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REVIEW ARTICLE

Applications of cyclodextrins: formation of inclusion complexes and their characterization

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Abstract

Cyclodextrins (CDs) act as host molecules to form inclusion complexes rather nonspecifically with a wide variety of guest molecules. These complexation favorites the high solubility of different molecules invites. Cyclodextrin complexation offered the possibility to improve the aqueous solubility of variety molecules and not modification of its original structure. In the present review of the literature was carried out to characterize the formation of inclusion complexes by different techniques in the solid and in the solution state complexation. The applications of cyclodextrins have been explained neatly and legibly. So it has wide applications in many industries, especially in the food and pharmaceutical industries. The characterization of inclusion complexes was done with a purpose to determine the interaction of different molecules with cyclodextrins which confirm the formation of inclusion complexes. Its inclusion complexes were identified by Thermo-Analytical Methods, Scanning Electron Microscopy (SEM), X-Ray diffractometry Single, Crystal X-Ray Structure Analysis, Infra-Red (IR) Spectroscopy, Thin Layer Chromatography (T L C). The Inclusion complexation in solution state characterized by Electrochemistry, Polarography, Conductivity Polarimetry Solubility Studies, Nuclear Magnetic Resonance (NMR) Spectroscopy, Electron Spin Resonance (ESR), Ultraviolet/Visible (UV/VIS) Spectroscopy, Fluorescence Spectroscopy, Circular Dichroism (CD) Spectroscopy, pH-Potentiometry Titration and Microcalorimetry.

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides containing six (α-CD), seven (β-CD) or eight (γ-CD) α-1, 4-linked glucopyanose units[1]. Each cyclodextrin unit has a hydrophobic cavity which can act as a host for a hydrophobic guest molecule [2]. The main factors acting as driving force for forming complexes and also responsible for the stability of these complexes are hydrophobic forces, the sizes of the cavity of the molecules and guest properties [3]. In fact the formation of its inclusion complexes with a wide variety of host molecule is due to the physicochemical properties of the latter. Molecular encapsulation can occur

both in the solid state and solution state. In the solid state, guest molecules can be enclosed within the cavity or may be combined with the outside of the cyclodextrin molecule and the state of solution; there is balance between complex molecules customers and non-complexed [4, 5]. Cyclodextrins are useful functional excipients that have benefited from the attention and use in various fields [6].

Thanks to their no toxicity, the use of cyclodextrins field is very large. De many applications, which generally use the complexing ability of cyclodextrins with a large, number of organic and inorganic guest molecules, are described in the literature [7]. Inclusion complexes formed between the guest and cyclodextrin molecules can be characterized both in the solid and solution state by several techniques. Characterization of inclusion complexes was done in order to determine the interaction of guest molecules with cyclodextrins which confirm the formation of inclusion complexes [8].

The history, chemistry, methods of complexation and application of cyclodextrin (CD) into different areas, particularly in the pharmaceutical and food industry are explained in detail.

1. History

Cyclodextrins have been isolated for the first time by Villiers [9] in 1891, thanks to the experience of the starch degradation by microorganisms strain (Bacillus macerans amylase: cyclodextrinase). Villiers highlights two products (probably the α- and β-cyclodextrin) with physicochemical properties similar to those of cellulose.

Cyclodextrins have been characterized in 1903 by Schardinger [10] as cyclic oligosaccharides; this is why they are called Schardinger dextrin in the first publications dealing with cyclodextrins.

In 1938 Freudenberg et al.[11] have demonstrated that cyclodextrins are constructed from D-glucose units linked together by α linkages (1 → 4) glucosidic bond. Freudenberg et al.[12] have found that the cyclodextrins were capable of forming inclusion complexes and entirely determine the structure of the γ-cyclodextrin. In the 1950, groups of French [13] and that of Cramer [14] have worked on the synthesis and purification of cyclodextrin complexes.

The first patent on the application of cyclodextrins for the shaping of a biologically active compound was deposited by Freudenberg [15] in 1953. From that time, the study of cyclodextrins takes considerable growth: industrial production, synthesis of cyclodextrins modified synthesis of inclusion complexes.

In the years 1970-80, Szetjli [16, 17] also called 'godfather' of cyclodextrins, contributed importance in the field. Since 1970, there were just over 130,000 documents on cyclodextrins (publications, patents, abstracts).

Today, the production of the β-cyclodextrin is greater than 1000 T / year and his ongoing price to drop. Other natural or modified cyclodextrins are produced industrially.

2. Structural characteristics of cyclodextrins

Cyclodextrins are cyclic oligosaccharides reducing name of α-D glucopyanose obtained industrially by enzymatic degradation of amylose (linear form of the starch) with the enzyme cyclodextrin glucosyltransferase (CGTase). The most common cyclodextrins are α-, β- and γ-cyclodextrin containing respectively 6,7 and 8 D-glucopyranosiques units (Figure-1) linked by α-1,4. There are also larger cyclodextrins (called giant) that can contain up to 14 units glucopyranosiques [18, 19].

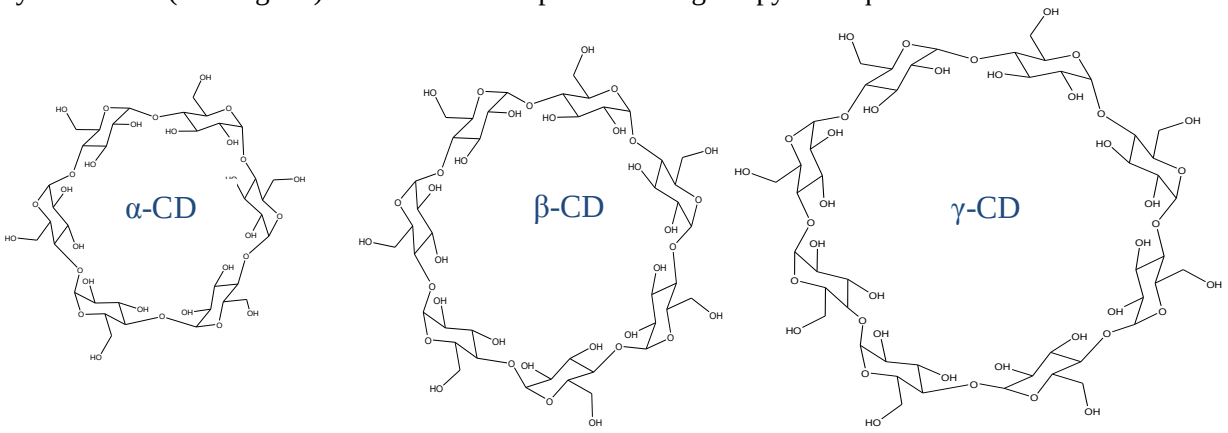


Figure.1. CD's chemical formula (α, β, γ).

Indeed, cyclodextrins have a cavity of about 5-8 Å diameter that allows them to include many organic compounds to form inclusion complexes in the solid state or in solution ,so, these cyclodextrins have a three dimensional structure in the form of a conical cylinder (Figure-2) whose wall is constituted by the glucose units, training chair C1-4 [20, 21].

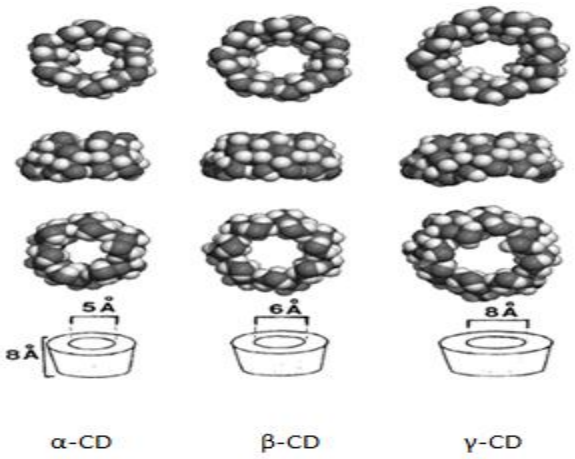


Figure.2. Simplified structure showing the tapered shape of cyclodextrins.

3. Physicochemical properties of α-, β- and γ-cyclodextrin

3.1. Polarity of cyclodextrins

As a result of the frusto-conical structure and the particular position of the hydroxyl, cyclodextrins are amphiphilic and thus have two distinct zones of polarity.

The exterior of the cavity and the pole ends are: This is essentially due to the hydroxyl and thus promotes the solubilization in very polar solvents. In contrast, the interior of the cavity where the only oxygen's interglucosiques is polar months (ether polarity type) and this area is more hydrophobic (contact surface with the guest molecule) [22].

3.2. Stability and solubility of cyclodextrins

Several factors such as temperature, solvent and pH were influenced stability cyclodextrins. They are stable in an alkaline medium but may undergo partial acid hydrolysis at a pH below 3.5 and at a temperature higher than 60 °C, producing glucose, and a series of acyclic maltosaccharides [23].

Coleman et al. were attributed the low solubility of β-CD having a symmetry axis 7, with the β-CD aggregation there between due to unfavorable interactions of hydrogen bonds with water [24].

Szejtli proposes that the intramolecular hydrogen bonds of hydroxyl β-CD (more rigid) are responsible for its low solubility [25]. The α-CD and γ-CD more flexible and having one, two or three glucose units inclined, do not contain this continuous belt of hydrogen bonds secondary hydroxyl (O2 and O3). And therefore favor the hydrogen bonds with the solvent, thereby increasing their aqueous solubility [26].

Alkylation of the hydroxyl of the β-CD significantly increases the solubility, and this phenomenon, a priori surprising, the subject of substantial research [27]. Eventually the cyclodextrins are soluble in water and polar aprotic solvents such as DMSO (dimethylsulfoxide), DMF (dimethylformamide) and pyridine.

Table.1. Physicochemical properties of the main native CDs.

Properties	α-CD	β-CD	γ-CD	References
Number of glucose units	6	7	8	-
Empirical formula (anhydrous)	$C_{36}H_{60}O_{30}$	$C_{42}H_{70}O_{35}$	$C_{48}H_{80}O_{40}$	-
Atomic mass (anhydrous) g / mol	972,85	1134,99	1297,14	-
Cavity length (Å)	7,9-8	7,9-8	7,9-8	28
Cavity diameter (Å)	4,7-5,3	6,0-6,6	7,5-8,4	28

Outside diameter (Å)	14,6-15	15,4-15,8	17,5-17,9	28
Volume of the cavity ($Å^3$)	174	262	427	28
pKa at 25 ° C	12,332	12,202	12,081	28
Melting point (°C)	275	280	275	-
Optical rotation αD at 25 °C (°)	149,5-150,5	162-163	176,9-177,9	-
Solubility (eau,25°C),mol/L	0,1211	0,0163	0,168	27
Solubility (eau,25°C), g/100 ml	14,5	1,85	23,2	-
Hydration CD,nH_2O	n = 6 to 7	n = 10 to 12	n = 7 to 13	-

4. Modified cyclodextrins

The aqueous solubility of natural CDs is low because of the strong intramolecular hydrogen bond in the crystal lattice [29]. Against by hydroxylation or methylation of the hydroxyl groups of β-cyclodextrin improves the solubility and the ability of including parent CD [30].The main types of chemical functionalization performed on cyclodextrins can be classified in the following way [31, 32]:

Etherification (alkyl derivatives and silylated)
Esterification (acyl derivatives and sulfonyl)
Halogenation
Substitution nucleophilic

5. Applications

5.1. Cosmetics, personal care and toiletry

In this sector, cyclodextrins involved in the stabilization, odor control and process improvements in the conversion of a liquid component in a solid form. The applications of cyclodextrins in this area include toothpaste, skin creams, liquid and solid fabric softeners, paper towels, tissues and underarm shields. In fact, the interaction of the guest with CDs produces a higher energy barrier to prevent volatilization, producing lasting fragrance [33].

5.2. Cyclodextrins in pharmaceutical industry

Cyclodextrins complexation uses are well known in the pharmaceutical industries that have been certified by several critics in recent years [34, 35, 36, 37]. Thanks to their non toxicities, the use of CDs is very important in the bioavailability [38], the active stabilization [39], odor or taste masking [40], reducing irritation [41] and uses handling equipment [42, 43]. Then the practical use of natural cyclodextrins as drug carriers is restricted to their low aqueous solubility. Table 2 lists cyclodextrin based commercially available pharmaceutical products [44, 45, 46].

Table.2. Commercially available pharmaceutical products based on cyclodextrin

Drug /Cyclodextrin	Trade name	Formulation and indications	Company (Country)
Voriconazole/SBE-β-CD	Vfend	IV solution, Fungal infections	Pfizer (USA, Europe)
Ziprasidone mesylate/SBE-β-CD	Geodon, Zeldox	IM solution, Antiscizophrenic	Pfizer (USA, Europe)
PGE1/α-CD	Prostavastin	Parenteral solutions, Chronic arterial	Ono(Japan)

		occlusive disease	
Alprostadil/α-CD	Caverject Dual	IV solution, symptomatic treatment of erectile dysfunction in adult males due to neurogenic, vasculogenic, psychogenic, or mixed etiology	Pfizer(Europe)
Nicotine/ β-CD	Nicorette	Sublingual tablets, stop smoking	Pfizer(Europe)
Nitroglycerine/ β-CD	Nitropen	Sublingual tablets, Coronary dilator	Nihon Kayaku(Japan
OP-1206/γ-CD	Opalmon	Tablets, Buerger's disease	Ono(Japan)
Diclofenac Na/HP-γ-CD	Voltaren	Eye drop solution, Nonsteroid Anti-inflammatory	Novartis(Europe)
Mitomycin/HP-β-CD	MitoExtra	IV infusion, Anti-inflammatory	Novartis(Europe)
Hydrocortisone/HP-β-CD	Dexocort	Solution, Mouth wash against aphta, gingivitis, etc.	Actavis(Europe)
Cloramphenicol/RM-β-CD	Clorocil	Eye drop solution, Antibiotic agent	Oftalder(Europe)
17β-Estradiol/RM-β-CD	Aerodiol	Liquid, Nasal spray	Servier(Europe)

Abbreviations: PGE1: prostaglandin E1; SBE-β-CD: sulfobutylether-β-cyclodextrin sodium salt; α-CD: α-cyclodextrin; β-CD: β-cyclodextrin; γ-CD: γ-cyclodextrin; HP-γ-CD: 2-hydroxypropyl-γ–cyclodextrin; HP-β-CD: 2-hydroxypropyl-β-cyclodextrin; RM-β-CD: randomly methylated-β-cyclodextrin; IV: intravenous; IM: intramuscular.

5.3. Cyclodextrins in textiles

The factors that influence the formation and decomposition of inclusion complexes, very important for the use of particularly complex and versatile for selecting the type of cyclodextrin (α, β, γ and δ), constitute the field several studies and are of particular interest to medical textiles with slow release medicine as well as the influencing factors on the transdermal permeability, very important for the use of cyclodextrins grafted onto textiles, for topical administration drugs [47].

In 2007, Perrin et al. were discovered another important application of textile finishing products is the use of cyclodextrin as equalizer dye. Given their external and internal polar hydrophobic, cyclodextrins are able to form inclusion complexes with dyes in aqueous media [48].

5.4. Cyclodextrins in agricultural and chemical industries

A wide variety of agricultural chemicals with cyclodextrins form complexes, including herbicides, insecticides, fungicides, repellents, pheromones and growth regulators.

Indeed, in the grain treated with certain amylases which degrade starch blocks seeds are inhibited. Initially, the plant grows more slowly, but later on this is largely compensated by an improvement of the growth plants producing a crop of 20 to 45% greater 28.Thereafter recent developments imply cyclodextrin glucanotransferases (CGTases) in 28, [49] plants. While in the chemical industry, cyclodextrins are widely used to separate the isomers and enantiomers, for catalyzing reactions to help various processes and to remove or detoxify waste. Also cyclodextrins are widely used in the separation of enantiomers by high performance liquid chromatography (HPLC) or gas chromatography (GC). There are other analytical applications can be found in the spectroscopic analysis.

But for nuclear magnetic resonance studies (NMR), the CDs may act as chiral shift agents and in that the circular Dichroism (chiral) selective modifying agents' spectra. Cyclodextrins may be used in electrochemistry to mask contaminant compounds, which allows for more precise determinations 28.

5.5. Cyclodextrins in environnemental

In the environmental field cyclodextrins play a very important role in terms of solubilization of organic contaminants, enrichment and the removal of organic pollutants and heavy metals from the soil, water and atmosphere [50]. CDs have been used in water treatment to increase stabilization of the action, encapsulation and adsorption of contaminants [51]. In 1999, Reid et al. discussed the soil test to determine the bioavailability of pollutants using CD and its derivatives. Cyclodextrins makes three benzimidazole fungicides (thiabendazole, carbendazim and fuberidazole) more soluble in water which causes the availability of its fungicides to soil. More CDs have the ability to increase the solubility of the hydrocarbon for the biodegradation, bioremediation and also reduce the toxicity resulting in increased microbial and plant growth [52]. Thus 90% of the toxic material is disappear [53].

5.6. Cyclodextrins in food technology

Cyclodextrins have found numerous applications in the food industry. They form inclusion complexes with a variety of molecules including fats, flavors and colorants. Also they are used to remove and hide the unwanted components and to salt out the desired components with time [54].

Cyclodextrins are also used to protect and relegate aromas. Natural and artificial flavors or oils are volatile liquids and complexation with cyclodextrins provides a promising alternative to conventional encapsulation technologies for the protection of aromas. For example, complexation of sweeteners such as aspartame with cyclodextrin stabilizes and improves its taste. It also eliminates the bitter taste of other sweeteners such as stevioside and rubusoside. Flavonoids and terpenoids are good for health because of their antioxidant and antimicrobial properties but cannot be used as food because of their low solubility and bitter taste. Sumiyoshi [55] discussed improving the properties of these compounds by complexing with cyclodextrin.

5.7. Cyclodextrins in catalysis

Cyclodextrins and their derivatives are used in the field of catalytic chemistry. For example, Atwood [56] explained the use of α-cyclodextrin in the modified porphyrin reduction of Mn (III). Ye et al [57] found that the use of a derivative of β-cyclodextrin as a catalyst increases the benzyl alcohol conversion rate to aldehyde.

Because of their steric effects, cyclodextrins plays a significant role in the biocatalytic process by increasing the enantioselectivity. Leventis and Silvius [58] have shown that the cholesterol cyclodextrins accelerate the transfer rate between the lipid vesicles.

5.8. Cyclodextrins in analysis

In chromatography, cyclodextrins are used extensively in the separation of chiral molecules for their ability to distinguish between the position isomers, groups' fontionnels, homologues and enantiomers [59].

This property is in fact one of the most useful agents for a range of separations. They are still used as ligands chemically bonded or absorbed in the stationary phase or the mobile phase [60].
Currently, chiral separations are one of the most important areas of application of cyclodextrins and their derivatives [61].

5.9. Cyclodextrins in polymers, adhesives and coatings

Cyclodextrins are used as additives and blowing agents compatible with hot melt systems. They also increase the stiffness, adhesion of hot melt adhesives and interaction between the associative thickener polymer molecules in emulsion-type coatings such as paints tends to increase viscosity. So, in the literature, CDS can be used to counteract this adverse effect [49].

6. Study of the complexation of structure

In the case of cyclodextrins, the hydrophobicity of the cavity allows for the inclusion of molecules called "invited" whose hydrophobicity and size correspond to those of the cavity while the hydroxyl functions provide good solubilization of the complexes in water.
One or more molecules may be "encapsulated" in one, two and even three times by the cyclodextrin molecules.
The complex formation mechanism is governed essentially by a geometric factor thus the substrate should have a size compatible with the cavity of the hot. Meier et al. [62] studied the influence of the cavity of cyclodextrins on the inclusion of decanoate anion (C10), and they found that the association constant of C10-β-cyclodextrin complex is 10 times larger than that of C10-γ-cyclodextrin.
Therefore a certain proximity of the guest molecule to form a stable inclusion complex with the cavity of the host molecule. For example, naphthalene is too large for the α-cyclodextrin but forms a stable complex with β-cyclodextrin. Anthracene may enter in the γ-cyclodextrin. The most frequent inclusion complexes are of type 1: 1. That is to say a cyclodextrin molecule is a guest molecule. If a molecule is too large to penetrate completely within the cavity, the other left free extremity can then in turn be encapsulated by another cyclodextrin molecule. This leads in this case to the formation of a complex type 2: 1 [63]. The literature is rich in examples of the types of complex 2: 2 [64], 3: 1 [65], 3: 2 [66] or 4: 5 [67]. It is also possible that it is one molecule of cyclodextrin, which interacts with several molecules to form complex 1: 2 [68] or 1: 3 [69].

8. Factors influencing the complexation of organic molecules by cyclodextrins

8.1. Factors related to the nature of the organic molecules

Glycosidic bridges give the cyclodextrin cavity similar ethanol polarity [70]. With this relatively apolar environment of the cavity, cyclodextrins are able to host hydrophobic molecules. These molecules have a greater affinity for the cyclodextrin cavity that for the aqueous phases [71].
Depending on the pH, the weak acids or weak bases exist in several ionic forms in solution. These different forms of the solute do not have the same physical and chemical characteristics (solubility, hydrophobicity). Therefore, the affinity of the cyclodextrin will not be the same for each of the forms. The complexation of cyclodextrins with ionized molecules must necessarily take into account the influence of pH [72], [73].
An important parameter for the complexation of the organic molecules is their size relative to that of the cavity of the cyclodextrin. The relative sizes of the cavity of the cyclodextrin and the substrate often also affect the stoichiometry of the complex.
As the size of the guest molecule is adjusted relative to that of the cavity, over the complex formed are stable (no contact with the cyclodextrin cavity, more interactions of Van Deer Waal's) [74]. However, complexation can also depend on other factors such as the molecular surface of the contaminant or its orientation in the cavity.

8.2. Factors related to the characteristics of cyclodextrins

The modified cyclodextrins show more capacity than the native forms solubilize organic compounds [75]. Among the modified cyclodextrins, and by way of example, methyl-β-cyclodextrin (MCD) appears to have a higher efficacy than the hydroxypropyl-β-cyclodextrin (HPCD) to dissolve the chlorinated solvents [76]. This ability is probably due to the remarkable character of the hydrophobic cavity of the

MCD. Brusseau et al. [77] HPCD showed that the complexation ability to phenanthrene is greater than that of the complexation of the MCD.

8.3. Influence of chemical factors

The influence of a co solvent on the complexing of an organic molecule with a cyclodextrin depends on the nature and concentration of the organic solvent present.

The presence of ethanol with a percentage lower than 30% of total solvent decreases the formation of the inclusion complex in the aqueous phase as the case between testosterone and hydroxyl-β-cyclodextrin [78].

Brusseau et al [79], for example, used the water (50:50) for the complex decomplex CD / PAHs (Polycyclic Hydrocarbon Atomic). However, the same authors reported that 10% of cyclopentanol in solution promotes complexing anthracene with some cyclodextrins [80].

Although cyclodextrins can form inclusion compounds in organic solvents such as alcohols, dimethyl sulfoxide or dimethylformamide, the association is generally lower than that observed for the same compound in aqueous media [81].

As already indicated, the addition of an organic solvent may allow moving the equilibrium cyclodextrin / solute and modifying the solubility of the complexing agent and the solute.

Also the temperature has dual effect on the complex formed: first it generally increases the solubility but at the same time it changes its stability. Most complex begins to decompose to 50-60 ° C, although some are stable at higher temperatures, particularly if the molecule is highly hydrophobic.

Then the effect of temperature depends on the nature of the substrate and also that of the cyclodextrin [82].

The yield of the complexation between the α-cyclodextrin or γ-cyclodextrin and chlorogenic acid to decrease by 50 ±14% when the temperature increases from 3 to 37 ° C, whereas with the β-cyclodextrin, complexation increases with temperature up to 25 ° C (ΔH positive), it is stabilized between 25 and 40 ° C, and then decreases between 40 and 60 ° C (ΔH negative). For the β-cyclodextrin, the variation of the complex formation constant with the temperature is probably related to changes of the solvate complex [83].

The yield of the complexation of the imidazole derivatives of β-cyclodextrin decreases when the temperature rises of 8-70 ° C (ΔH = -14KJ / mol) [84]. Blashak and Warner [85] were studied the effect of temperature on the complexation of HAP (polycyclic aromatic hydrocarbons) with cyclodextrins. They found that the complex stability constant decreases significantly with temperature. Overall, it appears that a rise in temperature is often unfavorable to the formation of cyclodextrin complexes / organic molecules.

9. Examples of inclusion complexes

There are in the literature a large number of journals that were published on cyclodextrins and their applications in several areas, demonstrating the great interest in these molecules in life. Table 3 shows a partial list of these publications.

Table.3. A non comprehensive list of international journal reviews on cyclodextrins and their applications.

Author(s)	Year	Article
J. Szejtli	2004	Past, present, and future of cyclodextrin research, Pure Appl. Chem., 76, 1825–1845.
M.E Davis., M.E. Brewster	2004	Cyclodextrin-based pharmaceutics: past, present and future, Nature Rev. , 3, 1023–1035.
S.SHIMPI, B. CHAUHAN, P.SHIMPI	2005	Cyclodextrins: Application in different routes of drug administration, Acta. Pharm., 55, 139–156.

R. Challa, A.Ahuja, J. Ali, R.K. Khar	2005	Cyclodextrins in Drug Delivery: An Updated Review, AAPS Pharm. Sci. Tech, 6 (2), 329-357.
A. Madene, M. Jacquot, J. Scher, S. Desobry	2006 2006	Flavour encapsulation and controlled release – a review, International Journal of Food Science and Technology, 41, 1–21
Y. K. Joung, H.D. Park, N. Yui, K. D. Park	2007 2007	Supramolecular Structures with Cyclodextrins for Biomedical Applications, Biomaterials Research, 11(4), 162-169. Cyclodextrins as pharmaceutical solubilizers, Advanced Drug Delivery Reviews, 59, 645–666
A. RASHEED, C. K. ASHOK KUMAR, V. V. N. S. S. SRAVANTHI Y. He, P. Fu, X. Shen, H. Gao	2008 2008	Cyclodextrins as Drug Carrier Molecule: A Review, Sci Pharm., 76, 567–598 Cyclodextrin-based aggregates and characterization by microscopy, Micron, 39, 495–516
S. Vikesh, M. Rajashree, A. Ashok, V. Manvi Fakkirappa. H. Bricout, F. Hapiot, A. Ponchel , S. Tilloy, E. Monflier	2009 2009	Influence of β-Cyclodextrin Complexation on Ketoprofen Release from Matrix Formulation, International Journal of Pharmaceutical Sciences and Drug Research, 1(3), 195-202. Chemically Modified Cyclodextrins: An Attractive Class of Supramolecular Hosts for the Development of Aqueous Biphasic Catalytic Processes, Sustainability , 1, 924-945
M. M. Nitalikar, D. M. Sakarkar, P. V. Jain R. Singh, N. Bharti, J. Madan, S. N. Hiremath	2010 2010	The Cyclodextrins: A Review, Journal of Current Pharmaceutical Research, 10 (1), 01-06. Characterization of Cyclodextrin Inclusion Complexes – A Review, Journal of Pharmaceutical Science and Technology, 2 (3), 171-183.
A. Hidetoshi, M. Keiichi, H. Taishi A. Munin, F. Edwards-Lévy	2011 2011	Potential Use of Polyamidoamine Dendrimer Conjugates with Cyclodextrins as Novel Carriers for siRNA, Pharmaceuticals, 5, 61-78. Encapsulation of Natural Polyphenolic Compounds; a Review, Pharmaceuticals, 3, 793-829.
T. Loftsson, M.E. Brewster D. H. Jeremy, T. Schluep	2012 2012	Methods to Enhance Complexation Efficiency, Cyclodextrins as Functional Excipients, 101, 3019-1032. Cyclodextrin-Containing Polymers: Versatile

		Platforms of Drug Delivery Materials: Review Article, Journal of Drug Delivery, 1-17.
S. Kumar Das, R. Rajabalaya, S. David, Nasimul Gani, J. Khanam, A. Nanda	2013	Cyclodextrins-The Molecular Container, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 4, 1694-1720.
C. U. Shah, B. Nanavati	2013	Cyclodextrin based nanosponges for pharmaceutical use: A review, Acta. Pharm., 63, 335–358
H. M. Dardeer	2014	Importance of cyclodextrins into inclusion complexes, International Journal of Advanced Research: Research Article, 2, 414-428.
P.H. Khade, A. C. Talware, S. V. Kotwal, S. V. Jograna, S. S. Kakade, M. H. Shitole	2014	Cyclodextrin based nanosponges used as solubility enhancing agent, International Research Journal for Inventions in Pharmaceutical Sciences: Research Article, 2, 54-61.

10. Analytical techniques for characterization of cyclodextrin complexes

It is often use different methods of analysis that makes it easier to assess the formation of inclusion complexes. The results of it is analyzes must be combined and considered together because each method explores a particular characteristic of the inclusion complex. While the concurrent use of different methods of characterization promotes a better understanding of the host-guest interactions and support inclusion of the approach of the host by the guest. Then, the analysis techniques available are generally based on the detection of changes in physicochemical properties as a result of the formation of an inclusion complex as each technique has its own inconvenient which should be known and taken into account in consideration was late to evaluate the results of forming an inclusion complex [86].

The main techniques generally used for the characterization of complex inclusions are:

A) Inclusion complexation in solution characterized by:

Spectroscopic techniques:

(a) Ultraviolet/visible (UV)

(b) Circular Dichroism (CD)

(c) Fluorescence

(d) Nuclear Magnetic Resonance (NMR)

(e) Electron Spin Resonance (ESR)

Electroanalytical techniques:

(a) Polarography

(b) Voltammetry

(c) pH-Potentiometry Titration

(d) Conductimetry.

Separation techniques:

(a) High performance liquid chromatography (HPLC)

(b) Capillary electrophoresis (CE)

Polarimetry

Polarimetry Isotermal titration calorimetry (ITC)

Solubility studies

B) Inclusion complexation in the solid characterized by:

a) Thermo-analytical methods

b) Scanning Electron Microscopy (SEM)

c) X-ray diffractometry
b) Single Crystal X-ray Structure analysis
e) Infra-Red (IR)
A) Inclusion complexation in solution characterized by:

10. 1. Electroanalytical techniques

Electroanalytical characterization methods have been considered as a criterion to determine the stability constants of complexes of inclusion in solution [87]. Among these methods are those most widely used as the polarographic and voltammetric.

10.1.1. Polarography and voltammetry

Polarography and voltammetry have a powerful tool to know the nature of such complex inclusions as appropriate techniques for the study of cyclodextrin complexation with electroactive molecules breakfast [88, 89]. Also they are particularly useful to assess the association constants inclusion complexes at very low concentration levels [87.] In polarography, changes in the potential of a half-wave of the guest molecule can be observed as a result of the electronic redistribution occurring in the presence of cyclodextrins.

In addition, the complexation with cyclodextrins results in a reduction of the amount of current leads to a reduction of the guest molecule diffusion subsequently decreased when complexed with cyclodextrins [90].

It is also found, DPP (differential pulse polarography) and UV spectrophotometry which were used for the determination of stability constants of complexes of inclusion with nifedipine and micardipine cyclodextrins on the basis of the results of studies DPP authors postulated that the fraction of the guest molecule incorporated in the CD cavity is the aromatic nitro group in the case of nifedipine and the phenyl group in the case of nicardipine [91].

10. 1.2. pH-Potentiometry Titration

In general, the potentiometric measurements are used to determine the constants of association inclusion complexes (CD / drug). Thus, the values of constants associations inclusion complexes formed by equimolar hydroxypropyl-CD and the ionized forms of drugs acids flurbiprofen and ibuprofen were determined at different temperatures by potentiometric pH measurement [92].

Potentiometric measurements are also used in the complexation modes neutral and protonated forms of benzimidazole with β-CD were studied by various techniques, including the potentiometric titration [93].

These potentiometric measurements with a glass electrode were used to calculate the stability constants of different local anesthetics (tetracaine, lidocaine and prilocaine) with β-CD and γ-CD [94].

The results for tetracaine were in good agreement with those obtained by fluorescence spectroscopy, while complex prilocaine and lidocaine cannot be detected [95].

Different inclusion complexes have been characterized by various analytical techniques such as potentiometric titration, which confirmed the phenomenon of inclusion.

10.1. 3. Electrical conductivity

The electrical conductivity measurements were used to determine the complex of equilibrium constants between the CDS and a variety of ionic surfactants and other amphiphilic molecules constants.

E. Junquera et al [96] were used to assess the electrical conductivity CD complex binding constants β-cyclodextrin or hydroxypropyl-beta-cyclodextrin with dodecyltrimethylam-monium bromide in aqueous solution, consider the combination of against-ion surfactant with the inclusion and the variation of the molar ionic concentration of the complex conductivity. Conductivity measurements were performed to study the behavior of three tricyclic antidepressants (imipramine, desipramine and amitriptyline hydrochloride) in aqueous solutions in the absence and presence of β-CD [97]. Also, it has been studied in different acidity conditions the formation of inclusion complexes between the β-CD and the local anesthetic novocaine in aqueous solutions using the conductivity for the variation of the stability constant [98].

Conductimetric measurements showed that the addition of β-CD on the drug solution resulted in the displacement of the apparent critical micelle concentration of the drug to higher concentrations, because of the involvement of the monomers in the complexing CD, thereby confirming the formation of an inclusion complex [99].

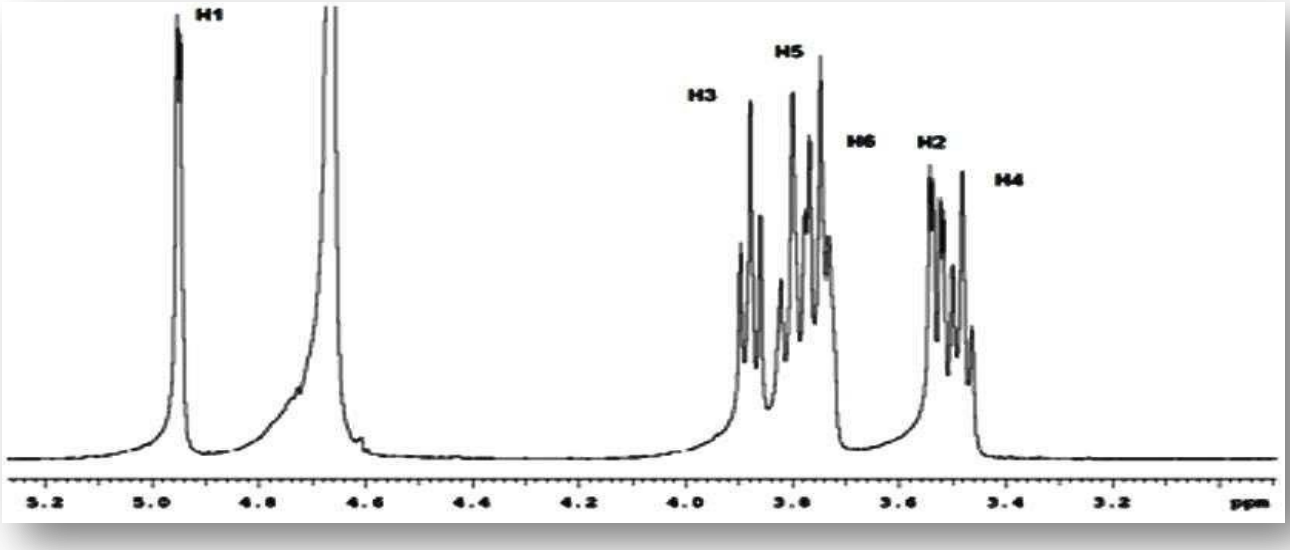
10.2. Spectroscopic techniques

NMR spectroscopy has been considered one of the most complete spectroscopic techniques because of its wide range of applications of the elucidation of the structure of survey structures on intra / intermolecular, that's why it has been extensively used in chemistry [100].

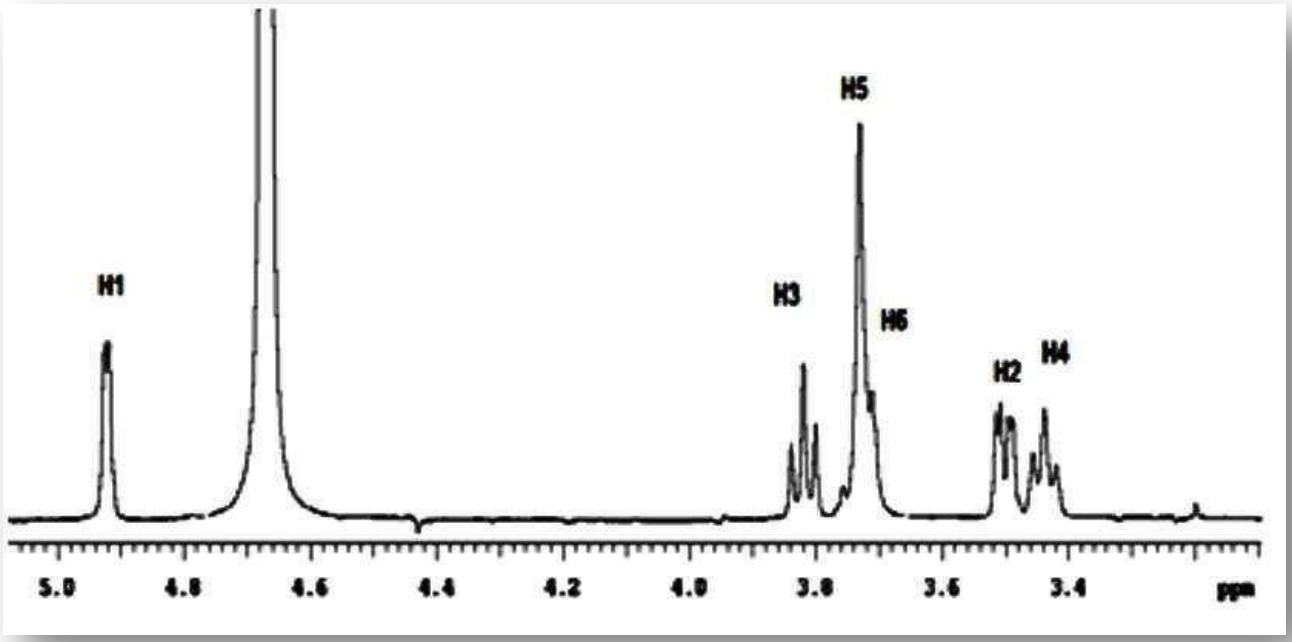
In general, NMR spectroscopy is used as a method of characterizing the observed difference in the changes in chemical shifts of the protons of the two species (of the host and the guest).

10.2.1. ^{1}H-NMR

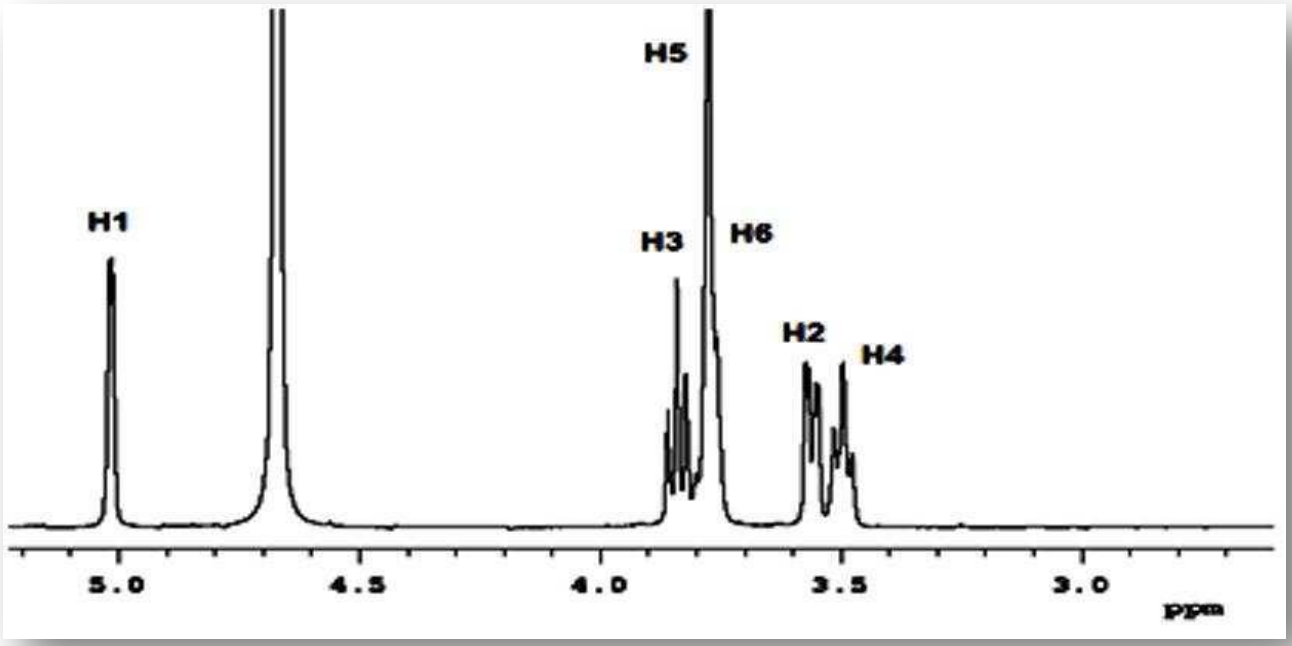
Thakkar and Demarco were started spectroscopic studies on CDs complexes by observing the change of the chemical shift of the H3 and H5 protons inside the cavity of α-CD, where the chemical is in the presence of molecules aromatic because of the anisotropic effect of the aromatic ring [101]. This change in chemical shift of the hydrogen atoms is due to the interaction between the host and the guest of the test of phenomenon or inclusion [102]. The following figure (Figure-3) shows the ^{1}H NMR of the three cyclodextrins (α, β, γ). One can see that the differences among the spectra of the three CDs, due to the proton shielding, are smaller than 0.1 ppm [102].



(a)



(b)



(c)

Figure.3. [1]H-NMR spectra of the natural cyclodextrins (298 K; 500 MHz; D2O; δHOD 4.67 ppm): (a) α-CD;(b) β-CD; (c) γ-CD.

It was concluded by Great banks and Pickford when ΔδH3> ΔδH5 inclusion is done partially and when ΔδH3 <ΔδH5, full inclusion occurs. Taking the example of the stability of the inclusion complex between the cyclodextrin and the natural Minoxidil (MNX) may be suggested by the analysis of the difference of the chemical shifts of protons before and after complexation [103]. Figure 4 shows the MNX structure and the protons assignment.

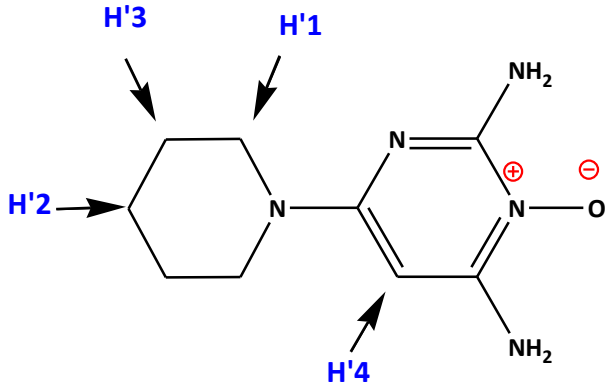


Figure.4. Structure of Minoxidil and their protons.

Tables 4 and 5 summarized the values of the chemical shifts of protons for this inclusion complex.

Table.4.CDs chemical shifts (δ) and their difference when in presence of MNX (Δδ = δcomplexed-δfree).

Proton Nature	α-CD	inclusion complex (MNX/αCD)		β-CD	inclusion complex (MNX/βCD)		γ-CD	inclusion complex (MNX/γCD)	
	δαCD	δαCD/ MNX	ΔδαCD/ MNX	δβCD	δβCD/M NX	ΔδβCD/ MNX	δγCD	δγCD/M NX	ΔδγCD/M NX
H1	4.960	4.955	-0.005	4.960	4.965	0.005	5.015	5.015	---
H2	3.530	3.540	0.010	3.545	3.550	0.005	3.560	3.555	-0.005
H3	3.910	3.890	-0.020	3.830	3.820	-0.010	3.860	3.850	-0.010
H4	3.490	3.480	-0.010	3.490	3.480	-0.010	3.495	3.490	-0.005
H5	3.765	3.750	-0.015	3.720	3.710	-0.010	3.800	3.800	---

Tableau.5. MNX chemical shifts (δ) and their difference when in presence of CDs (Δδ=δcomplexed-δfree)

Proton Nature	MNX	inclusion complex (MNX/αCD)		inclusion complex (MNX/βCD)		inclusion complex (MNX/γCD)	
	δMNX	δαCD/ MNX	ΔδαCD/ MNX	δβCD/M NX	ΔδβCD/ MNX	δγCD/M NX	ΔδγCD/M NX
H'1	3.340	3.360	0.020	3.420	0.080	3.340	---
H'2	1.535	1.550	0.015	1.570	0.035	1.535	---
H'3	1.455	1.490	0.035	1.520	0.065	1.455	---

MNX: Minoxidil, Δδ: difference of the chemical shifts, δ: chemical shifts, α: alphacyclodextrin, β: betacyclodextrin, γ: gammacyclodextrin.

One can see that ΔδβCD/MNX > ΔδαCD/MNX > ΔδγCD/MNX. Therefore, the complexation between β-CD and MNX is stronger than in α and β-CD. Considering the values of Δδ for H3 and H5, there is a partial inclusion in α and β-CD. For the complex βCD/MNX, the inclusion is total. These results were confirmed by other spectroscopic techniques.

10.2.2. ^{13}C NMR

^{13}C NMR spectroscopy is a similar method to identify the formation of inclusion complexes [104]. Their utility lies in understanding the phenomenon of inclusion complexes in aqueous solution. Besides, the cyclodextrin induced change in the ^{13}C chemical shift result predominantly from the electrical environment effect of the cyclodextrin cavity and in general ^{13}C inclusion shift may be mainly divided into hydrophobic and Vander Waals interaction shifts [105].

10.2.3. Ultraviolet/visible (UV)

The change in the UV visible absorption spectrum of a host molecule is the inclusion phenomenon [106], [107, 108]. During the spectral changes, the chromophore of the guest is transferred from an aqueous medium to the non-polar cyclodextrin. These changes must be due to a perturbation of the electronic energy levels of the guest caused either by direct interaction with the cyclodextrin, by the exclusion of solvation water molecules or by a combination of these two effects [109, 110]. Small shifts are observed on the UV spectra of the included guests, the method is often used to detect inclusion complexation [111, 112]. For example, evidence of the interaction between the cyclodextrin and the drug is in the presence of a hypsochromic or bathochromic shift in the absorption capacity without changing λ_{max}. It is often considered that the hydrogen bond is the main force behind the formation of an inclusion complex and it causes a decrease in the energy orbitals "n" and hypsochromic shift (blue shift). For example, 1.1 nm change was observed when hydrocortisone butyrate was complexed with (2, 6-di-O'Methyl) - β-cyclodextrin [113]. Besides, cleavage of the existing hydrogen bonds in the compound can lead to a bathochromic shift due to complexation. For example, 1.2 nm shifts was observed on complexing 1, 8-dihydroxy anthraquinone with γ-cyclodextrin [114].

10.2.4. Fluorescence

Fluorescence spectroscopy is a simple, fast and very sensitive method, particularly useful for investigating the formation in solution of CD inclusion complexes of fluorescent guests. In fact, an enhancement in fluorescence is generally observed upon inclusion of a fluorescent guest molecule into the CD cavity [115]. The inclusion complex formation generally leads to the change of excitation and emission wavelength of the drug [116]. Enhancement in fluorescence upon complexation with β-CD or its hydroxypropylated or methylated derivatives have been reported for example for naproxen [117, 118] and benzocaine [119]. Recognition of naproxen with maltoheptaose and maltohexaose has been studied by fluorescence spectroscopy. The results showed that both maltoheptaose and maltohexaose were able to form 1:1 pseudo-inclusion complexes with the drug about as stable as the true inclusion complexes with α-CD and β-CD [120, 121]. Further-more, studies on the location of the drug in the complex, performed with the fluorescence quenching method, indicated that it was completely penetrated into the CD cavity [122].

The fluorescence emission spectra confirmed that the solubility of the complex formed using β-CD and γ-CD was more than that of plain drug. Binding of the chromophore in a monomeric form with β-CD and γ-CD was also confirmed. The spectral change was maximum in γ- CD, less in β-CD and none in α-CD indicating that the substrate was included inside the cavity completely in case of γ-CD due to larger cavity size, and partly into the β-CD cavity and no inclusion in case of α-CD [123, 116].

10.2.5. Circular Dichroism (CD)

Circular dichroism can represent a powerful technique to prove the CD inclusion complexation of both chiral and non chiral guest molecules and obtain information about the structure of the complex in aqueous solution [86].

Both maxima of the circular dichroism spectrum of naproxen showed a variation in intensity and a shift in the presence of methyl-, hydroxypropyl- and hydroxyethyl- β-CDs; the most intense effect was observed in the presence of methyl- β-CD and attributed to a stronger interaction of such CD with the drug, i.e. to a more favorable fit between host and guest molecules [124].

Also the formation of inclusion complexes with cyclodextrin proved by changes in circular dichroism spectra (CD) and not just for the achiral guest molecules but also for chiral molecules guests. For example, the cyclodextrin spectra of β-CD complexes with naphthalene derivatives found a remarkable difference in the spectra between 1-substituted and 2-substituted naphthalene complexes, indicating that the steric effects of substituent on the formation of the complex is so strong that the complexation mode may be different for these guest molecules. They suggested that a positive CD band suggests an axial inclusion, while a negative CD band suggests an equatorial inclusion. According to this proposal, 2-substituted naphthalene is estimated to be included axially in the β-CD cavity [125].

10.2.6. Electron Spin Resonance (ESR)

Researchers use using electron paramagnetic resonance to investigate the phenomenon of inclusion in aqueous solution. Indeed, Electron Spin Resonance [125] is a useful method to investigate inclusion complexation with radicals in aqueous solutions. Since the hyperfine coupling constant of radicals is very sensitive to the medium polarity, its alterations due to its movement toward an environment less polar than water, such as the CD cavity, is indicative of the inclusion complex formation. For example, the inclusion complexes were prepared between Miconazole and cyclodextrins by freeze drying and kneading method and evaluated by Electron Spin Resonance. The electron microscopic pictures showed that the physical appearance and size of the complexes formed were completely different from that of Miconazole or corresponding cyclodextrins alone. The particle size of the inclusion complexes was much smaller than the parent cyclodextrins. The size of the HPβCD and α cyclodextrin (α-CD) was 256.7 and 50 μm, respectively, while that of the corresponding miconazole complexes was 2.3 and 5 μm [126]. Another example, ^{1}H NMR and ESR titrations were used to determine the stoichiometry and stability of the complexes of alpha-phenyl-N-tert-butylnitrone analogs and their superoxide spin adducts, respectively, with methylated β-CDs; after the superoxide radical spin trapping reaction, ESR titrations provided the stability constants of the corresponding CD-nitroxide complexes and indicated a bimodal inclusion of the nitroxide free radical spin adducts into the CD cavity [127].

10.3. Separation techniques

10.3.1. High performance liquid chromatography (HPLC)

This technique is suitable for chemically unstable compounds and for systems accompanying no spectral changes. In addition HPLC is a powerful tool to study the interactions of CDs and CD complexes with stationary phases, as well as to determine the stoichiometry and association constants of CD complexes in solutions. Cyclodextrins complexation have been successfully applied to rapid analysis of amoxicillin and clavulanic acid [128], multivitamin preparations [129], tricyclic antidepressant drugs [130], cephaloporins [131], tolfinamic acid [132], prostaglandin derivatives [133] by HPLC. However HPLC techniques can require large amounts of materials, may need extensive sample preparation, and require a strict control of experimental conditions to have good data reproducibility [134]. In HPLC experiments, CDs can be in the stationary phase, chemically bound to silica gel, or, more frequently, they can be added to the mobile phase: as a result of host–guest interactions, the retention time of the guest will change, becoming longer or shorter, depending on complexation occurring in the stationary or in the mobile phase, respectively (Fujimura method) [135, 136].

Hummel-Dreyer were used another method that requires a column equilibrated with an eluent carrying the host molecule; when the complexing agent is added to the eluting solution, the chromatogram shows a positive peak, due to the complexity, and a negative peak of the free molecule retention time, which corresponds to the amount of complexed host [137]. The stability constant is calculated by the variation of intensity of the negative (or positive) peak as the concentration of the ligand is increased. In the Hummel–Dreyer method the consumption of ligand is lower.

The advantages of HPLC method was that Ks values can be rapidly obtained by simple procedure with minimum quantity of guest molecule even when the significant spectral changes are not observed by complexation. This method is pertinent to various cyclodextrin complexes with weakly acidic or basic drug molecules, using a proper ion exchange support.

In study [138] of retention behavior of drug (D) and its cyclodextrin complex (D-Cyd) within ion exchange column, Cion, Cc and Ks are concentration distribution ratio of ionized D, that of D-Cyd and stability constant of 1:1 complex respectively, as defined by Eqs. (1-3).

$$\text{Cion} = \frac{(\text{D})\text{s}}{(\text{D})\text{m}} \quad (1)$$

$$\text{Cc} = \frac{(\text{D} - \text{Cyd})\text{s}}{(\text{D} - \text{Cyd})\text{m}} \quad (2)$$

$$\text{Ks} = \frac{(\text{D} - \text{Cyd})\text{m}}{(\text{D})\text{m}\,(\text{Cyd})\text{m}} \quad (3)$$

Where suffix s and m stand for stationary phase and mobile phase, respectively, and concentration of each species in parenthesis are in molar concentration unit. Based on these an equation was derived as:

$$\frac{(\text{Cyd})\text{m}}{\text{T0.} - \text{Tods}} = \frac{1}{\text{T0.} - \text{Tc}}(\text{Cyd})\text{m} + \frac{1}{\text{Ks}(\text{T0.} - \text{Tc})}\,, \ldots (4)$$

Where T0, T0., Tc and Tobs are retention time of non-retained band, that of D itself, that of D-Cyd complex, and that of D at a given concentration of cyclodextrin.

A plot of left hand term of Eq. (4) vs (Cyd)m gives both the Ks and Tc values from the slope and intercept. The Ks values determined by HPLC method were in fair agreement with those obtained by other methods, such as solubility and spectroscopic methods.

10.3.2. Capillary electrophoresis (CE)

The CE is considered a separation technique with high yield molecules in capillary tubes containing an electrolyte solution under the influence of an electric field. This technique offers significant advantages over other separation methods, such as high efficiency, which allows the observation of very fine enantioselective effects, high flexibility, high speed and miniaturization. In this technique, the separation is based on the difference in mobility of the ionic species in the affinity of the charged or uncharged molecules against charged electrolytes. The affinity of the molecules (charged or uncharged) based on electrostatic interactions, forces of Van Deer Waals and hydrogen bonds which are considered as affinity capillary electrophoresis (ACE). CDs played an important role in the development of various analytical methods, in particular as media modifiers for the separation of chiral compounds [139, 140]. In two different ways, it was used EC for estimating CD complex association constants with different guest molecules. In the Indirect Absorbance Detection (IAD) method, the back-ground electrolyte (BGE) contains the guest molecules and the mobility of the analyte (CD) is assessed as a function of the guest concentration; on the contrary, in the Direct Absorbance Detection (DAD) method, the guest is run as analyte and the CD is part of the BGE and the mobility of the guest is measured as a function of the CD concentration in the BGE. A general problem in the measurement of equilibrium constants by CE methods is that the effective mobility, and then the results obtained, depend not only on the concentration of the reactant in the BGE, but also on the shape and amplitude of the sample peak; this latter dependence gives rise to non-constant drift speeds within the moving sample, which, in extreme cases, could induce asymmetric peak shapes and erroneous results [141]. CE was used for the enantiomeric separation of the enantiomers of three alkaloids (vincamine, the vinpocetine and vincadifformine) which are complexed different cyclodextrins. The investigated CDs were the native ˜α, β˜, and γ-CDs and their hydroxypropylated, randomly methylated, carboxymethylated and sulfobutylated derivatives [142].

10.4. Solubility studies

The formation of the inclusion complex in solution is indicated in the guest solubility study according to the concentration of cyclodextrin, if the solubility of the guest increases with the concentration of cyclodextrin from which the complex formation [123, 143]. Vinpocetine aqueous solubility was evaluated in complexed and uncomplexed forms. Solubility studies were performed to evaluate the drug pH solubilization profile and to assess the effect of multicomponent complexation on Vinpocetine solubility. The effect of pH on the solubility and the dissolution Vinpocetine has been studied, and reported clearly

illustrated. From the solubility values obtained, it was predictable that the extent of Vinpocetine dissolution in the gastric environment would be high but the pH values generally found in the upper regions of the gastrointestinal tract, the solubility and dissolution of pure Vinpocetine will not be sufficient for complete dissolution of the doses administered. It was found that the solubility of pure vinpocetine (in uncomplexed form) in aqueous solution is less than the solubility of vinpocetine in multicomponent form (in complexed form) [123].

B) Inclusion complexation in the solid characterized by:

10.5. Thermo-analytical methods

The most commonly employed thermo analytical techniques for assessing the formation of CD inclusion complexes are differential scanning calorimetry and thermogravimetry. These techniques are usually the first to be considered when evaluating complex formations, because they are relatively simple and not time consuming [144].

10.5.1. Differential Scanning Calorimetry (DSC)

CDs curves obtained from differential scanning calorimetry (DSC) present endothermic events that correspond to dehydration. Thus, α-CD has two or three events, depending on the crystalline form, and β-CD and γ-CD have a broad peak around 120 and 150°C, respectively [145]. Complex formation analysis can be made by comparing the DSC curve of the complex with the CD used, the guest molecule and the physical mixture (CD plus guest molecule), prepared in the same proportion as the complex. The guest molecule is in crystalline form and its curve is represented by a well-defined narrow peak, corresponding to the melting point. The curve of the physical mixture is the sum of CD dehydration and the melting peak of guest molecule. When formation of the complex occurs, the melting peak is expected to disappear, shift or broaden due to the loss of the crystalline structure caused by encapsulament (Figure-5) [146].

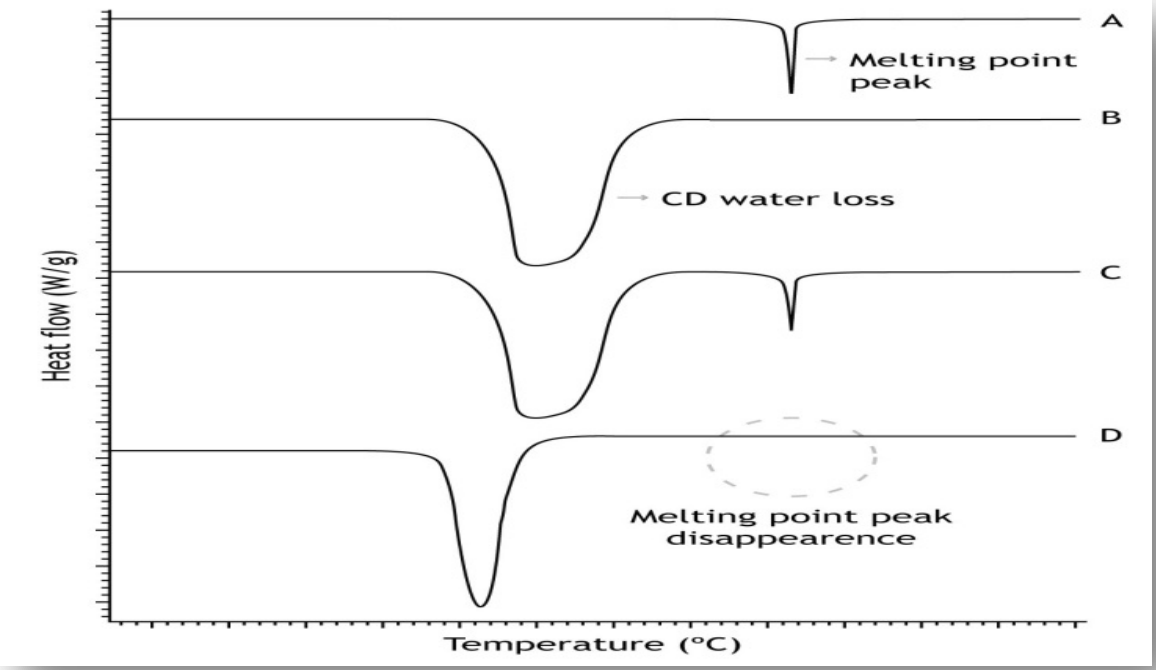


Figure.5. Hypothetical model of a DSC analysis for pure drug (A), CD (B), physical mixture (C) and complex (D).

Indeed, the presence of the melting peak indicates that there is still a free host molecule in the sample and when the partial complexation occurs, it is expected that the complex of the melting peak decreased from the physical mixture, resulting the interaction of the drug with the CD [147].

In some cases the physical mixture does not present the characteristic melting event of the guest molecule, because the heating of CD water can cause amorphization of the guest molecule [148]. In another situation, the peak may appear broader and shifted due to weak interactions between the guest molecule and CD [149]. The dehydration event of CD in the DSC curve of the complex can shift when water molecules are replaced in the cavity by guest molecules, resulting in a change in the energy state.

10.5.2. Thermogravimetric Analysis (TGA)

Natural CDs, when analyzed by thermogravimetry (TG) in a nitrogen atmosphere, show an initial weight loss due to water evaporation (adsorbed and of crystallization), and a second one from the degradation that occurs between 250 and 400°C, where they lose 70 to 80% of their weight [150]. The most common way to detect the formation of inclusion complexes using TG is to compare the temperature at the beginning (on set) of the degradation with that of the complex (Figure-6).

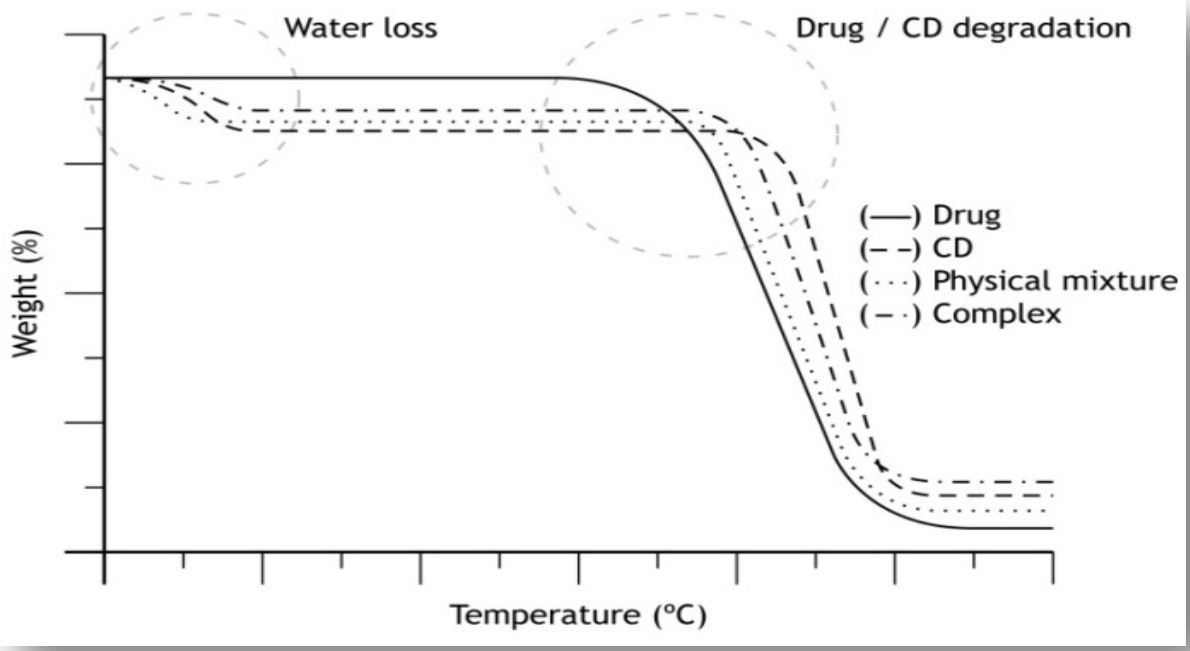


Figure.6.Hypothetical model for TG analysis of complexation of a drug with CDs(α, β, γ).

It is assumed that if complexation occurs, the degradation of the guest molecule takes place at higher temperatures, because the drug is protected by CD [151].

10.5.3. Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy [152, 153, 154, 155] is used to study the microscopic aspects of the raw material (cyclodextrin and the guest substances, respectively) and the product obtained by co-precipitation /evaporation [156, 157]. In fact, the SEM does not confirm the formation of the inclusion complex but it allows to determine the existence of a product in the preparation obtained, for example, in the case of the analysis of the retinoic acid SEM revealed that, while the β-cyclodextrin crystallizes in a relatively large number of polyhedral retinoic acid appears in the form of needles or elongated crystals [158]. As scanning electron microscopy (SEM) was used for the morphological study (particle size, shape) of the drug [159].

For example, the particle morphology of Ketoprofen, β-cyclodextrin (β-CD), its physical mixtures and solid complexes were evaluated by SEM photographs. Ketoprofen appeared as plate like crystals, tending to form aggregates. In contrast, a drastic change in the morphology and change in the crystalline nature

was observed in 1:1 freeze-dried, coprecipitated and kneaded products of both HPβCD and β-CD; it was revealed that there was an apparent interaction in the solid state [123, 152]. Finally, SEM analysis was performed to investigate the morphologies of pure drug and carriers and their combinations.

10.5.4. X-ray diffractometry (XRD)

The X-ray diffraction can be used to detect the formation of inclusion complex in the solid state [160,161]. Indeed, the comparison of diffractogram is only possible if the cyclodextrin as well as the guest molecules are treated under identical conditions as that of the assumed complex because cyclodextrin inclusion complex preparation processes such as freeze drying and grinding, may change the crystallinity of the pure substances and this may lead to different diffraction patterns [162, 158]. Also, the complex formation leads to the sharpening of the existing peaks, appearance of a few new peaks and shifting of certain peaks. For example: the inclusion complexes prepared by neutralization method for Naproxen [163].

As for the amorphous complex formation leads to the disappearance of certain peaks or peaks become less sharp than those of the pure compound or physical mixture. Take, for example spray drying complexes of acetaminophen, indomethacin, piroxicam and warfarin with β-CD and the dried gel with complexes of Naproxen β-CD [163, 104].

10.5.5. Single crystal X-ray structure analysis

To identify the detailed inclusion structure and mode of interaction they use the structural analysis of the crystal X-ray units. In fact, interaction between breakfast and guest molecules can be identified and the geometric relationship can be established. This information obtained during the analysis lead to know about the formation of inclusion complexes [152, 107].

10.5.6. Infra-Red (IR)

The estimation of the interactions between the cyclodextrin molecules and the guest in the solid state is determined by infrared spectroscopy.[152, 107]. In fact, the strips of cyclodextrins change slightly during the formation of the inclusion complex and if the fraction of the host molecules encapsulated in the complex is less than 25% while there are bands which could be made to that part of the molecules guests are easily masked by the bands cyclodextrins [108, 123, 164, 165]. The application of the Infra-red spectroscopy is limited to the guests having some characteristic bands, such as carbonyl or sulfonyl groups. Therefore, the hydrogen bond to the hydroxyl group causes the biggest change in the band stretching vibration. It was also shown that the cleavage of the hydrogen bond as a result of inclusion complexation results in the displacement of the absorption bands in the higher frequency. This is the case of the displacement of the aromatic carbon atom of stretching at 1272-1296 cm^{-1} in the case of β-CD complex, and the ester function of the section from 1183 to 1206 cm^{-1} in case dimethyl-β-cyclodextrin has been reported [113]. The hydrogen bond formation caused the OH bond of elongation, NH etc because of reduced elasticity. Therefore, the frequencies of the stretching vibration decreased. For example, when Piroxicam was complexed with β-CD, the band at 1180 cm^{-1} was shifted to 1154 cm^{-1} [123, 166].

Conclusion

Firstly, cyclodextrins have been described by de Villiers in 1891 and they have defined in full as a group of cyclic oligosaccharides having a hydrophilic surface and a hydrophobic central cavity. These unique properties have received widespread attention and use in the inclusions of complex formation with different guest molecules by inserting the cavity of cyclodextrins. Also, they have been used in many applications in many fields such as pharmaceutical, food, cosmetic, agricultural industry etc...

In this review, we are also focused on the physicochemical properties of cyclodextrins, inclusions phenomena and characterization of inclusion complexes. These methods of characterization are crucial to fully exploit the potential of CDs inclusions complexes and the study of literature showed that these techniques confirmed the formation of inclusion complexes in the solid and in the solution state.

To understand the mechanisms of the geometry of host-guest inclusion complex and especially to determine the accuracy of these stability constants must develop new techniques to obtain a complete and detailed description of the inclusion phenomenon.

Acknowledgments

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