Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2011, 3(6):822-828

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Role of Macromolecules in Chromatography: Cyclodextrines

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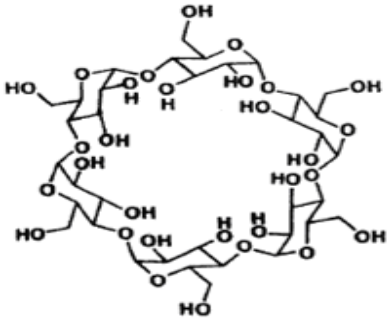
INTRODUCTION

Chromatographic separation techniques are principally based on the partition of analytes between stationary and mobile phases of different polarity. According to the characteristics of the stationary and mobile phases and the forces moving the mobile phase, chromatographic methods can be divided into gas solid (GSC), gas-liquid (GLC), thin-layer (TLC) and high-performance liquid (HPLC), supercritical fluid (SFC) chromatography and electrically driven separation methods, such as capillary electrophoresis (CE), capillary gel electrophoresis (GCE), micellar electrokinetic capillary chromatography (MEKC), capillary isoelectic focusing (CIEF), and capillary isotachophoresis (CITP)[1-3].

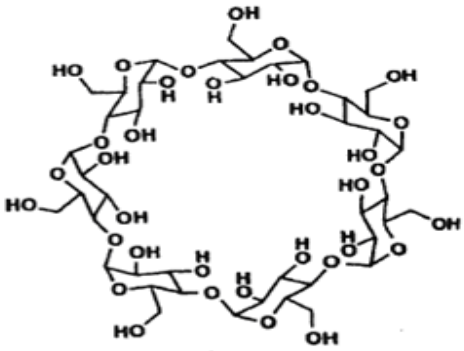
Cyclic oligosaccharides (cyclodextrins, CDs) can form inclusion complexes with a considerable number of organic and inorganic compounds. The formation of inclusion complexes can markedly modify the physicochemical parameters of the guest molecule (adsorption capacity, polarity, hydrophobicity, etc.). As the same physicochemical parameters govern the retention of analytes in a chromatographic system, the formation of inclusion complexes influences the retention behaviour of solutes. **As** the complex formation may modify the behaviour of individual analytes differently, the use of cyclodextrins in chromatography may result in modified retention behaviour and consequently better separation. As the strength of the complex formation between CDs and positional and optical isomers is generally different, the beneficial effect of CDs on the separation of these isomer classes is of paramount importance[4-6].

Cyclodextrins: Cyclodextrins (CDs) are cyclic, non-reducing oligosaccharides consisting of D-glucopyranose units bonded through α-1,4 linkages. The three major cyclodextrins are the following; the smallest is the α-CD (Schardinger's α-dextrin, cyclomaltohexose, cyclohexaglucan, cyclohexaamylose or C6A) with six glucose units, followed by ß-CD

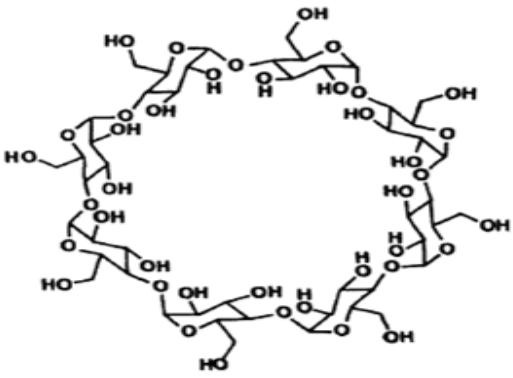
(Schardinger's ß-dextrin, cyclomaltoheptose, cycloheptaglucan, cycloheptaamylose or C7A) with seven glucose units and *γ*-CD (Schardinger's *γ*-dextrin, cyclomaltooctose, cyclooctoglucan, cyclooctaamylose or C8A) with eight glucose units. Unsubstituted native CDs are crystalline, non-hygroscopic, homogeneous substances, which are toruslike macrocycles built up from glucopyranose (glucose) units (Figure 1)[7-11].



α-Clodextrine



ß-Clodextrine



γ-Clodextrine

Figure 1 Schematic structures of α-, ß- and γ-Clodextrine

In CDs, the sugars adopt a $^4\mathbf{C_1}$ chair conformation and orient themselves so that the molecule forms a *toroidal truncated cone structure*. The cavity is lined by the hydrogen atoms and the glycosidic oxygen bridges. The non-bonding electron pairs of the glycosidic oxygen bridges are

directed towards the inside of the cavity to produce a high electron density and lend it some Lewis base character. The C-2 hydroxyl group of one glycopyranose unit can form a hydrogen bond with the C-3 hydroxyl group of the neighbouring glucopyranose unit. In the ß-CD molecule a complete secondary belt is formed by these hydrogen bonds, so that ß-CD is a rather rigid structure. This arrangement of hydrogen bonds can explain the observation that ß-CD has the lowest solubility of all native CDs. The lower solubility of ß-CD in water relative to α-and ***γ***-CDs may also be due to the marked structure of water arising from water-ß-CD interactions[12-15].

The hydrogen belt is incomplete in the α-CD molecule because one of the glycopyranose units is in a distorted position; therefore, instead of the six possible hydrogen bonds, only four can be formed. The γ-CD molecule has a more flexible structure and consequently is the most soluble of the three native CDs. The equilibrium constants for hydrogen-deuterium exchange in the secondary hydroxyl groups of α-**,** ß- and γ-CDs also indicate that the strongest hydrogen bond system is formed in the B-CD molecule.. Because of their torus-like geometry, relatively hydrophobic surface of the internal cavity and the hydrophilic character of external hydroxyl groups (Figure **2),** CD molecules easily form inclusion complexes with a wide variety of organic and inorganic molecules. This complex-forming capacity is the reason for their widespread application in chemistry and in separation science (Table 1)[16-19].

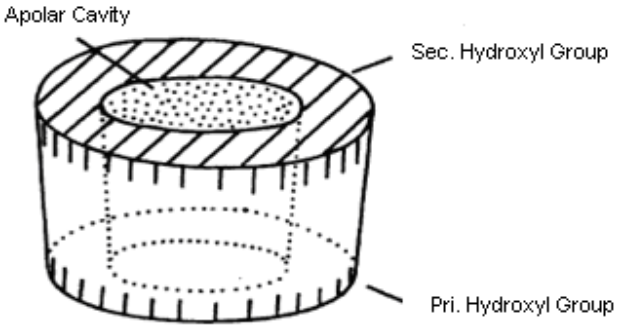


Figure 2 Functional scheme of Cyclodextrins torus.

Table 1 Use of Cyclodextrins in analytical separation methods.

Sr. No.	Chromatographic Method	Mode of Use of CDin the system
1	**GSC**	Deposit on inert support or immobilized
2	**GLC**	Chemically bonded selective component of a liquid stationary phase
3	**HPLC**	Chemically bonded stationary phase
4	**TLC**	Additive to the mobile phase
5	**SFC**	Chemically bonded stationary phase
6	**CZE**	Adddve to the background electrolyte
7	**MEKC**	Charged CD derivatives as transport agentdadditive to micellar electrolyte systems
8	**ITP**	Additive to the leading electrolyte

Chemistry of Cyclodextrins:

1. Selector Properties: As has been frequently demonstrated that cyclodextrins are capable of forming inclusion complexes with compounds having a size compatible with the dimensions of the cavity. Inclusion complexes are entities comprising two or more molecules; the ***'host'*** includes a ***'guest'*** molecule, totally or in part, by only physical forces, that is, without covalent

bonding. CDs are typical host molecules and may include a great variety of molecules having the size of one or two benzene rings, or even larger compounds, which have a side chain of comparable size, to form crystalline inclusion complexes. Complexation occurs when there is a steric compatibility between the CD cavity and the guest molecule and the affinity of the guest molecule for the CD cavity is higher than for the other components present ***(i.e.*** solvent). It has been established that, besides steric compatibility, hydrophobic interactions: *vander Waals interactive forces and hydrogen bonding* independently or in combination play a considerable role in the determination of the strength of inclusion complexes. The stoichiometry of inclusion compounds is usually 1:l ; however, complexes can be made of two or more guests (especially with the large γ-CD cavity) or with several CD molecules by the inclusion of different parts of a large guest molecule.

CDs have been effectively employed for chiral separations and for the improvement of a large number of separation processes in many chromatographic techniques, either as mobile-phase additives (dissolved in the mobile phase, as modifiers) or as stationary phases or stationary-phase additives. CDs offer numerous advantages. They are thermostable to a reasonable temperature which is important in GC; furtheremore, they are stable over very wide pH range (2-12) and do not absorb radiation in the region normally associated with *UV* detection (200-350 nm) facilitating their application in liquid chromatographic techniques[20].

2. Solubility of Cyclodextrins: The water solubility of native cyclodextrins shows anomalous behaviour. The solubility of ß-CD is only 1.85 g/lOO mL at ambient temperature, whereas the solubilities of α-CD and γ-CD are significantly higher, 14.5 g/100 mL and 23.2 g/lOO mL, respectively. In the presence of organic molecules, the solubility of native CDs generally decreases owing to complex formation[21].

3. Dielectric Properties: Dielectric constants of CD cavities have been established from the change in the fluorescence properties of occluded pyrene-3-carboxaldehyde. The estimated dielectric constants of CD cavities have been calculated as 48 and *55* for *ß*- and γ-CDs, respectively. These values can be explained by the assumption that the larger γ-CD cavity can possibly include more water molecules, thus providing a larger effective dielectric constant. The dielectric constants of native CD cavities on incorporating the toluidinyl group of 6-p-toluidinylnaphthalene-2-sulfonate at pH 5.3 and 25 ^{0}C have also been determined and were found to be 47.5, 52.0 and 70.0 for α-CD, *ß*-CD and γ-CD, respectively. It has been further established that the dielectric constant of the CD cavity depends not only on the inner diameter of the cavity but also on the size of the included guest molecule[22].

4. Thermal Properties: CDs and CD derivatives have been extensively used as stationary phases or stationary-phase additives in GC. As GC analyses are generally carried out at elevated temperatures, an exact knowledge of the thermal stability of CDs and CD derivatives under the GC conditions is of paramount importance. A considerable number of thermoanalytical methods have been employed for the study of thermostability of CDs and their corresponding inclusion complexes. The thermal degradations of native cyclodextrins and substituted ß-cyclodextrins were characterised by hot-stage microscopy (HSM), differential scanning calorimetry (DSC), thermogravimetry (TG) and X-ray powder diffractometry (XRD). The measurements indicated that cyclodextrins have no well-defined melting point, but from about 197 ^{0}C they begin to

decompose. It was further found that in an inert atmosphere each of them decomposed in a single major step (250-400 ^{0}C*)* leaving a residue which is thermally quite stable at higher temperatures. Furthermore, it has been frequently shown that the thermal stability of CD derivatives depends on the type, location and number of any substituents. In the case of amino- and phosphate-substituted cyclodextrins, the first degradation step is the same as for native CDs, then volatile by-products are formed by oxidation below 600 ^{0}C, the residue being a ceramic-like substance stable to 800°C. The charring process involves opening the CD rings followed by a chemical decomposition similar to that of cellulose with loss of the glucosidic structure and hydroxyl groups and a build up of carbonyl groups and aromatic structures. Carbon dioxide, water, levoglucosan and furans are the major volatiles evolved from cyclodextrin degradation in the same manner as from cellulose. Products derived from substituted CDs show that they do not simply behave as leaving groups but rather take part in the charring process.

Although chromatographic techniques mainly employ derivatised CD and not native CDs the number of studies dealing in detail with the thermal properties of CD derivatives is surprisingly low. This phenomenon is probably because CDs are used for practical purposes in chromatography (to improve separation of enantiomers and other hard to separate analytes) and the exact determination of the thermal properties of CDs has been considered as of secondary importance[23].

5. Chemically Modified Cyclodextrins: In CDs every glucopyranose unit has three free OH groups, two of which (on C-2 and C-3) are secondary and one (C-6) primary. As each of these free hydroxyl groups can be modified, by substituting the hydrogen atom or the hydroxyl group by a wide variety of substituents, the majority of simple synthetic reactions results in a considerable number of positional isomers. Hydroxyl protons can be exchanged by deuterium at the oxygen-hydrogen bonds or carbon-hydrogen bonds in *deuterated CD derivatives*. These molecules represent the smallest group of CD derivatives and have found application only in NMR studies of CDs.

- Some inorganic esters of CDs, such as nitrates, sulfates, phosphates ***etc.*** have also been synthesised; however, the organic (acetyl, benzoyl, propionyl, methyl and carbamoyl) esters have been more frequently used for practical industrial and analytical purposes.
- Ether derivatives are the most important CD derivatives from a practical point of view. These compounds can be prepared either by direct reaction with an alkylating agent or *via* an intermediate, such as sulfonate esters or deoxyhalogeno derivatives. This group equally contains neutral, anionic and cationic derivatives as well as silyl ethers.
- Deoxy cyclodextrins can be classified according to the mode of preparation into two groups: intermediaries which can be further derivatised, and end products containing thio-, amino-, substituted amino- or azido-substituents.
- Branched cyclodextrins (or second-generation cyclodextrins) can also be obtained by chemical synthesis but in most cases they are prepared by enzymatic reactions. Branched CDs can be divided into two categories. Homogeneous branched cyclodextrins have only glucose or malto-oligosaccharide side chains bound to the native CDs. Heterogeneous branched CDs have one or more galactose or mannose residues bonded either to each other or directly to the parent cyclodextrin rings. The solubilities of branched cyclodextrins in water, even in aqueous 80% ethanol or in aqueous 50% solutions of methanol, formaldehyde and ethylene glycol, are extremely high in comparison with their parent CDs.

It is well known that derivatisation changes the physical and chemical properties of the CDs, modifying their solubility, complex-forming capacity, thermal properties and chemical stability. Numerous CD derivatives have found application in chromatography. The aim may be the improvement of the solubility of the native CDs making possible an increase in their concentration in a chromatographic mobile phases, the enhancement of the stability of the host guest inclusion complexes by modifying the reactivity andor mobility of the guest molecule. Furthermore, derivatisation can be employed for the formation of insoluble and/or immobilised CD-containing structures, which can be used as stationary phases for liquid and gas chromatography[24].

6. Cyclodextrin Polymers: These compounds consist of two or more cyclodextrin rings covalently bonded to each other using a spacer molecule. According to the synthetic pathway they can be classified into *linear polymers*, which are generally water soluble, and *crosslinked polymers*, which are water insoluble. The water soluble CD polymers have a higher solubility than their parent cyclodextrins. Many examples have been reported of the solubilising effect of cyclodextrin polymers for poorly soluble drugs. As with native and derivatised CDs, these polymers have also found applications in chromatography. Water soluble polymers have been used as mobile phase additives in liquid chromatographic techniques and in electrically driven separation[25-28].

CONCLUSION

Cyclodextrin-base is the most popular materials used for the chiral resolution of racemic compounds. These Cyclodextrin-base (*Chiral Stationary Phase)* have a wide range of applications because they can be used successfully in all three mobile phase modes: normal, reversed, and polar organic. There are numerous examples of chiral separations on Cyclodextrin and Chiral Stationary Phase based on their derivatives. β-cyclodextrins are used to produce HPLC columns allowing chiral enantiomers separation. Cyclodextrins are able to form host-guest complexes with hydrophobic molecules given the unique nature imparted by their structure. As a result, these molecules have found a number of applications in a wide range of fields. Other than the above mentioned pharmaceutical applications for drug release, cyclodextrins can be employed in environmental protection: these molecules can effectively immobilise inside their rings toxic compounds, like trichloroethane or heavy metals, or can form complexes with stable substances, like trichlorfon (an organophosphorus insecticide), enhancing their decomposition. The ability of cyclodextrins to form complexes with hydrophobic molecule has led to their usage in supramolecular chemistry. In particular they have been used to synthesize certain mechanically-interlocked molecular architectures, such as rotaxanes and catenanes, by reacting the ends of the threaded guest. The application of cyclodextrin as supramolecular carrier is also possible in organometallic reactions. The mechanism of action probably takes place in the interfacial region. The application of cyclodextrins as supramolecular carrier is possible in various organometallic catalysis.

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