Fourier Transform Infrared Spectroscopy

Complex formation may be supported by IR spectroscopy either in the solid or the solution state in some cases, although the application of IR is limited to guests having characteristic absorption bands, such as carbonyl or sulfonyl groups. For example, the carbonyl stretching bands (*ca.* 1700 cm^{-1}) of parabens, are shifted about 40 cm^{-1} to a higher wave number by CD complexation since the intermolecular hydrogen bonding of the guests is ruptured, and the guests are dispersed in the CD cavity (Uekama et al. 1980). The band at 1690 cm^{-1}, characteristic of 4-biphenylacetic acid carbonyl stretching, appears as a lesser intensity band, also shifted to 1710 cm^{-1} in the inclusion complex sample with β-CD, due to the formation of a hydrogen bond between the secondary hydroxyl groups in β-CD and the carbonyl oxygen of the guest (Puglisi et al. 1990). In most cases, however, no change due to complex formation can be observed. Bands due to the included part of the guest molecule do shift or their intensities are altered, but since the mass of the guest molecule does not exceed 5%–15% of the mass of the complex, these alterations are usually obscured by the spectrum of the host. Therefore, no useful information can be obtained.

X-ray Crystallography

X-ray crystallography should be the ultimate tool for understanding crystalline complex structures. Many crystalline structures of complexes formed by natural CDs have been reported (Connors 1997; Caira and Dodds 1999). This technique is less useful for studying complexes formed with CD derivative since almost all the complexes formed with water-soluble CD derivatives are amorphous.

Nuclear Magnetic Resonance

NMR can be used in both solution and solid state. ^{1}H and ^{13}C-NMR have been used for studying space conformation of β-CD inclusion, and the formation of an inclusion complex can be evidenced by peak shifts of some of the drug protons and of the CD protons (Demirel et al. 2011). For the complexation between ketoconazole and β-CD, an upfield shift (0.082 ppm) in ^{1}H NMR spectroscopy of freeze-dried products confirmed that the aromatic groups of Ketoconazole were interacting with the β-CD.

COMPLEX FORMATION

Whether or not a compound can form inclusion complexes with CDs largely depends on the compound's size compatibility with the dimensions of the CD cavities. The stability of a complex also depends, however, on other properties of the guest molecule, such as its polarity. Compounds used medicinally usually are large molecules. Therefore, it is very commonly observed that the complexes form such that only certain groups or side chains penetrate into the carbohydrate channel.

The role of molecular dimensions is well demonstrated by complex formation with halogenated benzenes (Cohen and Lach 1963). 1:1 Complexes may be prepared from chloro-, bromo-, and iodobenzenes, but from chlorobenzene only with α-CD; from bromobenzene with α- and β-CDs, and from iodobenzene with β- and γ-CDs. A 1:2 (guest:CD) complex may be formed when the guest molecule is too large to find complete accommodation in one cavity and its other end is also amenable to complex formation. Some examples are the complexes of β-CD with prostaglandins, vitamin D3, and indomethacin.

Geometry, however, certainly is not the sole factor determining the stability of a complex. Antazoline and adiphenine should exhibit similar affinities for β-CD if only molecular dimension is considered. However, β-CD binds antazoline nearly twice as strongly as it does adiphenine. Cortisone acetate and testosterone also have common structural features. A similar affinity to β-CD would be expected, yet there is a definite preference for testosterone. The bulky side chain in cortisone acetate which encumbers the 17-hydroxyl group may be responsible for its low affinity for β-CD (Lach and Pauli 1966).