ORIGINAL ARTICLE

Preparation and characterization of inclusion complexes containing fixolide, a synthetic musk fragrance and cyclodextrins

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Abstract AHTN (7-Acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene), commercially known as fixolide or tonalide, is a synthetic fragrance widely used in replace of natural musk odor which is more expensive. It is a popular fragrance material added in the manufacturing of personal care and household products, such as perfumes, soaps, shampoos, detergents, and fabric softeners. AHTN is semivolatile and is degraded under light exposure and high temperature. This work focuses on the complexation of AHTN with cyclodextrins in the effort to stabilize the fragrance material. AHTN was complexed with β-cyclodextrin, methyl (MβCD), and hydroxypropyl (HPβCD) derivatives in the mole ratio 1:1, 1:2, and 1:3 guest:host, and the complexes formed by physical mixing, co-precipitation, kneading, and freeze-drying were analyzed by DSC and FTIR. Percent AHTN included in the complex was also determined by hexane extraction and GC analysis. It was found that no inclusion complex was formed in the physical mixture. When co-precipitation method was performed, only βCD could form inclusion complex with AHTN, while the other two derivatives could not. Using 1:2 AHTN:βCD, no free AHTN was left in the complex as evidenced by DSC and FTIR spectrum. In kneading and freeze-drying methods, complexes could be formed with all CDs tested. However, co-precipitation method with 1:2 AHTN:βCD and kneading method with 1:2 AHTN:MβCD provided the highest complex yield with highest amount of AHTN included in the complex. AHTN in the complex form was more stable against high temperature and UV exposure than its free form.

Keywords AHTN · Cyclodextrin · Fixolide · Fragrance · Synthetic musk

Introduction

The three major natural α-, β-, and γ-cyclodextrins (CDs) are cyclic oligosaccharides of 6–8 glucose units. Their truncated cone structure with a hydrophilic outer surface and a hydrophobic cavity can include a wide range of guest molecules via noncovalent interactions [1, 2]. This inclusion complex formation causes a change in physicochemical behavior of the guest such as solubility or stability. The complexes are thus being used in a diverse range of industrial applications.

Besides natural CDs, several derivatives have been chemically or enzymatically synthesized, most with modifications at the hydroxyl groups of the rim [3]. Examples are methylated-, hydroxypropylated-, and maltosyl-CDs with solubility better than parent compounds. As CD and CD derivatives possess different physico-chemical properties, their inclusion complexation with guest molecules could lead to different behavior of the guest.

AHTN (7-Acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene) (Fig. 1), commercially known as fixolide or tonalide, is a synthetic, polycyclic musk

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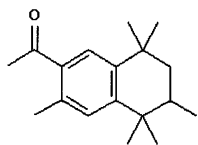University, Bangkok 10330, Thailand
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Fig. 1 Chemical structure of AHTN

fragrance widely used in the manufacturing of personal care and household products, such as perfumes, soaps, shampoos, detergents, and fabric softeners [4]. In recent years, polycyclic musks have become the most important commercial synthetic musks due in part to concern about the environment distribution and toxicological effects which lead to subsequent reduction in use of the nitro musks (e.g. musk xylene and musk ketone). In 1996, the worldwide production of polycyclic musks, 95% of which were HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-haxamethylcyclopenta-γ-2-benzopyran) and AHTN, was about 5,600 tons [5]. Although created to replace the more expensive and rare natural musks, polycyclic musks are not structurally or chemically similar to their natural counterparts. AHTN is semivolatile and is degraded under light exposure and high temperature. Its water solubility at 25 °C is only 1.25 mg/l. These unsatisfied properties give some limitations and drawbacks to its industrial use. The aim of the present study was to investigate the ability of CDs in the molecular inclusion of AHTN and in the improvement of AHTN performance. This is the first report on the complexation of AHTN with CDs.

Experimental

Materials

AHTN (product of Givaudan-Roure, Geneva, Switzerland) was a kind gift from the Thai-China Flavors and Fragrances Industry Co., Thailand. βCD, methyl-βCD (MβCD, randomly methylated with a degree of substitution (DS) of 1.7) and hydroxypropyl-βCD (HPβCD, with a DS of 0.91) were products from Ensuiko Sugar Refining Co. (Yokohama, Japan). Naphthalene and n-hexane of analytical grade were of Fluka and Fisher Scientific, respectively.

Preparation of solid complex

The complexes with three different mole ratios of AHTN:CDs (1:1, 1:2, and 1:3) were formed by different methods as followingly described. Three main

types of CDs: βCD, MβCD, and HPβCD were used as the host in complex formation.

By physical mixing

The physical mixtures of AHTN and CDs were prepared in 1:2 mole ratio. The amounts were mixed in a small plastic bag for 5 min at room temperature, then kept desiccated.

By kneading

Different amounts of AHTN and each type of βCD were mixed and grinded in a mortar for 15 min. Then 0.5 ml of ethanol:water (1:3) was gradually added and the mixture was kneaded for another 15 min until homogeneous paste was obtained. The paste was oven- dried at 40 °C for 24 h. The powder was kept desiccated.

By co-precipitation

Different amounts of CDs were dissolved in 30 ml of ethanol:water (1:3) at 55 °C, then 2 ml of AHTN in ethanol was added to give the desired mole ratio of AHTN:CD. The heater was switched off, then the mixture was magnetic stirred for 24 h. The precipitate was vacuum filtered, washed with 24 ml of ethanol:water (1:2), and dried at 40 °C for 24 h. The powder was kept desiccated.

By freeze-drying

Different amounts of CDs were dissolved in 27.5 ml of water, then 27.5 ml of AHTN in ethanol was gradually added. The mixture was magnetic stirred for 2 h before freeze-drying in a LYO-LAB Lyophilization Systems.

Analysis of solid complex

To examine complex formation, Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectrometry (FTIR) of different complexes were performed.

DSC

Approximately 3–4 mg of samples were placed in aluminium pans and heated at 10 °C/min in the temperature range of 30–250 °C. The measurements were carried out under dry nitrogen at the flow rate of 60 ml/min on a DSC 822e, Mettler Toledo. Thermal analysis apparatus.

FTIR

The spectra were recorded on Perkin Elmer Model 1760X FTIR spectrometer from KBr discs in the 400–4000 cm^{-1} region.

Extraction and determination of AHTN

AHTN was extracted from the complex using hexane and quantitatively determined by Gas chromatography [4]. One hundred milligram samples were dissolved in 30 ml water in 50 ml thick-walled screw cap tubes. *n*-Hexane (4 ml) was added together with 1 ml of naphthalene internal standard (10 mg/ml). The tubes were shaked vigorously for 1 min, then centrifuged at 2,500 rpm for 5 min. The upper phase was separated and the aqueous lower phase was re-extracted again with hexane. Two hexane extracted portions were pooled, and hexane was added to make final volume of 10 ml. GC analysis was performed on an Agilent GC apparatus, model 6890N. A 1 μl injection was analyzed using 1:40 split ratio, a OV-5 capillary column (5% Phenyl methyl siloxane, 30 m, 0.32 mm i.d., 0.25 μm film), helium (36 cm/s) as carrier gas, and a flame ionization detector. The inlet temperature was 250 °C. The oven temperature was programmed from 90 to 230 °C at flow rate of 10 °C/min, then hold for 2 min at 230 °C.

Determination of properties of the solid complex

Thermal and UV stability

For thermal stability, the complex was placed in a hot air oven at 30 °C, 50 °C, and 80 °C for 2 weeks. For UV stability, the complex was placed under the 30 w UV lamp at a distance of 12.0 inches for 24 h. At different time point, the sample was withdrawn for AHTN analysis by hexane extraction and GC measurement.

Results and discussion

The preparation of solid complex was carried out between AHTN (fixolide) and three types of βCD in the mole ratios of 1:1, 1:2, and 1:3. Physical mixing, co-precipitation, kneading, and freeze-drying were different methods employed. The complex mixtures were analyzed by DSC and FTIR to determine the methods and conditions which resulted in true complex formation. DSC represents a first choice analytical tool for an accurate physico-chemical characterization of guest-CD system in the solid state and is commonly used as a routine method for rapid preliminary qualitative investigation of thermal behavior of the single components, their physical mixtures, and the inclusion compound candidate prepared according to variety of standard procedures (e.g. co-precipitation, kneading, etc.) [6]. The DSC profiles of pure fixolide, βCD, and of the respective binary systems prepared by different methods in the melting range of the fragrance and dehydration of the carrier host (30–250 °C) are shown in Fig. 2. The thermogram of pure fixolide was typical of a crystalline anhydrous substance with a sharp endothermic peak at 57.5 °C corresponding to its melting point. βCD had no defined peak but formed broad endothermic around 121.6 °C. When thermograms of the mixtures were examined, free fixolide and
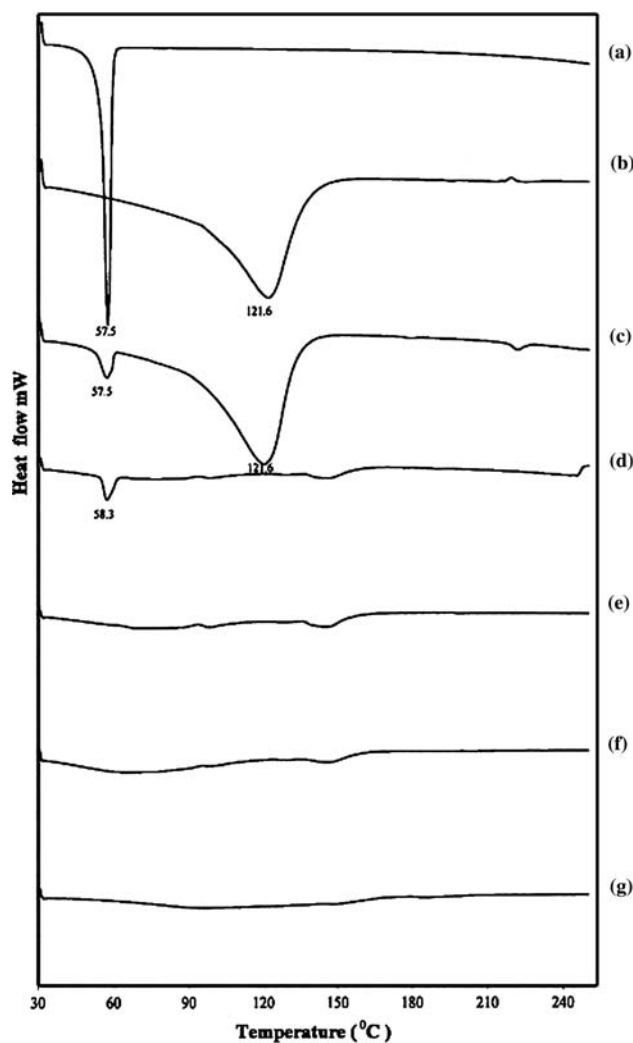

Fig. 2 DSC thermograms of (**a**) AHTN, (**b**) βCD, (**c**) 1:2 physical mixture, (**d**) 1:1 co-precipitation complex, (**e**) 1:2 co-precipitation complex, (**f**)1:2 kneading complex, and (**g**) 1:3 freeze-drying complex

βCD peaks were observed in the physical mixture 1:2 which indicates that no true complex was formed. Co-precipitation and kneading of 1:1 and freeze-drying of 1:2 mole ratios showed a small peaks of fixolide with no βCD peak which suggest the incomplete formation of the inclusion complex. The complete disappearance of fixolide and βCD peaks in the co-precipitation and kneading of 1:2 and 1:3 (not shown) and freeze-drying of 1:3 mole ratios was indicative of a strong interaction

Fig. 3 FTIR spectra of (**a**) AHTN, (**b**) βCD, (**c**) 1:2 physical mixture, (**d**) 1:2 co-precipitation complex, (**e**) 1:2 kneading complex, and (**f**) 1:3 freeze-drying complex

in the solid state which was likely attributed by an inclusion complex formation [7].

DSC profiles of AHTN:MβCD and AHTN:HPβCD of the three mole ratios prepared by different methods were also examined (data not shown). In co-precipitation, no precipitation was formed when MβCD and HPβCD were used in replace of βCD which should be due to high solubility of MβCD and HPβCD [3]. The result of DSC showed that inclusion complexes were formed between fixolide and MβCD when complexes were prepared by kneading and freeze-drying in 1:2 and 1:3 mole ratios. While with HPβCD, complete inclusion complexes were formed with kneading and freeze-drying in a 1:3 mole ratio.

Complex formation was also confirmed by FTIR. The major peak at 1683 cm^{-1} of the C=O stretching of carbonyl group was the important characteristic of AHTN (Fig. 3). βCD spectrum showed the significant OH bonding at 1645 cm^{-1}. The examples of AHTN:βCD are shown in Fig. 3. It should also be observed that the physical mixture of 1:2 AHTN:βCD still possessed the C=O peak of free AHTN. While no characteristic peak of AHTN was observed in all the complexes formed as earlier determined by DSC technique. These results pointed out that there was a change in electronic environment of AHTN when inclusion complex was formed.

To determine which method of complex formation and which type of CD were the most appropriate, the complex yield and the amount of AHTN included in the complexes which showed complete complex formation as determined by DSC and FTIR were compared (Table 1). The amount of AHTN included was determined by solvent extraction of the complex by
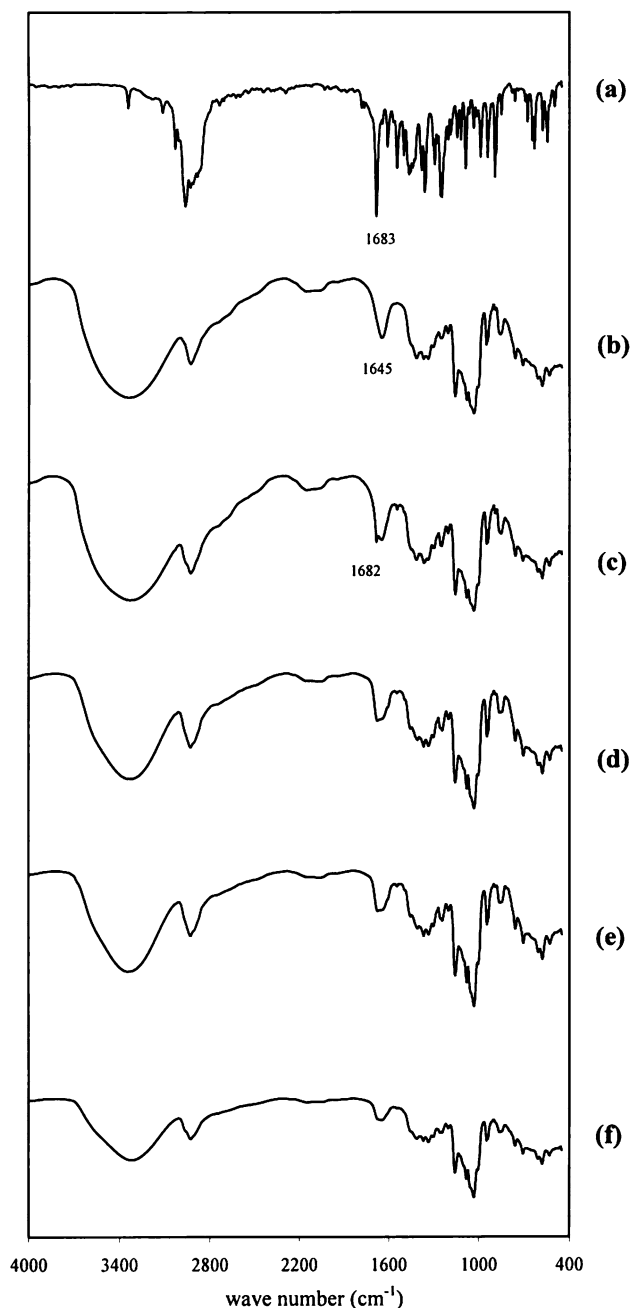
Table 1 Complex yield and AHTN included in CDs by different methods of complex formation

Method	AHTN:βCD	Complex yield (%)	AHTN included (mg/g complex)[b]
Co-precipitation[a]	1:2 βCD	73.63	96.93 ± 1.35
	1:3 βCD	67.86	62.66 ± 3.15
Kneading	1:2 βCD	75.43	59.62 ± 4.34
	1:2 MβCD	83.53	81.72 ± 2.71
	1:3 βCD	68.17	37.48 ± 4.37
	1:3 MβCD	76.35	56.36 ± 2.43
	1:3 HPβCD	71.59	53.14 ± 0.47
Freeze-drying	1:3 βCD	93.51	25.48 ± 1.24
	1:3 MβCD	84.75	58.42 ± 2.27
	1:3 HPβCD	70.99	35.36 ± 0.71

Complex yield (%) =

$$\frac{\text{total weight of dried complex}}{\text{weight of CD} + \text{weight of AHTN added}} \times 100$$

[a] no precipitate was obtained with MβCD and HPβCD by co-precipitation method

[b] all values were averaged from three extraction experiments
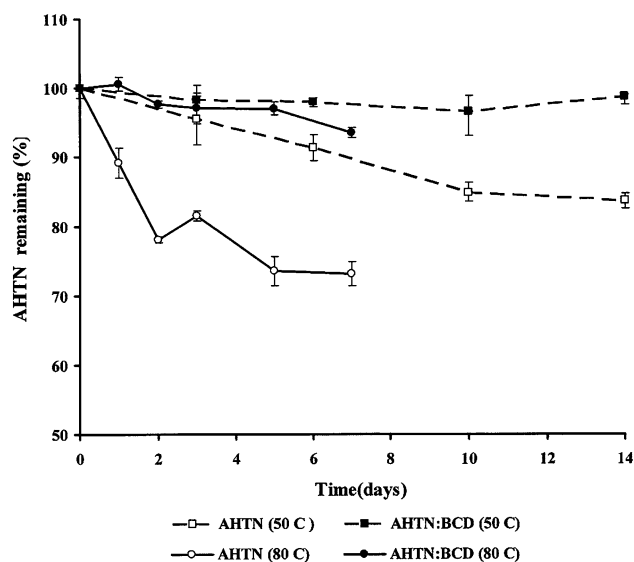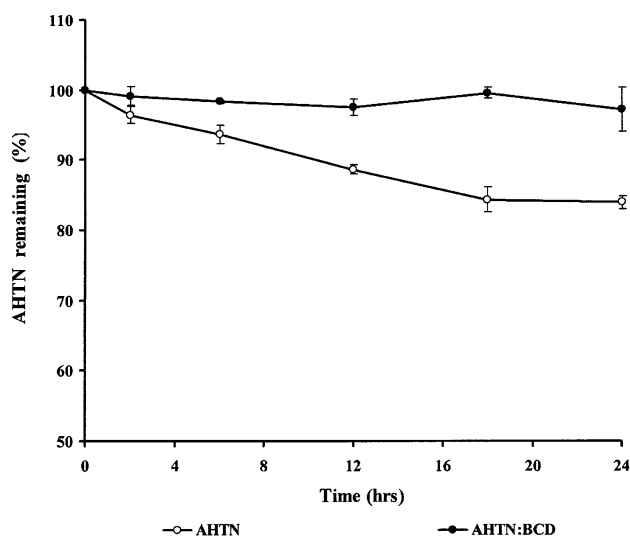
Fig. 4 Thermal stability test at 50 °C and 80 °C

Fig 5 UV stability test

n-hexane and quantified by GC [4]. From the result, it can be concluded that in the 1:2 mole ratio, AHTN:βCD complex prepared by co-precipitation was the most effective. And though 1:2 AHTN:MβCD prepared by kneading was nearly as good, the cost of MβCD is higher than βCD. It is possible that the polycyclic nature of AHTN structure fits well with the 1:2 mole ratio. Hara et al. [8] had demonstrated that the 1:1–1:3 mole ratios of different types of CD were appropriate for shorter to longer alkyl chain length unsaturated aldehyde odors. For aromatic guests smaller than AHTN e.g. menthol,

vanillin, carbaryl, guest:host ratio of 1:1 or 2:1 might be better [9, 10].

The complex 1:2 AHTN:βCD prepared by co-precipitation was tested for thermal and UV stabilities. It was found that AHTN in the complex form was more stable at 50 °C and 80 °C (Fig. 4) than its free form. At 50 °C when incubated for 14 days, the loss of the fragrance was 17% and 2% from the free and inclusion complex form, respectively. While stability at 30 °C was not significantly different for 14 days. For UV stability, AHTN in the complex form was about 10% more stable when exposed for 24 h (Fig. 5).

Conclusion

Cyclodextrins could form inclusion complex with AHTN. The best condition of complex formation was 1:2 mole ratio of AHTN:βCD prepared by co-precipitation. The complex yield and amount of AHTN included in the complex were analyzed. AHTN in the complex form was more stable to high temperature and UV exposure than in the free form.

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