Synthesis of Cyclodextrin-Based Polymers and Their Use as Debittering Agents

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Received 19 March 2007; accepted 10 July 2007
DOI 10.1002/app.27249
Published online 13 November 2007 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Cyclodextrins (CDs) and their derivatives are used to suppress unpleasant tastes and odors or to achieve a controlled release of certain food constituents. This article describes the synthesis by nonconventional methods of (1) crosslinked, insoluble CD polymers and (2) water-soluble, CD-grafted carboxymethylchitosan and carboxymethylcellulose. The CD polymers were obtained by the reaction of β-CD with one of the following crosslinking agents: epichlorohydrin, diphenyl carbonate, or hexamethylene diisocyanate. Their preparations were usually carried out under high-intensity ultrasound, which resulted in much shorter reaction times and narrower distributions of particle size (as determined by scanning electron microscopy measurements). A novel, insoluble CD polymer was obtained by reticulation under microwaves of propargyl-β-CD with 1,3-bis(azidomethyl)benzene through Huisgen 1,3-dipolar cycloaddition. Short columns packed with the insoluble polymers were found to efficiently sequester naringin from aqueous solutions; successively, they could be easily regenerated by a counter-current ethanol wash that also achieved an excellent recovery of the flavonoid. Differential scanning calorimetry thermograms showed that the crosslinked CD polymers formed inclusion complexes with naringin. The soluble polymers also interacted with bitter flavonoids of citrus fruits (naringin and limonin), as shown by the results of sensorial panel tests, in which they behaved as bitter-masking agents. J Appl Polym Sci 107: 2549–2557, 2008

Key words: host-guest systems; particle size distribution; stimuli-sensitive polymers; synthesis

INTRODUCTION

Cyclodextrins (CDs) present a hydrophilic external surface and a hydrophobic inner cavity, an unusual feature that determines their well-known ability to form in aqueous media stable inclusion complexes with guest molecules of low polarity. On these grounds, they have been recommended for applications in food processing[1] and as food additives with a variety of aims: to protect lipophilic food components that are sensitive to oxygen and light- or heat-induced degradation, to solubilize food colorings and vitamins, to stabilize fragrances, flavors, vitamins, and essential oils against unwanted changes, to suppress unpleasant odors or tastes, and to achieve a controlled release of certain food constituents.[2] The earliest attempt to use a CD for the purpose of suppressing bitter taste was reported in 1979 on ginseng extract.[3] Because taste is obviously a major determinant of food selection, taste modifiers are of great interest for both the food and pharmaceutical industries. A bitter taste is the main reason for the rejection of various food products,[4] although exceptions to this rule are rooted in many cultures: in some foods and beverages, such as coffee, beer, and wine, a certain degree of bitterness is expected. Bitterness, however, has proved a major limitation in the acceptance of commercial citrus juices. Two classes of chemical compounds, namely flavonoids and limonoids, are responsible for it, with a notable difference. Fruits with a high flavonoid content are bitter even when fresh. The peel (rind) of citrus fruits is very rich in some intensely bitter flavonoids such as naringin and neohesperidine. Naringin, however, is abundant in the unripe fruit, but its concentration decreases as the fruit ripens.[5,6] On the other hand, limonoids are present in the form of a nonbitter compound (limonoate-A-ring lactone), which on storage is converted to limonin and other bitter limonoids by the enzyme limonoate-D-ring lactone hydrolase. Hence, fresh citrus juice is not bitter but turns highly so in the course of storage at a rate that depends on the pH and storage temperature. A variety of procedures, some of them patented, have been developed to remove excess naringin and limonin from citrus juices:[7] adsorptive debittering,[8] selective extraction,[9] and CD treatments.[10] At the industrial level, filtration on polystyrene–divinylbenzene resins is currently the commonest method worldwide.[11]

Recently, several approaches have been tried to develop cheaper and more effective adsorbents based

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Contract grant sponsor: MIUR-COFIN 2004.
Contract grant sponsor: Ministero Università e Ricerca (MIUR), FIRB-2003 and PRIN-2004.

on natural polymers. Among these, polysaccharides such as chitin and starch and their derivatives deserve particular attention.[12,13] These biopolymers offer an interesting and attractive alternative as adsorbents because of their physicochemical characteristics and stability. Because of their macromolecular structure and polyfunctionality, they can form crosslinked networks, gels, and macroreticular resins.[14] If the degree of reticulation is high enough, the polymer matrix becomes mostly amorphous and insoluble in water and organic solvents. Mixed polymers with covalently linked CD units can be obtained by condensation with bifunctional or polyfunctional crosslinking agents such as aldehydes, ketones, isocyanates, and epoxides. β-CD polymers have been employed to make naringin-entrapping membranes,[15] which have found useful applications in the removal of bitter substances from citrus juices.[16]

A previous study of ours showed that water-soluble, CD-grafted chitosan derivatives determined a significant bitterness reduction in numerous bitter substances.[17] With the aim of further enhancing the bitterness-masking power of CDs and widening their application range, we prepared a series of macromolecular derivatives in which CDs were covalently bound to carboxymethylchitosan (CM-chitosan) and carboxymethylcellulose (CM-cellulose) and successfully tested them on limonin, naringin, and caffeine. However, as the introduction of these polymers as food additives would be subject to strict regulatory limitations, we thought this hurdle might be circumvented if insoluble, reticulated CD polymers displayed analogous properties. To this end, we synthesized a series of crosslinked, insoluble polymers obtained by the reaction of β-CD with diphenyl carbonate (DPC), epichlorohydrin (EP), or hexamethylene diisocyanate (HDI); these polymers were then tested as sequestering agents for naringin. The promoting effect of high-intensity ultrasound (US)[18] on these syntheses was generally considerable.[19] A novel reticulated CD polymer was prepared by Huisgen 1,3-dipolar cycloaddition under microwaves (MWs) by the reaction of a randomly propargylated β-CD with 1,3-bis(azidomethyl)benzene (AMB).

EXPERIMENTAL

Materials

Chemicals and Amberlite XAD-16 were purchased from Sigma–Aldrich Co. (Milan, Italy); CM-cellulose and low-viscosity chitosan were purchased from Fluka (Milan, Italy). Native CDs were kindly donated by Wacker Chemie (Munich, Germany).

Reactions were monitored by thin-layer chromatography (TLC) on Fluka F_{254} (0.25-mm) plates, which were visualized by heating after a spray of 5% sulfuric acid in ethanol. High-performance liquid chromatography (HPLC) analyses and separations were carried out on Waters instruments (Vimodrone-MI, Italy): a 1525EF pump, a 2996 diode array detector, and a 717 Plus autosampler.

^{1}H-NMR spectra were recorded on a Bruker 300 Avance (300 MHz) (Milan, Italy). $CDCl_3$ was used as the solvent, and $CHCl_3$ at $\delta = 7.27$ was used as the reference. Chemical shifts are given in parts per million. Electrospray ionization mass spectra were recorded on a Waters Micromass ZQ equipped with an electrospray ionization source, whereas matrix-assisted laser desorption/ionization mass spectra were acquired in the positive reflectron ion mode with delayed extraction on a Reflex III time-of-flight instrument (Bruker Daltonics, Bremen, Germany) equipped with a 337-nm nitrogen laser. The melting points were obtained with a Büchi SMP-20 (uncorrected (Assago-MI, Italy)). IR spectra were acquired with a Shimadzu 8001 Fourier transform infrared spectrophotometer (Milan, Italy). Differential scanning calorimetry (DSC) analysis was performed with a DSC/7 differential scanning calorimeter (Perkin-Elmer, Monza-MI, Italy) equipped with a TAC 7/DX instrument controller. The instrument was calibrated with indium for the melting point and heat of fusion. A heating rate of 20°C/min was employed in the 25–200°C temperature range. Standard aluminum sample pans (PerkinElmer) were used; an empty pan was used as the reference standard. Analyses were performed under a nitrogen purge; triple runs were carried out on each sample. Scanning electron microscopy (SEM) observations were made on gold-coated samples with a Leica Stereoscan S410 electron microscope (Wetzlar, Germany); the accelerating voltage was 15 kV, and the secondary electron detector was used. The sonochemical reactors used in this study were a probe with an immersion titanium horn[20] (20.4 kHz) and a titanium cavitating tube[21] (19.2 kHz). Both devices were developed in our laboratory in collaboration with Danacamerini Sas (Torino, Italy).

Synthesis

β-CD–CM-chitosan (**1**; ca. 15% γ-CD)

This adduct was prepared as described in a previous work.[22] To a 100-mL, round-bottom flask, 180 mg of CM-chitosan[23] (0.81 mmol), 790 mg of β-CD (0.70 mmol), 200 mg of γ-CD (0.15 mmol), 155 mg of *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDC; 0.81 mmol), a catalytic amount of 4-di(methylamino)-pyridine (DMAP), and water (40 mL) were added. The mixture was stirred at room temperature for 48 h. The product was precipitated with acetone and washed with ether to yield 620 mg of **1**.

β-CD–CM-cellulose (**2a**) and γ-CD–CM-cellulose (**2b**): general procedure

These adducts were prepared as described in our previous work.[22] To a 100-mL, round-bottom flask, 700 mg of CM-cellulose (ca. 2.5 mmol), the appropriate CD (0.9 equiv), 670 mg of EDC (3.5 mmol), a catalytic amount of DMAP, and water (50 mL) were added. The mixture was stirred at 45°C for 30 h. The product was precipitated with acetone (50 mL), filtered off, washed with methanol, and freeze-dried, yielding 905 mg of **2a** and 915 mg of **2b**.

β-CD–DPC (**3**)

In a 100-mL, double-necked flask, 4 g of β-CD (3.52 mmol) and 7.55 g (10 equiv, 35.25 mmol) of DPC were mixed, heated to 90°C, and sonicated (50 W) for 2 h. The reacted mixture was washed with water and successively with acetone. The product was evaporated to dryness: 2.6 g of **3** was obtained as a white powder.

IR (KBr, cm^{-1}): ν = 3312, 1754, 1655, 1273, 1030. SEM: average particle size, 30 μm. mp: decomposition over 300°C.

β-CD–EP (**4**)

The insoluble polymer was obtained with EP as previously described by Renard et al.[24] The polymer was a white powder.

IR (KBr, cm^{-1}): ν = 3400, 3000, 1452, 1253, 1040. SEM: average particle size, about 30 μm. mp: decomposition over 300°C.

β-CD–HDI (**5**)

A synthetic method described by Bhaskar et al.[25] was modified with a sonochemical reactor to reduce the reaction time. In our US cavitating tube, 1 g of β-CD (0.88 mmol) and 10 mL of anhydrous dimethylformamide (DMF) were placed, and then 1.2 mL of HDI (7.40 mmol) was added dropwise under sonication (30 W) at 20°C. The mixture was further sonicated for 90 min (60 W) at 50°C. The white precipitate was washed with 200 mL of MeOH and then filtered on a sintered glass Buchner funnel. The product was evaporated to dryness, yielding 1.48 g of **5**. The product was a white powder.

IR (KBr, cm^{-1}): ν = 3370, 1718, 1697, 1253, 1157, 1032. SEM: average particle size, about 100 μm. mp: decomposition over 300°C.

Propargyl-β-CD reticulated with AMB (β-CD–AMB or **6**).

6 was synthesized in four steps.

*6-O-Hepta-(*tert*-butyldimethylsilyl)–β-CD.* This was prepared as previously described.[26]

*2- and 3-O-Propargyl-6-O-hepta-(*tert*-butyldimethylsilyl)–β-CD.* In a flame-dried 100-mL, two-necked, round-bottom flask, 6-*O*-hepta-(*tert*-butyldimethylsilyl)–β-CD (5 g, 2.6 mmol) and LiH (124 mg, 15.6 mmol) were dissolved in anhydrous tetrahydrofuran/dimethyl sulfoxide (DMSO; 9 : 1; 60 mL). The mixture was magnetically stirred and heated for 2 h under reflux. After it cooled to room temperature, a solution of propargyl bromide (1.45 mL, 13 mmol) in tetrahydrofuran (3 mL) was added dropwise. The mixture was stirred for 6 h under reflux. For TLC, the eluent was $CHCl_3/CH_3OH$ (4 : 1). Finally, it was diluted with EtOAc, washed with water and brine, and dried (Na_2SO_4). The crude residue, purified by column chromatography ($CHCl_3/CH_3OH$: 19 : 1, 9 : 1, and 4 : 1), contained 2.64 g of di-, tri-, and tetra-*O*-propargylated derivatives. Their relative proportions were checked by electrospray ionization mass spectrometry analysis after cleavage of the of 6-OH protecting groups. Their relative abundance was estimated from respective peak areas (35, 50, and 15%). This product was a white powder.

IR (KBr, cm^{-1}): ν = 3420, 3325, 1473, 1254, 1086, 1040, 835. ^{1}H-NMR ($CDCl_3$) δ: 4.98 (7H, m, H-1), 4.5 (m, H-1′), 3.9 (14H, m, H-3, H-6b), 3.7–3.4 (28H, m, H-2, H-4, H-5, H-6a), 2.4 (m, H-3′), 0.88 (63H, s, *t*-But), 0.05 (42H, s, Si—CH_3). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: *m/z* calcd. for $[M + Na]^+$ 2032.0 (n = 2); 2070.0 (n = 3); 2108.0 (n = 4); found 2032.6.; 2070.4; 2108.3.

2- and 3-O-Propargyl-β-CD. To a 25-mL, two-necked, round-bottom flask equipped with a condenser and an optical-fiber thermometer, 2- and 3-*O*-propargyl-6-*O*-hepta-(*tert*-butyldimethylsilyl)–β-CD (2.64 g, 1.3 mmol), AcCl (2 mL, 2% in CH_3OH), and CH_2Cl_2 (10 mL) were added. MW irradiation of the mixture for 15 min under reflux caused the formation of a precipitate. After the addition of ether (50 mL), it was collected on a filter, washed with ether (40 mL), and dried *in vacuo*. The product (1.42 g) was obtained as a white powder (1.15 mmol, 88% yield).

IR (KBr, cm^{-1}): ν = 3420, 3325, 1658, 1159, 1082, 1032, 578. ^{1}H-NMR (D_2O) δ: 5.29 (m, H-1), 5.09–4.9 (m, H-1), 4.44 (m, H-1′), 4.08–3.84 (28 H, m, H-3, H-5, H-6a, H-6b), 3.68–3.54 (14H, m, H-2, H-4), 2.95 (m, H-3′). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: *m/z* calcd. for $[M + Na]^+$ 1233.4 (n = 2); 1271.4 (n = 3); 1309.4 (n = 4); found 1233.26; 1271.24; 1309.17.

*β-CD–AMB (**6**).* In a 25-mL, two-necked, round-bottom flask equipped with a condenser and an optical-fiber thermometer, 2- and 3-*O*-propargyl-β-CD (700 mg, 0.56 mmol) and AMB [158 mg (added in three equal portions at 10-min intervals), 0.84 mmol] were

TABLE I
Gradient Used for HPLC Analyses

	Time	Flow	A (%)	B (%)	Curve
1		1.00	95.0	5.0	
2	5.49	1.00	95.0	5.0	6
3	37.90	1.00	5.0	95.0	6
4	39.90	1.00	5.0	95.0	6
5	39.91	1.00	0.0	100.0	6
6	52.36	1.00	0.0	100.0	6

A = H_2O/TFA (0.1%); B = CH_3CN/TFA (0.1%).

dissolved in a 1 : 1 mixture of water and *t*-BuOH (7 mL each). A copper sulfate solution (1 M, 112 μL) was then added. The mixture was irradiated with MWs (200-W Constant) for 90 min, which caused the temperature to increase up to 90°C. After the reaction was completed, it was allowed to cool to room temperature and diluted with water (20 mL). The precipitate was collected by filtration and was washed with a cold solution of diethylenetriamine–pentaacetic acid sodium salt (20 mL) to remove copper and then with acetone. Product **6** (0.41 g) was obtained as a white powder.

IR (KBr, cm^{-1}): ν = 3420, 3325, 1658, 1159, 1082, 1032, 578.

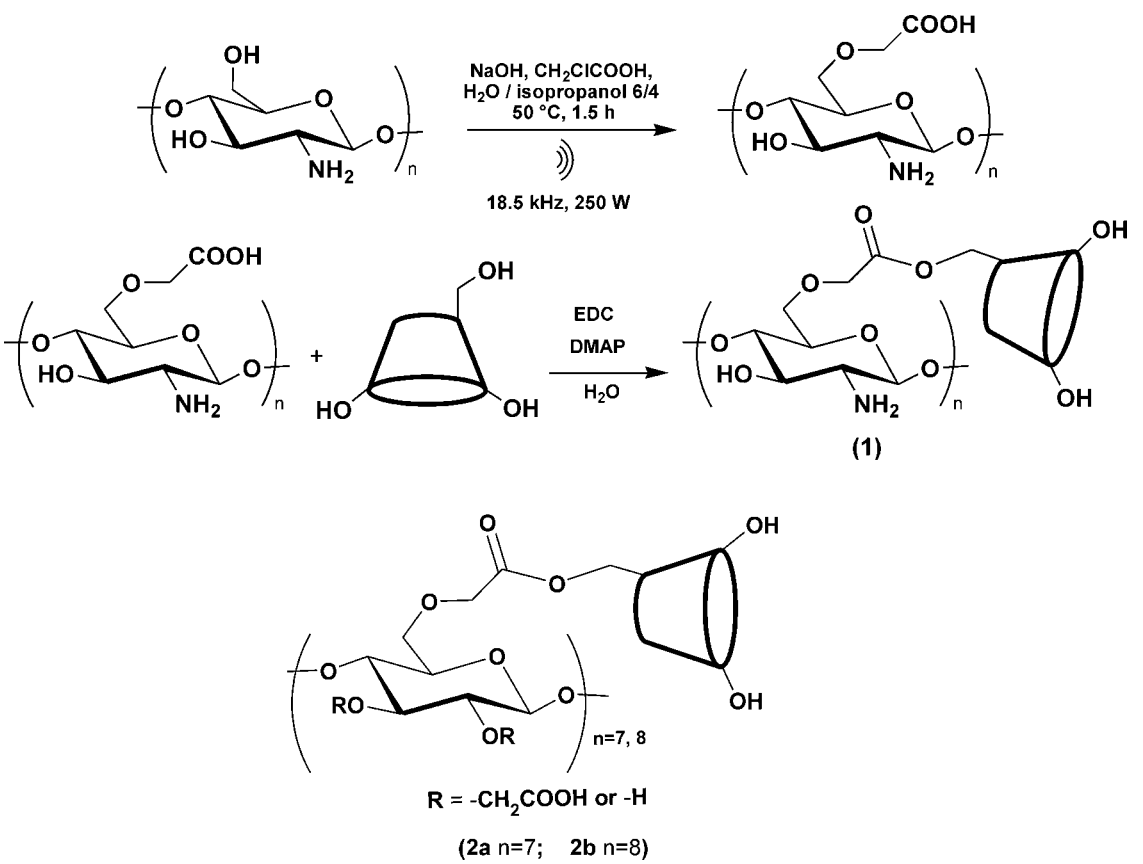
AMB

In a 25-mL, two-necked, round-bottom flask equipped with a condenser and an optical-fiber thermometer, 1,3-bis(bromomethyl)benzene (1 g, 3.79 mmol) and sodium azide (0.98 g, 15.1 mmol) were dissolved in DMF (10 mL)/H_2O (0.5 mL). The flask was irradiated for 5 min at 100 W, and this caused the temperature to increase up to 80°C. For TLC, the eluent was 9 : 1 hexane/EtOAc. The mixture was diluted with EtOAc, washed with water (3 × 30 mL) and brine, and finally dried (Na_2SO_4). AMB (0.47 g, 66%) was obtained as a yellow oil.

IR (KBr, cm^{-1}): ν = 2099, 1447, 1344, 1264, 706. ^{1}H-NMR ($CDCl_3$) δ: 7.45–7.40 (1H, m, H-5), 7.32–7.27 (3H, m, H-2,4,6), 4.38 (4H, s, H-Bz). SEM: average particle size, about 80 μm. mp: decomposition over 300°C.

Sensory evaluation

Twelve volunteers, six males and six females, 23–42 years old (mean 26.4 years), previously trained to evaluate the sensation of bitterness by comparison with standard caffeine solutions, tested 0.025% water solutions of limonin and naringin. In the first part of the experiment, dilutions of these compounds were



Scheme 1 Synthesis of CD–CM-chitosan and CD–CM-cellulose adducts.

TABLE II
Sensory Evaluation of Bitter Taste with Debittering Agents (Aqueous Solutions)

	Debittering agent [% (mean ± SD)]		
	Naringin (0.025%)	Limonin (0.025%)	Caffeine (0.05%)
β-CD–CM-chitosan (**1**)			
0.4%	1.00 ± 0.3	2.80 ± 0.2	1.20 ± 0.5
1.2%	0.00 ± 0.2	2.20 ± 0.3	1.00 ± 0.4
β-CD–CM-cellulose (**2a**)			
0.4%	2.5 ± 0.2	2.50 ± 0.2	3.20 ± 0.1
1.2%	1.80 ± 0.2	2.00 ± 0.2	2.80 ± 0.2
γ-CD–CM-cellulose (**2b**)			
0.4%	1.00 ± 0.2	2.00 ± 0.3	1.50 ± 0.3
1.2%	0.20 ± 0.2	1.80 ± 0.4	1.00 ± 0.4

prepared and tested. The panelists were asked to score bitterness on a six-point scale ranging from ''like water'' (0) to ''exceedingly bitter'' (5). When the sensation fell between two steps, the panelists were told to use half-point values. Six-milliliter aliquots to be tested were presented to the panelists in 90-mL poly(ethylene terephthalate) cups under codes that gave them no clue about the contents. The sample was not swallowed but was swirled for about 10 s inside the oral cavity, which was subsequently rinsed with low-salt mineral water. The order of presentation was from the least concentrated solution to the most concentrated one, and the interstimulus interval was 10 min. The aim of this preliminary step was to identify one limonin concentration and one naringin concentration (to be adopted for all subsequent tests) whose bitterness scores would conveniently fall in the middle of the scale, that is, near 3; the results pointed to 0.025% for both compounds.

In the second part of the experiment, samples containing bitter compounds with or without the addition of soluble CD derivatives were presented randomly, whereas a 0.025% naringin solution was available to panelists who wanted to recall its taste. Results were subjected to the one-way analysis of variance procedure (Excel, Office 2000) with a p value of 0.05. The Fisher test on variance revealed no significant differences among panelists with respect to these sensorial tests. Table I lists the results (averages of two separate runs conducted at a 1-week interval) as mean values side by side with standard deviations (SDs).

Naringin entrapping and recovery: general procedure

To a 100-mL beaker, 10 mL of H_2O was added to 1 g of polymer. Each polymer (**3–6**) was left to swell for 12 h before 16-mm columns were packed with it, and 80 mL of a 0.025% aqueous solution of naringin was poured on them and allowed to percolate. Filtered solutions from these adsorption trials were analyzed by HPLC [a Waters SunFire C18 3.5 μ 4.6 × 150 mm column; eluents A (H_2O/trifluoroacetic acid 0.1%) and B (CH_3CN/TFA 0.1%); flow rate = 1 mL/min] with a gradient as shown in Table I.

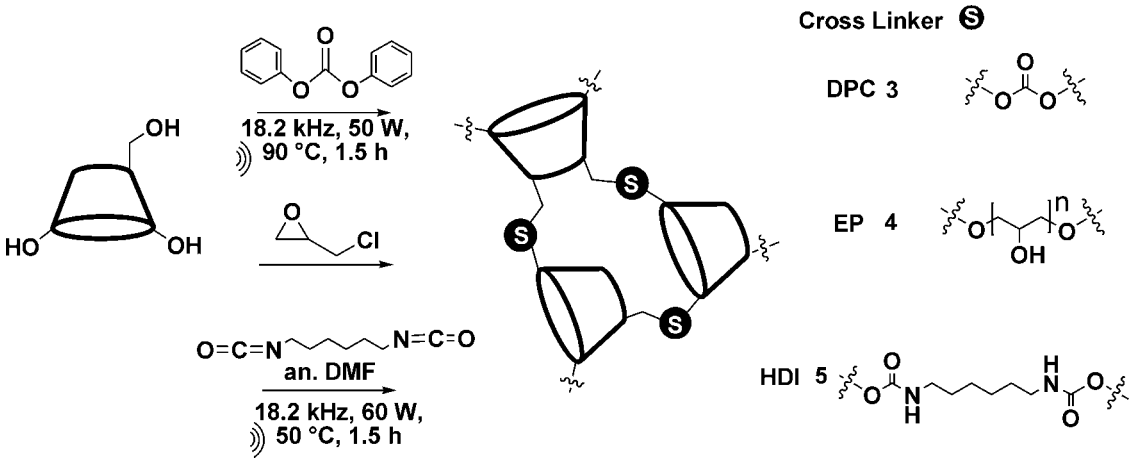
Analytical results were compared with those obtained in parallel filtrations through columns packed with Amberlite XAD-16, a polymeric adsorbent commonly used for fruit-juice upgrading.

Analysis by DSC confirmed that inclusion complexes with naringin had been formed in all cases (shown later in Figs. 6 and 7).

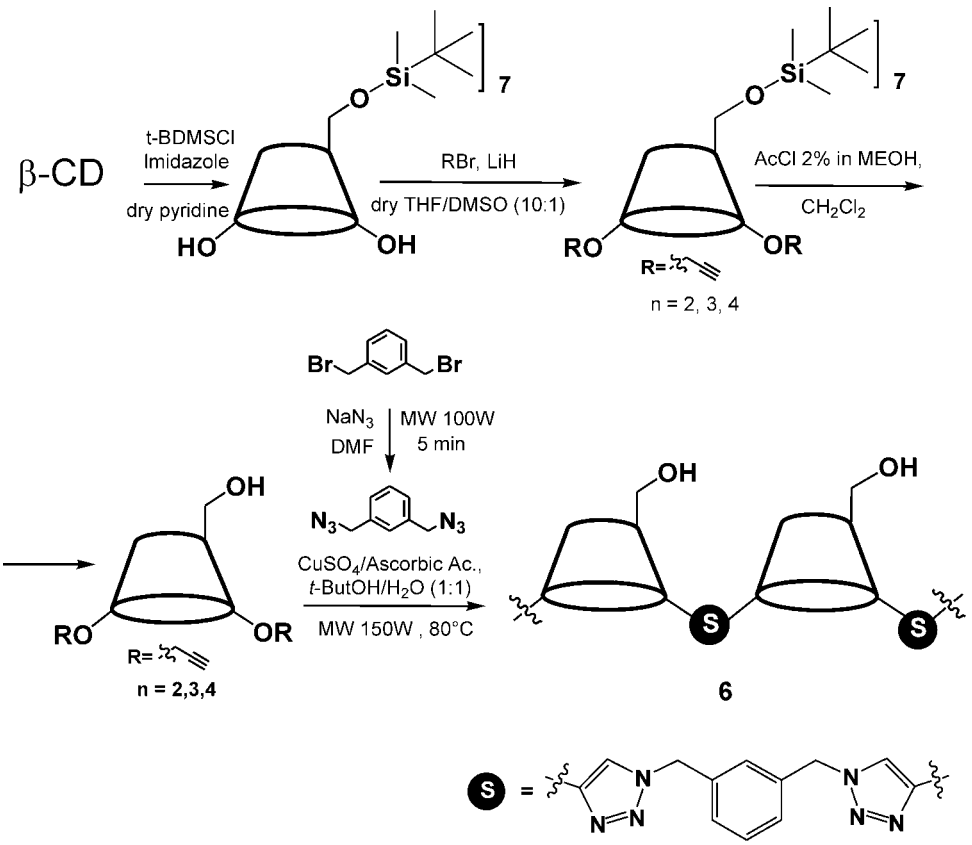
Polymers **3–6** were regenerated by a counter-current ethanol wash that also achieved an excellent recovery of naringin.

Preparation of binary mixtures

Binary mixtures of crosslinked β-CD polymers with naringin were prepared by the mixing of appropriate amounts of the solid components (10 : 1 w/w) in a glass mortar.



Scheme 2 Insoluble reticulated CD polymers.



Scheme 3 Synthesis of the β-CD–AMB polymer (crosslinked through the secondary OH groups).

RESULTS AND DISCUSSION

It is well known that CDs, as well as their derivatives and polymers, have a remarkable ability to form inclusion complexes with organic molecules through host–guest interactions. Bitter compounds are detected by human taste sensors in micromolar amounts. The sensation of bitterness is also more

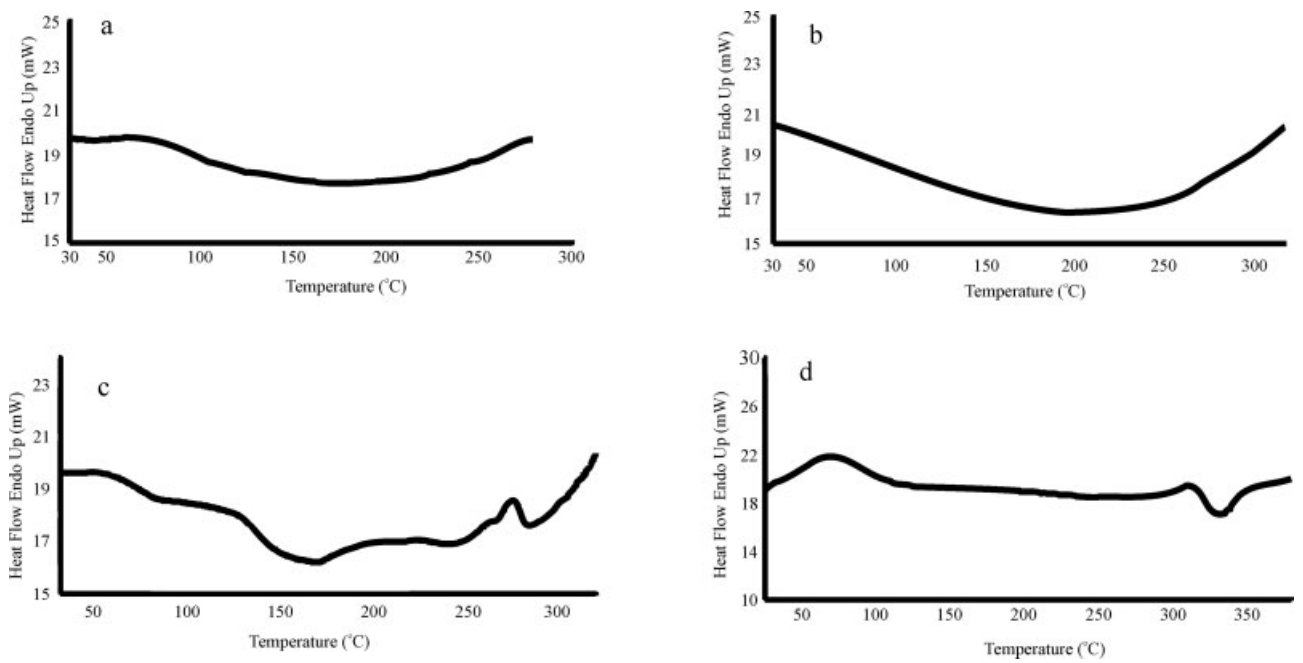


Figure 1 DSC curves of β-CD polymers: (a) β-CD–DPC (**3**), (b) β-CD–EP (**4**), (c) β-CD–HDI (**5**), and (d) β-CD–AMB (**6**).

prolonged than those caused by sweet, salty, or sour compounds.

We prepared and tested a few water-soluble, CD-grafted CM-chitosan and CM-cellulose derivatives as debittering agents (**1**, **2a**, and **2b**). CM-chitosan was prepared under sonochemical conditions, as reported in a previous article;[22] its coupling and those of CM-cellulose (Scheme 1) were obtained with a water-soluble carbodiimide.

Debittering activity was evaluated on naringin and limonin aqueous solutions (0.025%), with a standard aqueous solution of caffeine (0.05%) being used as a standard. A group of previously trained panelists were asked to score bitterness on a scale ranging from 0 (''tasting like water'') to 5 (''exceedingly bitter''). Each adduct was tested at two different concentrations (0.4 and 1.2%) for its ability to form inclusion complexes with bitter substances in an aqueous solution. Results, elaborated with the analysis of variance procedure, are shown in Table II side by side with the SDs.

CD polymerization

Four different polymers were synthesized with different crosslinkers (EP, HDI, DPC, and AMB), as shown in Scheme 2.

β-CD–DPC and β-CD–HDI were successfully synthesized under US with a probe with an immersion horn and a titanium cavitating tube, respectively. The yield of the EP polymer was not significantly affected by US irradiation because its precipitation in the form of a gel was the limiting step of the polymerization process.

The preparation of **6** required more steps (Scheme 3). First, 6-*O*-hepta-(*tert*-butyldimethylsilyl)–β-CD[25] was reacted at room temperature with excess propargyl bromide in the presence of lithium hydride to yield a mixture of di-, tri-, and tetra-*O*-propargylated derivatives. Silyl groups were cleaved off in a few minutes by an acid treatment under MWs. The mixture of propargyl derivatives was subjected to Huisgen 1,3-dipolar cycloaddition. This Cu(I)-catalyzed reaction between regiospecifically bound azides and terminal acetylenes gives 1,4-disubstituted 1,2,3-triazoles exclusively; it is simple from an experimental point of view and widely applicable. The catalyst is best prepared *in situ* by reduction of Cu(II) salts, which are less costly and often purer than CuI salts. Ascorbic acid and sodium ascorbate work very well as reducing agents. We performed both steps under MWs, cutting down the reaction times from hours to minutes.

Each polymer was ground in a mortar to yield particles of uniform diameter. Chemical structures were confirmed by IR spectra, melting points, and

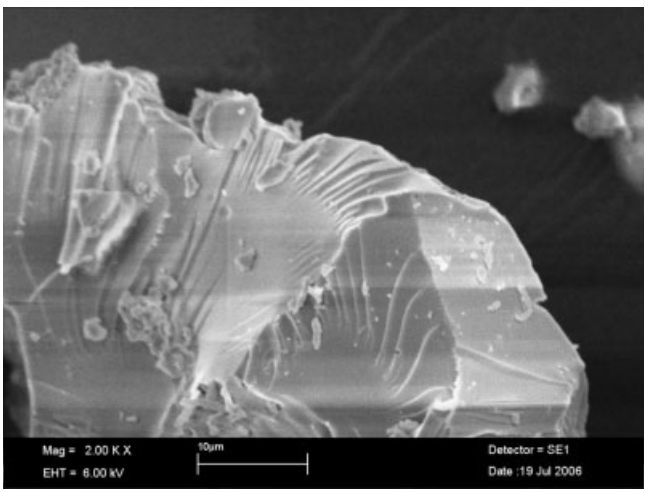


Figure 3 Surface section of the β-CD–EP polymer (**4**) examined by SEM.

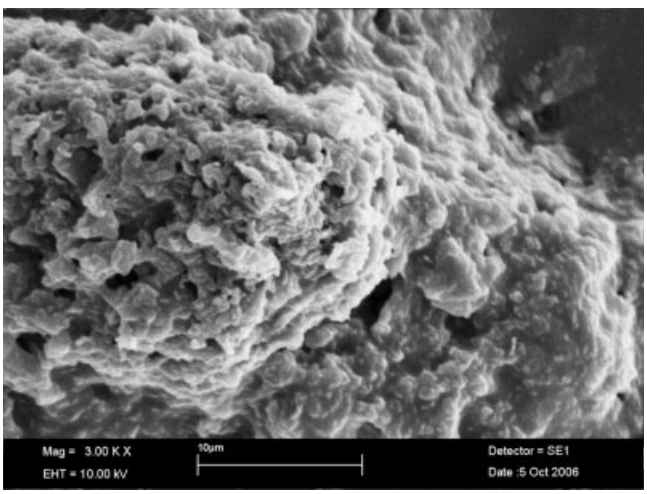


Figure 2 Surface section of the β-CD–DPC polymer (**3**) examined by SEM.

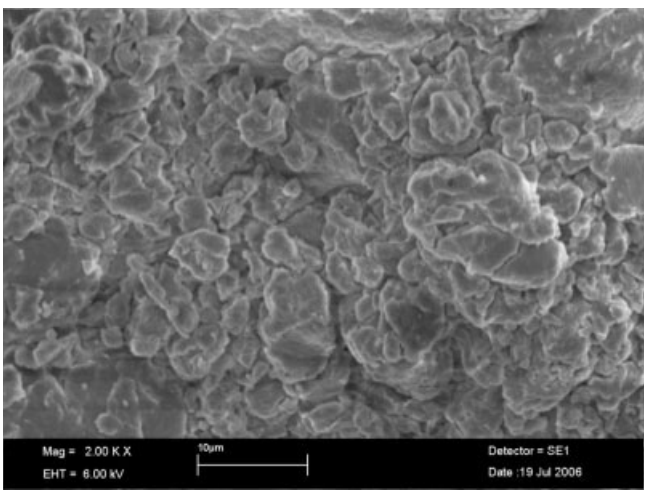


Figure 4 Surface section of the β-CD–HDI polymer (**5**) examined by SEM.

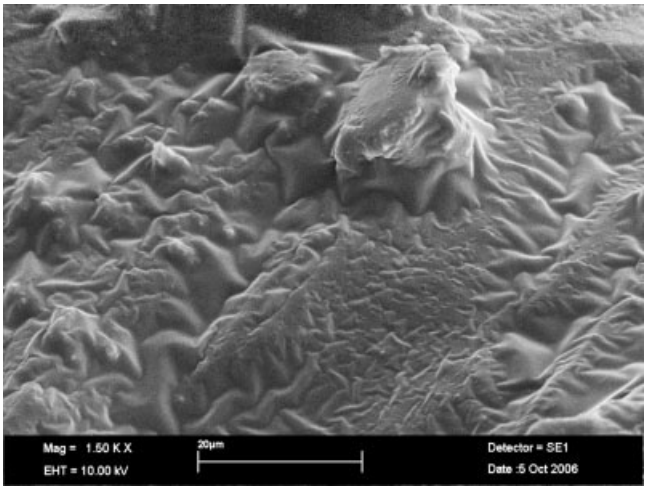


Figure 5 Surface section of the β-CD–AMB polymer (**6**) examined by SEM.

DSC. The differential scanning curves reported in Figure 1 show that no relevant thermal events took place up to 300°C for polymers **3** and **4**, whereas **6** decomposed above 300°C; they also confirmed the decomposition of **5** around 272°C.[24]

The surfaces of insoluble polymers, examined by SEM, showed spongy (**3**), lamellar (**4**), globular (**5**), and amorphous (**6**) structures (Figs. 2–5).

The four polymers were tested in fluidized bed columns for their ability to remove naringin from its aqueous solutions. These debittering tests on **3–6** were carried out as previously described in the Experimental section. Sequestered naringin was then recovered by elution with EtOH, which regenerated polymers in their original state, as judged by the reproducibility of a few successive cycles. Results obtained with the insoluble polymers (Table III) suggest that columns packed with these reticulated polymers could be used to selectively sequester bitter compounds from citrus juices. Polymer **6**, because EtOH swelled it to a viscous gel, needed a longer time to be regenerated on account of slow filtration.

Performances as naringin sequestrants of CD polymers **3–6** were actually better than the performance of commercial porous polymer beads of Amberlite XAD-16; moreover, they could be used for at least

TABLE III
Reduction of the Amount of Naringin (%) from Aqueous Solutions (0.025%) Determined by HPLC

CD–polymer	Reduction (%)
β-CD–DPC (**3**)	63
β-CD–EP (**4**)	>95
β-CD–HDI (**5**)	94
β-CD–AMB (**6**)	68
XAD-16	59

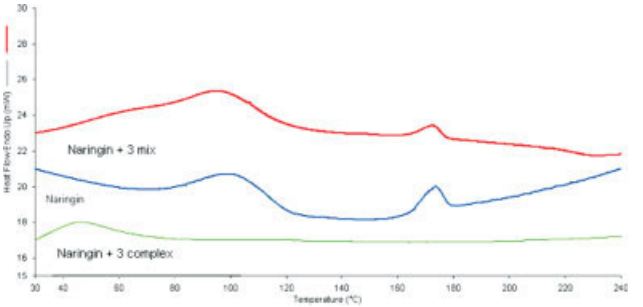


Figure 6 DSC thermograms of naringin, a naringin and β-CD–DPC (**3**) physical mixture, and a naringin and β-CD–DPC (**3**) complex. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

three entrapment/desorption cycles without appreciable loss of sequestering power.

All these crosslinked CD polymers were able to form inclusion complexes with naringin. As an example, we report in Figures 6 and 7 the thermograms of naringin and with two crosslinked CDs of polymers **3** and **6** after they had been saturated with naringin. Naringin showed two endothermic peaks, one at 83°C and one at 171°C, corresponding to the successive loss of two molecules of water (the compound crystallizes as a dihydrate). DSC thermograms of the naringin-saturated polymers did not show the DSC peaks corresponding to naringin, thus confirming its interaction with the two crosslinked CD polymers (**3** and **6**). On the contrary, the thermograms of binary mixtures prepared through grinding in a mortar did show the characteristic peaks of naringin.

CONCLUSIONS

This sensorial study compared the debittering activity of water-soluble, CD-grafted CM-chitosan and CM-cellulose derivatives. γ-CD–CM-cellulose (1.2%) gave the best results, as in its presence all tested

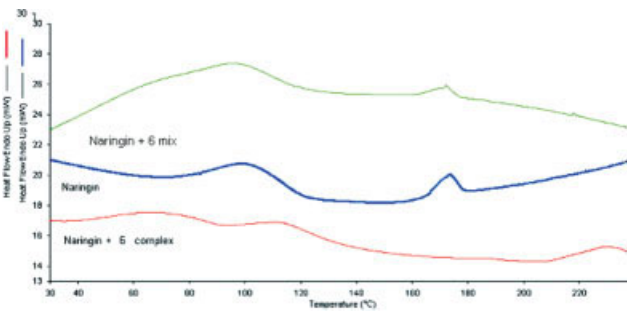


Figure 7 DSC thermograms of naringin, a naringin and β-CD–AMB (**6**) physical mixture, and a naringin and β-CD–AMB (**6**) complex. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

compounds (naringin, limonin, and caffeine) were perceived as less bitter; β-CD–CM-chitosan (1.2%) was the best debittering agent for naringin only.

Four crosslinked CD polymers prepared under nonconventional conditions (US- or MW-assisted reticulation) by the use of different crosslinkers were successfully employed to entrap naringin in a filtration column. Successively, they could be easily regenerated by a counter-current ethanol wash that also achieved an excellent recovery of the flavonoid. At least three entrapment/desorption cycles were possible without appreciable loss of sequestering power. DSC thermograms demonstrated the ability of the crosslinked CD polymers to form inclusion complexes with naringin.

The authors are indebted to the panelists and to E. Baranelli and F. Turci for their generous collaboration.

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