temperatures. [CA], 2 mole/1 mole CD; M/L—ratio 1:0.6. [SHP], 0.

at 3 h, and (b) rate and extent of reaction increases by raising the reaction temperature within the range studied.

4.3. Effect of material to liquor ratio

Fig. 4 shows the effect of material to liquor ratio (1:0–1:1.2) on the carboxyl content of β-CD citrate, when CA was allowed to esterifies β-CD using CA; 2 mole/1 mole CD, reaction time; 2 h at two different reaction temperature (100 and 120 °C). It is well know that, the material to liquor ratio plays an important function during the esterification process especially in a semidry state, since the amount of water is responsible for homogenization of the reacting ingredients as well as increasing the availability of CA molecules in the vicinity of β-CD. Also it is well established that the esterification process is carried out through (a) dehydration of water, (b) formation of anhydride intermediate and finally (c) the esterification reaction. Keeping the above in mind, the effect of material to liquor ratio started from zero water content to 1.2 ml of water containing 2 mole/1 mole β-CD was studied. It is clear from the data (Fig. 4) that, increasing the liquor from 0 to 0.6-ml water/1 g β-CD is accompanied by significant improvement in the carboxyl content. Further increase in the water content above this limit is accompanied by a decrease in the carboxyl content of β-CD citrate. Increasing water content from 0 to 0.6 leads to an increase in the homogeneity of the reactants and consequently enhancing the reactivity and accessibility of CA to esterify β-CD. Further increase in the water content above 0.6 ml caused a dilution of the reaction medium which needs either elevation of the reaction temperature from 100 to 120 °C

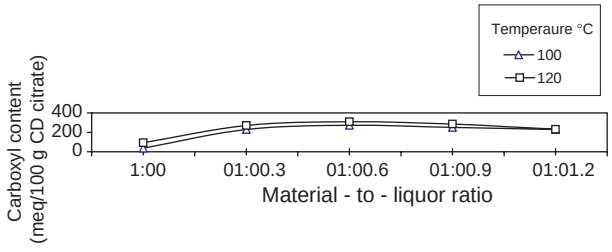


Fig. 4. Effect of material-to-liquor ratio on the carboxyl content of CD citrate at different temperatures. [CA], 2 mole/1 mole CD, time; 2 h, [SHP], 0.

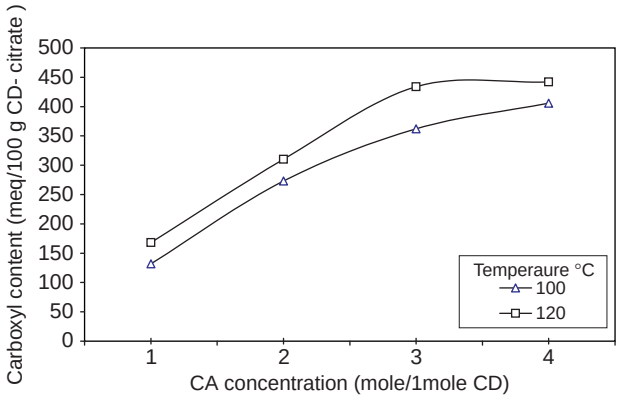


Fig. 5. Effect of [CA] on the the carboxyl content of CD—citrate at two different reaction temperatures. Time, 2 h; M/L, ratio; 1:0.6, [SHP]; 0, [CD], 1 mole.

or prolonging the reaction time to evaporate the water from the reaction medium.

4.4. Effect of CA concentration

The effect of CA concentration on the extent of esterification (expressed as mequiv carboxyl/100 β-CD citrate) is illustrated in Fig. 5. The esterification reaction was carried out using material-to-liquor ratio 1:0.6, reaction time; 3 h at two different reaction temperatures (100 and 120 °C). It is obvious that as a general, increasing CA concentration in the reaction medium is accompanied by a noticeable increase in the carboxyl content of the obtained β-CD citrate. This could be associated with higher availability of CA molecules in vicinity of CD molecules at higher concentration, leading to increasing the extent of esterification of β-CD. It could be concluded that, the optimum condition for preparation of β-CD citrate is CA; 2 mole/1 mole CD, reaction time; 2 h, reaction temperature; 100 °C, and material-to-liquor ratio 1:0.6.

5. Preparation of cyclodextrin/grafted chitosan

β-CD citrate with carboxyl content of 273 mequiv/100 g was prepared according to the optimum conditions. The prepared sample was used as a reactive CD to react with amino groups of chitosan for preparation of β-CD/grafted chitosan. Different parameters affecting the grafting reaction were studied briefly.

5.1. Effect of formic acid concentration

Fig. 6 shows the effect of formic concentration that is used for dissolving chitosan, as a function of graft yield %. It is evident from the data (Fig. 6) that, increasing formic acid concentration from 0 to 0.4 ml/1 g chitosan is accompanied by a significant increase in the graft yield % and reached maximum value at 0.4 ml formic acid/1 g chitosan at which chitosan is completely soluble in water. The enhancement in the grafting reaction, expressed as graft yield %, by increasing the formic acid incorporated in the reaction medium could be

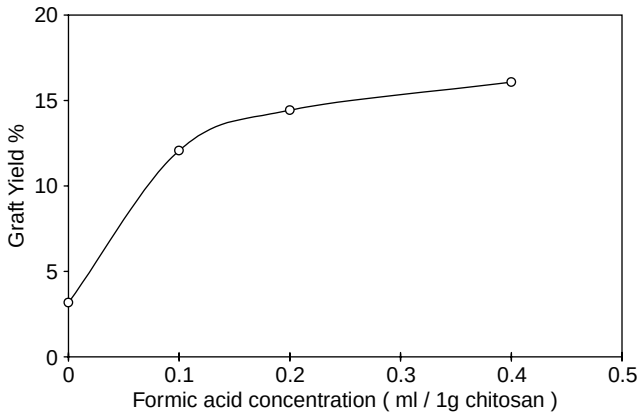


Fig. 6. Effect of [formic acid] on the graft yield % of CD/grafted chitosan CD—citrate; 0.15 g (1.34 mequiv free carboxyl); [Chitosan], 0.54 g (6.7 mequiv free amino), M/L, ratio; 1:20, M.Wt of chitosan; 50.000, temperature; 100 °C, time, 3 h.

associated with (1) greater solubility of chitosan, thereby increasing surface area of the chitosan molecules to react with β-CD-citrate, (2) increasing the homogeneity of the reacting ingredient, and (3) higher condensation efficiency.

5.2. Effect of β-CD citrate concentration

Fig. 7 shows the effect of β-CD citrate concentration (carboxyl content 273 mequiv/100 g) incorporated in the reaction medium (0.05–0.6 g, i.e. 0.446–5.36 mequiv carboxyl) on the graft yield percent at two different formic acid concentration (0 and 0.4 ml/1 g chitosan) using 0.54 g chitosan (6.7 mequiv free amino groups) at 100 °C for 3 h using material to liquor ratio of 1:20. It is clear from the data that, increasing β-CD citrate concentration incorporated in the reaction medium is accompanied by increasing the graft yield %, such increase in the G.Y. % may be due to increasing the number of available β-CD citrate molecules to react with chitosan. At the same time, increasing β-CD citrate concentration incorporated in the reaction medium in the presence and absence of formic acid (0 and 0.4 ml/1 g chitosan) shows two different values, since G.Y % at 0.4 ml of formic acid is higher than that in

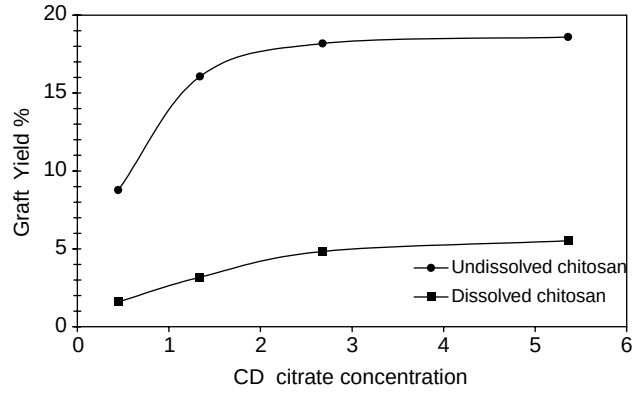


Fig. 7. Effect of [CD—citrate] on the graft yield of CD/grafted chitosan. [Chitosan]; 0.54 g (6.7 mequiv free amino), M/L, ratio; 1:20, temp.; 100 °C; time, 3 h; M.Wt of chitoasn, 50.000, [Formic acid]; 0.4 ml/1 g chitosan.

absence of formic acid, this could be associated with the condensation reaction was carried out on the surface of chitosan molecules, in absence of formic acid, while in the presence of formic acid, the chitosan molecules are completely soluble in water which increase the number of accessible amino groups to react with the carboxyl groups of β-CD citrate.

5.3. Effect of reaction temperature

It is well known that condensation reaction between the carboxyl groups of β-CD citrate and the amino groups of chitosan is highly dependent on the reaction temperature. Fig. 8 shows the effect of reaction temperature as a function of grafting percent, expressed as G.Y. %, when the grafting reaction was carried out in the presence and absence of formic acid. In the absence of formic acid, the grafting reaction is directly proportional to the reaction temperature, since raising the reaction temperature from 80 to 140 °C is accompanied by a significant increase in the G. Y. % from 1.72 to 14.84%, such increase in the G. Y. % may be due to (1) increasing the extent of diffusion of β-CD citrate molecules onto the vicinity of chitosan molecules, (2) increasing the swellability of chitosan molecules and (3) higher condensation efficiency. While in the presence of formic acid, raising the reaction temperature from 80 to 100 °C is accompanied by increasing the G.Y %. Further increase above this limit is followed by a decrease in the G.Y. %. This behavior could be attributed to formation of *N*-chitosan formate rather than reaction with β-CD citrate according to the following equation:

$$RH_3N^{+-}OOCH \xrightarrow{>100\ C} R-NHCOH + H_2O$$

5.4. Effect of material to liquor ratio

The relation between material to liquor ratio and the grafting reaction, expressed as G.Y. %, was observed in Fig. 9, when the grafting reaction was carried out using [chitosan], 0.54 g (6.7 mequiv Free amino group), β-CD citrate, 0.6 g (5.485 mequiv carboxyl) at 100 °C for 3 h. It is clear from the data (Fig. 9) that, using material to liquor ratio of 1:15 represents the optimum condition for the grafting reaction. Using liquor ratio higher than 1:15 leads to a decrement in the graft yield %, which could be ascribed to the dilution of the reaction medium.

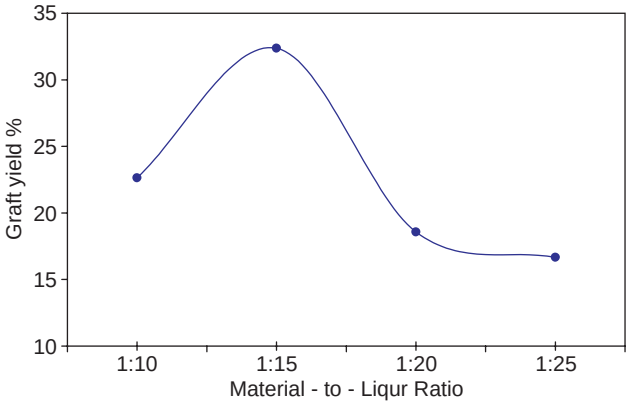


Fig. 9. Effect of material-to-liqur ratio on the graft yield % of CD/grafted chitosan. [CD-citrate]; 0.6 g (5.36 mequiv free carboxyl); [Chitosan], 0.54 g (6.7 mequiv free amino), M.Wt. of chitosan; 50.000, [Formic acid]; 0.4 ml/1 g chitosan; temperature, 100 °C; time, 3 h.

5.5. Effect of molecular weight of chitosan

The effect of the molecular weight of chitosan molecules incorporated in the reaction medium on the graft yield percent is shown in Fig. 10, when the grafting reaction was carried out using chitosan, 0.54 g (6.7 mequiv free amino group) of three different molecular weights (50,000, 30,000, 1500), β-CD citrate, 0.6 g (5.485 mequiv carboxyl), 0.4 ml of formic acid/1 g chitosan using material to liquor ratio 1:15 at 100 °C for 3 h. It is obvious from Fig. 10 that, the molecular weights of chitosan play an important role in the grafting process. The extent of the grafting (G.Y %) is inversely proportional to the molecular weight of chitosan, i.e. decreasing the molecular weight of chitosan is accompanied by a significant increase in the graft yield %, since the extent of the grafting % is obeying

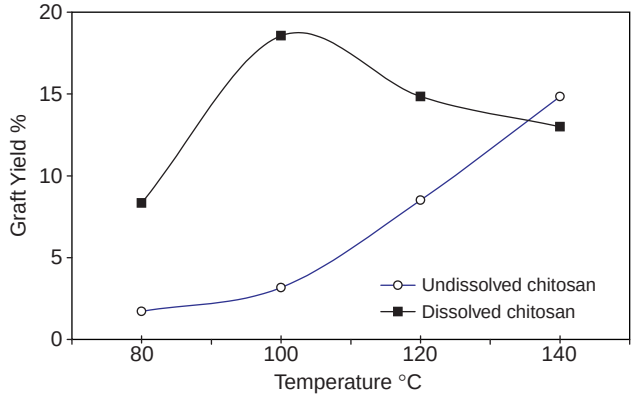


Fig. 8. Effect of temperature on the graft yield % of CD/grafted chitosan [CD—citrate]; 0.6 g (5.36 mequiv free carboxyl); [Chitosan], 0.54 g (6.7 mequiv free amino), M/L, ratio; 1:20, M.Wt. of chitosan; 50.000, [Formic acid]; 0.4 ml/1 g chitosan; time, 3 h.

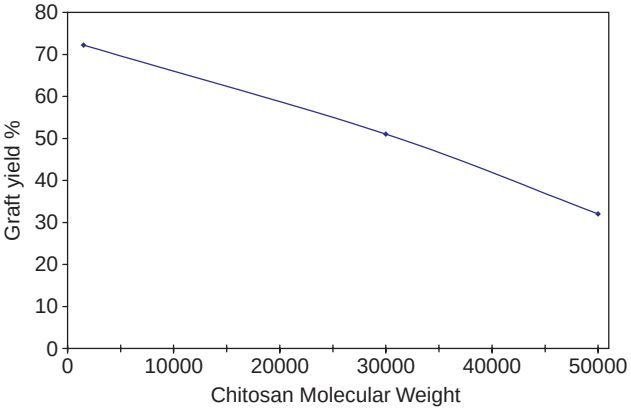


Fig. 10. Effect of molecular weight of chitosan on the graft yield % of CD/grafted chitosan. [CD-citrate]; 0.6 g (5.36 mequiv free carboxyl); [Chitosan], 0.54 g (6.7 mequiv free amino), M/L ratio; 1:15, [Formic acid]; 0.4 ml/1 g chitosan; temperature, 100 °C; time, 3 h.

Table 1
Minimal inhibition concentration of recorded pathogenic bacterial strains

Kind of microorganism	Strain	Minimal inhibition concentration MIC, mg						CD-citrate
		M.wt of chitosan			M.wt of chitosan linked CD			
		50,000	30,000	1500	50,000	30,000	1500	
Gram negative	*A. hydra*	25	25	12.5	12.5	12.5	12.5	R
	E. coli	50	50	12.5	25	12.5	12.5	R
	P. fragi	50	50	12.5	25	12.5	12.5	R
Gram positive	*S. aureus*	25	25	12.5	12.5	12.5	12.5	R
	B. megaterium	25	25	12.5	12.5	12.5	12.5	R
	B. cereus	25	25	12.5	12.5	12.5	12.5	R

R: resistance up to 100 mg.

the following trends: 1500 > 30,000 > 50,000. The increase in the graft yield % by decreasing the molecular weight of chitosan chains could be attributed to (1) lower viscosity, (2) higher mobility of chitosan molecules, (3) less sterric hindrance, and (4) higher accessibility of chitosan molecules to react with β-CD citrate.

5.5.1. Utilization of β-cyclodextrin-grafted chitosan as a new antimicrobial agent

Chitosan, a cationic antimicrobial agent (Lim & Hudson, 2003; Lim & Hudson, 2004), has been widely used, particularly for external disinfection and the target site of the cationic biocides is the cell envelope of bacteria.

Table 1 shows the antimicrobial activity, expressed as minimal inhibition concentration (MIC), of chitosan and β-CD/grafted chitosan against two kind of mcro-organisms, gram positive, such as *S. aureus, B. megaterium, B. cereus* and gram negative, such as *A. hydra, E. coli* and *P. fragis*.

The data indicated that, regardless of the chitosan used, the minimum inhibition concentration (MIC) decreases as the molecular weight of chitosan decrease. The mechanism behind the antimicrobial activity of chitosan can be summarizing as follow: (1) the cationic nature of chitosan binds with sialic acid in phospholipids, consequently restraining the movement of microbiological substance. (2) Chitosan molecules penetrate into the cells of micro-organisms and prevent the growth of the cells by prohibiting the transforming of DNA to RNA. Based on the above knowledge, the antimicrobial activity is generated from the free amino groups in chitosan in an aqueous acidic environment. It's well known that, the MIC of chitosan to impart antimicrobial properties depends upon the molecular weight, degree of deacetylation, concentration of chitosan and kind of functional groups introduced to the chitosan backbone by chemical modification. From the above finding, the enhancement in the antimicrobial activity of chitosan of lower molecular weight could be attributed to the penetration ability of chitosan to the cell wall of microorganisms and consequently enhancing the accessibility of the amino groups of chitosan to prohibit the growth of microorganism. This behavior reflects the decrement in the MIC by decreasing the M.Wt of chitosan.

The data indicates also that, β-CD citrate has no antimicrobial effect against the studied microorganisms. On the other hand, chemical modification of chitosan via it's coupling with β-CD citrate improves its antimicrobial activity, expressed in the decrement in the MIC, compared with chitosan. This behavior appears especially in chitosan of higher molecular weights. This behavior could be attributed to (1) enhancing the dissolution of chitosan and (2) the creation of carboxyl groups along with chitosan molecules serve in the dissolution of phospholipids area and causing leakage the intercellular components and finally the death of the microorganisms (Adams & Hall, 1988). The above finding illustrated too why CD/grafted chitosan is has antimicrobial activity against both gram positive and gram negative bacteria, while chitosan is a good antimicrobial agent for gram positive only.

6. Conclusions

We have synthesized β-cyclodextrin-grafted chitosan using a reaction between β-cyclodextrin citrate and chitosan for preparation of a new antimicrobial agent. β-CD citrate was prepared by esterifying β-CD with citric acid in the presence/or absence of SHP as a curing catalyst using a semi dry reaction method. It could be concluded that the optimum condition for preparation of a reactive β-CD-citrate is CA; 2 mole/1 mole CD, reaction time; 2 h, reaction temperature; 100 °C, and material-to-liquor ratio 1:0.6. β-CD citrate 'having carboxyl content of 273 mequiv/100 g ' was allowed to react with chitosan via a condensation reaction. It is also concluded that the molecular weight of chitosan, reaction temperature, and formic acid concentration are representing the major affecting factors for the condensation reaction.

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