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PROPERTIES AND APPLICATIONS OF CYCLODEXTRINS

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ABSTRACT

Cyclodextrins are enzymatically modified starches consisting of 6, 7, or 8 α-1,4-linked glucose monomers. The interior of the cyclodextrin is apolar and can associate with various organic molecules to form inclusion complexes. The cyclodextrins are chemically and physically stable, and they undergo the same reactions as other carbohydrates. The hydroxyl groups of the cyclodextrin can be substituted to change the solubility of cyclodextrin in water and in other solvents, and to change the binding strength between the cyclodextrin and the guest compound. Cyclodextrins and their derivatives have a wide variety of applications. Solubility of guest compounds can be altered to make them more soluble or less soluble. Cyclodextrins can be used to protect compounds against the effects of light, heat, and oxygen. Volatility of compounds can be reduced to give increased shelf life and reduced release of compounds into the environment. Cyclodextrins can be used as process aids to remove or isolate specific compounds from a mixture.

INTRODUCTION

Cyclodextrins are enzymatically-modified starches formed by the action of the enzyme glucosyltransferase (CGTase) upon starch. Three cyclodextrins are typically formed; alpha, beta, and gamma-cyclodextrins which contain 6, 7, and 8 α-1,4-linked glucose monomers, respectively. Cyclodextrins are ring or torus-shaped molecules and possess a hydrophobic cavity and a hydrophilic exterior. The hydrophobicity of the cavity allows cyclodextrin to associate with nonpolar organic molecules or por-

TABLE 1. Molecular Dimensions of Cyclodextrins [1]

CD Type	Volume, Å ^{3}	Diameter, Å
α-CD	174	4.7–5.3
β-CD	262	6.0–6.5
γ-CD	472	7.5–8.3

tions of organic molecules to form inclusion complexes. It is also possible for some organic molecules and some inorganic salts to interact with the hydroxyl groups of the cyclodextrins.

PHYSICAL PROPERTIES OF CYCLODEXTRINS

Molecular Dimensions of Cyclodextrins

Table 1 shows the molecular dimensions of cyclodextrins [1]. The different diameters and volumes of the cavities reflect the different numbers of glucose molecules in the ring of the cyclodextrins. The difference in cyclodextrin cavity sizes provides a basis for selectivity so that one may complex molecules of different sizes. For example, a phenyl ring fits very snugly into the cavity of α-cyclodextrin. This same phenyl ring also fits into the ring of β-cyclodextrin but some space is left unoccupied in which the phenyl ring can vibrate or wobble slightly. In the much larger cavity of γ-cyclodextrin, there is even more unoccupied space and the phenyl ring has more room to wobble. The molecule also is not as much in contact with the walls of the cavity of γ-cyclodextrin as with α- and β-cyclodextrin and as a result, it does not bind as strongly. In many cases, molecules that will complex with α-cyclodextrin will bind with β-cyclodextrin and molecules that will complex with γ-cyclodextrin will bind with β-cyclodextrin. Small molecules with four or fewer carbon atoms bind best with α-cyclodextrin and large molecules such as pyrene bind best with γ-cyclodextrin.

Solubility of Cyclodextrins

As the temperature increases, the aqueous solubility of the uncomplexed cyclodextrins increases (Table 2) [2]. Because of differences in the amount of strain in

TABLE 2. Aqueous Solubilities of Cyclodextrins [2]

	Solubility, grams/100 mL		
Temperature, °C	α-CD	β-CD	γ-CD
25	12.8	1.8	25.6
45	29.0	4.5	58.5
60	66.2	9.0	129.2

the rings of α-, β-, and γ-cyclodextrin, the orientation and degree of hydrogen bonding between the hydroxyl groups on C-2 and C-3 of adjacent glucose molecules are different in each of the cyclodextrins. The hydroxyl groups on C-2 and C-3 of the adjacent glucose units of β-cyclodextrin are oriented so that they interact very strongly with each other. As a result, they do not interact with water molecules in order to solvate the cyclodextrin molecule [3]. However, with the increased strain in the ring of α-cyclodextrin, these hydroxyl groups are positioned so that they interact less with each other than in β-cyclodextrin and can interact with molecules of water more than the hydroxyl groups of β-cyclodextrin. As a result, α-cyclodextrin is more soluble in water than β-cyclodextrin. γ-Cyclodextrin is even less constrained with less interaction between the hydroxyl groups of the adjacent glucose molecules in the ring. The hydroxyl groups in γ-cyclodextrin interact much more freely with water molecules than do the hydroxyl groups of α- or β-cyclodextrin which results in even greater solubility in water than the α- and β-cyclodextrins [4, 5].

The solubility of a complex of cyclodextrin may be very different than that of the uncomplexed cyclodextrin depending upon the guest molecule. Some compounds will form very insoluble complexes and others form highly soluble complexes, even more soluble than uncomplexed cyclodextrin. Because guests can orient and interact differently with the various cyclodextrins, the order of the solubility can be very different from that of the uncomplexed cyclodextrins. For example, a complex of a guest with γ-cyclodextrin may be more insoluble than a complex of that guest with either α- or β-cyclodextrin.

Table 3 shows the solubility of β-cyclodextrin in several solvents at room temperature (25°C) [2]. Although the solubility of the cyclodextrin is greater in some solvents than in water, complexation might not occur as readily in nonaqueous solvent because of the increased affinity of the guest for the nonaqueous solvent compared to its affinity for water.

β-Cyclodextrin is not soluble in most organic solvents, but is soluble in some solvent/water mixtures. In general, for most solvents, the solubility of the cyclodextrin decreases as the concentration of nonaqueous solvent increases. The exceptions

TABLE 3. Solubility of β-Cyclodextrin in Solvents [2]

Solvent	Solubility, %
Dimethylsulfoxide	41.0
Dimethylformamide	28.3
N-Methyl pyrrolidone	14.8
Ethylene glycol	7.0
Pyridine	3.5
Propylene glycol	0.5
Tetrahydrofuron	0
Methyl isobutyl ketone	0
Methyl isopropyl ketone	0
Acetone	0
Alcohols	0

are ethanol, propanol, and acetonitrile where a maximum solubility occurs at a 20 to 30% concentration of the organic solvent [6].

Thermal Stability

Figure 1 shows a thermogram obtained from differential scanning calorimetry (DSC) of β-cyclodextrin [2]. Thermograms of α-, β-, and γ-cyclodextrin are identical. Two peaks are seen. The first peak occurs at 100°C where energy is absorbed as water is evaporated from the crystals. The second peak occurs at 300°C. At this temperature, melting of the crystals and thermal decomposition of the cyclodextrin occur. The melting and decomposition cannot be separated from each other. Unlike starch, no peaks are observed between the two peaks. Cyclodextrins are single crystalline chemical entities and do not contain the variety of secondary and tertiary structures which are altered as the temperature increases as in starch.

CHEMICAL PROPERTIES OF CYCLODEXTRINS

Stability in Acids, Bases, and Peroxides

Although cyclodextrins are more resistant to acid hydrolysis than starch, strong acids, such as hydrochloric acid, and sulfuric acid hydrolyze cyclodextrins to yield a series or mixture of oligosaccharides ranging from an opened ring down to glucose. The rate of hydrolysis increases as the concentration of acid and temperature increase [7–9]. Hydrolysis is minimal or below the limits of detection in the presence of weak acids such as organic acids.

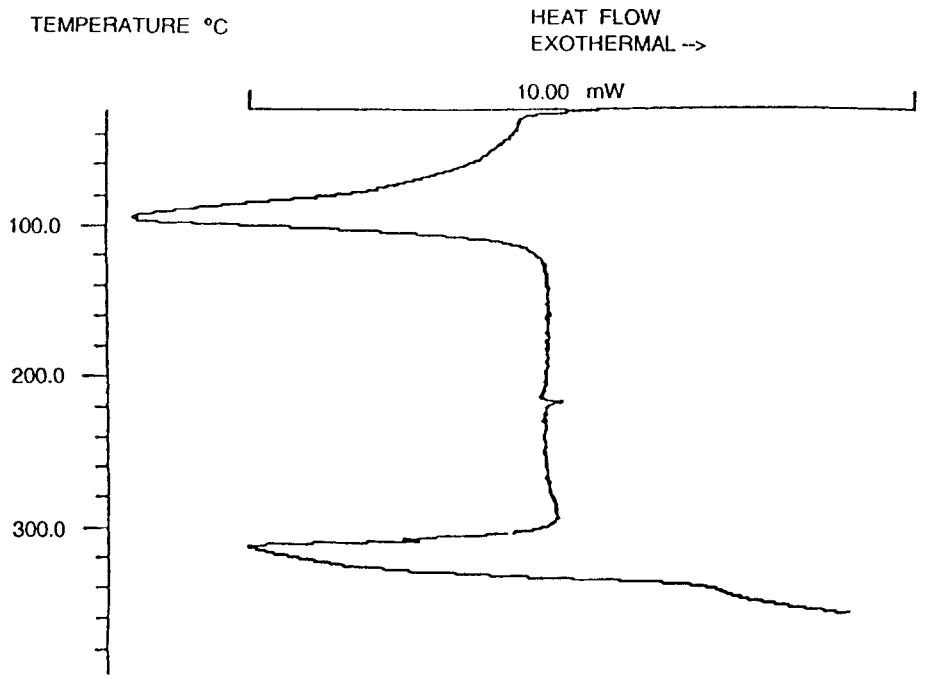

FIG. 1. Differential scanning calorimetry curve of β-cyclodextrin [2].

Cyclodextrins are not hydrolyzed by bases, even at elevated temperature. Exposure to a 0.35 N sodium hydroxide solution at 60°C results in no detectable hydrolysis of cyclodextrin.

Cyclodextrins will undergo oxidative reactions. The periodate reaction opens the glucose ring but no formaldehyde or formic acid is formed since no reducing groups are present [7]. β-Cyclodextrin is rapidly and completely oxidized at 50°C by hypochlorite at 5.25% of bleach solution. β-cyclodextrin is oxidized less rapidly by hydrogen peroxide. Only 11% of the cyclodextrin is oxidized after 2 hours at 50°C when cyclodextrin is exposed to 30% of hydrogen peroxide. Exposure of cyclodextrin to 50–200 ppm of hydrogen peroxide used to prevent microbial growth results in no detectable oxidation of the cyclodextrin.

Biodegradation of Cyclodextrins

α- and β-Cyclodextrins are more resistant to hydrolysis by α-amylase than starch [10–12]. Cyclodextrin glucosyltransferase can hydrolyze as well as form cyclodextrins. Enzymes such as β-amylases and glucoamylases requiring a reducing end group cannot hydrolyze cyclodextrins. Many α-amylases will not attack α- and β-cyclodextrins, and those which do will hydrolyze the cyclodextrin more slowly than starch. Unlike other cyclodextrins, γ-cyclodextrin is readily hydrolyzed by α-amylases [12]. Cyclodextrins are biodegradable. Many microorganisms found in soil can degrade cyclodextrins [13].

Toxicology of β-Cyclodextrins

Feeding studies have shown cyclodextrin to be nontoxic when presented orally. The acute LD_{50} value of β-cyclodextrin for mice is more than 12.5 g/kg, for rats is more than 18.8 g/kg, and for dogs is more than 5 g/kg [14, 15]. Upon injection, the β-cyclodextrin has been found to crystallize in the kidneys causing renal damage [16].

Rats were fed a diet containing 1.25, 2.5, 5.0, and 10.0% of β-cyclodextrin for 90 days [17]. There was no difference between the rats in the control group and the rats consuming β-cyclodextrin. No dose-related toxicological and pathological responses were found in the blood, serum, or organs. The rats consuming 5 and 10% of β-cyclodextrin had enlarged caeca. This is a normal response in rats fed materials that are slowly digested in the stomach and small intestine. The rats in the control group whose diets containing lactose also had enlarged caeca. A small amount of β-cyclodextrin, 0.1 to 0.3%, was found in the urine of rats fed diets containing 5 and 10% of β-cyclodextrin in the diet. No β-cyclodextrin was found in the urine of rats fed diets containing 1.25 and 2.5% of β-cyclodextrin. No evidence of abnormalities in the kidney was found upon autopsy of the rats at the conclusion of the study.

Beagle dogs were fed diets containing up to 5% of β-cyclodextrin for 1 year. No signs of systemic toxicity were observed in dogs fed any of the diets [18].

Rats were fed a diet containing 1.25, 2.5, or 5% of β-cyclodextrin for 1 year. A no effect dosage level of β-cyclodextrin was determined to be 1.25% for rats [18].

A three generation oral reproductive toxicity study was also performed on rats. The rats were fed a diet containing 0.31, 0.62, and 1.25% of β-cyclodextrin.

No adverse effects on fertility, reproductive performance, in utero fetal development, or physical pup development were found at any concentration [19].

Studies for dermal irritation [20], eye irritation [20], and inhalation [20] showed no detrimental effects. No mutagenic effect has been found using the Ames [21], chromosomal aberration [22], or drosophila recessive lethal tests [23].

MODIFIED CYCLODEXTRINS

Chemical or enzymatic modification of cyclodextrin has been found to enhance the properties of cyclodextrin by extending their usefulness in several applications. Solubility of β-cyclodextrin and its complexes have been a concern for many applications. By modification, the solubility of β-cyclodextrin and its complexes can be increased or decreased.

Modification of the cyclodextrins with either glucosyl, maltosyl, hydroxypropyl, hydroxyethyl, methyl, or sulfate groups increases the aqueous solubility of the cyclodextrins. Modified cyclodextrins with low aqueous solubility or insoluble in water can be achieved by adding aliphatic groups or short nonpolar groups to the cyclodextrin or by crosslinking cyclodextrins with a suitable crosslinker, such as epichlorohydrin, to form spherical beads of polymers. These modified cyclodextrins have the same functional properties as the unmodified cyclodextrins such as stabilization of guests, etc.

COMPLEXATION

Complexation with cyclodextrin is a molecular phenomenon usually involving only one guest molecule which interacts with the cavity of a cyclodextrin molecule to become entrapped. Encapsulation by other means usually involves more than one guest molecule being entrapped in the matrix.

Several explanations have been offered as the thermodynamic basis for forming complexes with cyclodextrins. One explanation involves the release of high energy water [24, 25]. The cavity of cyclodextrins holds a few molecules of water. Since water is polar and the cavity is apolar, this is not a very stable condition. In contrast, the guest molecule or portion which binds with the cavity is apolar. It can displace the water in the cavity, releasing the water from the cavity to the bulk solvent water which is more thermodynamically favorable. It is also more thermodynamically favorable for the apolar guest to associate with the apolar cavity than with the polar water. Some crystallographic studies have shown a change in the conformation of the ring of the cyclodextrin upon complexation [26]. As a result, release of conformational strain has been described as a driving force in complexation.

A variety of noncovalent forces such as van der Waals forces [27], hydrogen bonding [27], dipole–dipole interaction [28], London dispersion forces, and other hydrophobic interactions are responsible for the formation of a stable complex [29, 30]. Also, a force responsible for complexation for one series of molecules may not hold for another series of molecules. A single force cannot be found as the only factor responsible for complexation of all molecules. Several forces seem to be

involved in the complexation. As a result, it is difficult or impossible to predict how well a particular molecule might bind with cyclodextrins.

BENEFICIAL EFFECTS OF COMPLEXATION WITH CYCLODEXTRINS

Complexation of a guest with cyclodextrin can have one or more beneficial effects. These effects include increased solubility of the guest; stabilization of the guest to prevent volatilization, oxidation, and degradation due to exposure to light and heat; elimination or reduction of undesired tastes or odors; prevention of chemical reaction; directed chemical synthesis; and separation and isolation of various chemicals.

Iomeglamic acid, which has very low solubility in water (5.76 mg/100 mL at 22°C), has been used for radioscopy of the gallbladder. The aqueous solubility of iomeglamic acid was increased by 89-fold after complexing with β-cyclodextrin [31]. Ipriflavone, which is used to treat osteoporosis and osteomalacia, has poor bioavailability. The solubility of iprifalvone was enhanced more than 10-fold by complexing with β-cyclodextrin [32].

Vitamin A is readily oxidized when exposed to light and air. The vitamin A/β-cyclodextrin complex was exposed to air at room temperature for 24 hours, and the absorption spectrum of the complex was identical to that of fresh vitamin A. Under identical conditions, the absorption spectrum of uncomplexed vitamin A was shifted from 320 to 240 nm [33].

Stabilization of vitamin D_3 by cyclodextrin is illustrated by exposing vitamin D_3 and its complex with β-cyclodextrin to the air at 80°C [34, 35]. Vitamin D_3 was rapidly destroyed by the heat in 1 day. The cyclodextrin-completed vitamin decomposed at a slower rate. After 43 days, 49% of the original activity remained.

Benzoyl peroxide can be used both as a blowing agent and a curing agent in expandable adhesives [36]. Benzoyl peroxide is unstable and readily broken down to liberate oxygen as it is heated. Benzoyl peroxide was added to an expandable adhesive-sealant in both the free and complexed state. While mixing the free benzoyl peroxide to the melted formulation, the free benzoyl peroxide broke down rapidly as evidenced by bubbles forming. Very few bubbles were evolved while mixing the complexed benzoyl peroxide into the melted formulation. Upon heating the formulation to effect expansion and curing, the formulation containing the complex expanded more and cured better than the formulation in which benzoyl peroxide was free.

USES OF CYCLODEXTRINS AS PROCESS AIDS

Cyclodextrins can be used as process aids to remove specific components from a mixture of materials. The components form an inclusion complex with cyclodextrins. The complex can then be separated from the system by centrifugation or filtration. The components which have been removed may either be discarded or further purified, and the cyclodextrins can be recovered and recycled.

For most applications the complex of cyclodextrin can be heated in water to destabilize the complex, and the guest compound will form an immiscible phase

while the cyclodextrin remains in the aqueous phase. The solubility of the cyclodextrin in the aqueous phase depends on the cyclodextrin selected and the temperature. Additional processing of the cyclodextrin, such as carbon treatment, crystallization, etc., might be necessary for some applications before the cyclodextrin is recycled. If good separation of the guest from the cyclodextrin is achieved, the recycled cyclodextrin has virtually the same complexing ability as the virgin cyclodextrin [37].

Cholesterol is present in many foods of animal origin such as dairy products, eggs, lard, and tallow. In milk and eggs, cholesterol is suspended in an emulsion. When cyclodextrin was added to the system, cholesterol was complexed with cyclodextrin, forming an insoluble complex which was separated by centrifugation [38–40]. The cholesterol/cyclodextrin complex was further heated to separate cholesterol from cyclodextrin. The recovered cyclodextrin solution could be reused as is or could be cooled to crystallize the cyclodextrin for reuse [37].

Enzymatic browning of fruits and vegetables occurs when the fruit or vegetable is cut, bruised, abraded, or crushed. When this damage occurs, polyphenol oxidase, an enzyme present in fruits and vegetables, immediately begins to oxidize endogeneous polyphenolic compounds, such as chlorogenic acid and epicatechin. These polyphenols are oxidized to quinones and subsequently polymerized into brown pigments. This enzymatic browning reaction can be prevented or inhibited by treating the fruits or vegetables with cyclodextrin. The binding geometry, stoichiometry, and thermodynamics of the β-cyclodextrin inclusion complex with chlorogenic acid, the most abundant substrate for apple polyphenol oxidase, has been studied [41]. Complexation can be accomplished with a cyclodextrin monomer or cyclodextrin-containing polymer [42, 43]. Using the polymer, the polyphenolic compounds can be removed so that there is no substrate with which the polyphenol oxidase can react. Complexation with cyclodextrins renders the substrate inaccessible to polyphenol oxidase, and therefore inhibits the enzymatic browning reaction.

Naringen and limonin, which impart the bitter taste to citrus juices, can be removed by passing the juice through the polymer. Only naringen and limonin are removed. The oils, flavor components, and vitamins are not removed from the juice [44, 45]. Bitterness can also be masked by adding cyclodextrins to the juice [46].

Many beverages, such as coffee and tea, contain caffeine. The cyclodextrin-containing polymer was used to remove caffeine from coffee and tea [47]. Up to 65% of the caffeine in tea was removed by the polymer. Below 60°C, temperature did not have a great effect on removal of caffeine, but above 60°C the efficiency of removal of caffeine was reduced. The optimum pH for caffeine removal was pH 7.0. There was a decrease in efficiency of removal of caffeine above or below pH 7.0. Other components can also be removed depending on the source of tea or coffee.

Many natural products such as spices vary in potency depending upon where they are grown, time of harvest, and variation in climatic conditions from year to year or area to area. Extraction and complexation of the active ingredient with cyclodextrin produces a standard product whose potency is the same from batch to batch. The complex can be used as is or the active ingredient can be recovered from the complex.

Onion and garlic homogenate were mixed with β-cyclodextrin. The flavor of both onion and garlic complexes was more like that of fresh onion and garlic than the flavor of onion or garlic oil extracted by other means.

Many products from fermentation and enzyme reactions are recovered by extraction with organic solvents. A crude product is obtained which is further purified. In many cases the cyclodextrin is more selective than the organic solvent, and a purer product is obtained which requires less subsequent purification [48, 49].

Some isomeric hydrocarbons which have close boiling points and cannot be separated easily by distillation can selectively complex with cyclodextrins to achieve purification or enrichment of a particular component. Separation of xylene isomers and ethylbenzene [50, 51], trimethylbenzene [52], and nitrotoluene [53] has been reported. Immobilized cyclodextrin can also be used to effect a chromatographic separation of components, including chiral separation [54].

Cyclodextrins have been used to recover oil from tar sands and shale [55]. Tar sands were heated in the presence of an aqueous solution of β-cyclodextrin and stirred. After centrifugation, three components were isolated: oil, sand, and water. Maximum recovery of oil was obtained using a 2% solution of β-cyclodextrin. Increasing the concentrations of β-cyclodextrin did not increase the amount of oil recovered. About 90% of the oil in the tar sand was recovered. At the temperature used, binding of the oil to the cavity of cyclodextrin was strong enough to release the oil from the sand, but weak enough to allow easy release of oil from the complex so that a molecule of cyclodextrin could be used to recover several hydrocarbon molecules from oil. The β-cyclodextrin in the aqueous layer could be recovered and used to treat additional tar sands. Cyclodextrin gave better recovery of oil from the tar sands than did the surfactant tested.

USE OF CYCLODEXTRINS IN CAN COATING

Interior can coating compositions are applied to the interior surface of metal containers to form a protective barrier between the food or beverage materials and the metal surface of the container. Food and beverage products in the coated container have the potential of developing off flavors from the migration of trace impurities found in the coating materials.

Cyclodextrin can prevent the migration of the impurities from the can coating material into foods and beverages through inclusion complexation. The inclusion complexation with cyclodextrins occurs when a guest molecule enters the cavity of a cyclodextrin and becomes bound within the cyclodextrin. Once formed, the inclusion complex retains its guest molecule and prevents it from migrating out from the coating composition [56].

CONCLUSIONS

Cyclodextrins are chemically stable and can form inclusion complexes with various organic molecules. Several beneficial effects can be obtained by forming an inclusion complex with cyclodextrin. These effects include enhancing aqueous solubility for poorly soluble compounds; stabilizing labile compounds against heat, light, and oxygen; reducing or eliminating the volatility of compounds; and masking undesirable odor or taste.

Cyclodextrins can also be used as process aids to separate undesirable components or isolate desirable compounds from a system. The cyclodextrin and the material can be further separated, and the cyclodextrin can be reused.

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