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ENHANCED AQUEOUS SOLUBILITY OF PHENOLIC ANTIOXIDANTS USING MODIFIED β-CYCLODEXTRINS

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ABSTRACT

Phenolic antioxidants are useful additives with a possible role in cancer chemoprevention. This study describes inclusion complexation between phenolic antioxidants (butylated hydroxyanisole, BHA; butylated hydroxytoluene, BHT) and hydroxypropyl-ß-cyclodextrins (HPB) or hydroxyethyl-β-cyclodextrin (HEB) and their characterization by phase solubility analysis, X-ray diffraction and infra-red (IR) spectroscopy. The complexes were prepared by shaking an aqueous mixture of the antioxidant with each of the cyclodextrins (1:1 molar) at 40 °C for six days and lyophilizing the resulting clear solution. Each of the complexes dissolved instantaneously in water. Phase solubility analysis indicated a more pronounced increase in the aqueous solubility of BHA compared to that of BHT. X-ray diffraction patterns of the antioxidant-cyclodextrin complexes indicated a shift from crystalline pattern of the antioxidant to an amorphous pattern for the complexes. Also, the IR spectra of the BHA-cyclodextrin complexes indicated an almost complete disappearance or at least a shift in the -C-O-C- stretch (1200 cm^{-1}) compared to the corresponding stretch observed for BHA alone or a physical mixture (1:1) of BHA and each of the cyclodextrins. Furthermore, the sharp -OH absorption (3600 cm^{-1}) is retained in a physical mixture of BHT with either cyclodextrin (1:1) whereas this stretch is not observed in the IR spectra of either BHT-cyclodextrin complexes. These evidences indicate the formation of an inclusion complex between the antioxidants and each of the cyclodextrins.

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INTRODUCTION

Phenolic antioxidants (butylated hydroxyanisole, BHA; butylated hydroxytoluene, BHT) are common additives in food, cosmetics, and pharmaceuticals and have been investigated as possible cancer chemopreventive agents (1,2). It has been hypothesized that these agents function via induction of enzymes that protect against chemical carcinogenesis (3) or may act as highly efficient scavengers of free radicals thereby inhibiting lipid peroxidation (4). These agents exhibit low aqueous solubilities and in vivo studies investigating the enzyme inducing actions have usually relied on oral dietary administration of these antioxidants (2,5,15). Furthermore, studies on disposition of BHA and BHT have relied on intravenous (in PEG-Saline solution), oral administration, or intraperitoneal injection (6-8,16).

Cyclodextrins are cyclic oligomaltoses which have been shown to improve the solubility, dissolution rate, and solution stability of a number of drugs via inclusion complexation (9). Chemically modified cyclodextrins overcome the inherent low solubility characteristics of the natural cyclodextrins. Numerous reports have recently appeared evaluating hydroxyalkyl-ß-cyclodextrin as an additive in various formulations (10,11). An increase in the solubility of BHT to 3 mg/ml using hydroxypropyl-ß-cyclodextrin has been reported (12).

This study evaluates the increase in solubility of BHA and BHT by inclusion complexation with hydroxypropyl-ß-cyclodextrin (HPB) or hydroxyethyl-ß-cyclodextrin (HEB).

MATERIALS AND METHODS

CHEMICALS

BHA and BHT were obtained from Sigma Chemical Co. (St. Loius, MO). HPB and HEB were provided by Pharmatec, Inc. (Alachua, FL) with approximately 8% substitution in each case. IR-grade potassium bromide (KBr) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Double distilled water was used throughout the study.

PREPARATION OF THE COMPLEXES

The antioxidant-cyclodextrin complexes were prepared by mixing aqueous solutions of antioxidant and cyclodextrin (1:1 molar) at 40 °C for six days and the resulting solution lyophilized to yield a powdery mass which easily disintegrated upon gentle prodding with a spatula. BHT-cyclodextrin mixtures did not give clear solution at the end of six days and the supernatant was lyophilized to obtain the BHT-cyclodextrin complexes.

PHASE SOLUBILITY STUDIES

Phase solubility studies were carried out according to the method of Higuchi and Connors (13). Excess BHA or BHT was added to aqueous solutions containing various concentrations of cyclodextrins and gently shaken at 37 ± 1 °C in dark for six days. The clear supernatant was separated, diluted, and analyzed by UV spectrometry (Perkin-Elmer Lambda 3B) (BHA 290 nm; BHT 280 nm). The association constants for the inclusion complexes (K) were calculated from plots of antioxidant concentrations in supernatant versus cyclodextrin concentration, assuming 1:1 complexation, using the following formula:

$$K = \frac{\text{Slope}}{(\text{Intercept})(1\text{-Slope})}$$

X-RAY DIFFRACTION STUDIES

The X-ray diffraction patterns of the powder were obtained on a GE diffractometer with Cu-Kα radiation at 1.56 Å. The diffraction pattern were recorded on a strip chart recorder.

INFRA-RED SPECTROSCOPY

IR spectra were obtained on a Perkin-Elmer spectrometer with a pellet prepared by dispersing the sample in KBr (1:120) and compressing the mixture with a pressure of 10 tons for 2 min.

RESULTS AND DISCUSSION

Preliminary attempts to determine the solubility of the complexes involved gentle agitation of tubes containing BHA and HPB (1:1 molar) mixtures in water at 40 °C for several days in dark. It was found that the tubes containing BHA and

HPB mixtures gave a clear solution after six days whereas BHA alone did not completely dissolve in water. Hence, the BHA-cyclodextrin complexes were prepared by mixing 1:1 molar amounts of antioxidant and the cyclodextrin in water at 40 °C for six days. The clear solution was lyophilized and the resulting mass upon gentle prodding with a spatula gave a free flowing complex, which dissolved instantaneously in water. The BHT-cyclodextrin complexes were made in a similar manner except that some BHT remained undissolved at the end of six days. Therefore, the clear supernatant was decanted and lyophilized.

Phase solubility studies for either antioxidant (BHA or BHT) with HPB or HEB showed a linear increase in solubility with increasing concentration of cyclodextrins in 0 - 2mM cyclodextrin concentration range (Fig. 1). Such linear increases in solubilities have been categorized as type A_L curves (13). It is important to note that the antioxidants alone are sparingly water soluble and their solubilities increase significantly in the presence of either cyclodextrins (Table I).

The X-ray diffraction profiles of the BHA-cyclodextrin complexes (BHA-HPB and BHA-HEB) and BHT-cyclodextrin complexes (BHT-HPB and BHT-HEB) are presented in Fig. 2 and Fig. 3, respectively. The diffraction patterns of the complexes indicated a fundamental alteration in the physical characteristics of BHA and BHT from crystalline to amorphous nature, suggesting inclusion complex formation.

The information from IR spectra was interpreted using a reference text by Silverstein et al. (14). IR spectroscopy of BHA-HPB and BHA-HEB complexes indicated an almost complete disappearance or at least a shift in the -C-O-C- stretch (1200 cm^{-1}) and a significant attenuation of the methyl and aromatic -C-H stretchs between 3000 and 2800 cm^{-1}, over the corresponding stretches observed for BHA alone or a physical mixture (1:1) of BHA and cyclodextrins (Fig. 4a). This is consistent with X-ray diffraction data and strongly supports the encapsulation of BHA by the cyclodextrins.

Likewise, the IR spectra of both BHT-cyclodextrin complexes showed significant reduction in their aromatic -C-H stretches (near 2900 cm^{-1}) and the complete absence of absorption bands for out-of-plane bends of ring C-H bonds (near

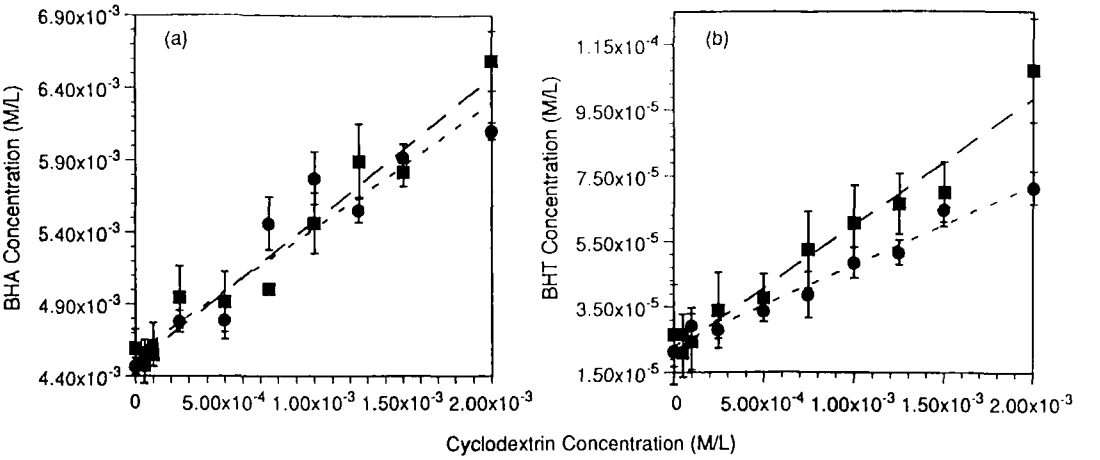

Fig. 1. (a) Phase Solubility Curves of BHA with HPB (■; r^2=0.9579, $p < 0.001$) and HEB (●; r^2=0.9176, $p < 0.001$). (b) Phase Solubility Curves of BHT with HPB (■; r^2=0.9653, $p < 0.001$) and HEB (●; r^2=0.9763, $p < 0.001$). (Each point represents the average and standard deviation of four experiments).

Table I

Summary of the Slopes and Association Constants from Phase Solubility Studies

Complex	Slope	Intercept	Association Constant (K, lit/mol) (x 10^3)
BHA-HPB	0.9932±0.0735[a]	4.52 x 10^{-3}	32.30
BHA-HEB	0.8702±0.0926[a]	4.56 x 10^{-3}	1.47
BHT-HPB	0.0386±0.0026[b]	2.15 x 10^{-5}	1.87
BHT-HEB	0.0245±0.0014[b]	2.33 x 10^{-5}	1.08

[a] $p>0.05$ (No significant difference), [b] $p>0.05$ (No significant difference)

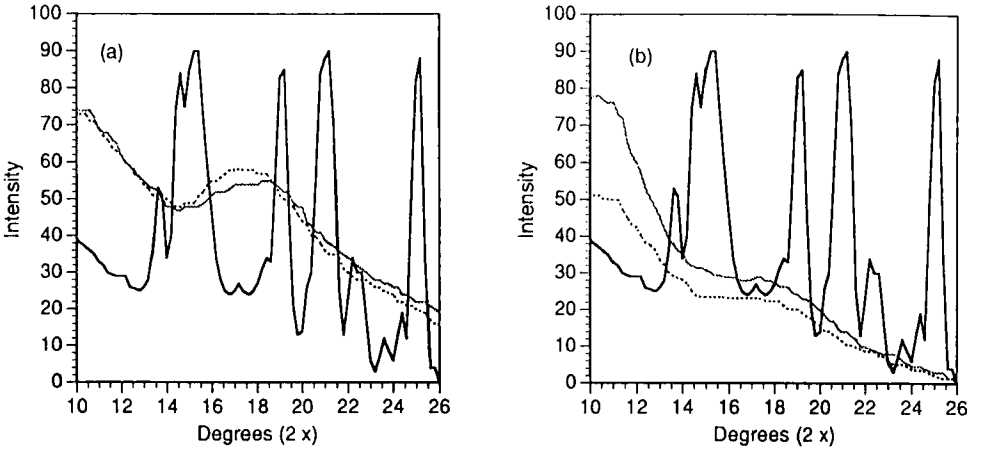

Fig. 2. (a) X-ray diffraction patterns of BHA (——), HPB (----), and BHA-HPB complex (•••••). (b) X-ray diffraction patterns of BHA (——), HEB (----), and BHA-HEB complex (•••••).

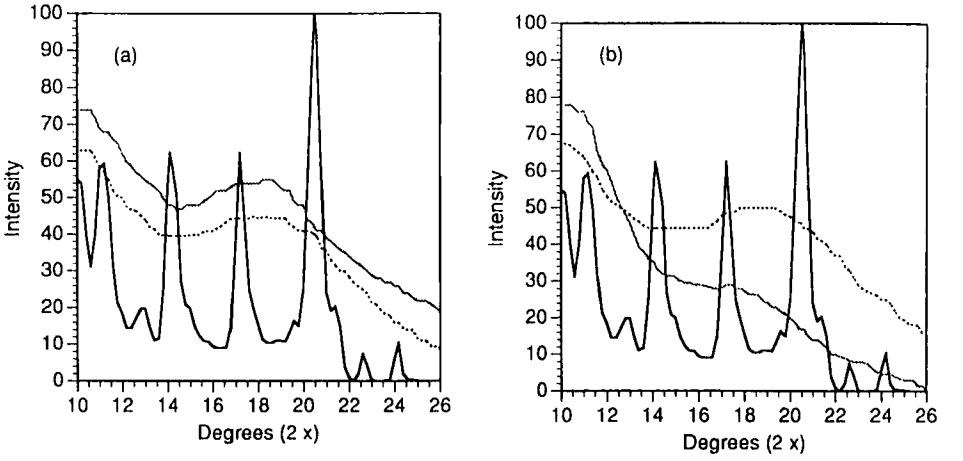

Fig. 3. (a) X-ray diffraction patterns of BHT (——), HPB (----), and BHT-HPB complex (•••••). (b) X-ray diffraction patterns of BHT (——), HEB (----), and BHT-HEB complex (•••••).

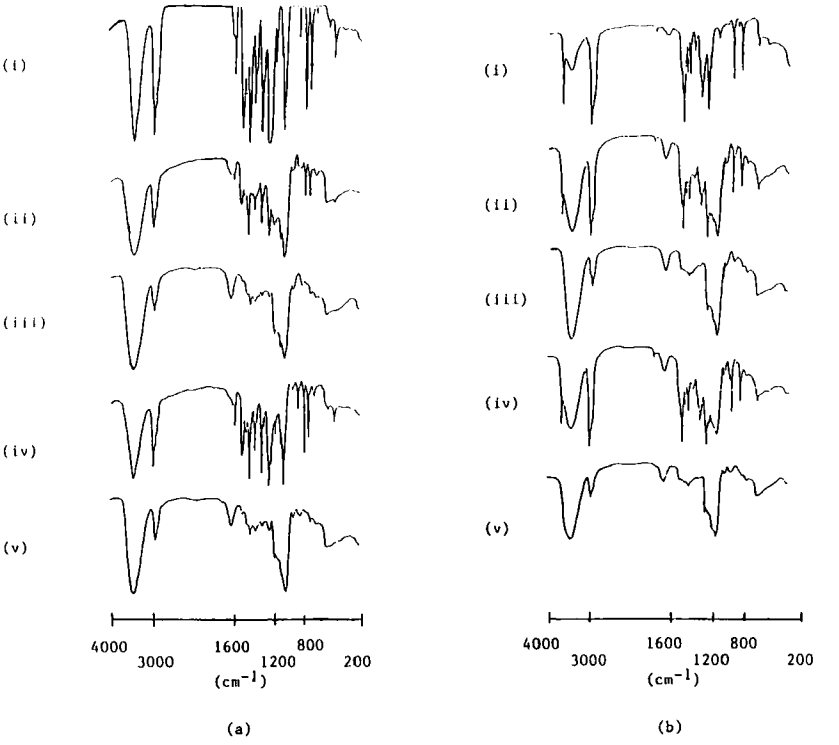

Fig. 4. (a) Infra-Red Spectra of (i) BHA; (ii) Physical mixture of BHA and HPB; (iii) BHA-HPB Complex; (iv) Physical mixture of BHA and HEB; (v) BHA-HEB Complex. (b) Infra-Red Spectra of (i) BHT; (ii) Physical mixture of BHT and HPB; (iii) BHT-HPB Complex; (iv) Physical mixture of BHT and HEB; (v) BHT-HEB Complex.

850 and 760 cm^{-1}) (Fig. 4b). Furthermore, the sharp -OH absorption (~3600 cm^{-1}) is retained in a physical mixture of BHT with either cyclodextrins (1:1) whereas this stretch is not apparent in the IR spectra of either BHT-cyclodextrin complexes. This again suggests the formation of the inclusion complex formation between BHT and the two hydroxyalkyl-ß-cyclodextrins.

The significant increase in aqueous solubilities, the complete transformation of the crystalline BHA (or BHT) to its amorphous state and the notable changes in the IR spectra of the antioxidants are strongly indicative of the formation of complexes with the modified ß-cyclodextrins. These complexes, after isolation,

dissolve instantaneously in water, thereby displaying favorable formulation characteristics; a property not exhibited by freeze dried BHA or BHT alone. These complexes have practical utility in formulating liquid and semisolid dosage forms containing oxidizable drugs/excipients. It is worth noting that the antioxidant activity is not likely to dminish by complexation since the inclusion process is a reversible phenomenon. The antioxidant would then expected to dissociate in relatively large volume of water (9).

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