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INCLUSION COMPLEXATION BY CYCLODEXTRIN : A NOVEL APPROACH TO IMPROVE SOLUBILITY AND BIOAVAILABILITY OF POORLY WATER SOLUBLE DRUG

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ABSTRACT

Cyclodextrins are cyclic oligosaccharides which have recently been recognized as useful pharmaceutical excipients. The outer surface of these doughnut-shaped molecules is hydrophilic, but they possess an axial open cavity, which is of hydrophobic character and capable of including other apolar molecules in case of geometric compatibility. This is the essence of molecular encapsulation by inclusion complex formation. Low solubility compounds show dissolution rate limited absorption and hence poor absorption, distribution and target organ delivery. Improvement of aqueous solubility in such a case is valuable goal to improve therapeutic efficacy. Complexation with CDs by different methods like physical mixing, kneading, spray drying, freeze drying, etc., has been reported to enhance the solubility, dissolution rate and bioavability of poorly water soluble drugs. This review aims to assess the use of cyclodextrins as complexing agents to enhance the solubility of poorly soluble drugs and hence to resolve the many issues associated with developing and commercializing poorly water soluble drugs.

Key words: Cyclodextrins, Solubility, Inclusion complexes

INTRODUCTION

Solubility problems are a genuine challenge for formulators since about 40% of marketed drugs are classified as “practically insoluble”. Solubilization of poorly soluble drugs is a frequently encountered challenge in screening studies of new chemical entities as well as in formulation design and development[10]. Any drug to be absorbed must be present in the form of an aqueous solution at the site of absorption. As Solubility & permeability is the deciding factor for the in-vivo absorption of the drug, these can be altered or modified by enhancement techniques. Many techniques had been developed to overcome these solubility problems such as, hydrotropy, alteration of pH of the drug microenvironment, solid dispersion, inclusion of complex by using cyclodextrins, microemulsion, solid solution, eutectic mixture and selective adsorption on insoluble carriers, evaporative precipitation into aqueous solution, use of surfactants, etc[10]

A drug delivery system is expected to deliver the required amount of drug to the targeted site for the necessary period of time, both efficiently and precisely. Different carrier materials are being constantly developed to overcome the undesirable properties of drug molecules. In pharmaceutical technology, cyclodextrins are one of the most versatile aids[11]. These are of a wide range of delivery devices from the most classical dosage forms to the newest drug carriers. Cyclodextrins offer an additional tool for the formulator to overcome some of the formulation and delivery limitations of some drugs[11]. The proposed review on Cyclodextrins (CDs) explores its utility as useful functional excipients that have enjoyed widespread attention and use in the pharmaceutical industry. The

bioadaptability and multi-functional characteristic of CDs make them capable of alleviating the undesirable properties of drug molecules in various routes of administration through the formation of inclusion complexes. Poor dissolution is a major problem of almost all BCS class II drugs, which leads to poor bioavailability. Such type of hosting with CDs helps in enhancing their aqueous solubility, dissolution, stability and bioavailability, thus proving CDs to be greatly applicable in the pharmaceutical, agrochemical, food and cosmetic industry.

The history, chemistry, complexation approches, applications of cyclodextrin (CD) in different areas of drug delivery, particularly in parenteral, oral, ophthalmic, nasal, dermal and rectal drug delivery systems are explained in review.

HISTORY OF CYCLODEXTRIN

Cyclodextrins are cyclic oligosaccharides consisting of six α-cyclodextrin, seven β-cyclodextrin, eight Ɣ-cyclodextrin or more glucopyranose units linked by α-(1,4) bonds. They are also known as cycloamyloses, cyclomaltoses and Schardinger dextrins . They are produced as a result of intramolecular transglycosylation reaction from degradation of starch by cyclodextrin glucanotransferase (CGTase) enzyme. [7]

In 1891 a French scientist, A. Villiers, described a bacterial digest that he had isolated from starch . Experimental results indicated that the substance was a dextrin and Villiers named it "cellulosine". Later an Austrian microbiologist, Franz Schardinger, described two crystalline compounds α-dextrin and β-dextrin which he had isolated from a bacterial digest of potato starch. Schardinger identified β-dextrin as Villiers' "cellulosine" . Now these compounds are commonly called cyclodextrins (i.e. α-cyclodextrin (αCD) and β-cyclodextrin (βCD)) or less commonly cyclomaltodextrins (i.e. cyclomaltohexaose and cyclomaltoheptaose) or cycloamyloses (i.e. cyclohexaamylose and cycloheptaamylose). γ-Cyclodextrin (γ CD; cyclomaltooctaose or cyclooctaamylose) was first described in 1935 by Freudenberg and Jacobi . In the years following these discoveries, large ring cyclodextrins (LR-CDs) were discovered . Presently only αCD, βCD and γCD, as well as some of their derivatives have advanced to the market. From 1935 to 1955 Freudenberg, Cramer and their co-workers identified the chemical structure of CDs, their general physicochemical properties and their abilities to form complexes. The biotechnological advances that occurred in the 1970's lead to dramatic improvements in production of highly pure αCD, βCD and γCD, transforming them from expensive chemical oddities to affordable industrial excipients.[11]

CHEMISTRY

CDs are 'bucket-like' or 'cone-like' rigid molecules, with a central core, the size of which differs in different cyclodextrin molecules. The arrangement of hydroxyl groups within the molecule gives the external surface hydrophilic characteristic, while internal surface of the core is hydrophobic. This type of chemistry of cyclodextrin allows the guest molecule to fit in the core and form an inclusion complex . Chemically they are cyclic oligosaccharides containing six (α-CD), seven (β-CD), eight (γ-CD), nine (δ-CD), ten (ε-CD) or more D-(+) glucopyranose units attached by α-(1, 4) glucosidic bonds[70]. Chemical structure of three main types of cyclodextrins α, β and γ are shown in fig. 1

The natural cyclodextrins, in particular β-cyclodextrin, are of limited aqueous solubility meaning that complexes resulting from interaction of lipophiles with these cyclodextrins can be of limited solubility resulting in precipitation of solid cyclodextrin complexes from water and other aqueous systems. In fact, the aqueous solubility of the natural cyclodextrins is much lower than that of comparable acyclic saccharides. This may be due to relatively strong intermolecular hydrogen bonding in the crystal state. Substitution of any of the hydrogen bond-forming hydroxyl groups, even by lipophilic methoxy functions, results in dramatic improvement in their aqueous solubility[70].

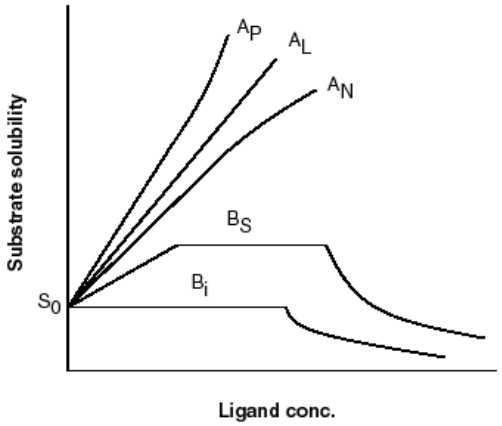
INCLUSION COMPLEX FORMATION

In aqueous solutions cyclodextrins are able to form inclusion complexes with many drugs by taking up a drug molecule, or more frequently some lipophilic moiety of the molecule, into the central cavity. No covalent bonds are formed or broken during the complex formation, and drug molecules in the complex are in rapid equilibrium with free molecules in the solution. The driving forces for the complex formation include release of enthalpy-rich water molecules from the cavity, electrostatic interactions, van der Waals' interactions, hydrophobic interactions, hydrogen bonding, release of conformational strain and charge-transfer interactions. The physicochemical properties of free drug molecules are different from those bound to the cyclodextrin molecules. Likewise, the physicochemical properties of free cyclodextrin molecules are different from those in the complex. In theory, any methodology that can be used to observe these changes in additive physicochemical properties may be utilised to determine the stoichiometry of the complexes formed and the numerical values of their stability constants. These include changes in solubility, chemical reactivity, UV/VIS absorbance,

fluorescence, drug retention (e.g., in liquid chromatography), pKa values, potentiometric measurements and chemical stability, nuclear magnetic resonance (NMR) chemical shifts and effects on drug permeability through artificial membranes.[6] Furthermore, because complexation will influence the physicochemical properties of the aqueous complexation media, methods that monitor these media changes can be applied to study the complexation; for example, measurements of conductivity changes, determinations of freezing point depression, viscosity measurements and calorimetric titrations. However, only few of these methods can be applied to obtain structural information on drug/cyclodextrin complexes.

PHASE SOLUBILITY ANALYSIS[6,70]

Higuchi and Connors have classified complexes based their effect on substrate solubility as indicated by phase-solubility profiles (Figure). A-type phase-solubility profiles are obtained when the solubility of the substrate (i.e. drug) increases with increasing ligand (i.e. cyclodextrin) concentration. When the complex is first order with respect to ligand and first or higher order with respect to substrate then AL-type phase-solubility profiles is obtained. If the complex is first order with respect to the substrate but second or higher order with respect to the ligand then AP-type phasesolubility profiles is obtained. AN-type phase-solubility profiles can be difficult to interpret. B-type phase-solubility profiles indicate formation of complexes with limited solubility in the aqueous complexation medium.



In general, the water-soluble cyclodextrin derivatives form A-type phase-solubility profiles while the less soluble natural cyclodextrins frequently form B-type profiles. Most drug/cyclodextrin complexes are thought to be inclusion complexes but cyclodextrins are also known to form non-inclusion complexes and complex aggregates capable to dissolve drugs through micelle-like structures. The phase-solubility profiles do not verify formation of inclusion complexes. They only describe how the increasing cyclodextrin concentration influences drug solubility. The most common type of cyclodextrin complexes is the 1:1 drug/cyclodextrin complex (D/CD) where one drug molecule (D) forms a complex with one cyclodextrin molecule (CD):

$$D + CD \overset{K_{1:1}}{\longleftrightarrow} D/CD$$

The value of K1:1 is most often between 50 and 2000 M^{-1} with a mean value of 129, 490 and 355 M^{-1} for α-,β-and Ƴ-cyclodextrin, respectively . Under such conditions an AL-type phase-solubility diagram, with slope less than unity, would be observed and the stability constant ($K_{1:1}$) of the complex can be calculated from the slope and the intrinsic solubility (S_0) of the drug in the aqueous complexation media (i.e. drug solubility when no cyclodextrin is present) and is given in eq. 2

$$K_{1:1} = \frac{Slope}{So(1-Slope)}$$

For 1:1 drug/CD complexes the complexation efficiency (CE) can be calculated from the slope of the phase-solubility diagram (eq. 3) by

$$CE = \frac{D/CD}{CD} = S_0 . K^{1:1} = \frac{Slope}{S_o(1-Slope)}$$

The most common stoichometry of higher order D/CD complexes is the 1:2 D/CD complex resulting in Ap-type phase soluability diagram. Consecutive complexation is assumed where 1:2 complex (eq. 4) is formed when one additional cyclodextrin molecule forms a complex with an existing 1:1 complex. The value of $K_{1:1}$frequently lies between 10–500 μ^{-1} and is lower than that of $K_{1:1}$ (50–2000 μ^{-1}).

$$D/CD + CD \overset{K\ 1:2}{\longleftrightarrow} CD/D_2$$

The various methods that are used to prepare D/CD complexes include solution method, co-precipitation method, neutralisation method, slurry method, kneading method, grinding method etc and water is essential for the successful complex formation. In solution, the cyclodextrin complexes are prepared by addition of excess amount of drug to an aqueous

cyclodextrin solution. The suspension formed is equilibrated at desired temperature and then centrifuged to form clear D/CD complex solution. For preparation of solid complexes water is removed from the aqueous D/CD complex by evaporation or sublimation. For a variety of reasons, such as isotonicity of parenteral formulations and formulation bulk of solid dosage forms, it is important to include as little cyclodextrin as possible in a pharmaceutical formulation. The complexation efficiency can be enhanced by various methods and are shown in tab. 2.

INCLUSION COMPLEXATION TECHNIQUES [4,10]:

Many techniques are used to form complexes with cyclodextrin, like grinding, kneading, co-precipitation, solid dispersion, neutralization, spray drying, freeze drying, melting, etc.

1. Physical blending /Grinding method :

Inclusion complexes can be prepared by simply grinding/ triturating the drug with cyclodextrin in mortar, on small scale. Whereas on large scale, the preparation of complexes is based on extensive blending of the drug with cyclodextrin in a rapid mass granulator usually for 30 minutes. Homogenous blending physical mixture of the drug and cyclodextrine followed by passing the mixture through an appropriate sieve to get the desired product. These powdered physical mixtures are then stored in the room at controlled temperatures and humidity conditions[4,10].

2. Kneading method

This method is based on impregnating the CDs with little amount of water or hydroalcoholic solutions to converted into a paste. The drug is then added to the above paste and kneaded for a specified time. The kneaded mixture is then dried and passed through no. 80 sieve to get desired product. In laboratory scale kneading can be achieved by using a morter and pestle . In large scale the kneading can be done by utilizing the extruders and other machines. This is the most common and simple method used to prepare the inclusion complexes and it presents very low cost of production[4,10].

3. Melting

Excess quantity of drug melted, mixed with powdered cyclodextrin, after cooling excess quantity of quest is removed by washing with weak complex forming solvent. The method restricted to sublimable guest like menthol[10].

4. Co-evaporation method

In this method, drug and cyclodextrin are dissolved in ethanol and in water separately. Both the solutions are mixed and stirred to attain equilibrium. The resulting solution is evaporated to dryness preferably under vacuum[4].

5. *Co-precipitation*:

Cyclodextrin is dissolved in water and the guest is added while stirring the cyclodextrin solution. By heating, more cyclodextrin can be dissolved (20%) if the guest can tolerate the higher temperature. The cyclodextrin and guest solution must be cooled under stirring before a precipitate is formed. The precipitate can be collected by decanting, centrifugation or filtration and washed[4].

6. Neutralization method:

Drug and cyclodextrin are separately dissolved in 0.1 N sodium hydroxide, mixed and stirred for about half an hour, pH is recorded and 0.1 N HCl is added drop wise with stirring until pH reaches 7.5, where upon complexes precipitates. The residue is filtered and washed until free from chlorine, It is dried at 250°C for 24 h. and stored in desiccators[10].

7. Lyophilization/ Freeze drying technique:

To get a porous, amorphous powder with high degree of interaction between drug and cyclodextrin, lyophilization/freeze drying technique is considered as a suitable. Here, the solvent system from the solution is eliminated through a primary freezing and subsequent drying of the solution containing both drug and cyclodextrin at reduced pressure. Thermolabile substances can be successfully made into complex form by this method .The required stoichiometric quantity of drug were added to aqueous solution of cyclodextrin and this suspension stirred magnetically for 24 hours, and resulting mixture is freeze dried at 60°C for 24 hours[4].

8. Spray drying:

In this method, first monophasic solution of drug and cyclodextrin is prepared using a suitable solvent. The solution is then stirred to attain equilibrium following which the solvent is removed by spray drying. In this method, host solution prepared generally in ethanol: water 50% v/v. To this guest is added and resulting mixture is stirred for 24 hr. at room temperature and solution is spray dried by observing following conditions-air flow rate, atomizing air pressure, inlet temperature, outlet temperature, flow rate of solution etc. Product obtained by passing through 63-160 micrometer granulometric sieve[4].

9. Solution-enhanced dispersion by the Supercritical fluids (SEDS)

SEDS is novel, single step method, which can produce solid drug-cyclodextrin complexes. The optimization of processing conditions is essential in order to achieve the optimum complexation efficiency and to compare with drug-cyclodextrin complexation methods described earlier in the literature (e.g. kneading, freeze drying, spray drying etc). Advantages over other methods are (a) Preparation of solid-cyclodextrin complexes in single step process, (b) Achievement of high complexation efficiency (avoidance of excess cyclodextrin in powder). (c) Possibility to minimize the contact of drug with cyclodextrin during the process. (d) Achievement of enhanced dissolution rate of the drug (which is comparable to the dissolution behavior of micronized drug-cyclodextrin complex)[4].

10. Microwave irradiation method

This technique involves the microwave irradiation reaction between drug and complexing agent using a microwave oven. The drug and CD in definite molar ratio are dissolved in a mixture of water and organic solvent in a specified proportion into a round bottom flask. The mixture is reacted for short time of about one to two minutes at 60 ºc in the microwave oven. After the reaction completes, adequate amount of solvent mixture is added to the above reaction mixture to remove the residual, uncomplexed free drug and CD. The precipitate so obtained is separated using whatman filter paper, and dried in vaccum oven at 40 ºc for 48 hrs. Microwave irradiation method is a novel method for industrial scale preparation due to its major advantage of shorter reaction time and higher yield of the product[4,10].

TECHNIQUES FOR CHARACTERIZATION OF INCLUSION COMPLEXATION:[9]

Inclusion complexes formed between the guest and cyclodextrin molecules can be characterized both in the solid and solution state by the following techniques:-

(*A) Inclusion complexation in the solid state characterized by*

(i) Thermo-analytical methods.
(ii) Scanning Electron Microscopy (SEM).
(iii) X-ray diffractiometry and single crystal X-ray structure analysis.
(iv) Wettability and dissolution tests.
(v) Infra-Red (I R) spectroscopy.
(vi) Thin Layer Chromatography (T L C)

(B) Inclusion complexation in solution state characterized by

(i) Electrochemistry.
(a) Polarography.
(b) Conductivity
(c) Polarimetry

(ii) Solubility studies.

(iii) Spectroscopy methods.
(a) Nuclear Magnetic Resonance (NMR) spectroscopy.
(b) Electron Spin Resonance (ESR).
(c) Ultraviolet/Visible (UV/VIS) spectroscopy.
(d) Fluorescence spectroscopy.
(e) Circular Dichroism (CD) spectroscopy.

(iv) Microcalorimetry.

(A) ***Inclusion complexation in the solid state characterized by :[9,10]***

1. ***Thermo-analytical methods :-***

Thermo-analytical methods determine whether the guest substance undergoes some change before the thermic degradation of cyclodextrin. The change of the guest substance may be melting, evaporation, decomposition, oxidation or polymorphic transition. The change of the guest substance indicates the complex formation. The effect of cyclodextrins on the thermogram obtained by DTA and DSC were observed for broadening, shifting and appearance of new peaks or disappearance of certain peaks. Changes in the weight loss were evaluated to provide supporting evidence for the formation of inclusion complexes. The nature of the drug and cyclodextrins used and method of preparation of complex have been found to influence the above finding considerably. If the interaction between the drug and the excipient is weak, the shift in the endothermic peak is very small. The formation of inclusion complex of Salbutamol with cyclodextrins by various methods was evaluated using DSC. The DSC endotherm of Salbutamol at 158°C was shifted to 150°C in the physical mixture showing a weak interaction. But the freeze dried complex showed no peak around 157°C indicating the formation of a true inclusion complex.

2. Scanning Electron Microscopy (SEM) : -

Scanning Electron Microscopy 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 .The difference in crystallization state of the raw material and the product seen under electron microscope indicates the formation of the inclusion complexes, even if there is a clear difference in crystallization state of the raw material and the product obtained by co-precipitation. This method is

inadequate to affirm inclusion complex formation. The particle morphology of Ketoprofen, β-cyclodextrin(βCD), its physical mixtures and solid complexes were evaluated by SEM. SEM analysis was performed to investigate the morphologies of pure drug and carriers and their combinations.

3. X-ray diffractiometry and single crystal X-ray structure analysis:-

Powder X-ray diffractiometry may be used to detect inclusion complexation in the solid state. When the guest molecules are liquid since liquid have no diffraction pattern of their own, then the diffraction pattern of a newly formed substance clearly differs from that of uncomplexed cyclodextrin. This difference of diffraction pattern indicates the complex formation . When the guest compound is a solid substance, a comparison has to be made between the diffractogram of the assumed complex and that of the mechanical mixture of the guest and cyclodextrin molecules. Comparison of the 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. A diffraction pattern of a physical mixture is often the sum of those of each component, while the diffraction pattern of cyclodextrin complexes are apparently different from each constituent and lead to a "new" solid phase with different diffractograms . Diffraction peaks for a mixture of compounds are useful in determining the chemical decomposition and complex formation. The complex formation of drug with cyclodextrin alters the diffraction patterns and also changes the crystalline nature of the drug. The complex formation leads to the sharpening of the existing peaks, appearance of a few new peaks and shifting of certain peaks.

Single crystal X-ray structure analysis may be used to determine the detailed inclusion structure and mode of interaction. The interaction between the host and guest molecules can be identified and the precise geometrical relationship can be established. This information obtained during the analysis lead to know about the formation of inclusion complexes.

4.Wettability and Dissolution tests : -

The wetting of the solid phase by a solvent is always the first step of any dissolution process. Cyclodextrin complexations of the lipophilic drug often improve the wettability in water considerably, but also simple addition of β-cylodextrin to non-wettable solid enhances their wettability. Three methods to characterize the wettability of solid cyclodextrin formulations include the measurement of the contact angles, powder sedimentation studies which may be carried out by layering equal amounts of the samples onto the surface of water, following their sedimentation photographically and the last method demonstrates the upward migration of a coloured front of three open tubes containing the guest compound, a mixture of the guest compound with cyclodextrin and the inclusion complex, respectively, as function of the time. When an assumed complex is dispersed in water, very rapid dissolution rate tests are based on this observation. The most often used dissolution tests are the rotating disk method and the dispersed amount technique . In the rotating disk method, the solid cyclodextrin formulations are pressed into tablets with exact identical surfaces for the samples and these tablets are placed on a rotating disc apparatus in an aqueous solution. At appropriate intervals samples are removed and analyzed for the guest content. The dispersed amount technique is a similar method but instead of a tablet, a powder is used.

5. Infra-Red (I R) spectroscopy : -

Infra-Red spectroscopy is used to estimate the interaction between cyclodextrin and the guest molecules in the solid state . Cyclodextrin bands often change only slightly upon complex formation and if the fraction of the guest molecules encapsulated in the complex is less than 25%, bands which could be assigned to the included part of the guest molecules are easily masked by the bands of the spectrum of cyclodextrin . The technique is not generally suitable to detect the inclusion complexes and is less clarifying than other methods . The application of the Infra-red spectroscopy is limited to the guests having some characteristic bands, such as carbonyl or sulfonyl groups . Infra-red spectral studies give information regarding the involvement of hydrogen in various functional groups. This generally shifts the absorbance bands to the lower frequency, increases the intensity and widens the band caused by stretching vibration of the group involved in the formation of the hydrogen bonds. Hydrogen bond at the hydroxyl group causes the largest shift of the stretching vibration band. It has been observed that cleavage of the hydrogen bonding due to inclusion complexation results in the shift of absorbance bands to higher frequency.

6. Thin Layer Chromatography(T L C):

In Thin Layer Chromatography, the Rf values of a guest molecule diminishes to considerable extent and

this helps in identifying the complex formation between guest and host molecule . Inclusion complexation between guest and host molecules is a reversible process. Consequently, the complex may separate completely in guest and host molecules during the chromatographic process and only the spots of the guest and host molecules are found on the TLC-plate

(B) Inclusion complexation in solution characterized by [9]:

1. Electrochemistry:-

(a) Polarography : -

Polarography is a suitable method to study inclusion complexation if the electron distribution of a complexed electroactive guest molecule in aqueous solution is different from that in the non-complexed state in aqueous solution .

(b) Conductivity: -

Conductivity measurement may be used to characterize inclusion complexation. Anionic surfactants having different polar heads, different tail configurations, the same Na+ counter ion and their solution conductivities are dramatically affected by inclusion complex formation with cyclodextrins. When these ionic surfactant form inclusion complexes with cyclodextrins, the amphiphilicity of the former often leads to strong associative species that dramatically affect solution conductivities .

(c) Polarimetry :-

Specific rotation [α] is the inherent characteristic property of an optically active species. A polarimetric study was conducted as a supporting tool for the complex formation because βCD is optically active in nature (α-[D]-glucopyranose units).

2. Solubility studies: -

In the solubility studies changes in solubility of the guest are plotted as afunction of the cyclodextrin concentration, if the solubility of a potential guest increases with increasing cyclodextrin concentration; complex formation in solution is indicated.

3. Spectroscopic methods:

(a) Nuclear Magnetic Resonance (NMR) spectroscopy:- The most direct evidence for the inclusion of a guest into a cyclodextrin cavity in solution is obtained by ^{1}H-NMR spectroscopy. ^{1}H-NMR may also be used to determine the direction of penetration of guest molecules into the cyclodextrin cavity.The H-3 and H-5 atoms of cyclodextrin, which are directed towards the interior of the cyclodextrin will show a significant upfield shift. if inclusion does indeed occur and the H-1, H-2 and H-4 atoms, located on the exterior of the cavity will show only marginal upfield shifts. The spectrum of the guest molecule may also be changed upon inclusion complex formation . A similar method to investigate inclusion complex formation is ^{13}C-NMR spectroscopy . It is often used to gain insight into the inclusion modes of inclusion complexes in aqueous solution. 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 .

(b) Electron Spin Resonance (ESR):-

Electron Spin Resonance is a useful method to investigate inclusion complexation with radicals in aqueous solutions. The hyperfine coupling constant of radicals is known to be sensitive to the polarity of the medium. If the hyperfine coupling constant alters, the movement of a radical to an environment less polar than water is indicated and confirms the inclusion complex formation .

(c) Ultraviolet/Visible (UV/VIS) Spectroscopy: -

The complexation causes a change in the absorption spectrum of a guest molecule . 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 solvating water molecules or by a combination of these two effects . Small shifts are observed on the UV spectra of the included guests, the method is often used to detect inclusion complexation

Hypsochromic or bathochromic shift or increase in the absorptivity without change in the λmax have been considered as evidence for interaction between cyclodextrin and the drug in the formation of the complex. Hydrogen bonding can be considered as the main force behind the inclusion complex formation. As hydrogen bonding lowers the energy of 'n' orbitals, a hypsochromic shift (blue shift) is observed Cleavage of the existing hydrogen bonds in the compound can lead to a bathochromic shift due to complexation. An increase or decrease in the absorption intensity of UV band without change in its λmax is also reported in certain inclusion complexes.

(d) Fluorescence spectroscopy: -

When fluorescent molecules in aqueous solution are included in cyclodextrins, fluorescence spectra may be influenced which indicates the formation of inclusion complexes . The inclusion complex formation generally leads to the change of excitation and emission wavelength of the drug

(e) Circular Dichroism (CD) spectroscopy:
Circular Dichroism is a useful method to detect cyclodextrin inclusion complexes in aqueous solution. When an achiral guest molecule is included within the asymmetric locus of the cyclodextrin cavity which consists of chiral glucose units, new Circular Dichroism (CD) bands can be induced in the absorption bands of the optically inactive guest . Not only achiral guest molecules but also chiral guest molecules may show changes in circular Dichroism (CD) spectra upon the formation of inclusion complexes with cyclodextrin

4. Microcalorimetry: -
Changes in thermodynamic properties due to inclusion complexation, can be measured by microcalorimetry. These changes in enthalpy and entropy are associated with the change in the behaviour of water structure within the cavity, removal of the water from the cavity, restructuring of water around the guest molecule and release of water into the bulk. Other contributions to the overall energies of reaction are due to the restriction in rotation around the glycosidal linkages of the cyclodextrin when the guest molecule enters the cavity .

APPLICATIONS OF CYCLODEXTRIN[5,6,16,70]

1. Oral Drug Delivery
Oral route has been the most popular route for designing a drug delivery system. In the oral delivery system, the release of the drug is either dissolution controlled, diffusion controlled, osmotically controlled, density controlled or pH controlled. CDs have been used as an excipient to transport the drugs through an aqueous medium to the lipophillic absorption surface in the gastrointestinal tract, i.e., complexation with CDs has been used to enhance the dissolution rate of poorly water-soluble drugs. Hydrophilic CDs have been particularly useful in this regard. Rapid dissolving complexes with CDs have also been formulated for buccal and sublingual administration. In this type of drug delivery system, a rapid increase in the systemic drug concentration takes place along with the avoidance of systemic and hepatic first-pass metabolism[5].

2. Rectal drug delivery system
Recent studies have shown that rectal mucosa can be used as a potential site for delivering drugs which have a bitter and nauseous taste, have a high first-pass metabolism and degrade in the gastrointestinal pH. It is an ideal route to deliver drugs to the unconscious patients, children and infants. However, rectal mucosa offers a very limited area for drug absorption resulting in an erratic release of drugs. To overcome these problems, a number of excipients have been used, and amongst them CDs have been found to be quite useful. They should be non-irritating to the rectal mucosa .They should inhibit the reverse diffusion of drugs into the vehicle . They should have a low affinity for the suppository base[16].

3. Nasal drug delivery system
Highly potent drugs can be aimed for systemic delivery through nasal mucosa. This novel approach is beneficial for the drugs with a low oral bioavailability due to extensive gastro-intestinal breakdown and high hepatic first pass effect. For lipophilic drugs nasal delivery is possible if they can be dissolved in dosage form. CDs are safe and non-toxic, thus are effective excipent for nasal drug delivery as they can enhance either drug absorption by increasing the aqueous solubility or by enhancing the drug permeability or both. The hydrophilic CDs enhance membrane permeability by solubilizing some specific lipids from biological membrane through the rapid and reversible formation of inclusion complex. Beside this, they can decrease nasal toxicity and can act as a carrier for sustained release of drug across nasal mucosa. HP-β-CD and methylated β-CDs have been used mainly as solubilizers and absorption enhancers in nasal drug delivery system[70].

4. Trasdermal drug delivery system
The main barrier for dermal drug absorption through the skin is the outer most layer stratum corneum. Penetration enhancers like alcohols, fatty acids etc. are used to decrease its barrier properties. Cyclodextrins improves the solubility and stability of drugs in the topical preparations, enhances the transdermal absorption of drugs, sustains the drug release from the vehicle and avoids undesirable side effects associated with dermally applied drugs . The cyclodextrins enhance drug delivery through aqueous diffusion barriers, but not through lipophilic barriers like stratum corneum. Cyclodextrins alleviate drug induced skin irritation by lowering the extent of free drug resulting from inclusion equilibrium[16] .

5. Opthalmic Drug delivery
Locally applied drug formulations such as suspensions, oily drops, gels, ointments and solid inserts have been used, but most of these formulations give rise to unwanted side effects. (e.g. eye irritation and blurred vision) Cyclodextrins enhance drug permeability through biological membranes such as eye cornea and skin by disrupting the membrane, either by permeating into the

membrane or by extracting or complexing with some lipophilic components such as cholesterol and phospholipids from the membrane . Cyclodextrins work as an anti irritant by formation of inclusion complex and thereby masking the irritating drugs or by replacing the irritating additives from the formulation[5].

6. Cyclodextrins in novel drug delivery

a. Cyclodextrins in Liposomal drug delivery

In drug delivery, the concept of entrapping CD–drug complexes into liposomes combines the advantages of both CDs (such as increasing the solubility of drugs) and liposomes (such as targeting of drugs) into a single system and thus circumvents the problems associated with each system. Liposomes entrap hydrophilic drugs in the aqueous phase and hydrophobic drugs in the lipid bilayers and retain drugs *en route* to their destination.The fact that some lipophilic drugs may interfere with bilayer formation and stability limits the range and amount of valuable drugs that can be associated with liposomes. By forming water-soluble complexes, CDs would allow insoluble drugs to accommodate in the aqueous phase of vesicles and thus potentially increase drug-to-lipid mass ratio levels, enlarge the range of insoluble drugs amenable for encapsulation (i.e., membrane-destabilizing agents), allow drug targeting and reduce drug toxicity. Problems associated with intravenous administration of CD complexes such as their rapid removal into urine and toxicity to kidneys, especially after chronic use, can be circumvented by their entrapment in liposomes.When the concept of entrapping CD complexes into liposomes was applied to HP-β-CD complexes of dexamethasone, dehydroepiandrosterone, retinal and retinoic acid, the obtained dehydration-rehydration vesicles (DRV liposomes) retained their stability in the presence of blood plasma. Liposomal entrapment can also alter the pharmacokinetics of inclusion complexes. Liposomal entrapment drastically reduced the urinary loss of HP-βCD/drug complexes but augmented the uptake of the complexes by liver and spleen, where after liposomal disintegration in tissues, drugs were metabolized at rates dependent on the stability of the complexes[5,6].

b. Cyclodextrins in Microspheres

In the presence of a high percentage of highly soluble hydrophilic excipients, complexation may not improve the drug dissolution rate from microspheres. Nifedipine release from chitosan microspheres was slowed down on complexation with HP-β-CD in spite of the improved drug-loading efficiency. Since it is highly unlikely for CD molecules to diffuse out of the microspheres, even with a low stability constant, the complex must first release the free drug that can permeate out of the microspheres. Hence, the observed slow nifedipine release from the microspheres was reported to be due to lesser drug availability from the complex and also due to formation of hydrophilic chitosan/CD matrix layer around the lipophilic drug that further decreases the drug matrix permeability. Sustained hydrocortisone release with no enhancement of its dissolution rate was observed from chitosan microspheres containing its HP-β-CD complex. The sustained hydrocortisone release was reported to be due to formation of a layer adjacent to the interface by the slowly dissolving drug during the dissolution process that makes the microsphere surface increasingly hydrophobic[6].

c. Cyclodextrins in Microcapsules

It was suggested that cross-linked βCD microcapsules, because of their ability to retard the release of watersoluble drugs through semipermeable membranes, can act as release modulators to provide efficiently controlled release of drugs. Terephthaloyl chloride (TC) cross-linked β-CD microcapsules were found to complex pnitrophenol rapidly and the amount complexed increased as the size of the microcapsules decreased. TC cross-linked βCD microcapsules retarded the diffusion of propranolol hydrochloride through dialysis membrane. Double microcapsules, prepared by encapsulating methylene blue with different amounts of β-CD microcapsules inside a cross-linked human serum albumin (HSA), showed decreasing release rate of methylene blue with increasing amount of β-CD microcapsules[5,6].

d.Cyclodextrins in Nanoparticles

Nanoparticles are stable systems suitable to provide targeted drug delivery and to enhance the efficacy and bioavailability of poorly soluble drugs. However, the safety and efficacy of nanoparticles are limited by their very low drug loading and limited entrapment efficiency (with classical water emulsion polymerization procedures) that may lead to excessive administration of polymeric material. Two applications of CDs have been found very promising in the design of nanoparticles: one is increasing the loading capacity of nanoparticles and the other is spontaneous formation of either nanocapsules or nanospheres by nanoprecipitation of amphiphilic CDs diesters. Both the new techniques have been reported to be useful due to great interest of nanoparticles in oral and parenteral drug administration[5,6].

Table I: Physicochemical properties of cyclodextrin[70]

Characteristics	A	β	Γ
No. of glucose unit	6	7	8
Internal diameter (nm)	0.47-0.53	0.60-0.65	0.75-0.83
Depth of cavity (nm)	0.79	0.79	0.79
Molecular weight	972	1135	1297
Solubility in water (gm/100ml)	14.5	1.85	23.2
Cavity diameter in A°	4.7-5.3	6-6.5	7.5-8.5
Volume of cavity (approx) in A°	174	262	472
Crystal forms	Hexagonal plates	Monoclonic parallelogram	Quadratic prism
Crystal water (% w/w)	10.2	13.2-14.5	8.13-17.7
Number of water molecules taken by cavity	6	11	17
Hydrophobic interaction	CD-cavity	CD-cavity	CD-cavity
Hydrogen bond	Glucose – OH	Glucose – OH	Glucose – OH

Tab. 2. Complexation efficiency can be enhanced by following methods

EFFECT	CONCEQUENCES
Drug ionization	Unionized drugs do usually form more stable complexes than their ionic counterparts. However, ionization of a drug increases its apparent intrinsic solubility resulting in enhanced complexation.
Salt formation	It is sometimes possible to enhance the apparent intrinsic solubility of a drug through salt formation.
Complex-in- complex	It is sometimes possible to increase the apparent intrinsic solubility of a drug through formation of metal complexes.
The acid/base ternary complexes	It has been shown that certain organic hydroxy acids (such as citric acid) and certain organic bases are able to enhance the complexation efficiency by formation of ternary drug/cyclodextrin/acid or base complexes.
Polymer complexes	Water-soluble polymers form a ternary complex with drug/cyclodextrin complex thereby increasing the observed stability constant of the drug/cyclodextrin complex. This observed increase in the value of the constant increases the complexation efficiency.
Solubilization of cyclodextrin aggregates	Organic cations and anions are known to solubilize uncharged D/CD complexes that have limited aqueous solubility. This will enhance the complexation efficiency during preparation of solid drug/cyclodextrin complex powder.
Combination of two or more methods	Frequently the complexation efficiency can be enhanced even further by combining two or more of the above mentioned methods. For example drug ionization and the polymer method or solubilization of the cyclodextrin aggregates by adding both polymers and cations or anions to the aqueous complexation medium.

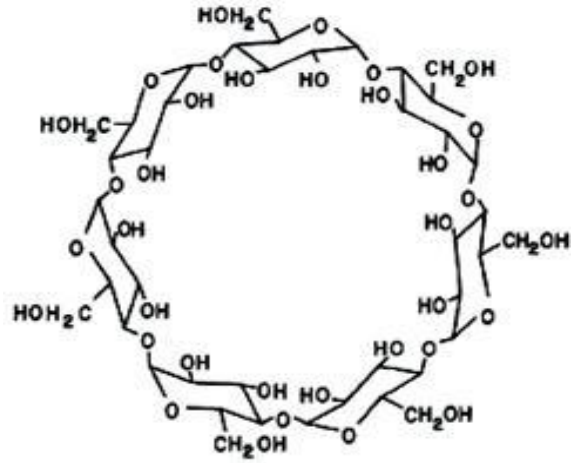


Figure. I: Chemical structure of cyclodextrin [6,70]

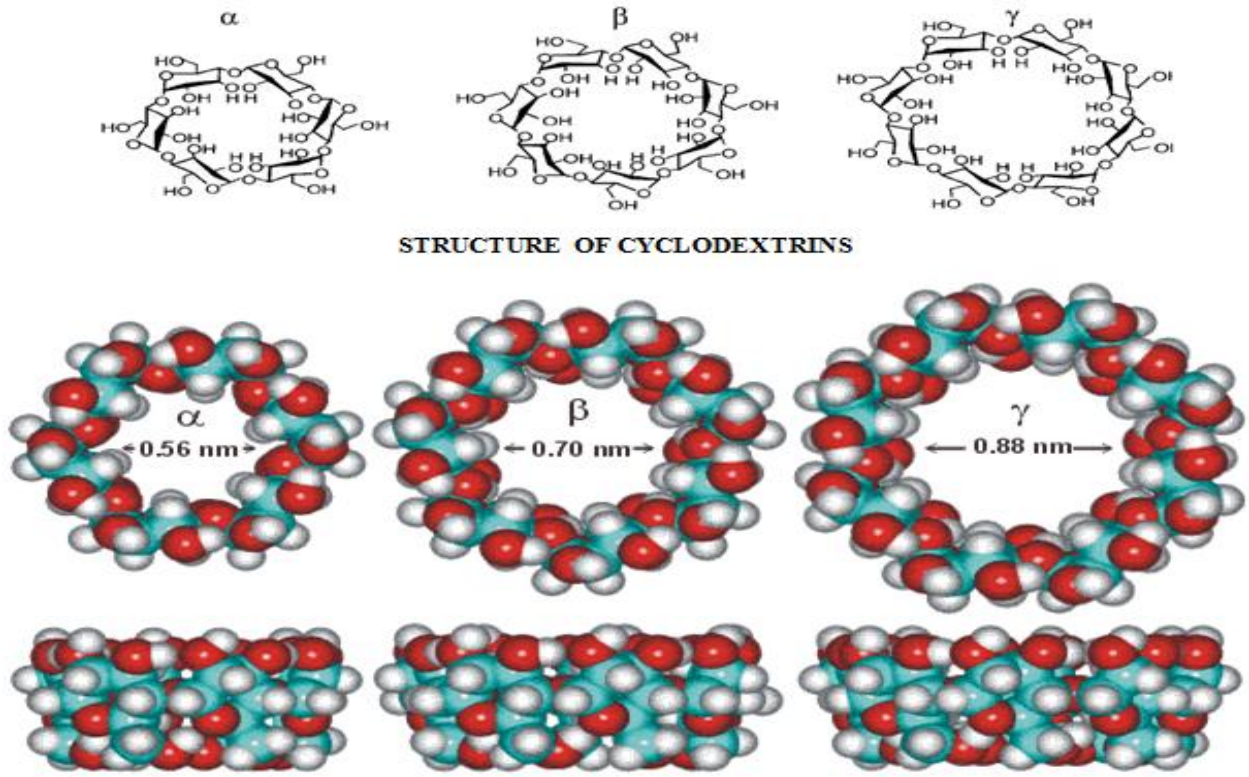


Figure.II Chemical structures of α-, β- and γ-cyclodextrin

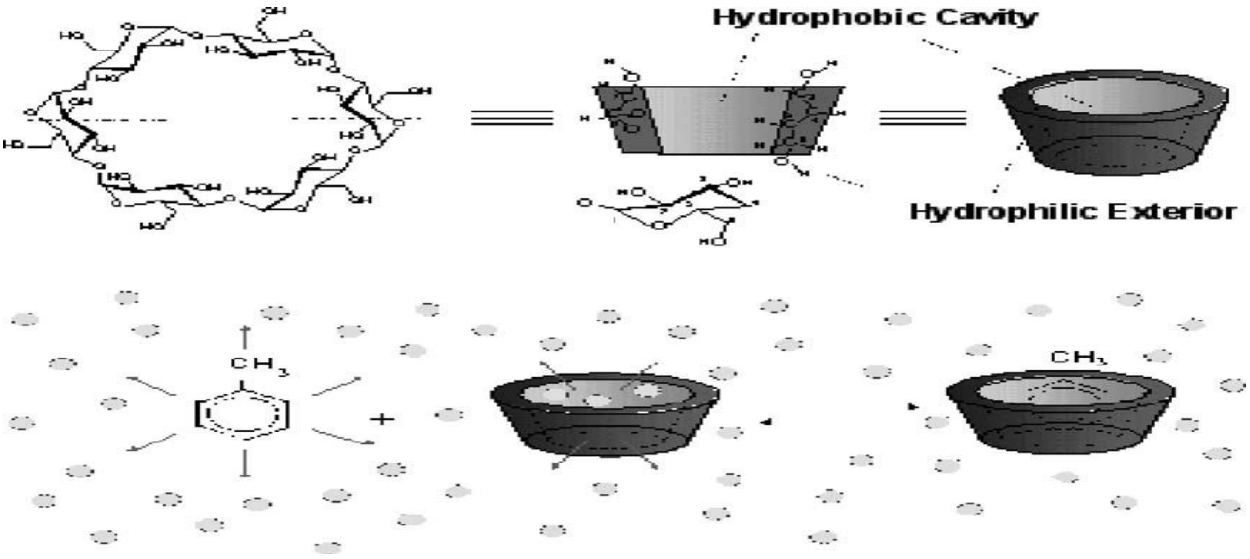


Figure III: Cyclodextrin structure and inclusion complex formation[7]

REFERENCES

1. Brewster ME, Loftsson T. Sci direct Adv Drug del reviews. 2007:59:645-666
2. Jain P, Goel A, Sharma S,Parmar M. Int J of pharma prof research. 2010:1(1)
3. Nasir A, Harikumar SL,Kaur A. Int Research J of Pharmacy. ISSN 2230-8407
4.Patil JS, Kadam DV, Marapur SC, Kamalapur MV. May-June 2010:2(2);Article 006,ISSN O976-044X
5. Gaurav tivari. J of Pharmacy and Bioallied sci. 2010 Apr-Jun; 2(2): 72–79.
6. Rasheed A, Ashok Kumar CK, Sravanthi VVNSS. sci pharma. 2008; 76: 567-598
7.Martine EM Del Valle. Elsevier, Process biochemistry
8.Jozsef Szejtli. Pure appl. Chem.,2004:76:10:1825-1845
9.Singh R, Bharti N, Madan J, Hiremathetal SN. J of pharmaceutical sci and tech. 2010:2(3): 171-183
10.N.Kanaka Durga Devil, A.Prameela Rani, Muneer Javed.M, K.Sai Kumar, J.Kaushik, V.Sowjanya . An Int res J. 2010,1(3), 155-165:ISSN 2229-5402
11.Nitalikar MM, Sakarkar DM, Jain PJ. J of current pharmaceutical res . 2012:10(1): 01-06
12. Chaudhary A, Nagaich U, Gulati N,Sharma VK, Khosa RL, J of adv pharmacy education and res. 2011: 2(1), 32-67: ISSN 2249-3379
13. Eastburn SD, Tao BY. Biotechnol Adv. 1994;12:325–39.
14. Szetjli J. Chem Rev. 1998;98:1743–53.
15. Stella VJ, Rajewski RA. Pharm Res 1997;14:556–5567.
16. Matsuda H, Arima H. Adv Drug Deliv Rev 1999;36:81–99.
17. Loftsson T. J Incl Phenom Macrocycl Chem. 2002; 44: 63–67. doi:10.1023/A:1023088423667
18.Brewster ME, Loftsson T. Adv Drug Del Rev. 2007; 59: 645–666. doi:10.1016/j.addr.2007.05.012
19.Roux M, Perly B, Djedaini Pilard F. Eur Biophys J. 2007; 36: 861–867. doi:10.1007/s00249-007-0207-6
20. Loftsson T, Duchene D. Int J of Pharm. 2007; 329: 1–11. doi:10.1016/j.ijpharm.2006.10.044
21. Hakkarainen B, Fujita K, Immel S, Kenne L, Sandstrom C. Carbohydr Res. 2005; 340: 1539–1545. doi:10.1016/j.carres.2005.03.016
22. Irie T, Uekama K.J Pharm Sci. 1997; 86:147–162. doi:10.1021/js960213f
23. Gould S, Scott RC. Food Chem Toxicol. 2005; 43: 1451–1459. doi:10.1016/j.fct.2005.03.007
24. Szente l, Szejtli J, Kis Gl.J Pharm Sci. 1998; 87: 778–781.doi:10.1021/js9704341
25. Liu L, Guo QX. J Incl Phenom Macroc Chem. 2002; 42: 1–14. doi:10.1023/A:1014520830813
26. Hirose K. J Incl Phenom Macroc Chem. 2001; 39: 193–209. doi:10.1023/A:1011117412693
27. Figueiras A, Carvalho RA, Ribeiro L, Labandeira JJT, Veiga FJB. Eur J Pharm and Biopharm. 2007; 67: 531–539. doi:10.1016/j.ejpb.2007.03.005
28. Higuchi T, Connors KA. Adv Anal Chem Instrum. 1965; 4: 117–212.
29. Loftsson T, Masson M, Brewster ME. J Pharm Sci. 2004; 93: 1091–1099. doi:10.1002/jps.20047
30. Connors KA. Chem Rev. 1997; 97: 1325–1357. doi:10.1021/cr960371r
31. Rao VM, Stella VJ.J Pharm Sci. 2003; 92: 927–932. doi:10.1002/jps.10341
32.Hedges AR. Chem Rev. 1998; 98: 2035–2044.doi:10.1021/cr970014w
33. Loftsson T, Masson M, Sigurjonsdottir JF. STP Pharma Sci. 1999; 9: 237–242.
34.Loftsson T, Sigfusson SD, Sigurosson HH, Masson M. STP Pharma Sci. 2003; 13: 125–131.
35. Irie T, Wakamatsu K, Arima H, Aritomi H, Uekama K. Int J Pharm. 1992; 84: 129–139. doi:10.1016/0378-5173(92)90053-5
36. Loftsson T, Masson M. Int J Pharm. 2001; 225: 15–30. doi:10.1016/S0378-5173(01)00761-X
37. Tasic LM, Jovanovic MD, Djuric ZR. J Pharm Pharmacol. 1992; 44: 52–55. PMid:1350629
38. Londhe V, Nagarsenker M.Indian J Pharm Sci. 1999; 61: 237–240.
39. Becket G, Schep LJ, Tan MY.Int J Pharm. 1999; 179: 65–71. doi:10.1016/S0378-5173(98)00382-2
40.Qian L, Guan Y, Xiao H. Int J Pharm. 2008; 357: 244–251. doi:10.1016/j.ijpharm.2008.01.018
41. Challa R, Ahuja A, Ali J, Khar R.. AAPS Pharm SciTech. 2005; 6: 329–357. doi:10.1208/pt060243
42. Van Dorne H. Eur J Pharm Biopharm. 1993; 39: 133–139.
43. Loftsson T, Vogensen S, Brewster ME, Konraosdottir F. J Pharm Sci. 2007; 10: 2532–2546. doi:10.1002/jps.20992
44. Uekama K. Chem Pharm Bull. 2004; 8: 900–915. doi:10.1248/cpb.52.900
45. Uekama K, Fujinaga T, Hirayama F, Otagiri M, Yamasaki M, Seo H, Hashimoto T, Tsuruoka M. J Pharm Sci. 1983; 72: 1338–1341. doi:10.1002/jps.2600721125
46. Miyake K, Arima H, Hiramaya F.Pharm Dev Technol. 2000; 5: 399–407. doi:10.1081/PDT-100100556
47.Rajewski RA, Stella VJ. J Pharm Sci. 1996; 85: 1142–1168. doi:10.1021/js960075u
48.Nicolazzi C, Venard V, Le Faou A, Finance C. Antiviral Res. 2002; 54: 121–127. doi:10.1016/S0166-3542(01)00218-2
49.Uekama K, Hirayama F, Irie T. Chem Rev. 1998; 98: 2045–2076. doi:10.1021/cr970025p
50.Ueda H, Ou D, Endo T, Nagase H, Tomono K, Nagai T. Drug Dev Ind Pharm. 1998; 24: 863–867. doi:10.3109/03639049809088532
51.Lutka A, Koziara J. Acta Pol Pharm. 2000; 57: 369–374. PMid:11126028
52. T. Loftssan and M.E. Brewter. J. Pharm. Sci. 85(10):1017- 1025(1996)
53.R.A. Rajewski and V.J. Stella. J. Pharm. Sci.85(11):1142- 1169(1996).
54. D. Duchene, C. Vaution and F. Glomot. Drug Dev. Ind. Pharm.12(11-13): 2193-2215(1988).

55. A.H.A. Marzouqi, I. Shehatta, B. Jobe and A. Dowaidar. J. Pharm.Sci. 95(2): 292-304(2006).
56.L. Wang, X. Jiang, W. Xu and C. Li. Int. J. Pharm. 341(1-2): 58-67(2007).
57.P.T. Tayade and P.R. Vavia. Indian J. Pharm. Sci. 68(2):164-170(2006).
58.G.S. Jadhav and P.R. Vavia. Int. J. Pharm. 352(1-2): 5-16(2008).
59. D. Amididouche, H. Darrouzet, D. Duchene and M.C. Poelman. Int. J. Pharm. 54(2): 175- 179(1989).
60.F. Maestrelli, M.L.G. Rodriguez, A.M. Rabasco and P. Mura. Int. J. Pharm. 298(1): 55-67(2005).
61. S. Scalia, R. Tursilli, N. Sala and V., Iannuccelli. Int. J. Pharm. 320(1-2): 79-85(2006).
62. D.V. Derle, B.S.H. Sagar, R.S. Kotwal, R.D., Ingole and S.S. Chavhan. Indian Drugs. 43(8): 625-629(2006).
63. M. Bencini, E. Ranucci , P. Ferruti, F. Trotta, E. Donalisio, M. Cornagilia, D. Lembo and R., Cavalli. J. Controlled Release. 126(1): 17- 25(2008).
64. D.D. Chow and A.K. Karara. Int. J. Pharm. 28(2-3): 95-101(1986).
65. N. Erden and N. Celebi. Int. J. Pharm. 48(1-3): 83- 89(1988).
66. H.M.C. Marques, J. Hadgraft and I.W., Kellaway. Int. J. Pharm. 63(3): 259—266(1990).
67. J. Manosroi, M.G. Apriyani, K. Foe and A. Manosroi. Int. J. Pharm. 293(1-2): 235-240(2005).
68. S. Baboota, M. Dhaliwal, K. Kohli and J. Ali. Indian J. Pharm. Sci. 67(2): 226-229(2005).
69. T. Takagi. Molecular Pharmaceutics. 3(6), 631–643 (2006)
70. A. Magnúsdóttir, M. Másson and T. Loftsson. J. Incl. Phenom. Macroc.Chem. 44, 213-218, 2002.