

Account of analytical techniques employed for the determination of thermodynamics of inclusion complexation of drugs with cyclodextrins

R Chadha*, N Kashid and A Saini

Pharmaceutical Chemistry Division,
University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160 014

Inclusion complexation of drugs with cyclodextrins is evolved in the recent past as an important phenomenon to increase the solubility, stability, and bioavailability of drugs along with masking of other undesirable properties, thus making the drug candidate more palatable. Cyclodextrins are known to form non-covalent bonded inclusion complexes with variety of drugs in both solution and solid state with different physical, chemical and biological properties than parent drug or cyclodextrins. Determination of the stability constants of the inclusion complexes is very important. A high value of equilibrium constant (>5000 kg/mol) may lead to very slow release of drug from complex while low value (<200 kg/mol) reduces the effect that inclusion complexation have on the bioavailability of the drug. Wide varieties of analytical techniques are employed to study complexation reaction in solution and solid state. Microcalorimetry is the most precise method for directly measuring the thermodynamic properties of the complex formation. The thermodynamic parameters give a macroscopic picture of the inclusion process.

Keywords: Analytical techniques, Cyclodextrins, Stability constants

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Introduction

Cyclodextrins (cyds) are cyclic (α -1,4)-linked oligosaccharides of α -D-glucopyranose, containing a relatively hydrophobic central cavity and hydrophilic outer surface. Owing to lack of free rotation about the bonds connecting the glucopyranose units the cyclodextrins are not perfectly cylindrical molecules but are toroidal or cone-shaped. Based on this architecture, primary hydroxyl groups are located on the narrow side of the torus, while secondary hydroxyl groups are located on the wider edge. The β , δ -cyclodextrins form intramolecular hydrogen bonds between secondary OH groups, which detract from hydrogen bond formation with surrounding water molecules, resulting in less negative heats of hydration. Thus, intramolecular hydrogen bonding can result in relatively unfavourable enthalpies of solution and low aqueous solubilities. Substitution of any of the hydrogen bond forming hydroxyl groups even by hydrophobic moieties such as, methoxy and ethoxy functions result in dramatic increase in water solubility. The central cavity of the cyclodextrin molecule is lined with skeletal carbons and ethereal

oxygens of the glucose residues. It is, therefore, lipophilic. The polarity of the cavity has been estimated to be similar to that of aqueous ethanolic solution. It provides the lipophilic microenvironment into which suitably sized drug molecules may enter and be induced. No covalent bonds are broken or formed during drug-cyclodextrin complex formation, and in the aqueous solutions the complexes are readily dissociated¹⁻³.

In the recent years, pharmaceutical modification by inclusion complexation with cyclodextrins has been extensively developed to increase aqueous solubility of poorly water soluble non-steroidal anti-inflammatory drugs (nimesulide^{4,5}, piroxicam⁶, indomethacin⁷), steroids⁸, rofecoxib⁹, griseofulvin¹⁰, antidiabetic drugs (tolbutamide¹¹, gliclazide¹²), and pyrene¹³. Complexation of drugs also increase their bioavailability and stability to photo- or chemical degradation (DY9760e¹⁴, insulin¹⁵, antitumour agents like, DuP937¹⁶, hydrocortisone-17-butyrate¹⁷) stability towards heat (diclofenac sodium¹⁸, dipyridamole¹⁹) has been reported.

In addition, cyclodextrins can be used to reduce or prevent the gastrointestinal or ocular irritation (e.g. flufenamic acid²⁰, thioctic acid²¹, naproxen²², thalidomide²³) or eliminate unpleasant smells or tastes

*Author for correspondence
E-mail: renukchadha@rediffmail.com

(e.g. ibuprofen, naproxen and bile acids), prevent the drug-drug or drug additive interactions or even to convert oils and liquid drugs into microcrystalline and amorphous powders. Simple salt formation does not provide real advantage in terms of absorption because in some cases even the salts (e.g. salts of acidic drugs²⁴, diclofenac sodium and other inorganic salts²⁵) are poorly soluble or for some other reasons inadequate, thus cyclodextrin complexation plays an important role in such cases. Hydrophobic cyclodextrins are also used for sustained release of highly water soluble drugs (e.g. molsidomine²⁶, captopril²⁷, diltiazem²⁸).

Inclusion complexes can be prepared by various methods²⁹. One of the best methods consists in dissolving the cyclodextrin and drug in water or in organo-aqueous solvent the inclusion compound precipitating simultaneously or after cooling the medium. The mixture can also be freeze dried or spray dried. Another method is the kneading method which consists in adding the active ingredient to the slurry of cyclodextrin and then kneading thoroughly to obtain a paste which is then dried. If the substance to be included is insoluble in water, it is dissolved in an organic solvent and added with agitation to a hot aqueous solution of cyclodextrin. Crystallization takes place within following hours or days. In liquid medium the formation of an inclusion is governed by an equilibrium reaction between the host and guest molecules.

Free drug molecules are in the equilibrium with the molecules bound within cyclodextrin cavity. Measurement of the stability constant (K_s) or equilibrium constants (K) of the drug- cyclodextrin complexes is important, since this is an index of changes in physico-chemical properties of a compound upon inclusion. Therefore the determination of stability constants of inclusion complexes is very important. A high value of equilibrium constant (>5000 kg/mol) may lead to very slow release of drug from complex while very low value (<200 kg/mol) reduces the effect that inclusion complexation has on bioavailability of drug^{30,31}.

Methods for determining K values are based on titrating the changes in the guest molecule, i.e., drug molecule with the cyclodextrin and then the concentration dependencies. Additive properties that can be titrated to provide information on the K values, include aqueous solubility, chemical reactivity, molar absorptivity, and other optical properties, phase

solubility measurements, NMR chemical shifts, pH metric methods, calorimetric titration, freezing point depression, LC chromatographic retention times. The stability constant K_s is better expressed as $K_{m:n}$ to indicate the stoichiometric ratio of the complex. It can be written as:



where D is the drug and cyd is the cyclodextrin, so that,

$$K_{m:n} = \frac{[D_m \text{cyd}_n]}{[D]^m [\text{cyd}]^n} \quad \dots(2)$$

In addition, a dissociation constant can be defined as:

$$K_d = \frac{1}{K_s} \text{ or } \frac{1}{K_{m:n}} \quad \dots(3)$$

The thermodynamic parameters³², i.e. the standard free energy ΔG , the standard enthalpy change ΔH , the standard entropy change ΔS , can be obtained from the temperature dependence of the stability constant for cyclodextrin complex. The complex formation is almost always associated with a relatively large negative ΔH and ΔS that can be either positive or negative. Also the complex formation is independent of the chemical properties of the drug, i.e., guest molecules. The association of binding constants with substrate polarizability suggests that van der Waals forces are important in complex formation. Other forces, important in the complex formation, include hydrogen bonding, hydrophobic interactions, release of ring strain in cyclodextrin molecule, and changes in solvent-surface tensions.

There are several techniques for the determination of thermodynamic parameters for complexation reaction. These are described here supported by citations.

Phase Solubility Method

Phase-solubility analysis is useful and widely applied analytical approach in this context³³. This technique is based on the equilibrium solubility of a chemical substance in a given solvent, at a given temperature and is quantity characteristics of the substance that may therefore be used as a criterion of purity and identity. The equilibrium solubility of a

pure compound is as much a characteristic physical property as the melting point. It involves an examination of the effect of a solubilizer, i.e., ligand or cyclodextrin (*cyd*) on the drug solubilized (*D*). Experimentally an excess drug is added to vials containing a constant volume of solution of cyclodextrin with successively increasing concentration of cyclodextrin. The presence of solid drugs in these systems is necessary to maximize the thermodynamic activity of the dissolved substrate. The vials are mixed at constant temperature until equilibrium is established (which frequently takes about one week). The solid drug is then removed and the solution assayed for the total concentration of *D*. A phase solubility diagram is constructed by plotting the total molar concentration of *D* on the y-axis and the total molar concentration of cyclodextrin added on the x-axis. Phase-solubility diagrams thus prepared fall into two main categories, A- and B- types (Fig. 1). A-type curves are indicative for the formation of soluble inclusion complexes, while B-type behaviour is suggestive of the formation of inclusion complexes of poor solubility.

A B_S -type response denotes complexes of limited solubility and B_I -curve are indicative of insoluble complexes. The A-curves are subdivided into A_L -linear increase of drug solubility as a function of cyclodextrin concentration, A_P -(positively deviating isotherm) and A_N -(negatively deviating isotherm) subtypes.

Hydrocortisone butyrate³⁴ shows B_S -type phase

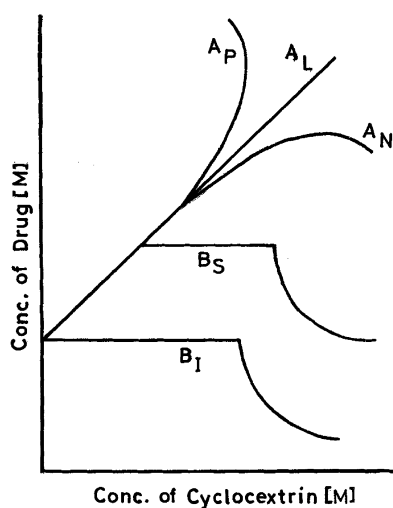


Figure 1 — Phase solubility relationships of drugs with different cyclodextrins

solubility curve with γ -cyclodextrin, whereas A_L -type phase solubility curve with α -cyclodextrin, β -cyclodextrin and dimethyl β -cyclodextrin³³. Nimesulide⁵ gives A_L -type solubility curve with β -cyclodextrin and hydroxy propyl- β -cyclodextrin, while progesterone⁹ shows A_L -type solubility curve with α -cyclodextrin but B_S -type with β - and γ -cyclodextrin. Bropirimine³⁵ and salbutamol³⁶ gave A_L -type while gliclazide¹² gave B_S -type curve with β -cyclodextrin and DY-9760e gave A_P -type curve with β -cyclodextrin.

β -cyclodextrin often gives rise to B-type curves due to poor water solubility of ligand itself, the chemically modified cyclodextrins including hydroxy propyl β -cyclodextrin and sulphobutyl ether β -cyclodextrin usually produce the soluble complexes (i.e., A-type systems). A_L -type diagrams are first order with respect to cyclodextrin (*cyd*) and may be first or the higher order with respect to the drug (*D*), i.e., D_1cyd , D_2cyd , D_3cyd ,... D_mcyd . If the slope of an A_L -type system is greater than one, higher order complexes are indicated. A slope of less than one does not necessarily exclude higher order complexation but 1:1 complexation is usually assumed in the absence of other information. A_P -type system suggests the formation of higher order complexes with respect to the ligand at higher ligand concentrations, i.e., D_1cyd_2 , D_1cyd_3 ,... D_1cyd_n . The stoichiometry of A_P -type systems can be evaluated by curve fitting. A_N -type systems are problematic and difficult to interpret. The negative deviation from linearity may be associated with ligand-induced changes in the dielectric constant of the solvent or self-association of the ligands at high cyclodextrin concentrations.

These phase-solubility systems not only allow a qualitative assessment of the complexes formed but may also can be used to derive the equilibrium constants. The equilibrium constant K for the formation of $[D_mcyd_n]$ can be represented by:

$$K = \frac{[D_mcyd_n]}{[D]^m [cyd]^n} \quad \dots(4)$$

Given that:

$$[D] = D_o, \quad D_t = D_o + m[D_mcyd_n] \quad \text{and} \quad cyd_t = cyd + n[D_mcyd_n]$$

then the values of $[D_mcyd_n]$ and $[cyd]$ can be obtained by,

$$[D_mcyd_n] = \frac{D_t - D_o}{m}, \quad \dots(5)$$

$$[cyd] = cyd_t - n[D_mcyd_n], \quad \dots(6)$$

where D_o is the equilibrium solubility of D , i.e., in the absence of solubilizer, D_t is the total concentration of D complexed and uncomplexed and cyd_t is the total concentration of cyd . For phase-solubility systems that are first order with respect to the cyclodextrin ($n = 1$), the following equation may be derived:

$$D_t = \frac{mK D_o^m cyd_t}{1 + K D_o^m} + D_o. \quad \dots(7)$$

A plot of D_t vs cyd_t for the formation of D_mcyd should give a straight line with y-intercept representing D_o and the slope being:

$$\text{Slope} = \frac{mK D_o^m}{1 + K D_o^m}. \quad \dots(8)$$

Therefore, if m is known, K can be calculated. If $m = 1$ (i.e., a 1:1 drug: cyclodextrin complex forms), the following equation can be applied:

$$K_{1:1} = \frac{\text{slope}}{D_o(1-\text{slope})}. \quad \dots(9)$$

Thus, from the slope and intercept of phase-solubility diagram the apparent stability constant can be determined. The only disadvantage of the method is that it is time consuming.

Spectroscopic Methods

UV- Visible Spectroscopy

Modification of UV spectra in the presence of cyclodextrins provides evidence of the formation of an inclusion complex. As cyclodextrin concentrations increase the spectra show a shift of the maximum

absorption to lower wavelengths and a decrease in molar extinction coefficient⁶. Generally the spectral changes observed are similar to effect caused by changes in polarity of solvent, thereby suggesting that chromophore of the guest is transferred from an aqueous medium to non-polar cyclodextrin cavity. These changes must be due to a perturbation of electronic energy levels of guest compound either by direct interaction with cyclodextrin, by exclusion of solvating water molecules or by combination of these two effects. Although frequently, only small shifts are observed on UV spectra of included guests the method is often used to detect inclusion complexation.

The influence of dimethyl- β -cyclodextrin on the absorption spectrum of anthrapyrazole antitumour agent⁶ DuP937 was studied. As cyclodextrin concentration increased the absorption spectrum changed significantly, bringing about an enhancement in absorption upon addition of dimethyl- β -cyclodextrin. This absorptivity change can be used in calculating the association constants using, the Benesi-Hilderbrand equation:

$$\frac{b}{\Delta A} = \frac{1}{S_t K_c \Delta \epsilon [DM\beta cyd]} + \frac{1}{D_t K_c \Delta \epsilon}, \quad \dots(10)$$

where b is the path length, ΔA the absorbance change, D_t the total substrate concentration, K_c the association constant, $\Delta \epsilon$ the molar absorptivity change and $[DM\beta cyd]$ the ligand concentration.

Cyclodextrin complexes of drugs like, piroxicam^{6,37}, thioctic acid²¹, bropirimine³⁰, purine nucleosides³⁸, and anthracycline antibiotics doxorubicin (Dx) and daunorubicin (Dr)³⁹, pyridine derivatives⁴⁰, chlorambucil⁴¹ are studied by this technique. When solutions of bropirimine were spectrophotometrically titrated with increasing amounts of β -cyclodextrin, a decrease in the intensity of the maximum absorbance of the drug was observed which indicated the formation of complex.

In the case of purine nucleosides, UV spectrophotometry was used to explore the structure binding relationship, inclusion complex geometry, cavity microenvironment, and 1:1 stoichiometry of complex. Absorption spectra of both anthracyclines Dx and Dr in alkaline solution shift towards the spectra obtained in acidic and neutral media upon

increasing the γ -cyclodextrin concentration. This indicated that phenolic hydroxyl groups (C_6 and C_{11}) with a pK_a value of around 10 undergo the less deprotonation in the presence of γ -cyclodextrin⁴². Thus the pK_a value of phenolic group increases upon the addition of γ -cyclodextrin. This result also suggest that the aglycone moiety of anthracycline is included in γ -cyclodextrin cavity. Absorption measurements performed with α - and β -cyclodextrin showed no significant change compared with the spectra of free *Dx* or *Dr*. The location of maxima as well as molar extinctions of compounds did not alter indicating that α - and β -cyclodextrin cavity size in comparison with γ -cyclodextrin is too small to include lipophilic aglycone parts of *Dx* and *Dr*. Stability constants can also be determined by UV method when suitable absorptivity changes of anthracyclines occur in the presence of different cyclodextrin concentration.

Infra-red Spectroscopy

IR spectroscopy is used to access the interaction between cyclodextrin and guest molecules in solid state. Technique is not generally suitable to detect inclusion complexes and is less clarifying than other methods. Cyclodextrin bands often change only slightly upon complex formation and if the fraction of guest molecules encapsulated in the complex is less than 25 per cent, 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 application of IR spectroscopy is limited to guests having some characteristic bands such as carbonyl or sulphonyl groups. Some of the drugs whose inclusion complexation with cyclodextrin have been studied by IR technique are nimesulide⁵, indomethacin⁷, bropirimine³⁵, ibuprofen⁴³, famotidine⁴⁴, tolbutamide⁴⁵, 4-substituted benzoic acid and benzaldehyde drugs⁴⁶, ibuprofen⁴⁷, phenothiazine⁴⁸, amylobarbitone⁴⁹, allopurinol⁵⁰, piroxicam⁵¹, and N-diclofenac⁵². The higher frequency shift of carbonyl group observed in IR spectra of inclusion compounds of β -cyclodextrin with benzoic acid drugs (like benzoic acid, *p*-anisic acid, *p*-toluic acid, *p*-chloro benzoic acid, *p*-nitro benzoic acid) can be ascribed to destruction of strong H-bonding structure in uncomplexed drugs upon formation of inclusion complexes with β -cyclodextrin. In IR spectra of Ibuprofen⁴³ characteristic carbonyl stretching band at 1709 cm^{-1} which appeared in both drug and physical mixture samples was shifted to 1736 cm^{-1} with markedly decreased intensity in inclusion complex

sample due to breakdown of intermolecular H-bonding associated with ibuprofen molecules and establishing of less weak forces in complex system. In IR of tolbutamide⁴⁵ carbonyl stretching at 1710 cm^{-1} for drug shifted to 1725 cm^{-1} together with broadening of band in inclusion complex. This shift was due to formation of H-bonds between drug and host molecule as well as dissociation of intermolecular H-bond between two molecules of tolbutamide. Restriction on bending and increased stretching vibration in β -cyclodextrin cavity are probably responsible for broadening of band⁵³.

Absorption peaks characteristic of amino groups of famotidine in range of $3220\text{--}3500\text{ cm}^{-1}$ have disappeared from spectrum of inclusion complex. Further, a shift in position of C=N stretching bands in region of $1528\text{--}1636\text{ cm}^{-1}$ is observed in IR spectra of complex. These changes have resulted from inclusion of famotidine within cavity of β -cyclodextrin and dissociation of intra-molecular H-bands of famotidine between guanidine nitrogen and thiazole nitrogen through this complexation.

NMR Spectroscopy

NMR spectroscopy has been used to investigate the stoichiometries and association constants of pharmacologically important drug-cyclodextrin complexes⁵⁴. It is useful technique in the analysis of structural and dynamic properties of complexes, both in aqueous solution and in solid state. ^1H -NMR may also be used to determine the direction of penetration of guest molecules into cyclodextrin cavity³⁸. The formation of inclusion complexes with cyclodextrins is accompanied by significant downfield shift of protons of guest molecule due to the hydrophobic nature of the cavity, while those of affected host protons upfield, thereby throwing light on the interactions, position, and orientation of the guest molecule⁵⁵. This effect was employed to determine the association constant (K_c). Inclusion of aromatic guests in the cyclodextrin cavity induce the upfield shifts of the H3 and H5 protons of the cyclodextrin which are located on the interior of the cavity due to the ring currents of aromatic guests and H-1, H-2 and H-4 atoms, located on the exterior of cavity show only marginal up field shifts. Alternatively, if association takes place at exterior of torus, H-1, H-2, and H-4 shall be strongly shielded⁵⁶. The magnitude of the upfield shifts of cyclodextrin's H-3 and H-5 protons can be used as a measure of complex stability as well as a depth of inclusion⁵⁷.

The association constants could be determined by holding the concentration of drug constant while the concentration of the cyclodextrin was varied. The value $\Delta\delta_{\text{obs}}$ was plotted against the concentration of cyclodextrin and fitted to the following equation⁵³ using a nonlinear least squares method.

$$\Delta\delta_{\text{obs}} = \Delta\delta_{\infty} \times \frac{1}{2} \left(\frac{[D]}{[D]} + \frac{1}{K_c[D]} + 1 \right) - \left\{ \frac{1}{4} \left(\frac{[D]}{[D]} + \frac{1}{K_c[D]} + 1 \right)^2 - \frac{[D]}{[D]} \right\}^{1/2}, \quad \dots(11)$$

where $\Delta\delta_{\text{obs}}$ is the change in chemical shift of a proton of drug due to addition of a certain concentration of cyclodextrin, and $\Delta\delta_{\infty}$ is the limiting value of this change for infinite cyclodextrin concentration.

Various drugs, whose inclusion complexes with cyclodextrins are determined by this technique are gliclazide¹², DY9760e (cytoprotective agent)¹⁴, insulin¹⁵, DuP937¹⁶, purine nucleosides³⁸, 4-substituted benzoic acid and benzaldehyde drug⁴⁶, amylobarbitone⁴⁹, flavanoids⁵⁸, riboflavin⁵⁹, melatonin⁶⁰, sulphamethizole⁶¹, piroxicam⁶², 6-p-toluidinyl naphthalene-2-sulphonate⁶³, pheromone constituents of olive fruit fly⁶⁴, prostaglandin E₁⁶⁵, 8-anilino-naphthalene-1-sulphonate (ANS)⁶⁶, tenilsetam⁶⁷, indomethacin^{68,69}, naproxen⁷⁰, ofloxacin⁷¹, mebendazole⁷², flutamide⁷³, and bropirimine⁷⁴.

¹H-NMR was used to characterize the β -cyclodextrin complex of piroxicam⁶² in terms of stoichiometry, association constant and geometry of complex in aqueous solution. 1:1 stoichiometry of complex was determined by continuous variation method. Significant NOE_s were observed between inner proton of β -cyclodextrin and protons of both aromatic rings of piroxicam sodium salt, a result indicating that two isomeric 1:1 complexes must be present in solution within range of concentrations investigated. Results also showed that upon dilution of the complex percentage of drug that is associated rapidly reduces until complete dissociation takes place.

In the case of 4-substituted benzoic acid and benzaldehyde drugs⁴⁶ ¹H-NMR spectra of pure β -cyclodextrin was compared with β -cyclodextrin in sample of inclusion compound with chlorobenzoic acid. In pure β -cyclodextrin proton signals of H-5 and H-3 appeared at 3.865 and 3.955ppm, respectively. They shifted to 3.770 and 3.887 ppm in sample of inclusion compound. By NMR spectroscopy it was also possible to determine the position of aromatic ring of drug molecule in inclusion compound.

In the case of 8-anilino-naphthalene-1-sulphonate (ANS)⁶⁶, the proton signals of ANS shifted by adding β -cyclodextrin. In the presence of β -cyclodextrin, signals due to 6-H and 3-H shifted downfield. The upfield shifts of signals due to 4-H, 3-H and 5-H protons, and protons of benzene ring are especially prominent. Comparison of ¹H-NMR spectra of pure β -cyclodextrin with that of β -cyclodextrin in the presence of ANS revealed that signals due to protons of all types in β -cyclodextrin molecule shifted upfield in the presence of ANS. Shift of signal due to 5-H was most prominent, followed by those due to 6-H and 3-H in this order. The signals due to 1H, 2H and 4H outside the cavity shifted only slightly upfield in the presence of ANS. These upfield shifts of signals of β -cyclodextrin appear to result mainly from magnetic anisotropy of benzene or naphthalene ring of ANS molecule. From NMR spectra it is also apparent that coplanarity of aromatic rings of ANS increases more in inclusion complex than in the uncomplexed form.

¹³C-NMR spectroscopy has also found application in investigation of intermolecular interactions. It is often used to gain insight into inclusion modes of inclusion complexes in aqueous solution. Cyclodextrin induced change in ¹³C-chemical shift results predominantly from electrical environmental effects of cyclodextrin cavity and in general ¹³C inclusion shifts may be mainly divided into hydrophobic and van der Waals interaction shifts⁷⁵. Generally, carbon atoms of guest molecules which are deeper in the cavity seem to undergo shielding, whereas those closer to the wider cavity of cyclodextrin tend to be deshielded by complexation^{57,76}. Inoue *et al.*⁷⁷ have on the basis of reaction field theory interpreted these findings as consequence of the difference in dielectric environment. ¹H-NMR investigation of cyclodextrin inclusion complexation may technically be hindered, if complex is slightly soluble in D₂O. ¹³C-NMR

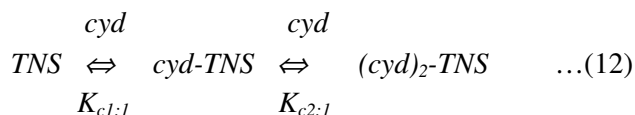
spectra may be obtained in aqueous solution which is a great advantage, however, higher concentrations should be required than in $^1\text{H-NMR}$. Examples of drugs-cyclodextrin complexes studied by this technique are gliclazide¹², DY9760e¹⁴, phenothiazine⁴⁸, pheromone constituents of olive fruit fly⁶⁴, prostaglandin E_1 ⁷⁸, barbiturates⁷⁹, naproxen⁸⁰, and piroxicam⁸¹.

Fluorescence Spectroscopy

Fluorimetry can be used for the calculation of stability constant in various ways, including changes in the fluorescence polarization⁸². Such changes reflect the relaxation time of molecules, thus providing direct evidence for complex formation and reduction (through quenching) in fluorescence intensity of guest molecule on inclusion in the cyclodextrin cavity. In some cases inclusion reactions might be slow, it is essential that equilibrium (indicated by a fluorescence reading that remain unchanged with time) is reached before the measurement of fluorescence intensity. Upon inclusion of the drug in cyclodextrin, fluorescence spectra may be influenced.

An enhancement in the fluorescence is clearly demonstrated by fluorescence spectra of pyrene phospholipids¹³, riboflavin⁵⁹, naphthalene^{63,66}, naproxen⁸³ and piroxicam⁸⁴ in the absence and in the presence of cyclodextrins in aqueous solution. 6-p-toluidinyl naphthalene-2 sulphonate (TNS)⁶³ is known to be a fluorescent probe for exploring the hydrophobic regions. The fluorescence of this probe is quenched (decreased) in water, but in the hydrophobic environment such as, proteins, it is augmented significantly with shifts of emission maxima towards shorter wavelength and with higher quantum yield of TNS fluorescence. TNS fluorescence increases markedly when α - or β -cyclodextrin were added to aqueous solution^{85,86}. Fluorescence has been thought to originate by including TNS in the cyclodextrin molecule cavity. Consequently the changes of fluorescence spectra offer evidence of hydrophobicity of cavity. Thus, hydrophobic bonding is suggested as an important driving force in formation of inclusion complex between dye and cyclodextrin.

Taking advantage of the effect that fluorescence intensity of TNS, increases in the presence of β -cyclodextrin, formation constant K_c was estimated. β -cyclodextrin forms two kinds of complexes with TNS having molar ratios of 1:1 and successively 2:1 as shown in Eq. (12)



Formation constants $K_{c1:1}$ and $K_{c2:1}$ for 1:1 and 2:1 complexes respectively, are shown by Eq. (13 and 14), respectively.

$$K_{c1:1} = \frac{[\text{cyd-TNS}]}{[\text{cyd}][\text{TNS}]} \quad \dots (13)$$

$$K_{c2:1} = \frac{[(\text{cyd})_2\text{-TNS}]}{[\text{cyd}][\text{cyd-TNS}]} \quad \dots(14)$$

Fluorescence intensity observed (F) is a sum of those of free TNS (F_t), 1:1 complex (F_1) and 2:1 complex (F_2). If the large excess β -cyclodextrin is added to the TNS, Eq. (16) is valid.

$$F = F_t + F_1 + F_2, \quad \dots(15)$$

$$F = \frac{F_T}{1 + C_o K_{c1:1} + C_o^2 K_{c1:1} K_{c2:1}} + \frac{F_{\alpha 1}}{1 + \frac{C_o K_{c1:1}}{C_o K_{c1:1}}} + \frac{F_{\alpha 2}}{1 + \frac{C_o K_{c1:1}}{C_o K_{c1:1}} + \frac{1}{C_o K_{c1:1} K_{c2:1}}} \quad \dots(16)$$

Here, F_T , $F_{\alpha 1:1}$, and $F_{\alpha 2:1}$ denote the fluorescence intensities which should be observed when all the TNS is free, all the TNS has formed 1:1 complex and all the TNS has formed 2:1 complex respectively. C_o represents total concentration of β -cyclodextrin.

Formation constants $K_{c1:1}$ and $K_{c2:1}$ can be estimated from Eq. (16), using a non-linear least square program. The Von't Hoff plots for 1:1 and 2:1 complexes were obtained by plotting $\log K$ against the absolute temperature. The changes in the enthalpy (ΔH°) and entropy (ΔS°), accompanying the complexation were determined from slope and

intercept of straight line, respectively. Similarly, inclusion complexes of 8-anilino-naphthalene-1-sulphonate⁶⁶ with β -cyclodextrin has been studied by this method. Formation constants, enthalpy and entropy accompanying the complexation were determined in the same way. From these studies, it was found that the complexation is accompanied by a negative change in the enthalpy and a positive change in the entropy, although these changes are small. These results suggest that van der Waals-London-dispersion force and hydrophobic interactions are mainly responsible for inclusion complex formation.

Circular Dichroism

It is a useful method to detect cyclodextrin inclusion complexes in aqueous solution. When an achiral guest molecule is included within asymmetric locus of cyclodextrin cavity which consists of chiral glucose units, new circular dichroism bands can be induced in the absorption bands of optically inactive guests and extrinsic cotton effects are generated in the wavelength region of guest chromophores. Not only the achiral guest molecules but also the chiral guest molecules may show the changes in circular dichroism spectra upon formation of inclusion complexes with cyclodextrins⁸⁷. The changes of the (induced) circular dichroism of the guest molecules, following the binding to cyclodextrins, may be largely dependent on geometry of the host and the guest molecules⁸⁸. The circular dichroism spectra of β -cyclodextrins complexes with naphthalene⁸⁹ derivatives has been found to have a remarkable difference in the spectra between 1-substituted and 2-substituted naphthalene complexes, indicating that steric effects of substituents on the formation of complex is so strong that complexation mode may be different for these guest molecules. They suggested that positive circular dichroism band shows an axial inclusion (parallel to the Z-axis of cyclodextrin cavity), while a negative circular dichroism band suggests an equatorial inclusion. According to this proposal, 2-substituted naphthalene is estimated to be included axially in the β -cyclodextrin cavity.

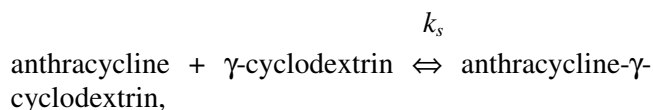
On the addition of β -cyclodextrin the positive and negative circular dichroism peaks of hydrocortisone³⁴ shifted to a longer wavelength, thereby indicating that the steroid molecule may be located in the hydrophobic cyclodextrin cavity. This technique has also been used to determine the complex formation constants as in case of the cyclodextrin inclusion complexes of purine nucleosides³⁸. In this, circular

dichroism spectra were recorded at various temperatures. The concentration of purine nucleosides was controlled such that maximum absorbance ($A_{\max}$) was below 2. Molar ellipticity differences, $\Delta\theta$, were recorded at wavelength at which differences were maximized as a function of cyclodextrin concentration. Complex formation constants were obtained from plots of $1/\Delta\theta$ vs $1/[\text{cyclodextrin}]$ according to Eq. (17)⁹⁰.

$$\frac{1}{\Delta\theta} = \frac{1}{\Delta\theta_{AB}K_c[\text{cyd}]} + \frac{1}{\Delta\theta_{AB}}, \quad \dots(17)$$

where $\Delta\theta_{AB}$ is the difference in the molar extinction coefficients for right and left circularly polarized light between free and complexed purine nucleoside. K_c is the complex formation constant.

This technique has been used to determine the stability constant of inclusion complexes of doxorubicin and daunorubicin with cyclodextrins. Complexation of these anthracycline drugs with cyclodextrin can be illustrated as:



where K_s represents stability constant of inclusion complex and is defined by Eq. (18).

$$K_s = \frac{[\text{Anthracycline-}\gamma\text{cyd}]}{[\text{Anthracycline}][\gamma\text{cyd}]}. \quad \dots(18)$$

Stability constant can be determined when suitable ellipticity changes of anthracycline in the presence of different cyclodextrin concentrations occur. K_s values are calculated according to Scott's equation:

$$\frac{[\text{Anthracycline}][\gamma\text{cyd}]}{d} = -\frac{1}{\Delta\epsilon}[\gamma\text{cyd}] + \frac{1}{K_s\Delta\epsilon}, \quad \dots(19)$$

where d is the change in the ellipticity of anthracycline by addition of γ -cyclodextrin, $\Delta\epsilon$

represents the differences between molar ellipticities for free and complexed drug. Since the values of stability constants obtained by this method are in good agreement with those obtained from degradation studies, thus circular dichroism is a suitable analytical technique to determine inclusion complex stability constants.

Electrochemical Methods

pH Potentiometric Titration Method

If the guest compound has a prototropic function, potentiometric titration method can be used to detect inclusion complex formation. Due to the fact that cyclodextrins usually favour unionized guest molecules having a higher hydrophobicity, rather than the ionized ones, pK_a value of the acidic guest molecule is usually increased, while those of the basic ones is usually decreased by binding to cyclodextrins. This technique has been used to determine the inclusion complexes of cyclodextrins with various drugs like nimesulide⁴, thioctic acid²¹, barbiturates⁹¹, substituted cyclohexane carboxylic acids and phenyl alkanolic acids⁹², diclofenac⁹³, ketoprofen⁹⁴, naproxen⁹⁵, and ibuprofen⁹⁶. This method proved to be applicable for β -cyclodextrin-barbiturate system⁹¹, where both of the unionized and ionized barbiturate molecule form 1:1 complexes. Results obtained were in fair agreement with those obtained by ultra violet, circular dichroism and solubility studies.

Advantage of this method is that two equilibrium constants, those for unionized and ionized substrates, can be obtained by simple procedure even when significant spectral changes do not occur by complexation. Since both the ionized and unionized barbiturate forms complex with β -cyclodextrin in same molar ratio, i.e., 1:1.

Values K_d and K_d' are the first step dissociation constants of non-complexed and complexed barbituric acid, respectively, as follows:

$$K_d = \frac{[B^-][H^+]}{[BH]} \quad \dots(20)$$

$$K_d' = \frac{[B^- \cdot \text{cyd}][H^+]}{[BH \cdot \text{cyd}]} \quad \dots(21)$$

Values K_{dun} and K_{di} are the dissociation constants of inclusion complexes of unionized and ionized barbituric acid, respectively.

$$K_{dun} = \frac{[BH][\text{cyd}]}{[BH \cdot \text{cyd}]} \quad \dots(22)$$

$$K_{di} = \frac{[B^-][\text{cyd}]}{[B^- \cdot \text{cyd}]} \quad \dots(23)$$

These equilibrium constants hold the following relation

$$K_d \cdot K_{dun} = K_d' \cdot K_{di} \quad \dots(24)$$

At half neutralization point there is relationship

$$[B^-] + [B^- \cdot \text{cyd}] = [BH] + [BH \cdot \text{cyd}] \quad \dots(25)$$

From Eqs (20-25), H^+ concentration at half neutralization $[H]_h$ can be expressed as:

$$[H^+]_h = K_d' + \frac{K_d'(K_{di} - K_{dun})}{K_{dun} + [\text{cyd}]} \quad \dots(26)$$

Equation (26) is hyperbolic with respect to $[H^+]_h$ and $[\text{cyclodextrin}]$ and finally can be reduced to $[H^+]_h = K_d'$ when $[\text{cyclodextrin}] = \text{excess}$. Since Eq. (26) is held in any concentration of β -cyclodextrin, following equation can be expressed between two arbitrarily selected cyclodextrin concentration, which are designated as $[\text{cyd}]_1$ and $[\text{cyd}]_2$, respectively:

$$\frac{\Delta[\text{cyd}]}{\Delta[H^+]_h} = \frac{K_{dun} + [\text{cyd}]}{K_d'(K_{dun} - K_{di})} \quad \dots(27)$$

where $\Delta[H^+]_h$ is the difference of $[H^+]_h$'s at two $[cyd]$'s and $\Delta[cyd] = [cyd]_1 - [cyd]_2$ when $[cyd]_1$ is held constant and $[cyd]_2$ is varied, linear relation should be obtained between.

$$\frac{\Delta[cyd]}{\Delta[H^+]_h} \quad \text{and} \quad [cyd]_2.$$

Referring to Eq. (27), K_{dum} is determined from linear relationship by (intercept) / (slope) and K_d' can be calculated from Eq. (28).

$$K_{d'} = \frac{K_{dum} + [cyd]_2}{(\text{intercept})} + K_d. \quad \dots(28)$$

Value K_d is obtainable by usual titration method in the absence of β -cyclodextrin and K_{di} can be determined by Eq. (24), substituting the obtained equilibrium constants.

Equilibrium constants of various barbiturate- β -cyclodextrin systems determined by potentiometric titration indicate that all the K_d values are larger than $K_{d'}$ values, indicating that protolytic dissociation of barbituric acid is suppressed by inclusion. Ratio of $K_d/K_{d'}$, magnitude of suppression is when 5-substituents include phenyl or cyclohexyl ring. Longer the substituent chain, stronger the inclusion.

Potentiometric titration gives values fairly consistent with those by other methods. Advantages of this method include the following²¹:

- (i) One pH-potentiometric experiment at each temperature, without buffering the solution, gives a complete thermodynamic description of the system, i.e., dissociation constants of the drug, association constants of both ionic and non-ionic species of the drug with cyclodextrin, and the stoichiometry of the complex;
- (ii) The characterization of the cyclodextrin:D and cyclodextrin:D⁻ complexes, not available through UV-VIS measurements, may be studied from the pH-potentiometric experiments;
- (iii) The potentiometric technique accurately allows for the study of cyclodextrin-drug systems for which the experimental properties of the drug in aqueous solution (such as the absorbance or the

pH) are not very much affected by the presence or the absence of the cyclodextrin.

The only requirement of this method is that the association constants of the complexes formed by the nonionized and ionized form of the guest must be neither very similar nor very different. This method has been shown to be applicable to obtain the equilibrium constants of inclusion complexes between cyclodextrins and weakly acidic and basic guest molecules in aqueous solution.

Conductometric Method

Conductivity measurements may be used to detect inclusion complex⁹¹. Palepu et al.⁹⁸ have found that solution conductivities are dramatically affected by inclusion complex with cyclodextrins. When the anionic surfactants having different polar heads, different tail configurations and same Na⁺ counter ion form inclusion complexes with cyclodextrins, amphiphilicity of the former often leads to strong associative species that dramatically affect solution conductivities.

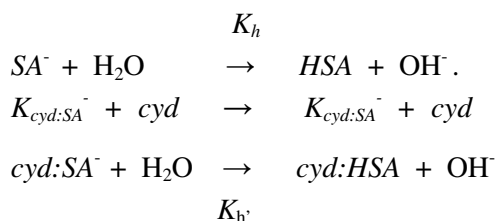
Encapsulation process of sodium salicylate (NaSA)⁹⁹ by β -cyclodextrin and 2,6-di-o-methyl β -cyclodextrin (DIMEB) in aqueous solution has been studied by conductivity experiments. In these experiments, conductivity data provides not only the stoichiometries but also association constants of cyd :NaSA inclusion complexes, obtained from non-linear regression fitting of conductivity data. These association constants are most important thermodynamic parameters regarding the bioavailability of the drug. The conductivity measurements were made for pure NaSA and for systems β -cyclodextrin + NaSA and DIMEB + NaSA.

- (i) As a function of NaSA concentration at different constant values of cyclodextrin.
- (ii) As a function of cyclodextrin concentration at several constant NaSA.
- (iii) As a function of cyclodextrin and NaSA at a constant stoichiometric ratio cyclodextrin/NaSA equal to 1.

Molar conductivity λ of pure NaSA decrease appreciably in the presence of DIMEB, the magnitude of this decrease being higher as long as the constant DIMEB concentration increases. This fact can be attributed to formation of DIMEB: SA⁻ complex. The SA⁻ species loose mobility as long as it is included in the cyclodextrin cavity and mobility of cyclodextrin:

SA⁻ complex is lower than that of SA⁻ alone. This loss of mobility justifies the decrease in λ . The same trend has been found in the system β -cyclodextrin+ NaSA. The stoichiometry A, of the complex can be determined as ratio (DIMEB)/(NaSA), where (NaSA) is constant drug concentration and (DIMEB) is cyclodextrin concentration at which a change of the slope of λ vs (DIMEB) is observed.

The encapsulation of drug molecule into cyclodextrin cavity is an equilibrium process governed by binding constant and equilibrium involved in the process can be shown as:



The constants K_h and $K_{h'}$ are hydrolysis constants of salicylate species SA⁻ in free and complexed form respectively, while $K_{\text{cyd:SA}^-}$ and $K_{\text{cyd:HSA}}$ are binding constants of inclusion complexes formed by cyclodextrin and non-protonated and protonated forms of HSA, respectively.

These association constants, together with stoichiometry, are important thermodynamic parameters necessary to characterize these cyclodextrin: drug inclusion complexes and regarding the bio-availability of drug.

Conductivity has been widely used to characterize a large amount of different host guest systems with drug molecule as the guest and cyclodextrin as the host^{95,100}.

Polarographic Method

It is a suitable method to study inclusion complexation if electron distribution of a complexed electroactive guest molecule in aqueous solution is different from that in non-complexed state in aqueous solution and if this change is polarographically detectable¹⁰¹. There are many examples of organic drug molecules that are electrochemically active¹⁰² hence the potential application of polarography^{103,104}. The effect of β -cyclodextrin on peak height and voltage of polarographic reduction of several esters of hydroxybenzoic acid¹⁰¹ has been investigated.

The presence of β -cyclodextrin markedly diminished the peak height of each of these molecules, i.e., the presence of β -cyclodextrin result in less conductance by each ester upon reduction. This decrease in the current conducted is due to decreased rate of diffusion of complexed ester as compared with ester alone. The half wave potential at which these reductions become more negative in the presence of β -cyclodextrin, i.e., species is more difficult to reduce. This change in potential is in order ethyl > propyl > butyl and is most likely due to electron redistribution that occur in the presence of β -cyclodextrin. The hydroxy benzoate molecule must reside in β -cyclodextrin cavity to such a degree that the presence of β -cyclodextrin ring causes an electron-distribution of carbonyl group, which results in change of reduction potential. The degree to which half-wave potential is shifted in the presence of β -cyclodextrin is an indication of complexing ability of electro-reductive guest molecule with a β -cyclodextrin host.

Thus, polarography is a useful technique not only for studying inclusion complexation phenomenon, but also for investigating the mode of interaction of the host and guest molecules.

Thermal Methods

Differential Scanning Calorimetry (DSC)

This method is used for determining the inclusion complexation in the solid state (e.g. salbutamol³⁶, phenothiazine⁴⁸, amylobarbitone⁴⁹, allopurinol⁵⁰, piroxicam⁵¹, flutamide⁷³, bropirimine⁷⁴, progesterone¹⁰⁵, danazol¹⁰⁶, oxazepam¹⁰⁷, nimesulide¹⁰⁸, alben-dazole¹⁰⁹, corticosteroid loteprednol etabonate¹¹⁰, hydrocortisone¹¹¹ and praziquantel¹¹²).

Although the results obtained by solubility studies strongly indicate the formation of a true complex of drug- β -cyclodextrin, they do not preclude the possibility that product is simply a physical mixture. Thus the thermal behavior of cyclodextrin inclusion compounds was studied by DSC in order to confirm the formation of a solid complex. When the guest molecules are incorporated in the cyclodextrin cavity or in the crystal lattice, their melting point, boiling point, and sublimation points usually shift to a different temperature or disappear within the temperature range where cyclodextrin lattice is decomposed. Becket *et al.*¹¹² have shown that DSC is the best method for detecting cyclodextrin complexes. β -cyclodextrin (apart from an endotherm due to water

loss) did not produce any interesting peaks when examined by DSC¹¹³.

Microcalorimetric Method

Changes in thermodynamic properties due to inclusion complexation in solid state were measured by microcalorimetry. These changes in enthalpy and entropy are associated with the change in behaviour of water following the complex formation and include a breakdown of water structure within the cavity, removal of the water from cavity, restructuring of water around the guest molecule and release of water into bulk. Other contributions to overall energies of reaction are due to restriction in rotation around glycosidal linkages of cyclodextrin when the guest molecule enters the cavity. The positive entropies suggest significant contribution from redistribution of water and large enthalpies should be consistent with dipole interactions and H-bond formation.

Calorimetric technique measures the heat directly, it is the only method which allows direct and accurate determination of binding constants, reaction stoichiometry (n), enthalpy (ΔH) and entropy (ΔS) of molecular interactions as heat is either generated or absorbed when substances bind or get complexed. Out of various methods described earlier, microcalorimetry is most important one for directly measuring the thermodynamic properties of complex formation¹⁰⁶. Thermodynamic properties give the macroscopic picture of inclusion process.

Some of the examples of drug-cyclodextrin complexes studied by this technique are diclofenac- Na^{31} , barbiturates⁷⁹, anionic, cationic and neutral chiral guests¹¹⁴, amine drugs with diphenyl methyl functionality¹¹⁵, naphthalene sulfonates¹¹⁶, diflunisal¹¹⁷, and pefloxacin mesylate¹¹⁸.

In the case of diclofenac Na^{31} , study has been undertaken to determine the enthalpic effect connected with interaction between diclofenac Na and β -cyclodextrin using the technique of solution calorimetry. This study also supports 1:1 stoichiometry and equilibrium constant for complex formation has been evaluated to be $1670 \text{ dm}^3/\text{mol}$. This equilibrium value is encouraging from manufacturing point of view and also compares well with earlier value determined, using potentiometric and spectrofluorimetric techniques.

X-Ray Diffraction

This method may be used to detect inclusion complexation in solid state. Method is specifically

useful in the case of liquid guest molecules, since liquids have no diffraction pattern of their own. When pattern of a newly formed substance clearly differs from that of un-complexed, cyclodextrin complex formation is indicated. When guest compound is a solid substance, a comparison has to be made between diffractogram of assumed complex and that of physical mixture of guest and cyclodextrin molecules. Comparison of diffractograms is only possible if cyclodextrin as well as guests are, before mixing, treated both under identical conditions as assumed complex because cyclodextrin inclusion complex preparation processes such as, freeze drying and grinding, may change the crystallinity of pure substances and may also lead to different diffraction pattern. A diffraction pattern of physical mixture is often the sum of those of each component, while diffraction patterns of cyclodextrin complexes are apparently different from each constituent and lead to a new solid phase with a different diffractogram. The inclusion complex of various drugs with cyclodextrin has been identified by this technique e.g. nimesulide⁵, flufenamic acid²⁰, ibuprofen⁴³, famotidine⁴⁴, tolbutamide⁴⁵, oxazepam¹⁰⁷, retinoic acid¹¹⁹, β -blockers¹²⁰, Ionidamine¹²¹, and nifedipine¹²².

The X-Ray diffractometry pattern of physical mixture of retinoic acid¹¹⁹ and β -cyclodextrin is approximately superposition of patterns of raw materials. On the other hand, co-precipitate product has a completely different pattern in which it is no longer possible to distinguish the characteristic peaks of retinoic acid and thus confirming the existence of new compound.

The X-ray diffraction analysis of powder samples revealed less crystallinity in complex system, as evidenced by fewer and broader peaks of lower intensity. Diffraction patterns obtained for complex and physical mixture indicate less crystalline nature of complex system⁴³.

High Performance Liquid Chromatography (HPLC)

This technique is suitable for chemically unstable compounds and for systems accompanying no spectral changes. Cyclodextrin complexations have been successfully applied to rapid analysis of amoxycillin and clavulanic acid¹²³, multivitamin preparations¹²⁴, tricyclic antidepressant drugs¹²⁵, cephalosporins¹²⁶, tolfenamic acid¹²⁷, prostaglandin derivatives¹²⁸ by HPLC. Retention times of prostaglandins¹²⁸ decrease significantly by addition of

α - or β -cyclodextrin into aqueous mobile phase on anion exchange support, because of the formation of soluble complexes in the mobile phase. Changes in the retention times of various drug molecules as a function of cyclodextrin concentration have been quantitatively treated to obtain stability constant values (K_s). Advantage of HPLC method was that K_s 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 study¹³⁰ of retention behavior of drug (D) and its cyclodextrin complex (D - cyd) within ion exchange column, C_{ion} , C_c , and K_s are concentration distribution ratio of ionized D , that of D - cyd and stability constant of 1:1 complex respectively, as defined by Eqs. (29-31).

$$C_{ion} = \frac{(D)_s}{(D)_m}, \quad \dots(29)$$

$$C_c = \frac{(D-cyd)_s}{(D-cyd)_m}, \quad \dots(30)$$

$$K_s = \frac{(D-cyd)_m}{(D)_m(cyd)_m}. \quad \dots(31)$$

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:

$$\frac{(cyd)_m}{T_o - T_{obs}} = \frac{1}{T_o - T_c} (cyd)_m + \frac{1}{K_s(T_o - T_c)}, \quad \dots(32)$$

where T_o , T_o , T_c and T_{obs} are the 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. (32) vs $(cyd)_m$ gives both the K_s and T_c values from the slope and intercept. The K_s values determined by HPLC method

were in fair agreement with those obtained by other methods, such as solubility and spectroscopic methods.

Scanning Electron Microscopy (SEM)

SEM is used to study microscopic aspects of raw materials (cyclodextrin and guest substances e.g. oxazepam¹⁰⁷, retinoic acid¹¹⁹, β -blockers¹²⁰) and product obtained by coprecipitation/evaporation. Even if there is a clear difference in crystallization state of raw material and product obtained by co-precipitation, this method is inadequate to affirm inclusion complex formation, but nevertheless helps to assess the existence of a single component in the preparations obtained, e.g., in the case of retinoic acid¹¹⁹ analysis by SEM revealed that, whereas β -cyclodextrin crystallizes in a relatively large polyhedral form retinoic acid appears as needles or elongated crystals. The product obtained after evaporation is amorphous. This amorphous form is characteristic of product obtained here and is not the consequence of evaporation process, because it has been very slow due to low temperature and in these conditions, it should normally not impede any crystallization.

Molecular Modeling Technique

The use of the molecular modeling techniques for the study of cyclodextrins was somewhat limited until recently due to the size and flexibility of such molecules, which represent a real challenge for computational methods. On the other hand the fact that these molecules are frequently studied experimentally in the aqueous media, imposes some restrictions on the use of theoretical approaches to the study of cyclodextrin inclusion complexes. However, in recent years, several studies reporting the use of quantum mechanics and molecular mechanics calculations for investigating the 3-D structure and properties of such complexes has increased dramatically¹²⁹. Accordingly the combination of experimental and computational studies has been recognized as a powerful tool for the study of geometry of complexation^{130,131}. The molecular modeling data has become an interesting tool to know the host-guest interactions in cyclodextrin technology, e.g., tolbutamide¹¹, salbutamol⁵⁷, sulphamethizole⁶¹, piroxicam¹³², ibuprofen¹³³, NSA¹³⁴, amine drugs with diphenyl methyl functionality¹³⁵, and ketoprofen¹³⁶.

The aromatic ring of salbutamol⁵⁷ was predicted to penetrate into the cavity of β -cyclodextrin molecule leaving the amino group externalized as was found

with COSMIC molecular modeling technique. However, according to molecular mechanics (MM2 and PM3) calculations, in the absence of solvent the introduction of aromatic ring of salbutamol is preferred to the introduction of tertiary-butyl group into the β -cyclodextrin cavity, but in aqueous solution the introduction of tertiary-butyl group into the cavity is preferred. The global minimum energy conformation of tolbutamide: β -cyclodextrin complexes¹¹ also illustrated by this method. The optimum structure was obtained when aromatic ring of the tolbutamide molecule was included in β -cyclodextrin cavity and tolbutamide is more likely to enter the larger cavity side of the β -cyclodextrin molecule. After the inclusion tolbutamide molecule become more elongated due to its various internal degrees of freedom. In the lowest energy conformer of sulphamethizole⁶¹, the thiadiazole group was predicted to be included in the cyclodextrin by entering its narrow end. The two possible complexes of ibuprofen¹³⁵ with β -cyclodextrin has been predicted by molecular modeling. In one complex the two methyl groups of drug were nearer the wider side of the β -cyclodextrin cavity and in the other, the two methyl groups of it were located near the narrower opening of the cone.

Conclusions

Cyclodextrins are used in pharmaceutical industry for various applications, but most importantly for enhancement of solubility and stability. They increase water solubility through inclusion complexation leading to consequently increased stability and/or bioavailability of water insoluble drugs. In the light of the above reported examples it can be observed that inclusion complexes can be detected quickly and studied elegantly in solid state as well as in aqueous solution. As a result of development, high resolution spectrometers it has become possible to make quantitative statements about the thermodynamic and kinetic parameters of host-guest interactions. X-ray structural analysis reflects the atomic details of the static complex in solid state. NMR spectroscopy allows its dynamic properties in solution.

Although inclusion of guest molecule can be followed by monitoring changes in its conductivity, polarographic properties, apparent changes in its basicity but most adventitious technique has been found to be solution calorimetry. Calorimetric technique is the only method which allows direct and

accurate determination of thermodynamic properties of complex formation such as binding constants (K), reaction stoichiometry (n), enthalpy (ΔH) and entropy (ΔS) as heat is either generated or absorbed. Thermodynamic properties give the macroscopic picture of inclusion process.

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