Journal of Advances and Scholarly Researches in Allied Education
Vol. 19, Issue No. 4, July-2022, ISSN 2230-7540

DOI:

The Synthesis and Characterisation of Hydroxypropyl Cyclodextrin Inclusion Complexes in Certain Medications and Fluorophores are Compared

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Abstract: Pharmaceutical and biochemical researchers have shown great interest in the development and study of hydroxypropyl cyclodextrin (HP-β-CD) inclusion complexes because of their potential to improve the solubility, stability, and bioavailability of different drugs and fluorophores that are not very water-soluble. This research compares the methods of synthesis, efficiency of encapsulation, and physical and chemical characteristics of HP-β-CD inclusion complexes made with various drugs and fluorophores. We synthesised inclusion complexes using kneading, freeze-drying, and co-precipitation procedures. Then, we characterised them using spectroscopic methods including FTIR, NMR, and UV-Vis, as well as thermal (DSC, TGA), and crystallographic (XRD) investigations. The effective encapsulation was confirmed by the creation of inclusion complexes, which resulted in notable alterations in spectral patterns, thermal stability, and crystalline structure. Complexation with fluorophores increased photostability and fluorescence intensity, whereas medications exhibited higher water solubility and regulated release behaviour. While the inclusion method stays the same (via non-covalent contacts within the hydrophobic cavity of HP-β-CD), the comparison analysis shows that the guest molecule's structural structure and polarity determine the level of complexation and functional enhancement. These results highlight the flexible use of HP-β-CD as a host molecule in drug delivery systems and fluorescence applications, which bodes well for its potential in pharmaceutical and biomedical formulations.

Keywords: Synthesis, , Characterisation Hydroxypropyl Cyclodextrin Medications, Fluorophores

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INTRODUCTION

The cyclic oligosaccharides known as cyclodextrins (CDs) may form inclusion complexes with various guest molecules. They are incredibly adaptable molecular hosts. The pharmaceutical and biological fields have taken notice of hydroxypropyl-β-cyclodextrin (HP-β-CD), a derivative of β-cyclodextrin, because of its improved water solubility, decreased toxicity, and higher complexation efficiency. The physicochemical characteristics of substances that are ordinarily weakly soluble can be enhanced by encapsulating them in cyclodextrins, thanks to their distinctive structural feature of a hydrophobic interior chamber and a hydrophilic outside surface. Because of its capacity to incorporate other substances, HP-β-CD is a good choice for use in medication administration systems and for improving the stability and performance of fluorophores in analytical and diagnostic procedures (Henriques, M. 2014). Especially in the realms of sensitive imaging technologies, targeted treatment, and contemporary drug development, the significance of these inclusion complexes has skyrocketed. Improved solubility, bioavailability, stability, and controlled

release behaviour for pharmaceuticals may be achieved by encapsulating pharmacological substances and fluorescent dyes using HP-β-CD, which forms non-covalent host-guest relationships. Dye encapsulation results in enhanced fluorescence intensity and photostability.

Low bioavailability and therapeutic ineffectiveness are consequences of many APIs' poor water solubility, a problem that the pharmaceutical industry has long battled. In addition to introducing toxicity or being incompatible with the human biological milieu, traditional methods of solubilisation such salt creation, pH adjustment, and the use of co-solvents frequently fail to offer long-term stability. On the other hand, HP-β-CD provides a safe and non-harmful method to enhance the solubility of drugs without altering their chemical composition. Numerous studies have shown its usefulness in increasing the solubility of hydrophobic pharmaceuticals, including anti-inflammatory agents, antifungal chemicals, and cardiovascular treatments. Patient compliance and treatment results can be greatly improved when the guest drug's physicochemical behaviour undergoes complexation. This includes changes to its dissolution rate, permeability, taste, and odour, among other things. Moreover, it is crucial for the management of chronic diseases and the reduction of dosage frequency that HP-γ-CD forms reversible inclusion complexes, which enable the drug's prolonged or regulated release (Szejtli, J. 1998).

Fluorophores are essential in molecular diagnostics, bioimaging, and environmental sensing, all of which rely on fluorescence-based detection and imaging. Nevertheless, a lot of fluorophores have issues with photobleaching, insolubility in water, and instability in living things. In order to overcome these restrictions, the encapsulation of fluorophores within HP-β-CD inclusion complexes has demonstrated encouraging outcomes. In addition to enhancing solubility, HP-β-CD inhibits photodegradation and undesirable interactions with external quenchers by creating a protective milieu surrounding the fluorophore. Quantum yield and fluorescence lifetime are two examples of the improved fluorescent characteristics. This is especially helpful in biomedical settings where signal strength and stability are paramount, such flow cytometry, fluorescence microscopy, and FRET-based tests. The complexation also causes the fluorophore's absorption or emission spectra to change, which gives designers more leeway when making multi-fluorophore detection systems (Robert, O. 2003).

Common methods for synthesising HP-β-CD inclusion complexes include co-precipitation, kneading, freeze-drying, and solvent evaporation. The guest molecule's properties and the complex's intended application dictate the preferred technique, each of which offers its own set of benefits. Preliminary experiments frequently employ co-precipitation and kneading because they are easy and inexpensive. However, complexes that are appropriate for pharmaceutical use are produced by freeze-drying, which is known for its great stability and purity. The effectiveness of complex formation is highly dependent on the solvent system, temperature, and pH that is chosen. After synthesis, the inclusion complexes are studied by a battery of analytical methods to determine if complexation has occurred and, if so, how the materials behave structurally and thermally. Common techniques used in this field include FTIR, NMR, DSC, TGA, XRD, and UV-Visible spectroscopy (ultraviolet), powder X-ray diffraction, and thermogravimetric analysis. These methods are useful for detecting the telltale signs of successful inclusion, which include alterations to thermal characteristics, optical behaviour, crystalline structure, and molecular vibrations (Uekama, K. 1997).

STRUCTURE AND CHARACTERISTICS OF CYCLODEXTRINS

The cycling oligosaccharides that make up cyclodextrins are α-(1,4) glucopyranose subunits. Schardinger 1 also places them in the category of cycloamylases, cyclomaltoses, and dextrins. Degradation of the cyclodextrin glucanotransferase (CG Tase) enzyme results in its formation by intramolecular transglycosylation. The three primary CDs share a torus with Macro-Rings and are crystalline, homogeneous, non-hygroscopic solids composed of glucopyranose units. There are six glucopyranose units in α-cyclodextrin, seven in β-cyclodextrin, and eight in β-cyclodextrin (Hedges, A. R. 1998).

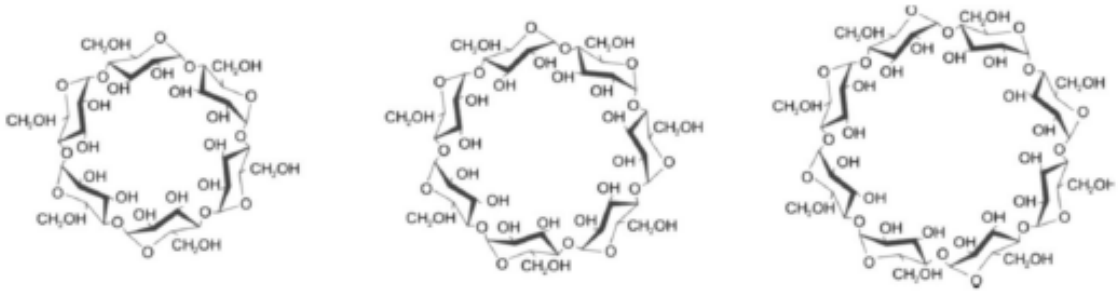

Figure 1: Chemical structure of α-, β- and γ-cyclodextrins

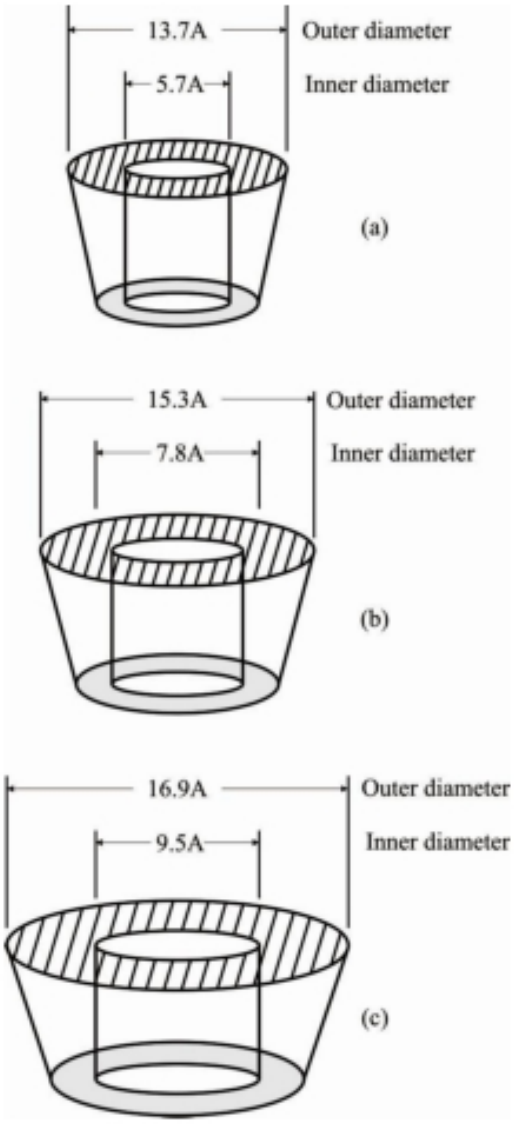

Figure 2: Shape of (a) α-cyclodextrin (b) β-cyclodextrin and (c) γ-cyclodextrin

Table 1: Properties of Cyclodextrins

Property	α- cyclodextrin	β- cyclodextrin	γ- cyclodextrin
Number of glucopyranose units	6	7	8
Molecular Weight (g/mol)	972	1135	1297
Solubility in water at 25°C(%W/V)	14.5	1.85	23.2
Outer diameter (Å)	14.6	15.4	17.5
Cavity diameter (Å)	4.7 – 5.3	6.0 – 6.5	7.5 – 8.3
Height of tours (Å)	7.9	7.9	7.9
Cavity volume ($Å^3$)	174	262	427

Cyclodextrins have main hydroxy groups (C6) on one side of the band and Apolar C3 and C5 hydrogeals and etheral oxygen on the other. The X-ray structures point to these groups being positioned on the ring's narrow edge. The outcome is a microheterogeneous environment known as a hydrophobic matrix, created when an apolar cavity dissolves a hydrophilic exterior molecule (Chen, Y. 2002). Countless crystal structural investigations have provided evidence for cyclodextrins in solution. The more typical type of cyclodextrin crystallisation is a cage structure, although channel structures are also possible. The crystal structures show that the expected 'round' shape is adopted by complexes of cyclodextrins with all glucopyranoses. Additionally, studies with linear maltohexaoses, a parallel double helix, have demonstrated that α-cyclodextrin exhibits the highest degree of steric strain due to cyclisation.

INCLUSION COMPLEXES

It is interesting to note that cyclodextrins have the inherent capacity to form inclusion complexes with various compounds9–13. Solid inclusion complexes (host-guest complexes) can be formed between cyclodextrins and a wide range of molecular complexes in solid, liquid, and gaseous states1. A guest molecule is contained in the cavity of the cyclodextrin host molecule in these molecules. Molecules that are both hosts and guests undergo dimensional change during complex formation. Complex inclusions can be accessed by non-polar moieties of enough size through the cyclodextrin lipophilic cavity's microwave (Goselin, P. 2000). The development of the inclusion complex does not involve the breaking or formation of any covalent bonds. The vast majority of the enthalpy-rich cavity water molecules are being released during complex formation. Reduced cyclodextrin ring strain is the outcome of an apolar-apolar interaction made possible because hydrophobic guest molecules displace water molecules in the solution.

Rather from being a one-way street, binding of guests to host cyclodextrin is an ongoing process. Both the stability of the host-guest complex and the selectivity of the local interactions between surface atoms determine the degree of binding. Complexes can be formed in either solutions or crystals; however, solutions are best dissolved in water. When deciding whether cyclodextrin may form complexes, two factors are critical. A steric component that, cyclodextrin-dependent, does not outweigh the guest molecule or certain functional groups of your visitor by an excessive amount. The findings will be inaccurate if the visitor is either too short or too tall, as the cyclodextrin cavity calibration depends on their height. The second most important component is the thermodynamic interaction among the various components (cyclodextrin, guest, solvent). A net energetic driving force that is favourable to the complex's development must be produced in order to get the visitor into cyclodextrin (Nigam, P. 2001).

- Translocation of polar water molecules including apolar cyclodextrin.
- More hydrogen bonds are formed as the displaced water flows back into the bigger lake.
- The visitor's inherent fear of water and aquatic life will be mitigated in this manner.
- The apolar cyclodextrin cavity is reached by the visitor through an increased contact of the hydrophobics.

This cavity allows for the integration of several hydrophobic guest molecules into cyclodextrins. One, two, or three cyclodextrins can hold one or two complementary molecules. The "host" and "guest" molecules in an inclusion complex are actually one molecule each. When exposed to water, the host's cavity can accommodate molecules or fragments of microphobic substances. Medications in crystalline CD complexes

are also not always stoichiometric. Due to the lack of covalent connections between the host and guest molecules, the cyclodextrin physiological complexes dissolve rapidly (Santimone, M. 2001).

FACTORS AFFECTING THE COMPLEXATION OF CYCLODEXTRINS TO HOSTS/DRUG MOLECULES"

A number of variables affect the process of cyclodextrin/drug complex formation, including

Types of CD

There will be interference between the kind of CD and the formation and efficiency of includes complexes. In their study on various CDs, including beta cyclodextrin (β-CD), hydroxypropyl beta cyclodextrin (HP β-CD), and methyle beta cyclodextrin (Mβ-CD), Castillo et al. discovered that these substitutes are more soluble than beta cyclodextrin 30. This finding has implications for numerous drugs, including albendazole, mebendazole, and ricobenzole. The impact of α-CD, -CD, α-CD, and HP-CD on Fenuprofen was shown to be most stabilised at -CD and HP-CD complexes, according to Diaz et al. (31). Mura and colleagues supposedly found that Mβ-CD, instead of β-CD 32, enhanced the efficiency of Ketoprofen solubility. In their study, Nesna et al. discovered that some CDs, specifically α-CD, β-CD, and β-CD, had a strong preference for binding to Cocaine in water (Martyn, M. A. 1995).

Cavity Size

In order for the medicine to fit inside the α-CD, the cavity needs to be sufficiently changed. The cave size of -CD as a gliclazide compound has been studied by Arias-Blanco and colleagues, however the size of α-CD as a Gliclazide ring34 is inappropriate. Ueda et al. investigated the impact of the ɘ-CD on digitoxin and discovered that the cavity's interior contributes to a partial enhancement of solubility. Akasaka and his colleagues have demonstrated, via studying the effects of macro-cyclical compounds such as α-CD, β-CD, µ-CD, and Ś-CD, that reasonably stable molecules may be obtained by shrinking α-CD, CD, and bigger µ-CD and β-CD36 macrocyclic complexes. In their study, Mura et al. examined how α-CD, -CD, and Ţ-CD affected ibuprofen. They discovered that -Cd, α-CD, and α-CD were the most effective in increasing the drug's dissolving rate. The solubility of the medications has been observed to decrease due to the absence of the promethazine ring in a CD cavity, according to Lutka and colleagues' studies on the interaction between prochloro-methazine and γ-CD, HP γ-CD, and Dimethyl beta cyclodextricine (DM γ-CD) (Stalcup, A. M. 2000).

Effect of Methods of Preparation

The phenomena of drug complexation with CDs are affected by preparation procedures. Several techniques may be employed to modify the drug-CD complexation, such as co-grinding, kneading, solid dispersion, solvent evaporation, co-precipitation, sprinkling, and freezing. The drug and CD qualities determine how well the complexing works. For medications like Albendazole, Mebendazole, Ribendazole, and Ketoprofen with CDs like -CD, HP -CD, and M-CD, Castillo and colleagues found that a freeze-drying approach alone was far more successful than a co-precipitation method for complex formation. In contrast to kneaded precipitation and sealed warming, Mura et al. found that ketoprofen with -CD and DM -CD exhibited greater dissolution. Researchers from Mura et al. were able to examine how α-CD, β-CD, and œ-CD

affected ibuproxam. They found that kneading was ineffective, whereas spray drying and sealed heat treatments improved complexation when treating α-CD, β-CD, and œ-CD. When the ratio is adjusted to 1:440, solid dispersion accomplishes complexation more efficiently than kneading or spray secretion, respectively. Methoxybutyrate, HP-CD, and -CD have all been documented by Palmieri et al (Connors, K. A. 1997). In contrast to kneaded studies and the spray-drying study, Moyano et al. found that DMβ-CD significantly enhanced the solubility of oxyazepam in their testing. Pose-Vilarnovo and colleagues are using the freeze-drying technique to observe a higher dissolution rate for sulfamethoxazole complexes integrated with β-CD and HPβ-CD. The product of improved solubility for grinding, physical mixing, and kneading was discovered when Mitrevej et al. examined the impact of β-CD on Glibenclamide. The research conducted by Senoferjan et al. on the development and assessment of tenoxicam β-CD shown superior efficacy in dissolving in water and neutralising complex compounds compared to the traditional methods of solvent and kneading.

pH and Ionisation State

Research conducted by Nagase and colleagues has shown that DY-9760E and the sulfobutyl-ether beta cyclodextrin (SBE—CD) exhibit substantial interactions in the acidic area at pH~445. Jain et al. found that NSC-639829 had an increased solubility at pH~1 when treated with SBE—CD. The solubility of ETH-615 with spontaneously mechanised beta cyclodextrin (R M -CD) (HTMA -CD) was enhanced in a research by Loftsson et al. including a number of CDs, including HP-CD, RM-CD, SBE—CD, Carboxy methyl beta cyclodextrin (CM-CD), and 2-hydroxy3-trimethyl-ammony beta cyclodextrin (CD). The complex stability constants are low with the very polar drug at pH~5, which means it can't penetrate the cavity as well for CD, but they're high with the less polar anionic version at pH~1047. In their study 48, Dalmora and colleagues showed that -CD may be successfully complexed at low pH. This is shown by piroxicam. McCandless et al. discovered that at pH ~4, the solubility of Levemopamil HCl (in mg/mL) is increased thrice (7.88 to 25.62), and at pH ~10.6, it is three25 times more soluble (0.0036-1.37). According to Kim and colleagues' study on Ziprasidone mesylate with SBE-CD, complexion preferred ion-pair over dissociated ion form50. In their investigations with Sulindac and CD, Tros de Ilarduya et al. verified that complexation with non-ionized drug 51 is simpler. In the inclusion complex formation of Mebendazole and HP-CD, Diaz et al. found that the unionised form was less protected than the ionised form (Stella, V. J. 2003).

Temperature

Temperature variation has a minor influence on the stability constant44, according to Nagase and colleagues' study of the effect of SBE-α-CD on DY-9760E and Sulfobutyl-ether-beta cyclodextrin. The apparent stability constant of the Sulindac-α-CD inclusion complex was shown to diminish as the temperature increased, according to Tros de Ilarduya et al. Zarzycki and colleagues found that when phenolphthalein was bound to α-CD, the association constant decreased as the temperature decreased. According to Jain et al., when the temperature increases, the complex's stability reduces.

Degree of Substitution

Complexation, as well as the shape and quantity of substitutions on the parent CD molecule, can have a

substantial impact on the physico-chemical properties of CDs. An HP-β-CD or any other β-CD derivative is not unique in terms of the "degree of substitution." Due to the possibility of hydroxypropyl groups occupying different locations, the physico-chemical properties of samples with the same level of HP-β-CD substitution may differ from those of the parent CD molecule under various circumstances. You need to understand the following terms used to identify CD cleanliness since they have a significant impact on the ultimate quality and marketability of the medicinal product.

THERMO-ANALYTICAL METHODS”

The most common thermo-analytical methods for assessing the production of CD-like complexes are differential scan calorimetry and thermogravimetry. Because they are both simple and time-consuming, these strategies are usually tried and true in complicated training assessments.

Differential Scanning Calorimetry

Differential calorimetry (DSC) CD curves show endothermic events that are consistent with dehydration. The number of instances for α-CD is two or three, and the crystalline type determines the size of the peak at 120°C for β-CD and 150°C for β-CD. A complex formulation that has been studied and includes the guest molecule and the physical combination (CD + the guest molecule) may be compared to the DSC curve. A small temperature peak, corresponding to the guest molecule's melting point, is visible in its crystal structure. The melting point of the guest molecule and CD dehydration form its physical mixture curve. The encapsulation-induced crystalline structure will either enlarge, alter, or disappear in the complex formation 116. This melting peak's existence indicates that the sample has at least one free host molecule; during partial complexation, the complex of this peak should decrease from the physical mixture, allowing the medication to interact with the CD.

There are situations where the physical mix does not result in the guest molecule's typical melting. To put it simply, the guest molecule can get amorphized when a CD complex is heated. A false impression of growth and motion can be produced by the guest molecule's weak interactions with CD. A change in energy state occurs as a result of a shift in the dehydration event of CDs in the complex DSC curve, which occurs when water molecules are replaced by guest molecules in the cavity (Frioriksdottir, H. 1993).

Thermogravimetric Analysis

Thermogravimetry (TG) in a nitrogen environment causes an initial weight loss from evaporation of adsorbed and crystallised water, according to the Natural CD. Degradation between 250 and 400°C accounts for a second weight loss of 70 to 80%, according to the CD. It is common practice to compare the complex's degradation rate to the initial (start) temperature of degradation while looking for TG inclusion complex formation. Since the medicine is CD-protected, it is anticipated that the guest molecule decays at higher temperatures during complexation.

Scanning Electron Microscopy

The product created by co-precipitation/evaporation and the source material (cyclodextrin and guest compounds) are analysed microscopically using Electron Scanning Microscopy (SEM). The results of

scanning electron microscopy (SEM) investigations on retinoic acid have shown, for instance, that although β-cyclodextrin crystallises in a large amount of polyherded retinoic acid as needles or elongated crystals, SEM cannot confirm the formation of the inclusion complex but can locate the substance's presence. The morphological examination, which includes measuring the size and form of the medication particles, was carried out using scanning electron microscopy (SEM). The shape of Ketoprofen particles, β-cyclodextrin (β-CD), physical combinations, and solid complexes was evaluated using SEM photos, for example. Ketoprofen often crystallised into plate-like clumps. In the solid state, there is a clear correlation between the morphological change and the transition to crystalline nature observed in 1:1 freeze-dried, co-precipited, and kneaded products from both HPβCD and β-CD. The last step was to use scanning electron microscopy (SEM) to examine the morphologies of both the pure medication and the carriers in combination.

X-ray Diffractometry

By using X-ray diffraction (XRD), the formation of an integration complex in a solid state may be detected. In fact, in order to compare diffractograms, the cyclodextrin and guest molecules must be treated in the same way as the assumed complex. This is because the inclusion of cyclodextrin changes the crystalline structure and causes different diffraction patterns due to the complex preparation procedures, including grinding and freezing. Some peaks are created or altered, while others are sharpened, as a result of dynamic formation. As an example, the neutralisation technique was used to create inclusion complexes for Naproxen. Compared to the physical mixture or pure component, the amorphous complex formation exhibits a less pronounced disappearance of some peaks. An example would be the β-CD spray-drying complexes of acetaminophen, Indomethacin, piroxicam, and warfarin, as well as the β-CD dried gel complexes of naproxen.

Single Crystal X-ray Structure Analysis

The inclusion's precise structure and interaction mode are defined by the structural study of the crystal X-ray units. Indeed, a geometric connection may be defined by describing the interaction of host and guest molecules. New understandings on the evolution of integration complexes are aided by this study (Irie, T. 1998).

Fourier Transform Infra-Red Spectroscopy

Infrared spectroscopy is used to evaluate the solid-state interactions between the visitor and the cyclodextrin molecules. Cyclodextrins rapidly obstruct any potential bands for that part of the molecules, and the complex encapsulates less than 25% of the host molecules. Guests with certain distinguishing bands, such carbonyl or sulfonyl groups, are not allowed to use the infrared spectroscopy. The influence on the stretch band's vibration is, thus, most strongly influenced by the hydrogen bond to the hydroxyl group. Further evidence from inclusion complexing points to hydrogen bond breakage as a mechanism for the absorbent bands' higher-frequency mobility. The ester characteristic of the 1183 cm-1 to 1206 cm-1 observed region is present in the β-CD complex and dimethyl-β-cyclodextrin, as is the displacement of the aromatic carbon atom from 1272 cm-1 to 1296 cm-1. Elongation of OH, NH, etc., causes hydrogen bonds to form. The result was a dampening of the prolonged vibration's frequency. As an example, by complexing

Piroxicam with γ-CD, the band shifted to 1154 cm-1 at 1180 cm-1.

CONCLUSION

The research on inclusion complexes involving hydroxypropyl cyclodextrin (HP-β-CD) and certain drugs and fluorophores shows how HP-β-CD may improve the physical and chemical characteristics of various guest molecules. This sentence shows how HP-β-CD can be used for many different things since inclusion complexes can be successfully synthesised using typical procedures including kneading, freeze-drying, and co-precipitation. The presence of stable complexes has been verified by spectroscopic, thermal, and crystallographic characterisation, which has revealed significant changes in solubility, thermal behaviour, and crystal structure. Improving bioavailability and therapeutic efficacy, encapsulation of pharmaceutical substances inside HP-β-CD results in greater water solubility, higher stability, and, in certain instances, enhanced controlled release. Complexes containing fluorophores are advantageous for imaging and diagnostics because inclusion improves photostability and fluorescence emission. While hydrophobic interactions and host-guest compatibility form the basis of the inclusion process, the study found that the encapsulated compound's unique chemical composition and polarity determined the extent to which performance was enhanced. Hence, the wide range of pharmaceutical and biomedical applications of HP-β-CD is supported by its efficiency and robustness as a platform for the development of drug delivery systems and fluorescence-based technologies.

References

1. Pinho, E., Grootveld, M., Soares, G., & Henriques, M. (2014). Cyclodextrins as encapsulation agents for plant bioactive compounds. Carbohydrate Polymers, 101, 121–135.
2. Szejtli, J. (1998). Introduction and general overview of cyclodextrin chemistry. Chemical Reviews, 98, 1743–1753.
3. Thorsteinn, L., & Robert, O. (2003). Cyclodextrins in solid dosage forms: Drug delivery oral. Business Briefing: Pharmaceutical Technology, 176–180.
4. Irie, T., & Uekama, K. (1997). Pharmaceutical applications of cyclodextrins: Toxicological issues and safety evaluation. Journal of Pharmaceutical Sciences, 86(2), 147–162.
5. Hedges, A. R. (1998). Industrial applications of cyclodextrins. Chemical Reviews, 98(5), 2035–2044.
6. Lu, X. N., & Chen, Y. (2002). Chiral separation of amino acids derivatized with fluorescein-5-isothiocyanate by capillary electrophoresis and laser-induced fluorescence detection using mixed selectors of β-cyclodextrin and sodium taurocholate. Journal of Chromatography A, 955(1), 133–140.
7. Baudin, C., Pean, C., Perly, B., & Goselin, P. (2000). Inclusion of organic pollutants in cyclodextrin and derivatives. International Journal of Environmental Analytical Chemistry, 77, 233–242.
8. Kumar, R., Dahiya, J. S., Singh, D., & Nigam, P. (2001). Biotransformation of cholesterol using Lactobacillus bulgaricus in a glucose-controlled bioreactor. Bioresource Technology, 78(2), 209–211.

9. Koukiekolo, R., Desseaux, V., Moreau, Y., Marchis, M. G., & Santimone, M. (2001). Mechanism of porcine pancreatic α-amylase: Inhibition of amylose and maltopentaose hydrolysis by α-, β-, and γ-cyclodextrins. European Journal of Biochemistry, 268(3), 841–848.

10. Muñoz-Botella, S., Castillo, B. D., & Martyn, M. A. (1995). Cyclodextrin properties and applications of inclusion complex formation. Ars Pharmaceutica, 36, 187–198.

11. Schneiderman, E., & Stalcup, A. M. (2000). Cyclodextrins: A versatile tool in separation science. Journal of Chromatography B, 745(1), 83–102.

12. Connors, K. A. (1997). The stability of cyclodextrin complexes in solution. Chemical Reviews, 97(5), 1325–1358.

13. Rao, V. M., & Stella, V. J. (2003). When can cyclodextrins be considered for solubilization purposes? Journal of Pharmaceutical Sciences, 92, 927–932.

14. Loftsson, T., Olafsdottir, B. J., & Frioriksdottir, H. (1993). Cyclodextrin complexation of NSAIDs: Physicochemical characteristics. European Journal of Pharmaceutical Sciences, 1, 95–101.

15. Uekama, K., Hirayama, F., & Irie, T. (1998). Cyclodextrin drug carrier systems. Chemical Reviews, 98(5), 2045–2076.