Enhancement of Plant Essential Oils' Aqueous Solubility and Stability Using Alpha and Beta Cyclodextrin

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Sodium benzoate has been shown to produce benzene in combination with ascorbic acid. This has led to research for safe alternatives from plant essential oils and parabens that have shown some antimicrobial activity, but many of these compounds exhibit poor solubility in aqueous solutions. Cyclodextrins can increase the solubility of many compounds. This work aimed to investigate the solubility of 23 plant essential oils and 4 parabens in water and an apple juice medium. Four of these compounds were chosen for their low aqueous solubility to determine if complexing the compound with α- and β-cyclodextrin would increase solubility. Three of the complexes were dissolved in an acidified aqueous solution and then studied in glass and polyethylene terephthalate (PET) to determine if storage material would affect the stability. Solubility of the 27 compounds in distilled water ranged from 1.6 mg/L to 2460.6 mg/L and the solubility of 18 of the compounds decreased from 2.5 to 84.7% in apple juice medium (pH = 3.4, 12−13 °Brix). Complexation with cyclodextrin dramatically increased the solubility of the compounds, up to 10-fold. Packaging material had no effect on concentration of compounds present over 7 days. Cyclodextrins were able to increase solubility of these compounds to more suitable concentrations, and may lead to viable natural alternatives to sodium benzoate.

KEYWORDS: Antimicrobials; cyclodextrin; complex; phase solubility; essential oils; parabens

INTRODUCTION

In November 2005, the Food and Drug Administration (FDA) received a small study from a laboratory indicating that low levels of benzene were present in some soft drinks that contained benzoate salts and ascorbic acid (*1*). Benzene, a known human carcinogen and neurotoxin, was being produced by sodium benzoate reacting with ascorbic acid to produce free benzene (*2*). Although the FDA has no standards for allowed levels of benzene, they have adopted the standards that the U.S. Environmental Protection Agency (EPA) holds for drinking water, which dictates that the maximum contaminant level (MCL) should be below 5 μg/L. The study's results were posted in April 2006 reporting that, out of the 100 beverages tested, four soft drinks and one fruit drink contained benzene in levels above 5 ppb in aqueous solution (*1*). In 2007, a second study by the same researchers found levels ranging from not-detectable to 88.9 ppb (*1*).

Sodium benzoate, the sodium salt of benzoic acid, is present as protonated benzoic acid below its pK_a (4.19) in aqueous environments. A wide variety of foods and beverages use sodium benzoate as an antimicrobial additive. Due to its broad availability and low cost, it is used in many different types of foods, including carbonated and still beverages. The usage level ranges from 0.05% to 0.10% (*3*).

There is interest from beverage companies to identify one or more natural preservatives suitable for replacing sodium benzoate in beverages. Unfortunately, many of the candidate compounds have low aqueous solubility. The formation of inclusion complexes has important effects on the physicochemical properties of host molecules. Some beneficial changes induced by complexation include the following: alteration of guest's solubility, stabilization against effects of light, heat, and oxidation, reduction of guest's physiological responses, and reduction of volatility (*4*). The most common industrial use of inclusion complex formation with cyclodextrins (CDs) is to increase solubility of functional ingredients. Generally, the lower the water solubility of a compound, the greater the relative solubility increases gained by complexation (*5*). Inclusion complex formation may also stabilize compounds. Once the cavity is occupied by a molecule, other reactive molecules are excluded from occupying the cavity at the same time, preventing interaction and reaction. In addition, steric hindrance prevents the interactions with exposed portions of the guest molecule (*4*). Furthermore, complexation can reduce the rate of photodegradation of some light sensitive compounds (*6*).

Wacker Biochem submitted to the U.S. Food and Drug Administration (FDA) an independent Generally Recognized As Safe (GRAS) determination for β-CD as a flavor carrier or protectant. The FDA did not question the self-affirmed GRAS status and assigned it the GRAS Notice No. GRN 000074 in October 2001 (*7*). Wacker Biochem also submitted to the FDA an

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Table 1. Solubility of Test Compounds in Water and Apple Juice Media (AJM) ($n = 3$)[a]

| | solubility (mg/L) | | |
test compound	aqueous	AJM[b]	solubility change (%)
nonanoic lactone	1.6 ± 0.8	2.7 ± 0.7	38.8
β-pinene	4.5 ± 1.0	c	c
benzyl cinnamate	14.1 ± 4.5	2.2 ± 0.2	−84.7
limonene	33.3 ± 5.4	c	c
cyclohexanebutyric acid	43.9 ± 0.3	41.4 ± 11.3	−5.8
methyl nonanoate	48.5 ± 18.2	31.3 ± 1.1	−35.6
benzyl paraben	49.9 ± 14.9	39.2 ± 8.2	−21.4
propyl benzoate	53.9 ± 3.8	116.6 ± 13.9	53.8
trans,trans-2,4-decadienal	55.7 ± 1.0	54.3 ± 0.8	−2.5
perillaldehyde	134.6 ± 4.4	113.7 ± 5.0	−15.6
butyl paraben	147.1 ± 10.5	148.1 ± 12.1	0.7
undecalactone	173.9 ± 45.0	153.7 ± 21.9	−11.6
eugenol methyl ester	282.9 ± 46.1	c	c
citronellol	294.1 ± 83.7	298.1 ± 38.5	1.3
citral	362.6 ± 7.8	386.5 ± 230.2	6.2
methyl *trans*-cinnamaldehyde	388.8 ± 46.5	332.4 ± 61.0	−14.5
propyl paraben	426.0 ± 39.7	367.5 ± 30.4	−13.7
undecanal	566.0 ± 193.5	575.9 ± 245.4	1.7
cinnamic acid	573.1 ± 40.3	414.5 ± 17.7	−27.7
cinnamaldehyde	589.9 ± 49.1	406.8 ± 92.5	−31.1
o-methoxycinnamaldehyde	602.9 ± 2.7	498.3 ± 72.1	−17.4
decanal	640.5 ± 82.5	c	c
pulegone	808.7 ± 130.1	670.8 ± 15.4	−17.1
ethyl paraben	940.9 ± 280.6	758.1 ± 101.1	−19.4
thymol	1097.0 ± 117.7	1119.8 ± 94.4	2.0
eugenol	1282.2 ± 241.9	1211.3 ± 289.5	−5.5
sorbic acid	1514.5 ± 179.9	1274.6 ± 195.8	−15.8
methyl paraben	2460.6 ± 65.1	1992.8 ± 206.1	−19.0

[a] Solubility was determined by shaking an excess of test compound in water or AJM for 24 h at 25 °C and 250 rpm, filtering with 0.45 μm syringe filters, and measuring absorbance of the solution. Standard curves were prepared for all compounds in the appropriate media. Values are mean ± SE. [b] AJM: 10% apple juice solution between 12 and 13 °Brix, acidified with malic acid to pH of 3.4. [c] Components of the AJM interfered with the compounds' maximum absorbance wavelength.

independent GRAS determination for α-CD as a carrier or stabilizer for flavors (flavor adjuvant), colors, vitamins and fatty acids, and to improve mouth-feel in beverages. The FDA also did not question the self-affirmed GRAS status and assigned it the GRAS Notice No. GRN 000155 in June 2004 (*8*).

To identify a natural compound that may be an effective benzoate replacement, 28 compounds were evaluated to determine their aqueous solubility. In addition, the compounds' solubility in an apple juice medium (AJM) was also determined. Twenty-three of the compounds evaluated are components of plant essential oils (**Table 1**). Essential oils (EOs) are aromatic oily liquids obtained from plants and plant products such as fruits, seeds, and leaves. EOs have exhibited antiviral, antibacterial, antimycotic, antitoxigenic, antiparasitic, and insecticidal properties (*9*, *10*). The remaining five compounds included in this study are a group of alkyl esters of *p*-hydroxybenzoic acid, commonly referred to as parabens (**Table 1**). Methyl paraben and propyl paraben are directly added to commercial food systems as antimicrobial agents (*11*). Parabens, although not typically thought of as natural, have been identified from several natural sources including flowers and bacteria (*12*−*15*). The interest in these compounds in the development of a beverage preservative arises because some are already in commercial use as food preservatives.

Furthermore, the formation of α- and β-CD complexes with four of the selected test compounds (*o*-methoxycinnamaldehyde, *trans,trans*-2,4-decadienal, cinnamic acid, and citronellol) through phase solubility analyses was investigated to determine if the compounds' complexation with cyclodextrins improves aqueous solubility and to determine the maximum amount of guest compound that can be complexed. Solid complexes were

formed to obtain a physical complex and to characterize the weight percent attributable to the test compounds. Finally, a study was carried out to compare the storage stability of three α-CD complexes (*o*-methoxycinnamaldehyde, *trans,trans*-2,4-decadienal, and citronellol) in aqueous solutions, stored in glass and polyethylene terephthalate (PET) containers.

The purpose of this experiment was to test the solubility of a wide range of potential antimicrobial compounds in both water and an apple juice medium, test the ability of cyclodextrins to increase this solubility, and test the effect of packaging material on concentration of the complexes over time in solution.

MATERIALS AND METHODS

Preparation of Apple Juice Media. The AJM consisted of 100 mL of apple juice (100% Pure Apple Juice, Motts, Rye Brook, NY, USA), 46.8 g of α-D-glucose (Acros Organics, Geel, Belgium), 59.4 g of fructose (Fisher Scientific, Fair Lawn, NJ, USA), and 1.8 g of sucrose (Sigma, St. Louis, MO, USA) in a 1 L beaker. The mixture was then brought to 1 L with distilled water. The mixture was stirred at room temperature until all ingredients were dissolved. The pH of the mixture was adjusted to 3.4 with 1 M malic acid (food grade, Sigma). The Brix of the mixture was verified to be between 12 and 13 °Brix (Reichert Abbe 3L refractometer, Depew, NY, USA). The mixture was filtered using a 0.45 μm microcellulose bottletop filter (Nalgene, Rochester, NY, USA).

Solubility of Test Compounds. Saturated solutions of the compounds (all compounds from Sigma) in water were prepared by adding 200 mg of compound into flasks with 100 mL of distilled water for a concentration of 2000 mg/L, in triplicate. The solubility of methyl paraben was greater than 2000 mg/L, so the same procedure was followed with the exception that the saturated solution was concentrated to 3000 mg/L. Flasks with the concentrated solutions were capped with plastic stoppers and mechanically shaken for 24 h at 25 °C and 250 rpm. The solutions were

then taken up into 10 mL latex-free syringes, and filtered through 0.45 μm microcellulose filters (Whatman, Florham Park, NJ, USA) into 20 mL test tubes to await analysis.

Standard curves were prepared for all compounds by dissolving them in ethanol followed by serial dilution with distilled water. UV absorption spectrophotometry was performed to quantify the content of compound in solution with a Shimadzu UV-2101PC UV−vis scanning spectrophotometer (Kyoto, Japan). Samples were placed in quartz cuvettes and scanned from 190 to 400 nm. Distilled water was used as a reference. The maximum absorbance was recorded for each compound. Linear regression was then used to calculate the concentration of test compound in water.

The evaluation of the test compounds' solubilities in AJM was performed using the same procedure described for the aqueous solubility determination with the exception that the distilled water was replaced with the AJM. AJM was used as a reference to negate the effects of absorbance of the test medium. Standard curves were prepared for all compounds by dissolving them in ethanol and serially diluting them with the AJM.

Phase Solubility Analysis. Due to their low aqueous solubilities, phase solubility studies were performed on citronellol, *o*-methoxycinnamaldehyde, *trans,trans*-2,4-decadienal and *trans*-cinnamic acid complexed with α- and β-CDs (α-cyclodextrin, CAVAMAX W6 and β-cyclodextrin, CAVAMAX W7 were supplied by Wacker Fine Chemicals, Munich, Germany). Various concentrations (10, 40, 70, 100, and 130 mmol/L) of α-CD were added to 50 mL polypropylene conical tubes. A 0 mmol/L α-CD sample was prepared to reference the solubility of the test compound by itself. The tubes were filled to a volume of 20 mL with distilled water. Tubes were tightly capped and mechanically shaken for 24 h at 25 °C and 250 rpm (Innova 4230, refrigerated shaker). After 24 h, the tubes were taken out of the shaker and each test compound was added to the aqueous-CD solution in excess. One hundred milligrams of each test compound was added to the aqueous cyclodextrin solution for a concentration of 5000 mg/L. The tubes were recapped and mechanically shaken for 48 h at 25 °C and 250 rpm. After 48 h the shaker was turned off and the tubes were left stationary for 24 h at 25 °C to allow any excess test compound to settle out of solution. The solution was extracted using a 10 mL syringe and filtered with a 0.45 μm microcellulose filter. One milliliter of sample was diluted with 1 mL of ethanol in order to dissociate the complex. UV absorption spectrophotometry was performed with a Shimadzu UV-2101PC UV−vis scanning spectrophotometer to quantify the content of compound in solution. A 50% aqueous ethanol solution was used as a blank. Standard curves were prepared for each test compound in 50% aqueous ethanol. Linear regression analysis was used to quantify the concentration of test compound in each solution.

For phase solubility analysis with β-cyclodextrin, the same procedure was followed with the exception that the concentrations of β-CD added were different due to β-CD's solubility. β-CD was added to four tubes at 4 mmol/L, 8 mmol/L, 12 mmol/L and 16 mmol/L. β-CD was added to each tube appropriately and then filled with distilled water up to a volume of 20 mL. A 0 mmol/L β-CD sample was prepared to reference the solubility of the test compound by itself. The rest of the procedure was carried out as described above. The procedure was done in triplicate (*n* = 3).

The molar ratios of the complexes in solution were calculated at the highest solubility increase shown by the phase solubility curves. The calculated moles of guest at the highest point of the phase solubility chart were divided by the moles of α- or β-CD at that same point. The result is a guest to host molar ratio expressed in moles of guest [G] to moles of host [H]; [G]:[H].

Solid Complexes. Solid complexes were prepared for *o*-methoxycinnamaldehyde, *trans,trans*-2,4-decadienal, cinnamic acid, and citronellol complexed with α-CD. The concentration of CD which showed the largest increase in solubility for each compound in the phase solubility analysis was added to 1 L of distilled water. The aqueous-CD solution was shaken for 24 h at 25 °C and 250 rpm (Innova 4230, New Brunswick Scientific, Edison, NJ). An excess of 5000 mg of test compound was added to the solution and placed back on the shaker for 48 h at 25 °C and 250 rpm. After 48 h, the solution was filtered and placed in wide shallow dishes covered with plastic wrap and frozen to −18 °C. The samples were freeze-dried over four days (Sentry Freezemobile 12SL, Virtis, Gardiner, NY). The solid samples were stored in closed bottles inside a desiccator until further analysis.

UV absorption spectrophotometry was used to determine the percentage of test compound found in the solid complex. A concentration of 1000 mg/L

Table 2. United States Pharmacopeia Descriptive Terms for Solubility of Chemical Compounds

descriptive term	concn (mg/L)
very soluble	>1,000,000
freely soluble	1,000,000−100,000
soluble	100,000−33,000
sparingly soluble	33,000−10,000
slightly soluble	10,000−1,000
very slightly soluble	1,000−100
practically insoluble/insoluble	100−0

of solid complex was diluted in distilled water. One milliliter of the complex solution was diluted in 1 mL of ethanol to dissociate the complex and have a final 50% aqueous ethanol solution. The maximum absorbance at each compounds' specific wavelength was plotted against a standard curve prepared in the same 50% aqueous ethanol solution. The weight percent (%) was calculated by dividing the concentration of guest by concentration of complex added to solution.

Stability of Complexes in Glass and PET. An acidified aqueous solution was prepared by adding ascorbic acid to distilled water until pH 3.4 was reached. Solutions of each α-CD complex in acidified water were prepared by adding 100 mg of complex to 100 mL of acidified water for a concentration of 1000 mg/L. Each solution was divided into two equal parts. Fifty milliliters of each solution was stored in a glass Erlenmeyer flask, and the other 50 mL was stored in PET containers in triplicate. Samples were stored on the benchtop of a laboratory exposed to regular fluorescent lighting. Before analysis, each sample was filtered through a 0.45 μm microcellulose filter tip and diluted for a final concentration of 50% ethanol (v/v). Standard curves were prepared by dissolving the guest compounds in ethanol and serially diluting them with distilled acidified water. UV absorption spectrophotometry was performed with a Shimadzu UV-2101PC UV−vis scanning spectrophotometer from 190 to 400 nm. A 50% ethanol−50% acidified water (v/v) solution was used as a reference. The maximum absorbance was recorded for each compound. Linear regression analysis was used to calculate the concentration of test compounds in solution. The procedure was performed on day 0 of storage and replicated at day 2, day 4, and day 7.

RESULTS AND DISCUSSION

Solubility. The standard curves produced by UV absorbance had R^2 values ranging from 0.85 to 1.00. The aqueous solubility calculated for each of the compounds evaluated is shown in **Table 1**, organized from the lowest to the highest water solubility. It can be noted that the values range from 1.6 mg/L for γ-nonalactone to 2460.6 mg/L for methyl paraben. Using the United States Pharmacopeia (*16*) solubility descriptors, we can classify the compounds evaluated in three categories: practically insoluble, very slightly soluble, and slightly soluble. **Table 2** depicts the USP solubility descriptive terms converted to milligrams of compound per liter of solvent. With aqueous solubilities of less than 100 mg/L, the following group of compounds can be described as practically insoluble in water: nonanoic lactone, β-pinene, benzyl cinnamate, (*R*)-limonene, cyclohexanebutyric acid, methyl nonanoate, benzyl paraben, propyl benzoate, and *trans,trans*-2,4-decadienal. The group of very slightly (100 mg/L to 1000 mg/L) water-soluble compounds tested includes perillaldehyde, butyl paraben, undecalactone, citronellol, eugenol methyl ester, citral, methyl, *trans*-cinnamaldehyde, propyl paraben, undecanal, cinnamic acid, cinnamaldehyde, *o*-methoxycinnamaldehyde, decanal, pulegone, and ethyl paraben. Finally, the group of slightly soluble test compounds (1,000 mg/L to 10,000 mg/L) includes thymol, eugenol, sorbic acid, and methyl paraben. The aqueous solubilities of parabens were reported by Davidson (*11*). The results of this study found similar values and confirmed the trend that longer alkyl chains lower water solubility.

The results of solubility evaluations of the test compounds dissolved in the AJM are also shown in **Table 1**. The model system

interfered with the maximum absorbance wavelengths of decanal, eugenol methyl ester, limonene and pinene. For that reason, the solubility of those compounds dissolved in the model system could not be determined. The AJM is intended to mimic a commercial beverage with the two most influential factors being soluble solids content and pH. A direct comparison of the test compounds' solubilities in water and the beverage mixture is shown in **Table 1**. It can be noted that although the solubility values are similar to the aqueous solubility values, there are some general trends that can be differentiated. Of the 24 compounds evaluated, 18 compounds showed a decrease in maximum solubility in the AJM compared to water. This is consistent with the study by Terrance and LeMaguer (*16*), which reported that all terpenic essential oil components included in their study exhibited reduced aqueous solubility as the concentration of soluble solids increased.

It is well-known that the presence of soluble solids may reduce the solubility of organic compounds in water because water molecules that hydrate the soluble solids are no longer available to dissolve other compounds, in this case, the test compounds (*16*).

Table 3. Guest to Host Molar Ratios of Cyclodextrin Complexes (Moles of Guest: Moles of Cyclodextrin)[a]

cyclodextrin	*o*-methoxy-cinnamaldehyde	*trans,trans*-2,4-decadienal	cinnamic acid	citronellol
α	0.24	0.18	0.58	0.39
β	1.25	0.19	1.53	0.72

[a] The concentration (M) of test compounds was determined spectrophotometrically after shaking with various concentrations of cyclodextrins for 48 h at 25°C and 250 rpm. The molar ratio was determined at the highest solubility of the test compound.

The only compound that showed a significant increase of solubility in the beverage mixture was propyl benzoate with an increase of 53%. However, the increase in solubility was only from practically insoluble in water (53.9 mg/L) to slightly soluble in the beverage mixture (116.6 mg/L). γ-Nonalactone exhibited an increase in solubility from 1.6 mg/L in water to 2.7 mg/L in the beverage model system; however, the compound remained practically insoluble. Citral, thymol, undecanal, and citronellol showed very slight increases in solubility in the beverage mixture ranging between 1.3% and 6.1%. Nevertheless, considering the average standard deviation of the two analyses range between 9.5% and 30.5%, it can be concluded that the solubility values for these four compounds remained constant in both mediums.

Phase Solubility Analyses. Table 3 shows a summary of the guest molecule:cyclodextrin ratios. Molar ratios were calculated at the highest point of the phase solubility curves. **Figure 1** depicts the phase solubility diagrams of *o*-methoxycinnamaldehyde−CD complexes. The optimum uptake was at a concentration of 0.13 M α-CD aqueous solution. At the highest point of the solubility isotherm, the complex formation increased the solubility of *o*-methoxycinnamaldehyde from 505 mg/L to 5133 mg/L, a 10-fold increase. The moles of *o*-methoxycinnamaldehyde divided by the moles of α-CD denote a molar ratio of 0.24 or approximately 1:4 guest to host. An important observation is that *o*-methoxycinnamaldehyde was added to the system in excess at a concentration of 5000 mg/L. The average calculated guest content of 5133 mg/L is higher than the excess concentration added with a standard deviation of ±537 mg/L, giving insight that nearly all the compound present was complexed at a concentration of 0.13 M α-CD aqueous solution. This suggests that, if more compound was present in the system, the reaction could yield a more efficient complex than what was observed in this study. It can be noted
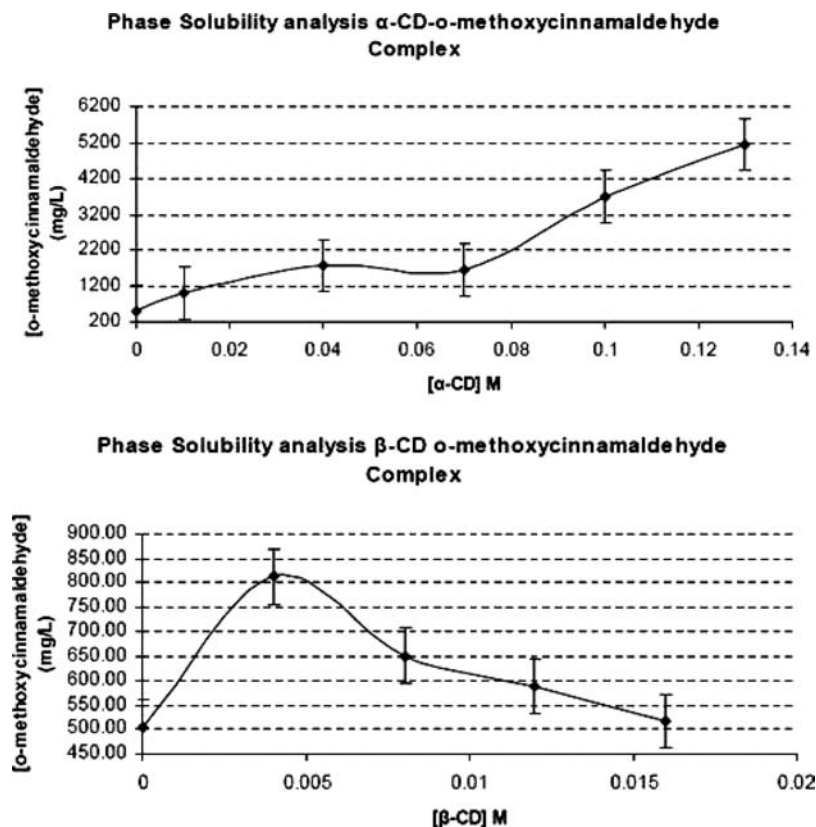

Phase Solubility analysis α-CD-o-methoxycinnamaldehyde Complex

Phase Solubility analysis β-CD o-methoxycinnamaldehyde Complex

Figure 1. Phase solubility diagram of *o*-methoxycinnamaldehyde/cyclodextrin complex. An excess of *o*-methoxycinnamaldehyde was added to cyclodextrin solutions of various concentrations. The mixture was shaken for 24 h and then filtered. The *o*-methoxycinnamaldehyde concentration was measured via UV absorbance. Error bars are pooled SE.

Table 4. Cyclodextrin Concentration (M) Demonstrating the Largest Solubility Increase of Test Compound[a]

cyclodextrin	o-methoxy-cinnamaldehyde	trans,trans-2,4-decadienal	cinnamic acid	citronellol
α	0.13	0.01	0.04	0.04
β	0.004	0.008	0.004	0.004

[a] The concentration (M) of test compounds was determined spectrophotometrically after shaking with various concentrations of cyclodextrins for 48 h at 25 °C and 250 rpm.

that the correlation of *o*-methoxycinnamaldehyde concentration with that of α-CD is linear ($R^2 = 0.91$). This linearity depicts a behavior in which *o*-methoxycinnamaldehyde presents an unlimited increase in solubility as the concentration of CD is increased. When this correlation is strictly linear, a complex of constant stoichiometry is formed (*17*).

Figure 1 also depicts the phase solubility diagram of *o*-methoxycinnamaldehyde-β-CD complex. The solubility increase limit of the complex was observed at a concentration of 0.004 M β-CD aqueous solution. At this point, the concentration of *o*-methoxycinnamaldehyde was 811 mg/L. From its initial solubility of 505 mg/L, the complex formation showed an increase in solubility of 1.6-fold. At a concentration of 0.004 M β-CD the molar ratio was 1.25 which is higher than 1:1 in favor of the guest. The phase solubility diagrams of decadienal, cinnamic acid, and citronellol looked similar to the *o*-methoxycinnamaldehyde-β-CD complex diagram with a single peak representing the concentration of CD that provided the maximum solubility.

When the correlation of guest concentration and CD concentration is not linear, the solubility increase deviates upward or downward. This happens when the solubility increases faster or slower than the concentration of CD and the guest to host ratio is not constant. The solubility limit of the complex can be observed when the curve reaches the end of the linearly increasing section, which results in a plateau. At this point increasing CD concentration results in no further increase in the guest solubility. When the concentration of guest in the system begins to fall, it signals that the maximum amount of guest present in the system is complexed, so the apparent solubility begins to decrease.

Table 4 lists the CD concentrations where maximum solubility of the test compounds was achieved. The maximum concentration of *trans,trans*-2,4-decadienal complexed by α-CD was 281.3 mg/L in an α-CD concentration of 0.01 M. From an initial solubility of 86.85 mg/L there was a solubility increase of 3.2-fold. The molar ratio of was 0.18, approximately 1:5 guest to host. The solubility increase limit was seen at 0.008 M for β-CD. From the initial solubility of 86.9 ± 36.9 mg/L the complex formation increased the compound's solubility to a maximum of 235.3 ± 36.9 mg/L for a 3.1-fold increase. The molar ratio of β-CD *trans,trans*-2,4-decadienal complex was 0.19 or approximately 1:5 guest to host. Both solubility isotherms showed nonlinear correlations.

Cyclodextrin Complexes. The maximum concentration of cinnamic acid complexed by α-CD was 3411 mg/L aqueous solution at 0.04 M α-CD. From a solubility of 527.1 ± 475.1 mg/L there was a 6.3-fold increase when complexed with 0.04 M α-CD. A molar ratio of 0.58 was calculated. The 1:2 molar ratio has been observed in other studies involving α-CD and cinnamic acid (*18*, *19*). In addition, literature shows results of 2518 mg/L for a cinnamate ion−α-cyclodextrin complex, although cosolvents were used to aid complex formation (*20*). With β-CD, the solubility increase limit was seen at 0.004 M with a concentration of 905.99 mg/L of cinnamic acid. The solubility increase from 527.13 mg/L was of 1.7-fold. The molar ratio calculated was approximately 2:1 (1.53). Both of the molar ratios calculated for

α- and β-CD−cinnamic acid complexes are comparable to values found in the literature (*20*, *21*).

The maximum concentration of citronellol complexed was 2437.6 mg/L at 0.04 M α-CD. The solubility of citronellol by itself was calculated at 300.03 mg/L, so the maximum amount complexed represents an 8-fold increase in solubility. At the highest point of the phase solubility curve, the molar ratio was calculated at 0.39, approximately 1:3 guest to host. The maximum concentration of citronellol complexed by β-CD was 449.43 mg/L. The optimal uptake of citronellol was at 0.004 M of β-CD. The formation of this complex increased the solubility of citronellol by 1.6 times. The average molar ratio calculated was close to 1:1 (0.72).

An increase in solubility of a poorly soluble substance as CD concentration increases indicates complex formation (*17*). In theory, the stoichiometry of CD complexes is characterized by constant guest:host ratios. However, in practice there are factors that modify the composition of the complexes. For instance, in solution the association/dissociation equilibrium and the size and shape of the guest allow a variety of guest to host ratios of complexes to coexist. The general rule is that for aqueous solutions the 1:1 complex is predominant (*17*). According to theoretical and past experimental observations, the fact that the molar ratios calculated in this study show α- and β-CD complexes with values lower than 1:1 is more likely to indicate that not all of the CD molecules present in the system were forming complexes as opposed to the idea that more than one cyclodextrin molecule was needed to fully complex the guest compound. It is also important to note that some recent studies have shown that cyclodextrins are able to form not only inclusion complexes but also more complicated structures that can increase solubility (*22*). Inclusion complexes have been confirmed in citronellol/β-CD, citronellol/α-CD, and cinnamic acid/β-CD (*23*, *24*). Further studies are needed incorporating the appropriate techniques (X-ray diffraction, ^{1}H NMR, etc.) to elucidate the true relationships between CD and the test compounds.

In all instances, the larger cavity of β-CD produced higher molar ratios, favoring the guest, than α-CD complexes. Moreover, α-CD was more effective for the purpose of increasing the aqueous solubility of the host despite the less efficient molar ratios. The higher solubility of α-CD allows less efficient complexes to still have larger increases in aqueous solubilities of the guests.

Solid Complexes. Given that α-CD complexes showed better results for the purpose of increasing the aqueous solubility of the test compounds, solid complexes were prepared for the four test compounds and α-CD complexes. A solid complex allows physical characterization and the determination of weight percentage (%) of host. The resulting complex of α-CD−*o*-methoxycinnamaldehyde resulted in a lightweight, flaky, white powder with very different physical appearance and color than the α-CD−*o*-methoxycinnamaldehyde physical mixture. The resulting complex of α-CD−*trans,trans*-2,4-decadienal was a lightweight powder with a uniform, but very slight, hint of yellow. α-CD−cinnamic acid and α-CD−citronellol complexes resulted both in lightweight, white powders.

The calculation of weight percentage (%) attributed to the guest compound in the complex was done by dividing the concentration of compound calculated by the total concentration of complex added to the system and multiplied by 100. The weight percentage calculation for α-CD−*o*-methoxycinnamaldehyde complex resulted in 3.7% of the complex's weight attributable to the compound. A low weight percentage is expected since the molar ratio indicated a 1:4 guest to host ratio. For the α-CD−*trans,trans*-2,4-decadienal complex a weight percentage of 6.7% resulted. Again a low weight percentage confirms a molar ratio
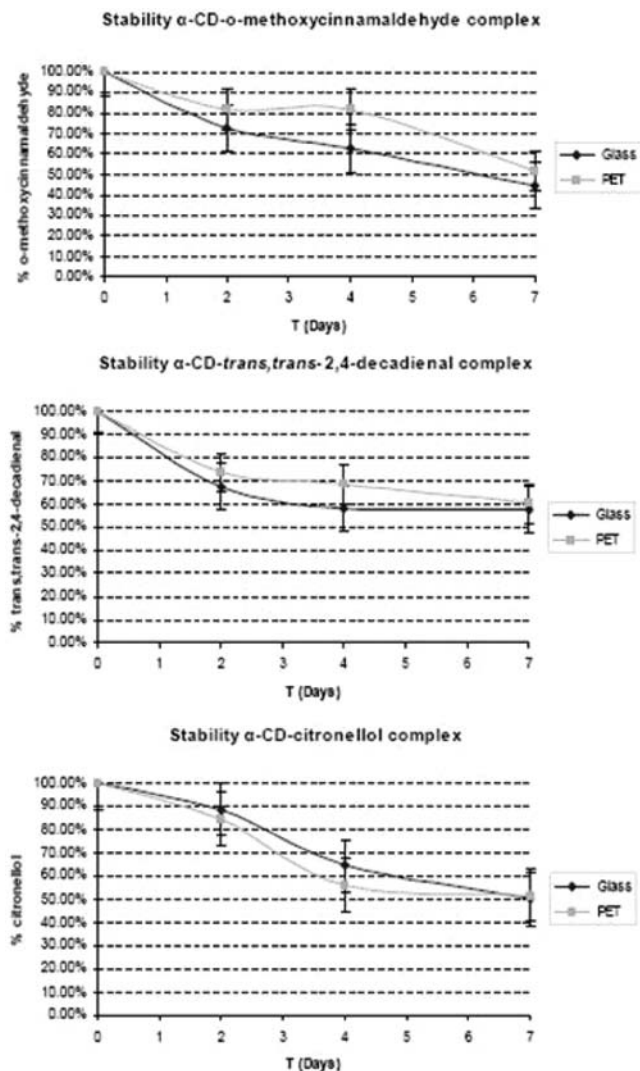
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Stability α-CD-o-methoxycinnamaldehyde complex

Stability α-CD-*trans,trans*-2,4-decadienal complex

Stability α-CD-citronellol complex

Figure 2. Stability of complexes detected by UV−vis spectrophotometry in acidified aqueous solution throughout a period of 7 days in either glass or PET. Samples were stored at room temperature. Error bars are pooled SE.

with a higher proportion of host than guest. The α-CD−cinnamic acid complex showed a weight percent of 8.9% of cinnamic acid. This value was confirmed with past work on α-CD−cinnamic acid complexes by Truong et al. (*25*) and Romano (*19*). Finally, the α-CD−citronellol complex resulted in 7.3% of weight attributable to citronellol.

Standard curves prepared to quantify the presence of each guest compound showed excellent linearity with correlation coefficients, R^2, between 0.98 and 0.99. Before analysis the solutions containing each complex were diluted to a final concentration containing 50% ethanol to achieve the complete dissociation of the complex (*26*).

Figure 2 shows the storage stability comparison of the complex in an acidified aqueous solution stored in a glass container compared to the same stored in a PET container. The initial *o*-methoxycinnamaldehyde concentration (day 0) of compound detected by this method was 40.3 mg/L. It can be noted that the solution stored in glass had a more constant decline in concentration of compound. The concentration of *o*-methoxycinnamaldehyde was higher in the solution stored in PET up until day 4. However, at day 7 the concentration of *o*-methoxycinnamaldehyde detected was very close in both containers with 18.0 mg/L in glass and 20.8 mg/L in PET. At the end of the analysis period, day 7, the concentration of *o*-methoxycinnamaldehyde had dropped by

55.3% in the solution stored in the glass container and by 48.3% in the solution stored in the PET container. **Figure 2** shows the storage stability comparison of the *trans,trans*-2,4-decadienal complex in acidified aqueous solution stored in glass and PET containers. The initial concentration was calculated to 84.6 mg/L. Both samples showed a steady decline with similar trend lines. By the end of the study the concentration of guest compound declined by 42.4% in the sample stored in glass and by 40.7% in the sample stored in PET.

The initial concentration of citronellol found at day 0 was 117.24 mg/L. The concentration decreased with very similar trend lines in both samples. By day 7 the concentration of citronellol found in the sample stored in glass had decreased by 49.9% to 58.7 mg/L. Similarly, the sample stored in PET had a decrease of 48.3% to 60.6 mg/L.

The concentration of guest compound of all the complexes in AJM showed a decrease in concentration ranging from 44%-63%. A similar study conducted by Ajisaka and others (*27*) that evaluated the stability of cyclodextrin−terpene complexes including β-CD−citronellol found comparable results. However, their complex solutions were stored in open beakers, which may lead to compound loss to the atmosphere. Literature reports that aromatic compounds complexed with cyclodextrin and packed under vacuum to lose 25%−30% of their active ingredient after being exposed to elevated temperatures of 150 °C for 24 h (*17*).

The results of the storage stability analysis of the samples stored in glass and PET packages did not show remarkable differences. It is well-known that thermoplastic polymers, including PET, have varying degrees of permeability to small molecules, such as volatile organic compounds. In beverages, sorption (also called scalping) is a common phenomenon of permeation where molecules from the product are taken up into (but not through) the package (*28*). The concentration of *d*-limonene in citrus juices was used by Mannheim and others (*29*) to demonstrate the absorption capacity of polyethylene showing that after 14 days at 25 °C it was 25% lower than the same samples stored in glass containers. Conversely, in this case, none of the complexes studied exhibited a behavior in which permeation into the polymer film could be attributed to its decrease in concentration since the decrease in guests' concentrations was very close in both methods of storage. It is possible that the complexation of the guest compounds makes up a molecule too large to be adsorbed by the polymer film.

Aldehydes and dienaldehydes such as *o*-methoxycinnamaldehyde and *trans,trans*-2,4-decadienal are frequently involved in self-condensation polymerization reactions that can be catalyzed by acid. In addition, these aldehydes may be susceptible to autoxidation reactions initiated by exposure to light or air (*30*). Degradation of the test compounds causes an inverse relationship between concentration of test compound and storage time. Further experimentation is needed to improve the stability of the complexes including using packaging to block harmful wavelengths of light, pairing with antioxidants to combat oxidation, and determining the effect of pH on complex stability.

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