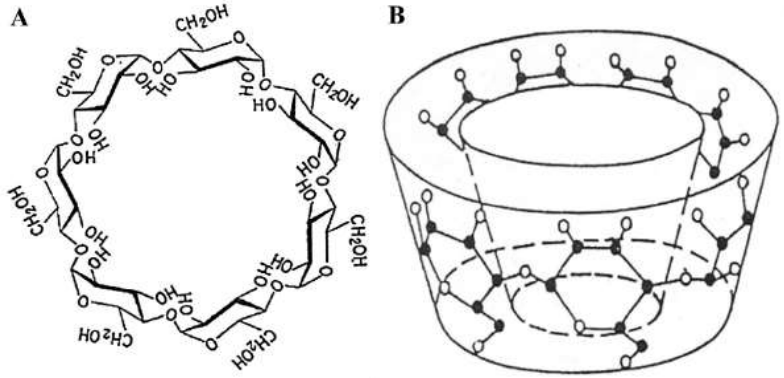
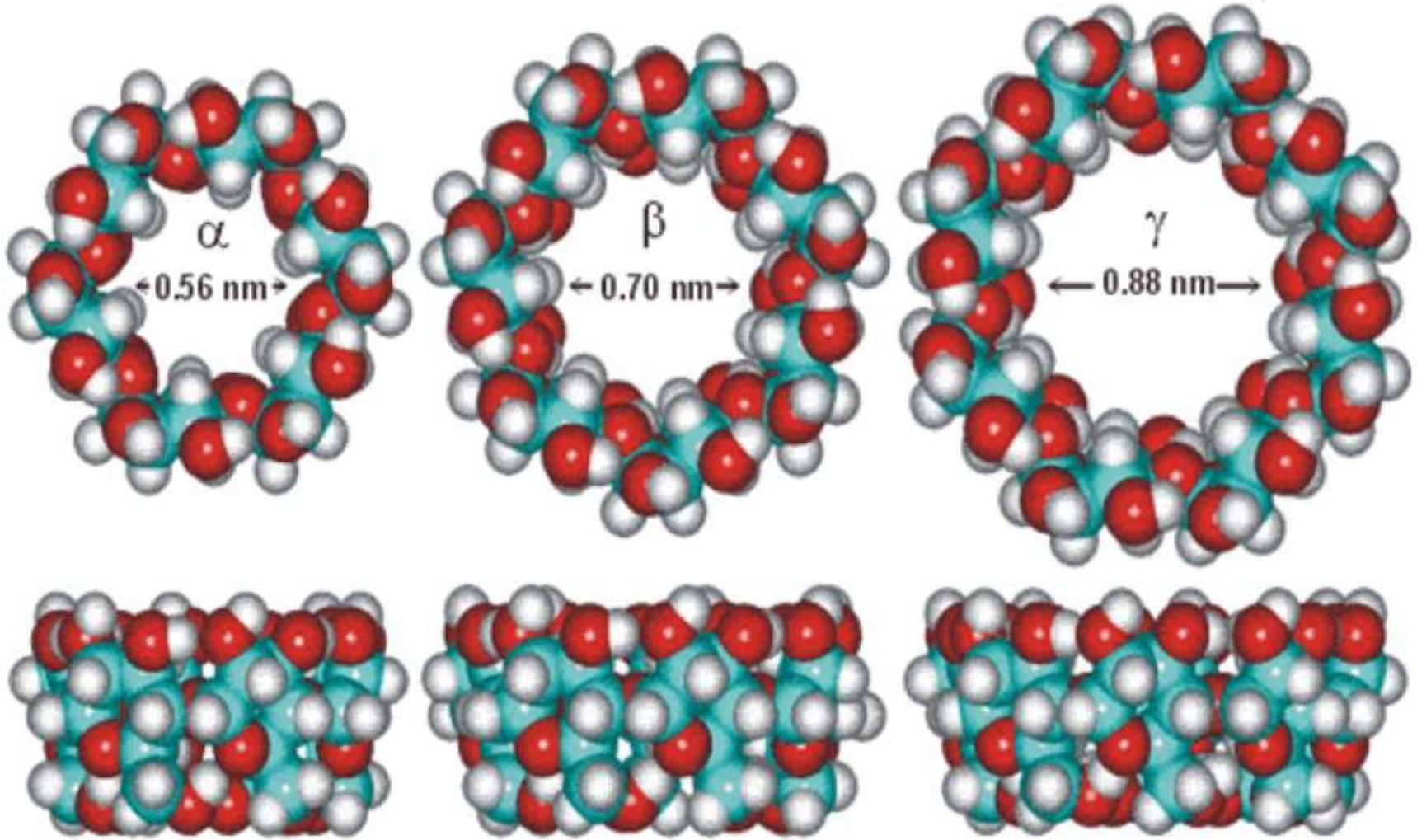
ΠΡΟΙΟΝΤΑ ΕΓΚΛΕΙΣΜΟΥ ΒΙΟΔΡΑΣΤΙΚΩΝ ΟΥΣΙΩΝ ΣΕ ΚΥΚΛΟΔΕΞΤΡΙΝΕΣ

ΦΥΣΙΚΕΣ ΚΥΚΛΟΔΕΞΤΡΙΝΕΣ





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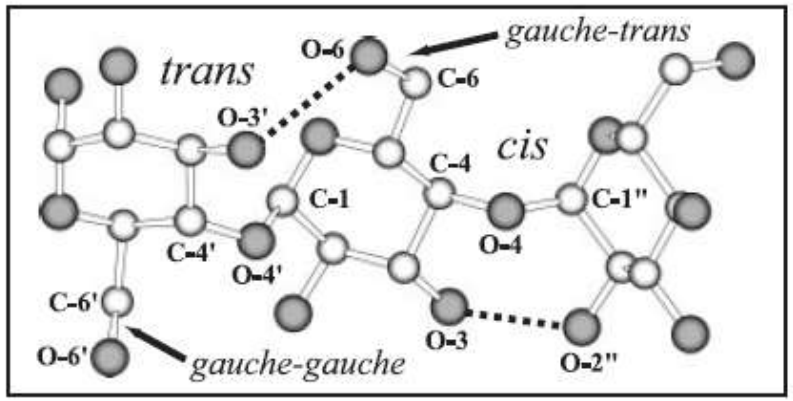
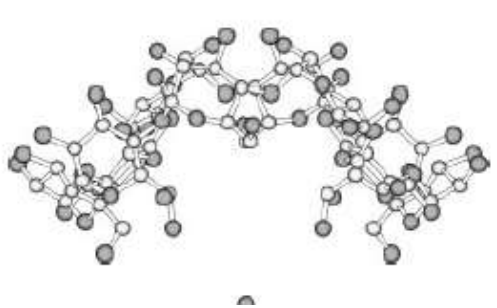
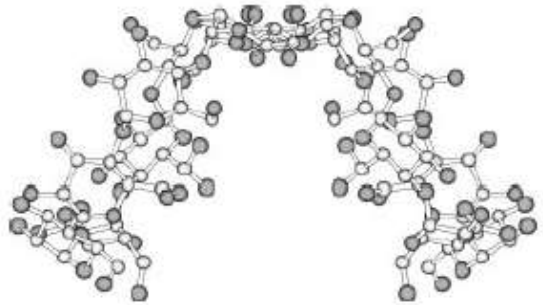
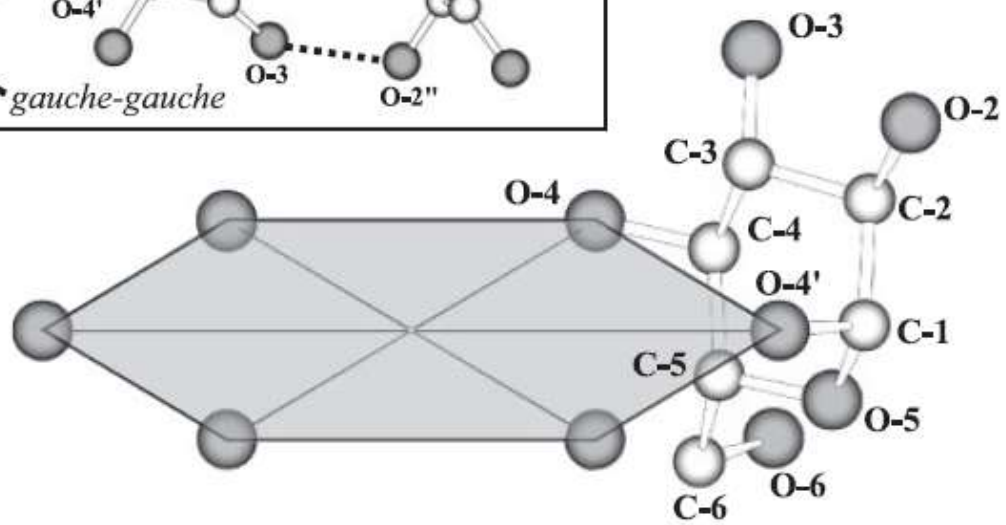








Ευκαμψία φυσικών κυκλοδεξτρινών

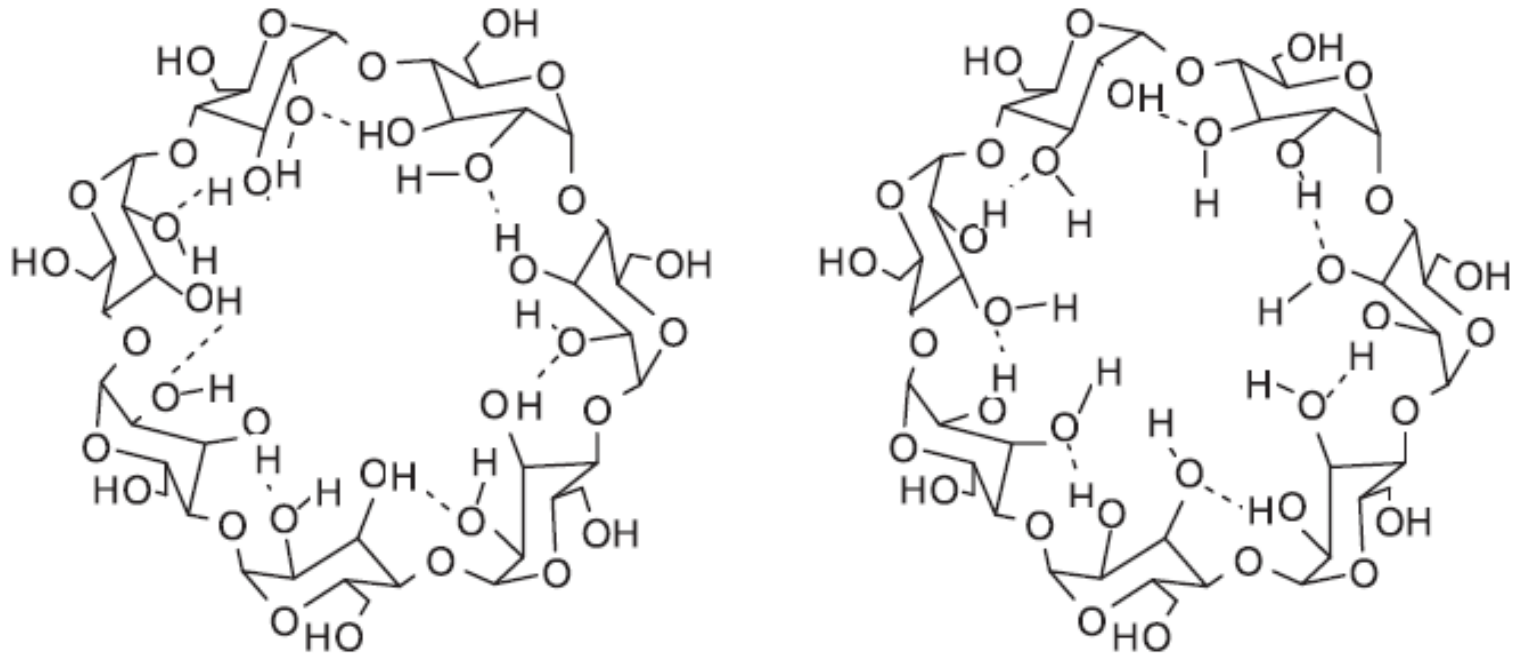


Fig. 1.7. Rapidly interconverting circular systems of hydrogen bonds in β-CyD 2 (flip-flop, HO2 → O3 simultaneously and reversibly changing to O3H → O2).

ΦΥΣΙΚΕΣ ΚΥΚΛΟΔΕΞΤΡΙΝΕΣ

Chemical–physical properties of native CDs.

	αCD	βCD	γCD
Number of glucopiranose units	6	7	8
Molecular weight	972	1135	1297
Solubility in water (% w/v) 25 °C	14.5	1.85	23.2
Inner cavity diameter (nm)	0.47–0.53	0.60–0.65	0.75–0.83
Outer cavity diameter (nm)	1.46	1.54	1.75
Cavity height (nm)	0.79	0.79	0.79
Cavity volume (nm^3)	0.174	0.262	0.472
Crystal water content (wt.%)	10.2	13.2–14.5	8.13–17.7
Water molecules in cavity	6	11	17
Melting temperature range (°C)	255–260	255–265	240–245

ΦΥΣΙΚΕΣ ΚΥΚΛΟΔΕΞΤΡΙΝΕΣ

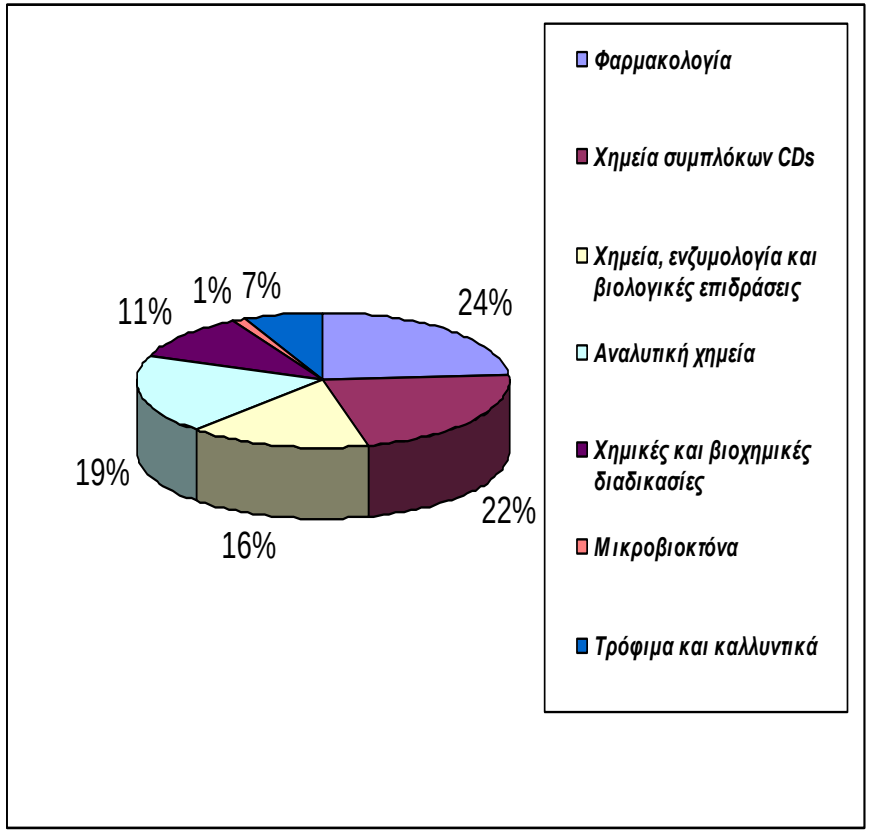
Regulatory status (2004)

	Food Approval			Pharmacopoeia Monographs		
	US	**Europe**	**Japan**	**USP/NF**	**Ph.Eur.**	**JP**
αCD	In Preparation	Planned	Yes	No	Yes	Yes
βCD	GRAS	Food Additive	Yes	Yes	Yes	Yes
γCD	GRAS	Pending	Yes	No	In Progress	Yes

ΕΦΑΡΜΟΓΕΣ ΚΥΚΛΟΔΕΞΤΡΙΝΕΣ

Πεδία εφαρμογών:

- ❑ Το 80-90% της παγκόσμιας παραγωγής σήμερα προορίζεται για την Βιομηχανία τροφίμων (σταθερότητα σε υψηλές θ επεξεργασίας, ανθεκτικότητα αρωμάτων σε οξείδωση, θερμική αποδιάταξη, σταθερότητα χρωματισμού, ευκολία χειρισμού, ένδειξη GRASS)[1]
- ❑ Βιομηχανία φαρμάκων (μορφή ταμπλέτας)[2],
- ❑ Αναλυτική χημεία (TLC,GC,HPLC)
- ❑ Βιοτεχνολογία (Βιομιμητικά μόρια)



Κατανομή των 1706 δημοσιεύσεων πάνω στην χημεία των CDs και εφαρμογών, που δημοσιεύτηκε στο Cyclodextrin News το 1996

1. Loftson, Expert Opin. Drug Deliv., 2005, **2**, 335-351
2. Sjetli, Chem. Rev., 1998, **98**, 1743-1753

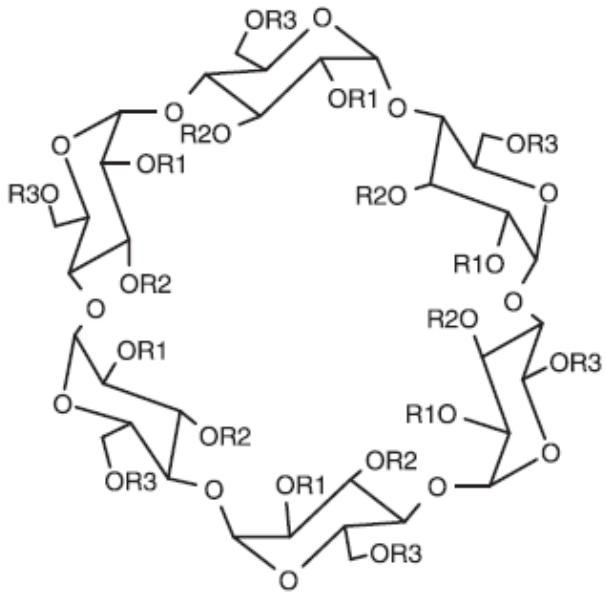
Η ΕΝΤΥΠΩΣΙΑΚΗ ΑΝΑΠΤΥΞΗ ΤΗΣ ΤΕΧΝΟΛΟΓΙΑΣ ΤΩΝ CDs ΟΦΕΙΛΕΤΑΙ:

- Είναι ημι-φυσικά προϊόντα. Απλή παραγωγή από ανανεώσιμες ύλες (άμυλο)
- Παραγωγή φιλική προς το περιβάλλον. Η β-CD παράγεται σε ποσότητες 1500 τόνων κατά έτος, ενώ η τιμή της δεν είναι απαγορευτική
- Μείωση των αρχικά υψηλών τιμών τους, άρα αποδεκτές για τους περισσότερους βιομηχανικούς σκοπούς
- Η ικανότητά τους σχηματισμού συμπλόκων με διάφορα μόρια. Ο μοριακός εγκλεισμός χρησιμοποιείται ήδη ευρέως σε πολλά βιομηχανικά προϊόντα, τεχνολογίες και αναλυτικές μεθόδους
- Οι τοξικές επιδράσεις τους είναι αμελητέες
- Οι κυκλοδεξτρίνες μπορούν να καταναλωθούν ως συστατικά τροφών (α- και β-CD) και να χρησιμοποιηθούν σε φάρμακα ή καλλυντικά[1]

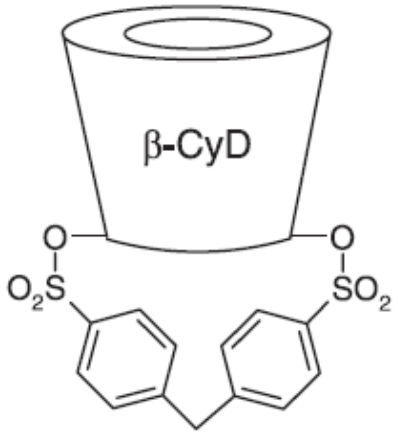
1.Szejtli, Encyclopedia of Nanoscience and Nanotechnology, 2004, 283-304

ΤΡΟΠΟΠΟΙΗΜΕΝΕΣ ΚΥΚΛΟΔΕΞΤΡΙΝΕΣ

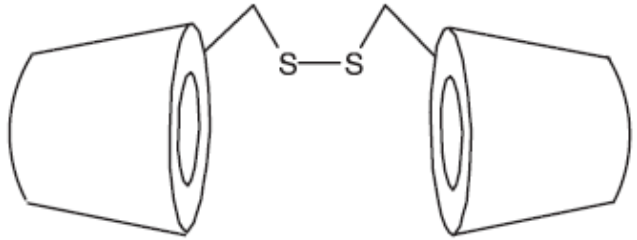
amphiphilic R1 = R2 = OH, R3 = $CH_2S(CH_2)_3C_6F_{13}$



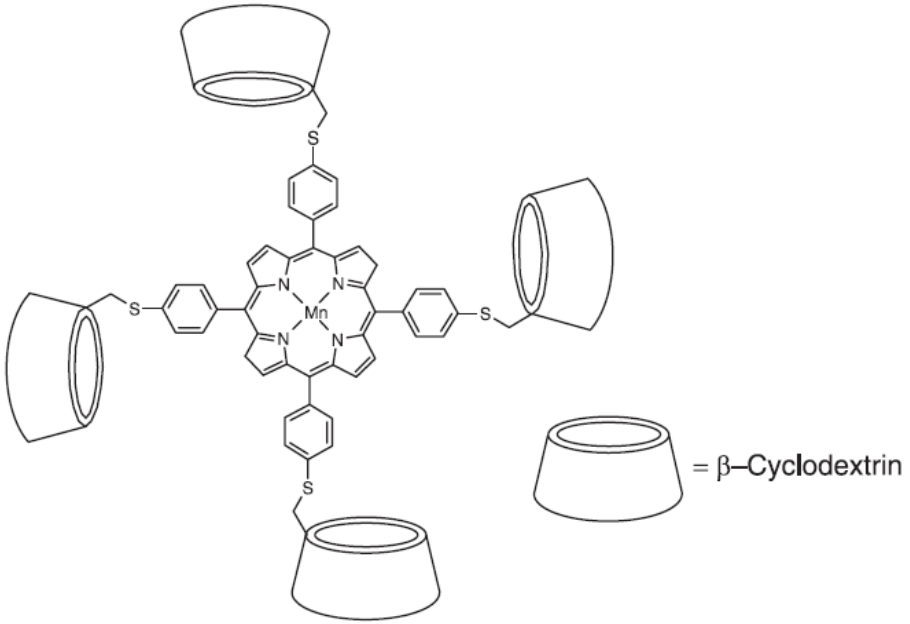
“capped”



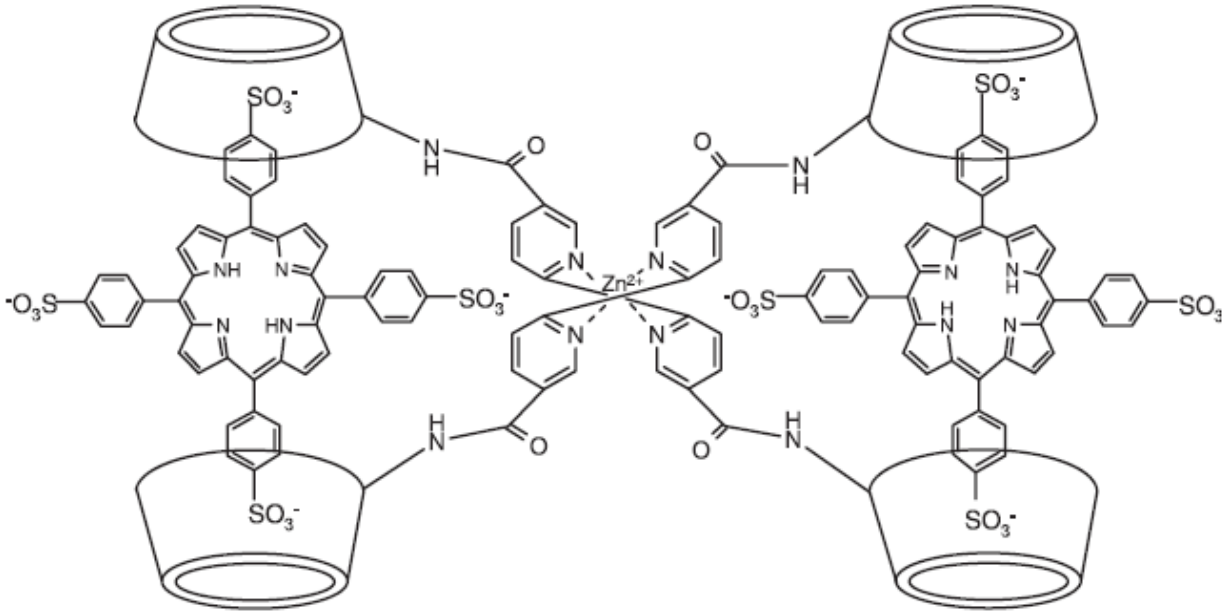
“bridged”



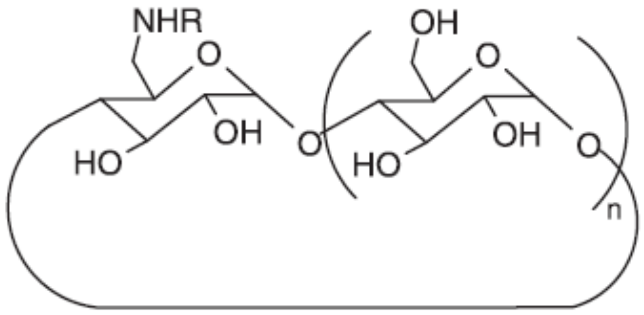
ΤΡΟΠΟΠΟΙΗΜΕΝΕΣ ΚΥΚΛΟΔΕΞΤΡΙΝΕΣ



2:2 complex formed by the CyD dimer
with porphyrin and zinc ion



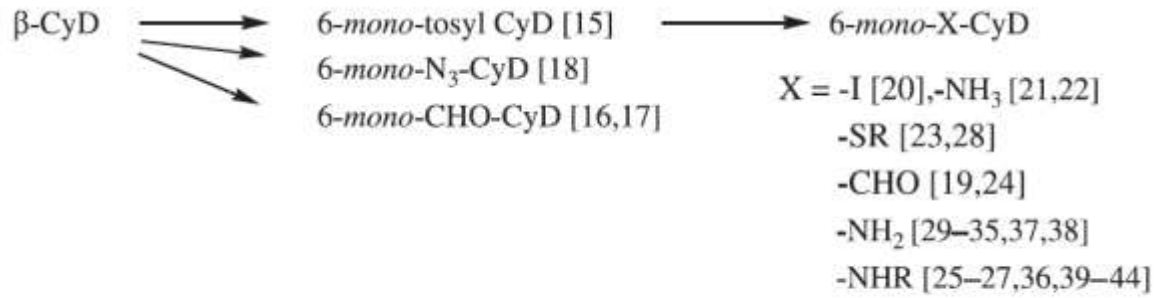
peptide appended



n = 6, 7 R = Glycyl-L-Phenylalanyl

ΤΡΟΠΟΠΟΙΗΜΕΝΕΣ ΚΥΚΛΟΔΕΞΤΡΙΝΕΣ

Modification Reactions at the Primary Side
Mono-modification at the C6-Position



Scheme 2.1. Mono-modification at the C6-position of *β*-CyD.

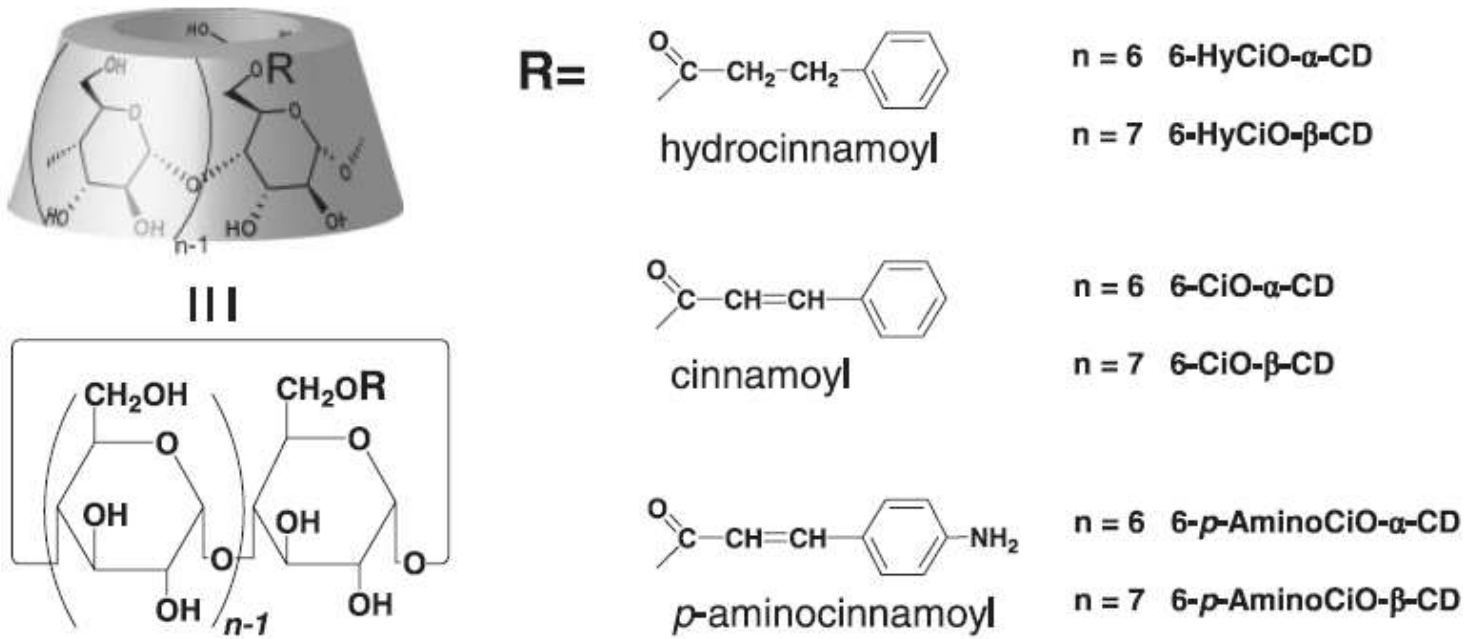


Fig. 3.4. Structures of hydrocinnamoyl- and cinnamoyl-modified CDs.

ΤΡΟΠΟΠΟΙΗΜΕΝΕΣ ΚΥΚΛΟΔΕΞΤΡΙΝΕΣ

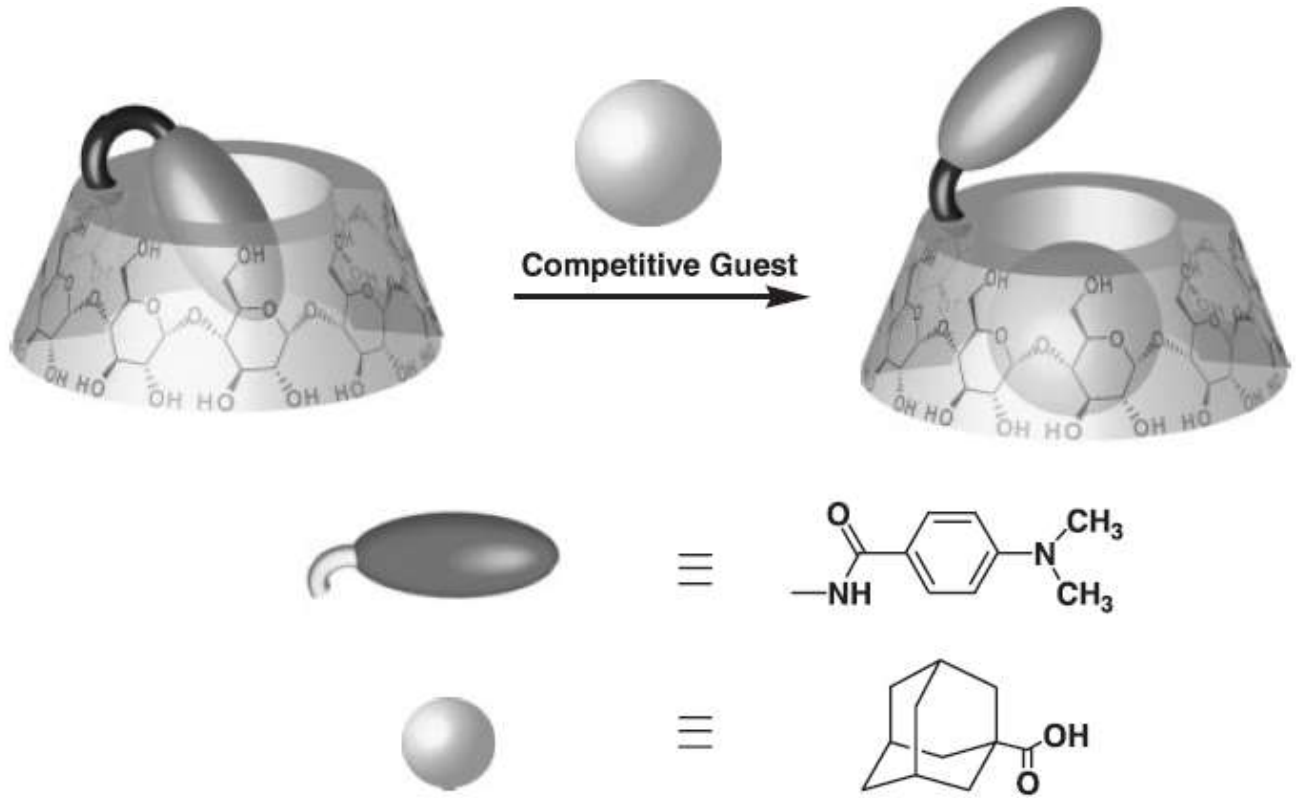


Fig. 3.3. Schematic representation of a fluorescent chemosensor involving an intramolecular complex bearing a chromophor.

ΤΡΟΠΟΠΟΙΗΜΕΝΕΣ ΚΥΚΛΟΔΕΞΤΡΙΝΕΣ
Supramolecular dimers

Fig. 3.9. Structures of Liu's intermolecular dimeric complexes formed by *p*-nitrobenzoyl-substituted *β*-CDs and *p*-hydroxy-benzoyl-substituted *β*-CDs [36, 37].

ΤΡΟΠΟΠΟΙΗΜΕΝΕΣ ΚΥΚΛΟΔΕΞΤΡΙΝΕΣ
Supramolecular trimers

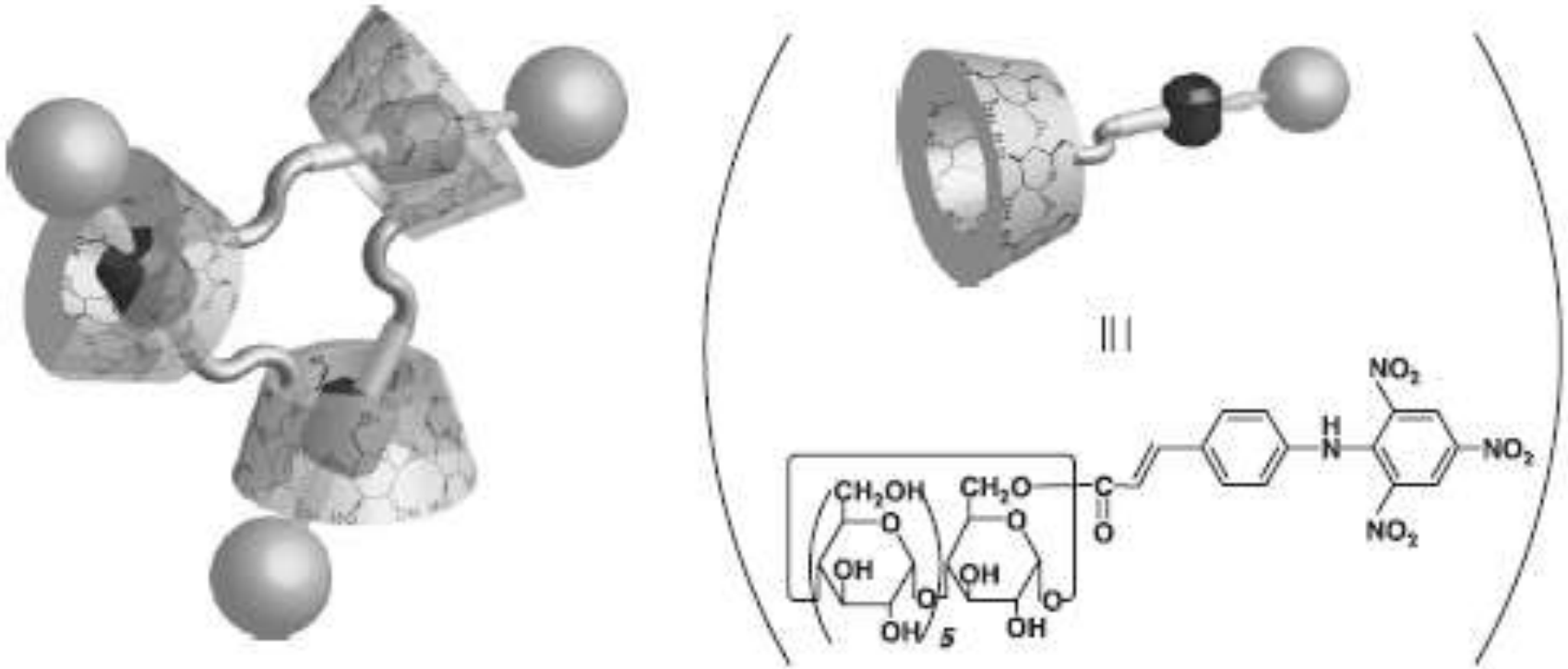


Fig. 3.12. Harada's daisy chain necklace: cyclic tri[2]rotaxane containing α-CD [41].

Supramolecular polymers

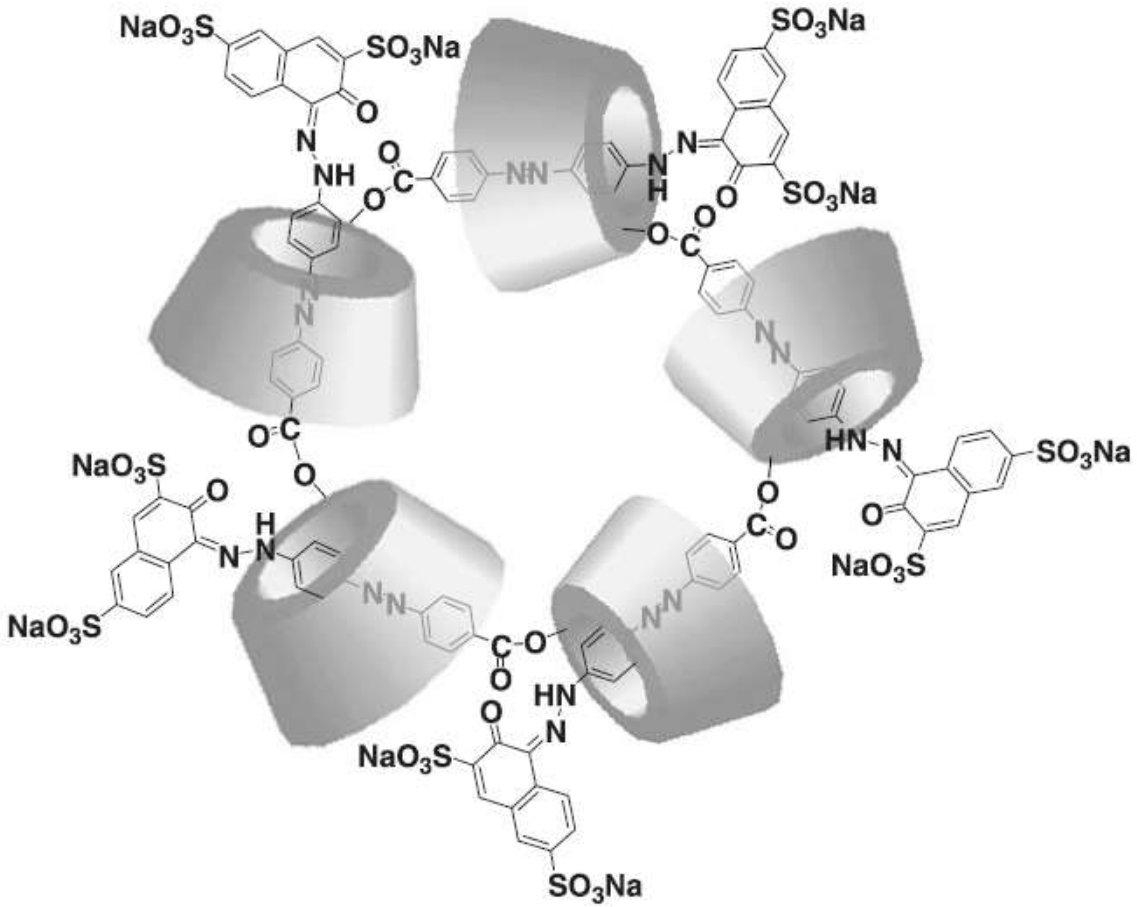


Fig. 3.15. Structure of Kaneda's cyclic tetra[2]rotaxane formed by permethylated α-CDs with an azobenzene derivative [32].

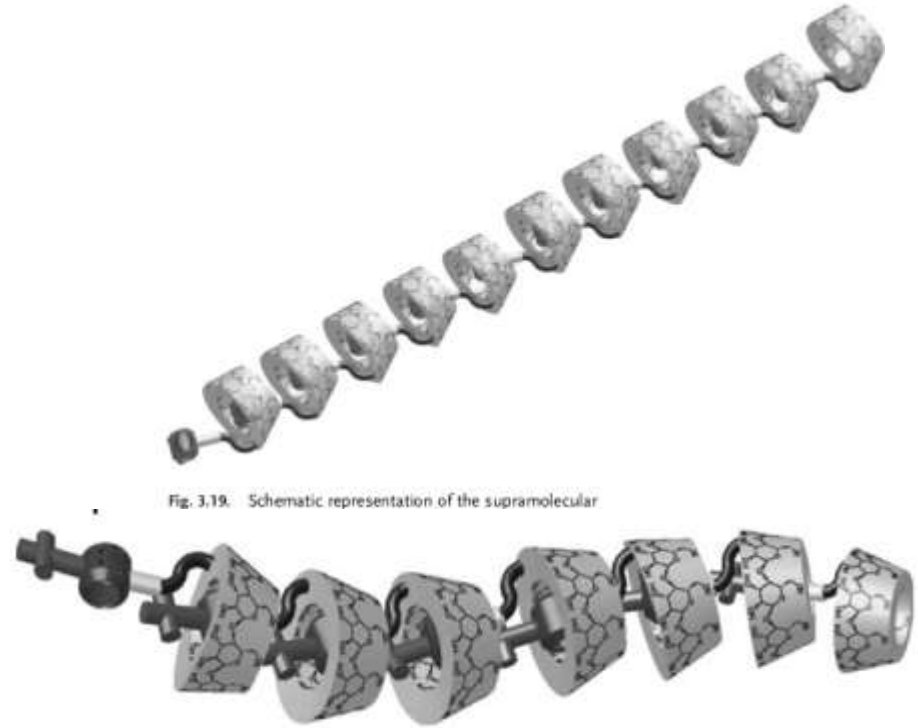


Fig. 3.19. Schematic representation of the supramolecular

Fig. 3.26. Proposed structure for the supramolecular polymer formed by 3-*p*-^{t}BocCiNH-α-CD in aqueous solution [45, 46].

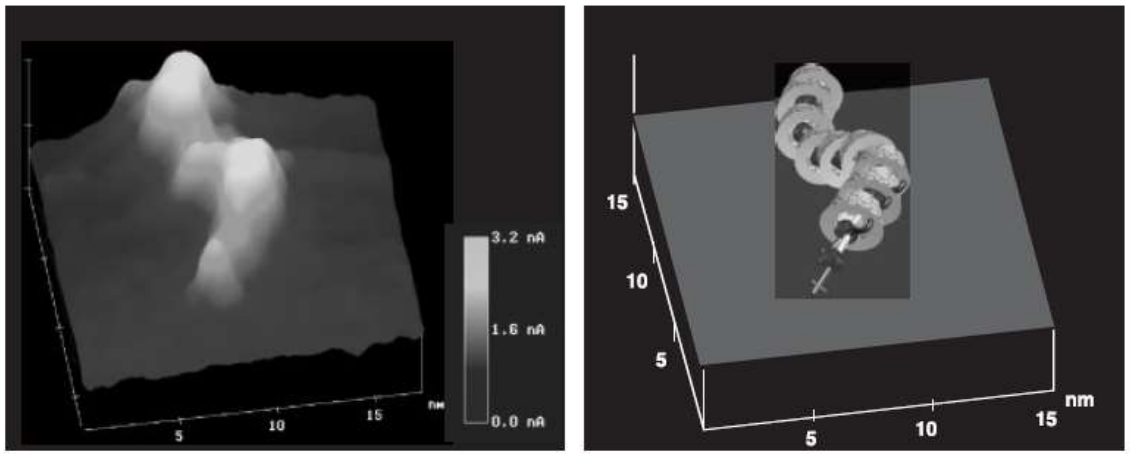
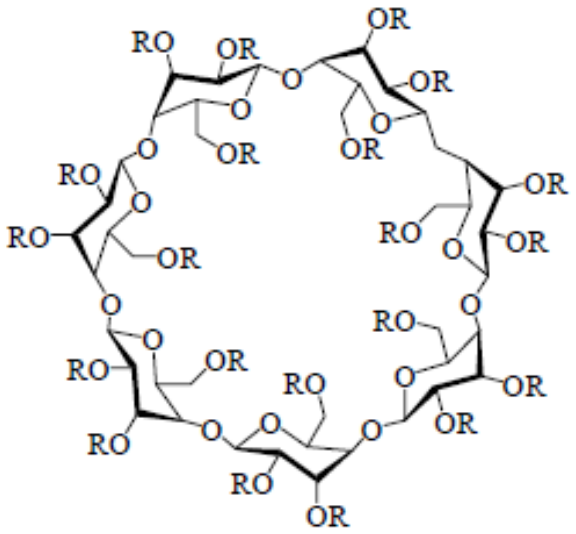


Fig. 3.25. STM image of 3-*p*-^{t}BocCiNH-α-CD from concentrated aqueous solution on a MoS_2 substrate (a) and its schematic structure (b).

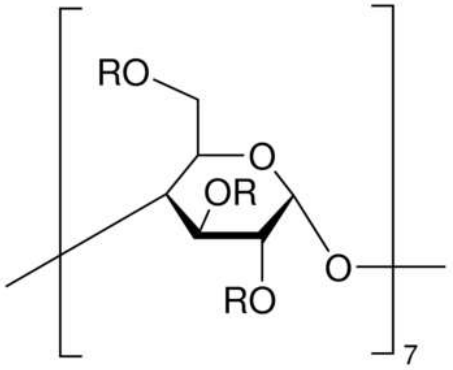
ΤΡΟΠΟΠΟΙΗΜΕΝΕΣ ΚΥΚΛΟΔΕΞΤΡΙΝΕΣ



Cyclodextrin	R = H or
β-Cyclodextrin	-H
2-Hydroxypropyl-β-cyclodextrin	$-CH_2CHOHCH_3$
Sulfobutylether β-cyclodextrin sodium salt	$-(CH_2)_4SO_3^-Na^+$
Randomly methylated β-cyclodextrin	$-CH_3$
Branched β-cyclodextrin	Glucosyl or maltosyl group

ΤΡΟΠΟΠΟΙΗΜΕΝΕΣ ΚΥΚΛΟΔΕΞΤΡΙΝΕΣ

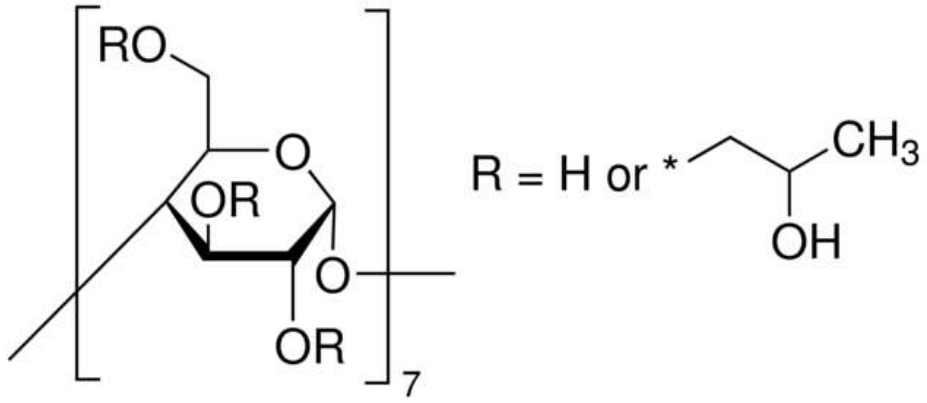
Methyl-β-cyclodextrin



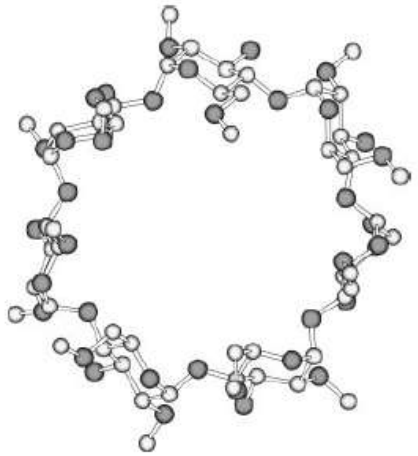
R = H or *−CH_3

average M_n 1310

(2-Hydroxypropyl)-β-cyclodextrin



heptakis(2,6-di-O-methyl)-β-CyD



heptakis(2,3,6-tri-O-methyl)-*β*-CyD

ΟΡΙΣΜΕΝΕΣ ΕΜΠΟΡΙΚΑ ΔΙΑΘΕΣΙΜΕΣ ΚΥΚΛΟΔΕΞΤΡΙΝΕΣ

Cyclodextrin	Substitution[a]	MW[b]	Solubility in water (mg/ml)[c]	Indicative bulk price[d] (USD/Kg)
α-Cyclodextrin	-	972	145	45
β-Cyclodextrin (βCD)	-	1135	18.5	5
2-Hydroxypropyl-β-cyclodextrin	0.65	1400	>600	300
Randomly methylated β-cyclodextrin	1.8	1312	>500	350
β-CD sulfobutyl ether sodium salt	0.9	2163	>500	-
γ-Cyclodextrin	-	1297	232	80
2-Hydroxypropyl-γ-cyclodextrin	0.6	1576	>500	400

[a] Average number of substituents per glucopyranose repeat unit.
[b] MW in Daltons.
[c] Solubility in pure water at approx. 25°C.
[d] Approximate bulk price given as the price of one kilogram in US dollars.

Expert Opin. Drug Deliv. 2, 335-351 (2005).

Review

Cyclodextrins in drug delivery

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Μέθοδοι παρασκευής προϊόντων εγκλεισμού ουσιών σε κυκλοδεξτρίνες

Various methods are used and widely reported for the formation of the cyclodextrin inclusion complexes.

The selection of the method and its effectiveness highly depends on the nature of the drug and/or cyclodextrin.

- ***Physical mixtures (PM)***
  - prepared mainly to perform comparative evaluations
  - Weighted amounts of drug and cyclodextrin (in molar ratios of 1:1 or 1:2 or other) are simply blended in a mortar during a time period of about 15 min (or 30 min or other)

Μέθοδοι παρασκευής προϊόντων εγκλεισμού ουσιών σε κυκλοδεξτρίνες

- ***Kneading method (KN)***
  Drug and cyclodextrin are accurately weighed in different stoichiometric ratios (e.g. 1:1, 2:1, 3:1 and 1:2) and are wetted with appropriate quantity of water so as to obtain a paste. Then they subjected to different trituration timings ranging from 15 min. to 1 hr (to study the effect of trituration time over the effective complexation). After that it was dried at 50 ^{o}C, for one day, crushed, sieved and stored at temperature of 25° ± 2.0° and relative humidity between 40-50%. For small scale mortar and pestle is used while for large scale rapid mass granulator was used. The kneading method was repeated at 75 ^{o}C.

Μέθοδοι παρασκευής προϊόντων εγκλεισμού ουσιών σε κυκλοδεξτρίνες

- ***Co-evaporation method (CE*)**

Drug and cyclodextrin (usually in equimolar ratio 1:1) are completely dissolved in a solution of ethanol and water (v/v = 1:20). The dispersion of drug in the aqueous cyclodextrin solution is mechanically shaken at room temperature and 100 rpm for a time period varying from 1h to 4 days to achieve equilibrium of the complexation reaction. After evaporation of the ethanol from the reaction mixture, the uncomplexed drug is removed by filtration. The filtrate is evaporated under reduced pressure to remove the solvent and dried in vacuum to give the drug/cyclodextrin complex.

Μέθοδοι παρασκευής προϊόντων εγκλεισμού ουσιών σε κυκλοδεξτρίνες

- ***Co-precipitation method (CP***)

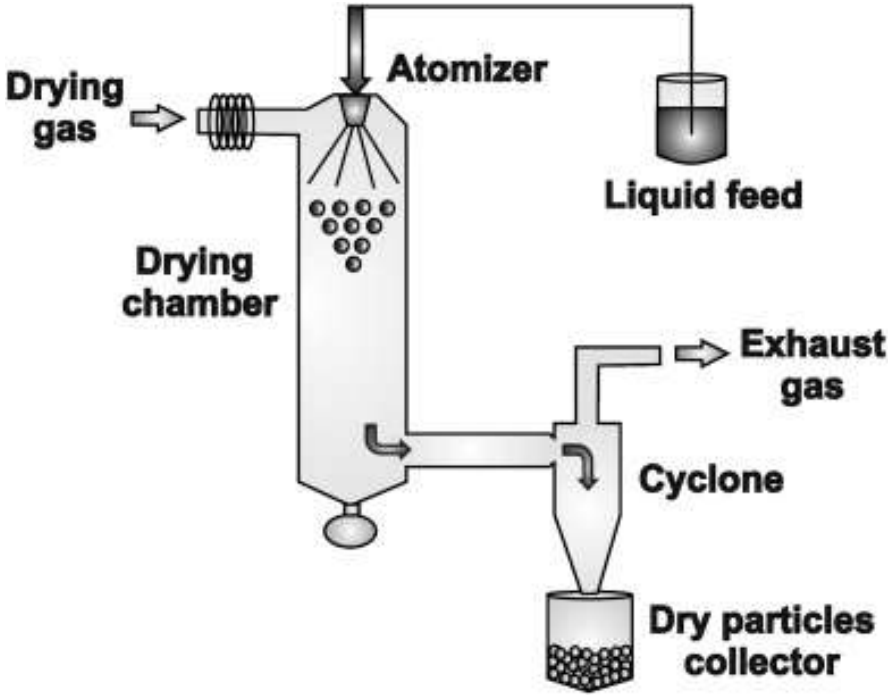
Drug is dissolved in minimum quantity of ethanol and is added drop wise to the solution of *β*-CD (in equimolar ratio 1:1 or other ratios like 1:2 etc) in minimum quantity of water previously maintained at 75 ^{o}C while stirring. Stirring is maintained for about 1 h at 75^{o}C. Then gradually it is cooled to room temperature while stirring. The precipitates are then filtered, dried and stored at temperature of 25° ± 2.0° and relative humidity between 40-50%.

The experiment is carried out both at small and large scale in various molar ratios

Μέθοδοι παρασκευής προϊόντων εγκλεισμού ουσιών σε κυκλοδεξτρίνες

- ***Spray-drying (SD)***

Spray-drying is carried out in a Spray-Dryer equipment. For this purpose, the guest is dissolved in an organic solvent (e.g. ethanol) and an amount of cyclodextrin (guest:cyclodextrin molar ratios 1:1 or 1:2) is separately dissolved in purified water. The solutions are next mixed for about 20 min by sonication to produce a clear solution which is spray-dried under specific conditions.



Μέθοδοι παρασκευής προϊόντων εγκλεισμού ουσιών σε κυκλοδεξτρίνες

- ***Crystallization (slow cooling or slow heating)***

The drug is mixed in an aqueous solution of native or methylated β-CD (equimolar ratio) and the mixture is stirred for a two hours period at 65-70 ˚C . In some cases ethanol is added (ethanol/water v/v = 1:20) in order to dissolve the insoluble drugs. Then the mixture is left to cool gradually to room temperature over a period of one or two weeks.

The solutions of guests in methylated CDs hosts (they present reverse solubility) are stirred for 30 min at 45˚C and then maintained at 50˚C for some days. At the end of these time periods transparent crystals of the complexes, suitable for X-ray data collection are obtained.

Παρασκευή προϊόντων εγκλεισμού ουσιών σε κυκλοδεξτρίνες

- Πόσα g *β*-κυκλοδεξτρίνης (*β*-CD) πρέπει να προσθέσουμε σε 5 ml νερού (δισαποσταγμένο) ώστε να προκύψει κορεσμένο διάλυμα;
- Πόση ποσότητα θυμόλης πρέπει να προσθέσουμε ώστε να προκύψει ισομοριακό (thymol:*β*-CD molar ratio 1:1) μείγμα;
- Πόση ποσότητα ευγενόλης πρέπει να προσθέσουμε ώστε να προκύψει ισομοριακό (eugenol:*β*-CD molar ratio 1:1) μείγμα;

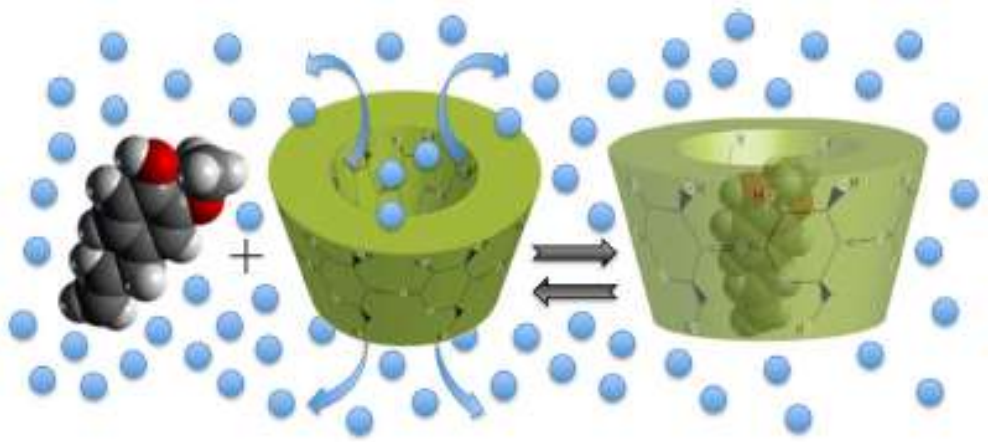
thymol

Properties	
Chemical formula	$C_{10}H_{14}O$
Molar mass	150.22 g·mol^{-1}
Density	0.96 g/cm^3
Melting point	49 to 51 °C (120 to 124 °F; 322 to 324 K)
Boiling point	232 °C (450 °F; 505 K)
Solubility in water	0.9 g/L (20 °C)[1]

eugenol

Properties	
Chemical formula	$C_{10}H_{12}O_2$
Molar mass	164.20 g·mol^{-1}
Density	1.06 g/cm^3
Melting point	−7.5 °C (18.5 °F; 265.6 K)
Boiling point	254 °C (489 °F; 527 K)
Acidity (pK_a)	10.19 at 25 °C
Magnetic susceptibility (χ)	−1.021 × 10^{-4} cm^3/mol

Εγκλεισμός σε κυκλοδεξτρίνες



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.

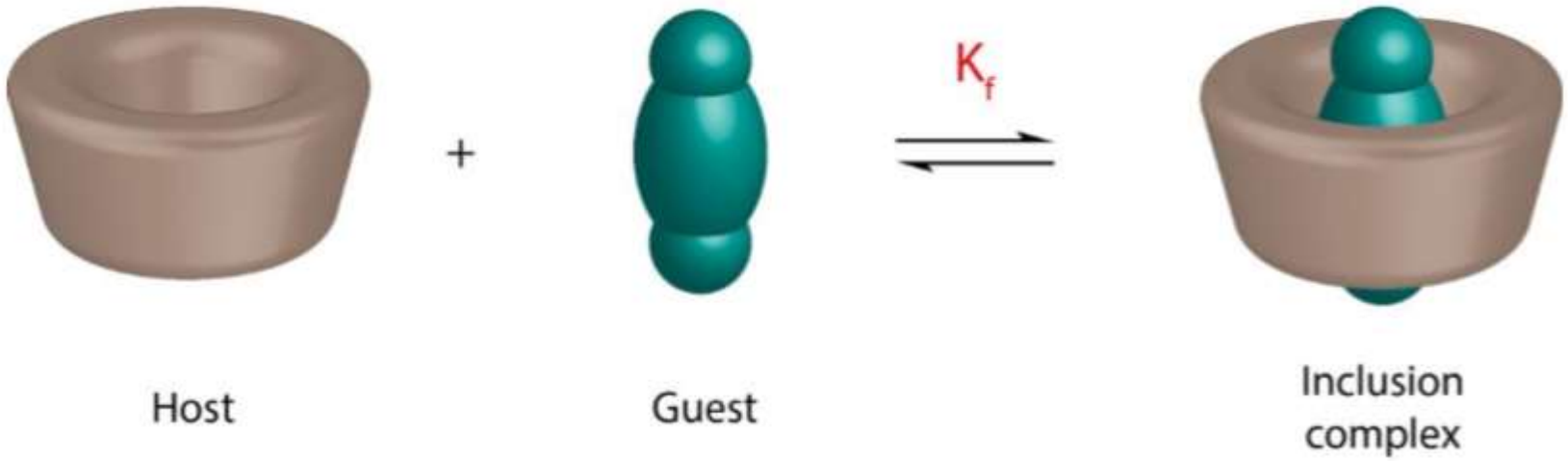
No covalent bonds are formed or broken during the complex formation and drug molecules in the complex are in rapid equilibrium with free molecules in the solution.

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.

Φυσικοχημικός χαρακτηρισμός των προϊόντων εγκλεισμού ουσιών σε κυκλοδεξτρίνες

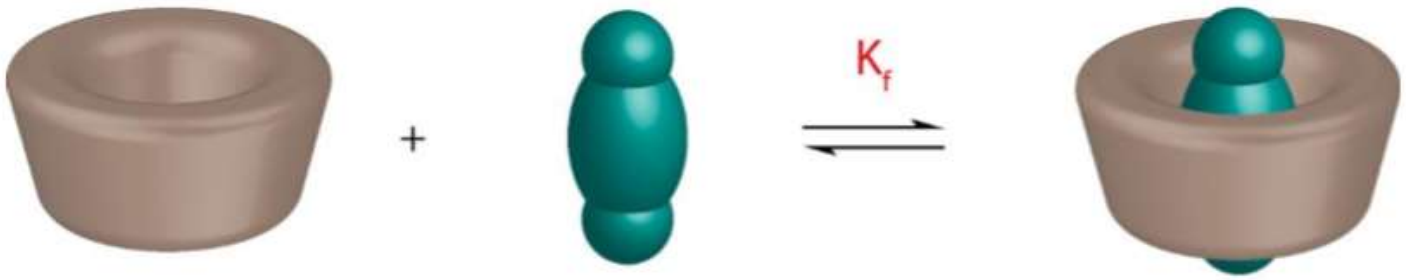
The starting crucial point for a suitable and careful characterization of an inclusion complex is to assess the value of the ***formation constant (K_f), also called stability or binding constant***. This task requires the application of the appropriate analytical method and technique.



Φυσικοχημικός χαρακτηρισμός των προϊόντων εγκλεισμού ουσιών σε κυκλοδεξτρίνες

K_f values are determined mainly to answer ***two different types of questions***.

- The first one, which deals with the encapsulation in an absolute mean, is, can a CD encapsulate the guest?
- The second question is a comparative one, that is, what is the binding strength? Which inclusion complex is more stable?



Φυσικοχημικός χαρακτηρισμός των προϊόντων εγκλεισμού ουσιών σε κυκλοδεξτρίνες

- 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 utilized to determine the stoichiometry of the complexes formed and the numerical values of their stability constants.
- In fact, to fully exploit the potential of CD inclusion complexes, ***it is important to have at disposal adequate analytical techniques for their suitable and careful characterization***. In particular, the determination of the stability constants of the inclusion complexes is a crucial point for the evaluation of their effectiveness, since the different possible effects related to the complex formation all rely on the stability of the complexes formed.

Analytical techniques to characterize drug–cyclodextrin complexes in solution

- The assessment of the formation of a drug–CD inclusion complex and its full characterization is not a simple task and often requires the ***use of different analytical methods, whose results have to be combined and examined together, since each method explores a particular feature of the inclusion complex***.
- The concomitant use of different techniques can allow a better and more in-depth understanding of host–guest interactions and help in selection of the most appropriate CD for a given guest molecule. The different available methods are generally based on the ***detection of the variation in any suitable physical or chemical property of the guest as a consequence of the inclusion complex formation***. Obviously, it is essential that ***the observed variation is large enough to be detected or estimated with sufficient precision***.
- Moreover, any measurement ***method suffers from its own drawbacks***, which should be well known and held into due consideration, in order to evaluate how much they can affect the reliability of the results.

Φυσικοχημικός χαρακτηρισμός των προϊόντων εγκλεισμού ουσιών σε κυκλοδεξτρίνες

The changes in the physicochemical properties of both host and guest molecules include:

- changes in solubility,
- changes in chemical reactivity,
- changes in UV/VIS absorbance,
- changes in fluorescence,
- NMR chemical shifts,
- changes in drug retention (e.g. in liquid chromatography),
- changes in pKa values,
- potentiometric measurements,
- changes in chemical stability and
- effects on drug permeability through artificial membranes.

Φυσικοχημικός χαρακτηρισμός των προϊόντων εγκλεισμού ουσιών σε κυκλοδεξτρίνες

Furthermore, since 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.

Main analytical techniques used for characterization of CD inclusion complexation

In solution:

- Spectroscopic techniques:
  - (a) Ultraviolet/visible (UV)
  - (b) Circular dichroism
  - (c) Fluorescence
  - (d) Nuclear magnetic resonance (NMR)
  - (e) Electron spin resonance (ESR).
- Electroanalytical techniques:
  - (a) Polarography
  - (b) Voltammetry
  - (c) Potentiometry
  - (d) Conductimetry.
- Separation techniques:
  - (a) High performance liquid chromatography (HPLC)
  - (b) Capillary electrophoresis (CE)
- Polarimetry
- Isotermal titration calorimetry (ITC)
- Phase solubility studies

In the solid state:

- Infrared spectroscopy (FTIR),
- X-ray diffraction (XRD),
  - Powder XRD
  - Single crystal XRD
- scanning electron microscopy (EM)
- differential scanning calorimetry (DSC).

Main analytical techniques used for characterization of CD inclusion complexation

- Applicability of each technique
- Advantages, inconvenient and limits of each applied method (improvement of the current methods and to the development of new techniques).

In Solution: Spectroscopic techniques

All the spectroscopic methods are based on the measurement of a variation, upon inclusion complex formation, in a given property (absorbance, NMR shift, fluorescence intensity, etc.) of the system.

Therefore, for their applicability, it is necessary that this ***variation can be detectable with sufficient precision***.

Moreover, these methods usually consist in working at a fixed guest concentration and varying the concentration of the CD host, thus requiring the preparation of a large number of sample solutions. This implies a considerable waste of time and material and it could be a disadvantage in the initial development phases.

Ultraviolet/visible (UV-Vis) spectroscopy

Simple, economic, fast and useful method of studying the formation of host–guest complexes in solution, ***when the complexation gives rise to a significant modification of the absorption spectrum of the guest molecule***.

Depending on the position of the drug chromophore, the transfer of the guest molecule from an aqueous medium to the non-polar CD cavity can modify its original UV absorption spectrum, due to partial or total replacement of the salvation shell of the molecule by the CD molecule, which leads to new solute environment interactions.

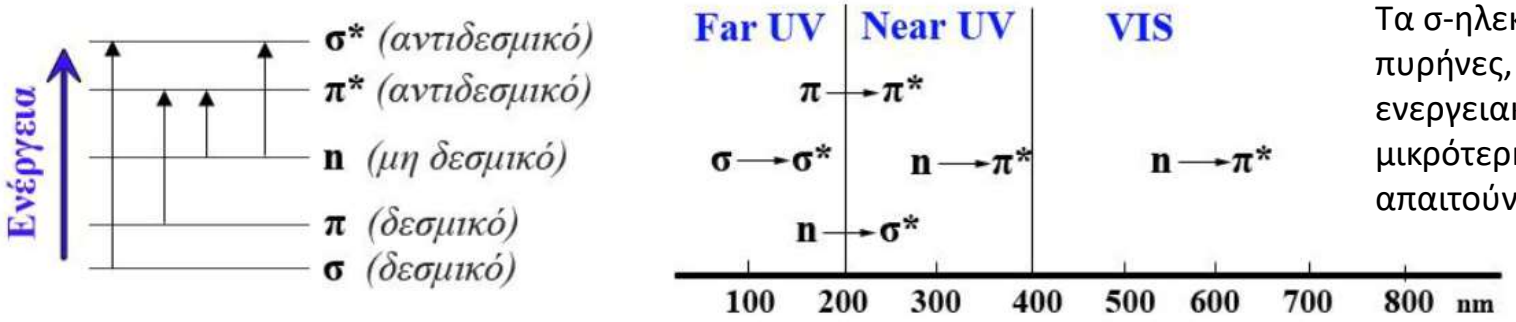
Modifications of the UV spectrum of a drug in presence of CDs can provide evidence of the formation of an inclusion complex. However, the method is ***not specific and suffers from the presence of interfering substances and it does not provide a direct evidence of the actual inclusion complex formation***.

Θεωρία των μοριακών τροχιακών (*Molecular Orbital Theory*)

- Ηλεκτρονιακές μεταβάσεις ανάμεσα στα μόρια των χημικών ενώσεων και ενεργειακές μεταβολές που συμβαίνουν κατά τη λήψη φασμάτων *UV-VIS*.

- Ο γραμμικός συνδυασμός δύο ατομικών τροχιακών δημιουργεί δύο μοριακά τροχιακά διαφορετικής ενέργειας, όπου το μοριακό τροχιακό χαμηλής ενέργειας ονομάζεται δεσμικό (*bonding orbital*) και αυτό με την υψηλότερη ενέργεια αντιδεσμικό (*antibonding orbital*). Για την πλειοψηφία των οργανικών μορίων τα ηλεκτρόνια κατατάσσονται σε τρεις κατηγορίες: τα δεσμικά σ- και π-ηλεκτρόνια, και τα n-ηλεκτρόνια ή μη δεσμικά (non bonding) που δεν παίρνουν μέρος στον σχηματισμό χημικών δεσμών και συγκρατούνται ασθενέστερα ώστε να μπορούν να υποστούν μεταπτώσεις.

- Η ποσότητα της ενέργειας που απαιτείται για τη διέγερση των ηλεκτρονίων από τη δεσμική στην αντιδεσμική κατάσταση αντιστοιχεί στην εμφάνιση απορροφήσεων στην UV και VIS περιοχή του φάσματος. Έτσι, η μετάβαση από ένα δεσμικό σ-ηλεκτρόνιο σε ένα αντιδεσμικό σ*-ηλεκτρόνιο ($\sigma \rightarrow \sigma*$) απαιτεί υψηλή ποσότητα ενέργειας της οποίας το μήκος κύματος εντοπίζεται στην άπω υπεριώδη περιοχή, ενώ οι διεγέρσεις του τύπου $\pi \rightarrow \pi*$, $n \rightarrow \sigma*$ και $n \rightarrow \pi*$ εμφανίζονται σε μεγαλύτερα μήκη κύματος, λόγω της μικρότερης διαφοράς ενέργειας, που εκτείνονται από τη διαχωριστική γραμμή της άπω υπεριώδους (far UV) και εγγύς υπεριώδους (near UV) ως την ορατή (VIS) περιοχή

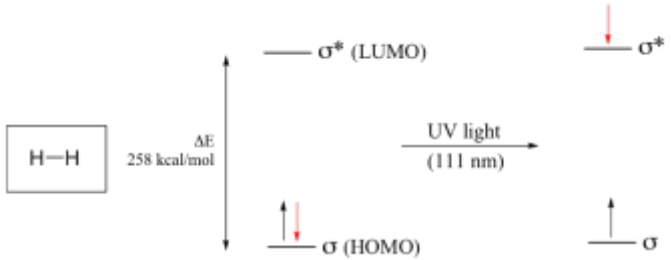


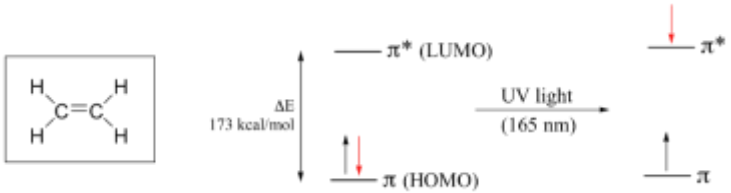
Ενεργειακό διάγραμμα ηλεκτρονιακών μοριακών τροχιακών
Τα σ-ηλεκτρόνια, που είναι πιο σταθερά και προσκολλημένα στους πυρήνες, απαιτούν μεγαλύτερη ενέργεια για να μεταβούν σε άλλα ενεργειακά επίπεδα, ενώ για τα π- και τα n-ηλεκτρόνια απαιτείται μικρότερη ενέργεια. Συνήθως, αλλά όχι πάντοτε, τα n-ηλεκτρόνια απαιτούν χαμηλότερη ενέργεια από τα π-ηλεκτρόνια.

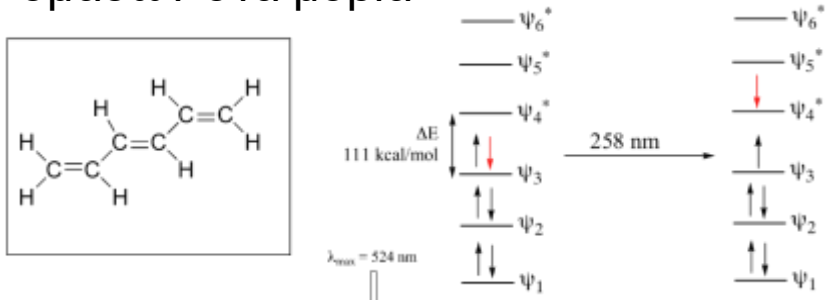
UV-Visible Absorption Spectra

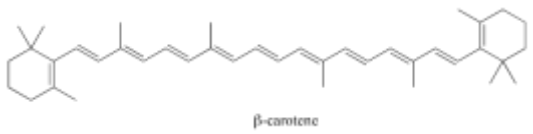
- Ultraviolet radiation having wavelengths less than 200 nm is difficult to handle, and is seldom used as a routine tool for structural analysis. ***Conjugation*** generally moves the absorption maxima to longer wavelengths, so conjugation becomes the major structural feature identified by this technique.

Θεωρία των μοριακών τροχιακών
(*Molecular Orbital Theory*)
Ύπαρξη συζυγιακών διπλών δεσμών ή χρωμοφόρων και αυξόχρωμων ομάδων στα μόρια

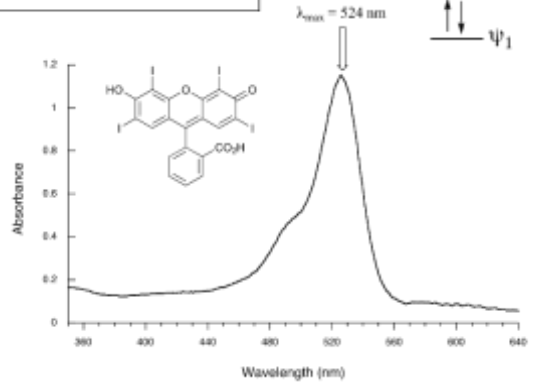








In molecules with extended π-systems, the HOMO-LUMO energy gap becomes so small that absorption occurs in the visible rather then the UV region of the electromagnetic spectrum. Beta-carotene, with its system of 11 conjugated double bonds, absorbs light with wavelengths in the blue region of the visible spectrum while allowing other visible wavelengths – mainly those in the red-yellow region - to be transmitted. This is why carrots are orange.

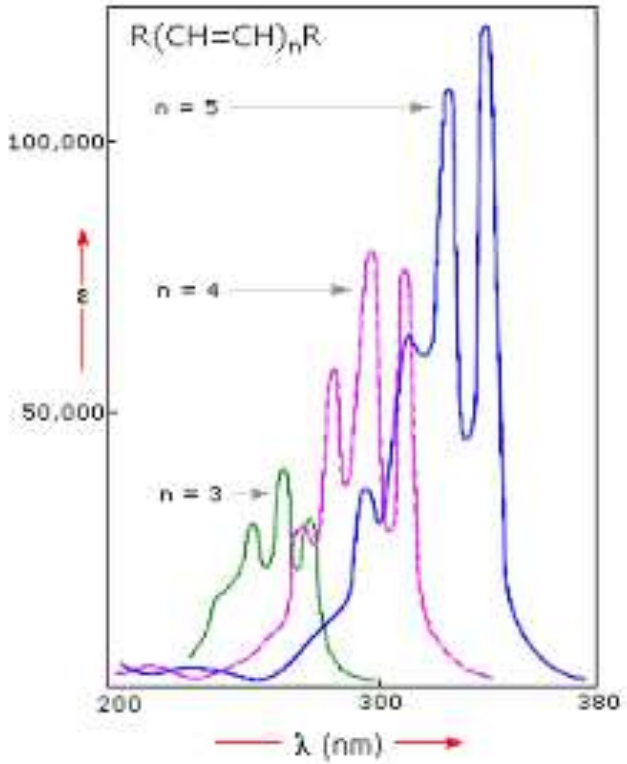


common food coloring Red #3: the extended system of conjugated pi bonds causes the molecule to absorb light in the visible range. Because the $\boldsymbol{\lambda}_{max}$ of 524 nm falls within the green region of the spectrum, the compound appears red to our eyes.

https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Supplemental_Modules_(Organic_Chemistry)/Spectroscopy/Ultraviolet_and_visible_spectroscopy

Ultraviolet/visible (UV-Vis) spectroscopy

Both **hypsochromic or bathochromic** shifts of the absorption maximum of the guest UV spectrum, and/or **increase or decrease** in its intensity can be observed as a consequence of the inclusion complex formation.

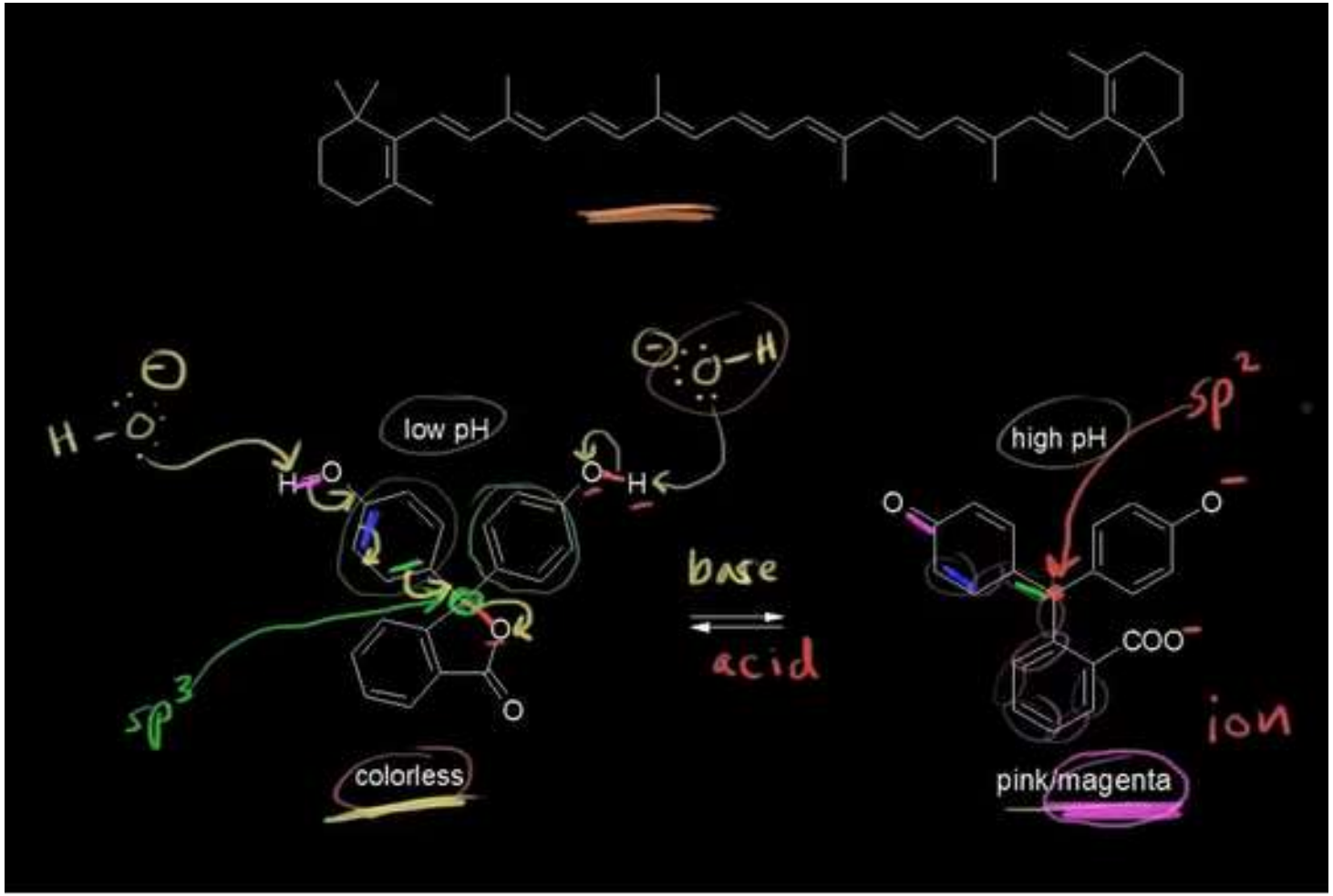


Terminology for Absorption Shifts

Nature of Shift	Descriptive Term
To Longer Wavelength	Bathochromic
To Shorter Wavelength	Hypsochromic
To Greater Absorbance	Hyperchromic
To Lower Absorbance	Hypochromic

UV-Vis spectroscopy

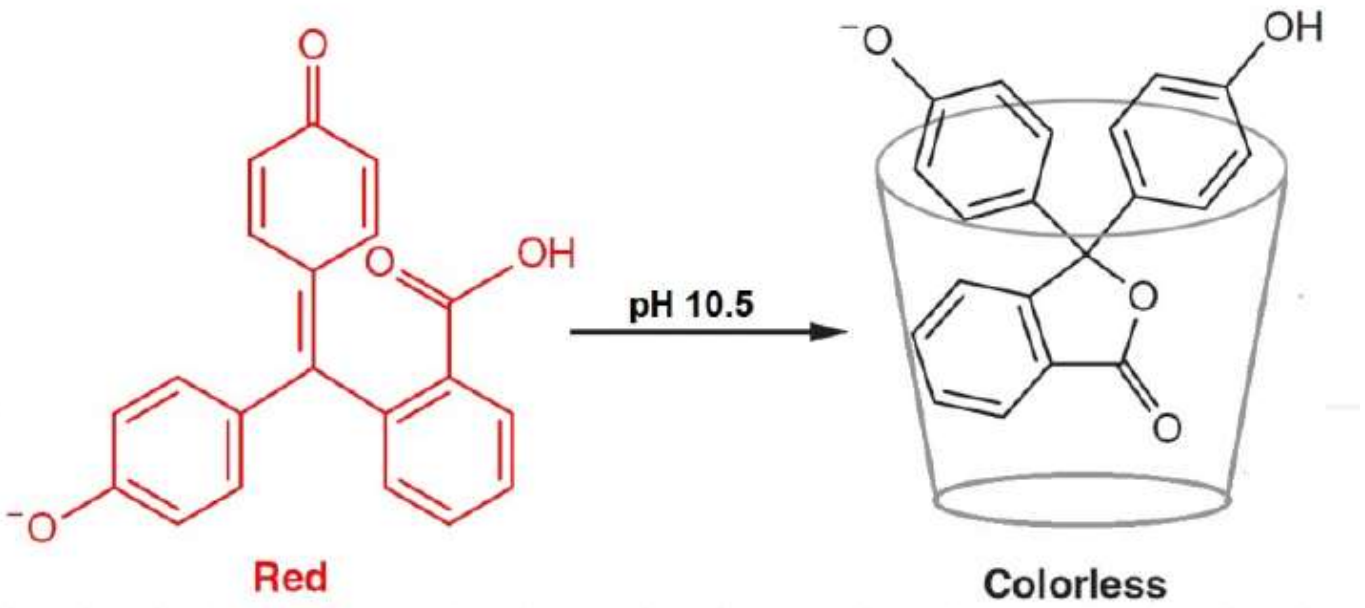
Phenolphthalein



Conjugation and color

UV-Vis spectroscopy

Taguchi (1986) has demonstrated that upon the binding of phenolphthalein to β-CyD cavity in aqueous solution at pH 10.5, the red-colored dianion form is rapidly transformed into a colorless lactonoid form. This effect and some other similar spectral changes may reflect the altered polarity of the cavity microenvironment and preferential or specific guest–host interactions and stabilization of the preferred form and suppression of the other form in equilibrium.



Proposed mechanism for the colour change of phenolphetalein in the presence of β-CyD

UV-Vis spectroscopy

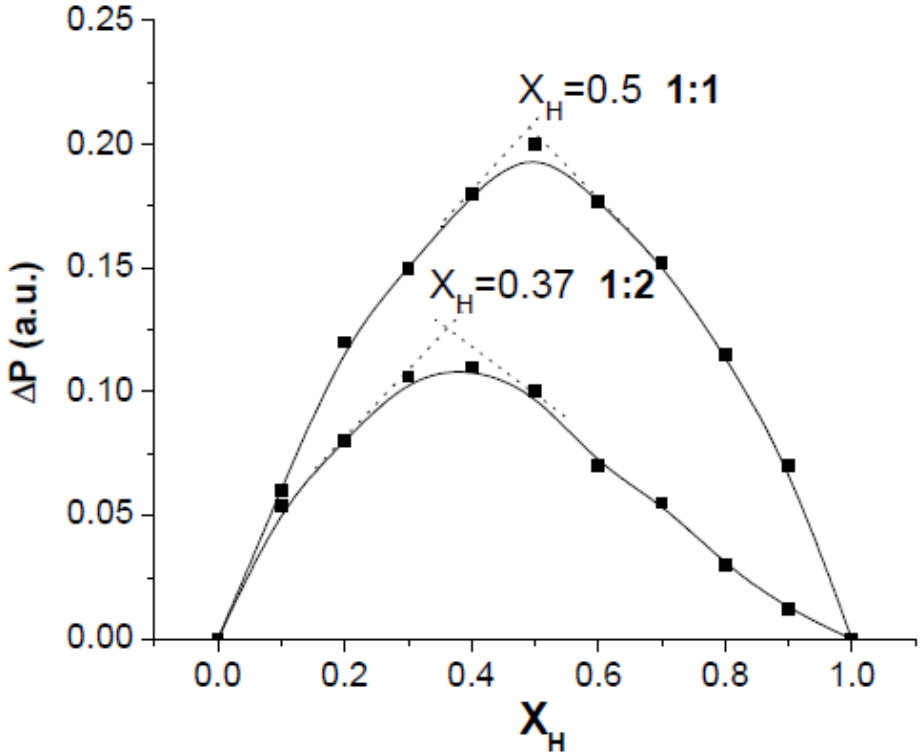
- **Stoichiometry** of the complex can be calculated from such spectral changes using, the mole ratio or the continuous variation methods (Job's plot)
- **Stability constant** of the complex (Ks) can be calculated from the spectral shift methods (Benesi–Hildebrand or Scott equation)

Determination of the stoichiometry. Job's method

- One of the first methods used for the determination of the stoichiometry of inclusion complexes was Job's method, also known as the continuous variation method (Job, 1928).
- The experiments use stock **solutions with equimolecular concentrations of H and G components**. The samples are prepared by **mixing different volumes of these two solutions in such a way that the total concentration [H]+[G] remains constant and the molar fraction of the guest, X_G varies in the range 0–1**.
- The **variation of the experimental measured property, ΔP, in presence of the host in respect with the value for the free guest is plotted vs. X_G or X_H. The value of X_G for which the plot presents the maximum deviation gives the stoichiometry of the inclusion complex** (X_G = 0.5 for 1:1 or 2:2 G:H complexes; X_H = 0.33 for 1:2 G:H complexes).
- In most cases, in a Job plot ΔP represents the change of the absorbance of the guest during addition of the host, ΔA (other properties correlated with the concentration of the complex, like the change in the NMR chemical shifts (Δδ) or the enthalpy changes (ΔH) can be used as well)

Determination of the stoichiometry. Job's method

- Two typical schematic Job's plots for 1:1 and 1:2 inclusion complexes Considering ΔP as the absorbance change, ΔA, for the complexes.
- In the case of 1:1 complexes, the maximum deviation is obtained for $X_H = 0.5$, while for the second type of complexes the maximum is reached for $X_H \sim 0.37$.



Determination of both the stoichiometry and the association constant

- The determination of the stoichiometry in the host–guest interaction is strongly correlated with the estimation of the association constant. Excepting Job's method which gives indications only on the stoichiometry of the inclusion complexes, for all other methods the following procedure is applied. ***Several stoichiometries are assumed and the experimental data are fitted to the corresponding linear or nonlinear models***.
- Starting with the equations of the assumed chemical equilibria, the general idea is to **monitor the changes of an experimental property** (Pobs) directly correlated with the concentration of the former or the new-formed species, **at gradual host addition**. The function Pobs = f(Ci, parameters), where Ci represents the equilibrium concentration of the species *i*, is called the binding isotherm. As for Job's plot, this property can be the absorbance (A), fluorescence quantum yield or fluorescence emission (Φ or F), ellipticity (θ) or NMR chemical shift (Δδ).

Determination of both the stoichiometry and the association constant

Equations corresponding to some widely used linear (eqs. 1, 2, 5) (Benesi & Hildebrand, 1949; Scott,1956) and nonlinear (eqs. 3, 4, 6–9) (Liu et al., 2001; Park et al., 2002) models.

- The most used equations are the Benesi-Hildebrand linear or double reciprocal equations. However, their reliability was the subject of many discussions, especially to differentiate the formation of 1:1 and 1:2 complexes.

Stoichiometry	Equations
1:1	$G + H \rightleftarrows GH$
	$\frac{1}{\Delta P_{obs}} = \frac{1}{(P_{GH} - P_{G_0})K_{11}[H]} + \frac{1}{(P_{GH} - P_{G_0})}$ (1)
	$\frac{[H][G]_0}{\Delta P_{obs}} = \frac{[G]_0}{P'_{GH}} + \frac{1}{K \cdot P'_{GH}}$ (2)
	$P_{obs} = \frac{P_{G_0} + P_{GH}K_{11}[H]}{1 + K_{11}[H]}$ (3)
	$\Delta P_{obs} = \frac{1}{2}([H] + [G] + \frac{1}{K_{11}}) - \sqrt{([H] + [G] + \frac{1}{K_{11}})^2 - 4[H][G]}$ (4)
1:2	$G + 2H \rightleftarrows GH_2$
	$\frac{1}{\Delta P_{obs}} = \frac{1}{(P_{GH} - P_{G_0})K_{12}[H]^2} + \frac{1}{(P_{GH} - P_{G_0})}$ (5)
	$P_{obs} = \frac{P_{G_0} + P_{GH_2}K_{12}[H]^2}{1 + K_{12}[H]^2}$ (6)
1:1+1:2	$G + H \rightleftarrows GH;\ GH + H \rightleftarrows GH_2$
	$P_{obs} = \frac{P_{G_0} + P_{GH}K_{11}[H] + P_{GH_2}K_{11}K_{12}[H]^2}{1 + K_{11}[H] + K_{11}K_{12}[H]^2}$ (7)
2:1	$G + H \rightleftarrows GH;\ GH + G \rightleftarrows G_2H$
	$P = P_{G_2H}\frac{1}{2}([G]_0 - [G] - K_{11}[H][G])$ (8)
	where $[G] = \frac{-(K_{11}[H] + 1) + \sqrt{(K_{11}[H] + 1)^2 + 8K_{11}K_{21}[H][G]_0}}{4K_{11}K_{21}[H]}$
2:2	$G + H \rightleftarrows GH;\ GH + GH \rightleftarrows G_2H_2$
	$P = P_{G_2H_2}\frac{1}{2}([G]_0 - [G] - K_{11}[H][G])$ (9)
	where $[G] = \frac{-(K_{11}[H] + 1) + \sqrt{(K_{11}[H] + 1)^2 + 8K_{11}^2K_{22}[H]^2[G]_0}}{4K_{11}^2K_{22}[H]^2}$

Table 1. Nonlinear and linear fitting models for the determination of the stoichiometry and

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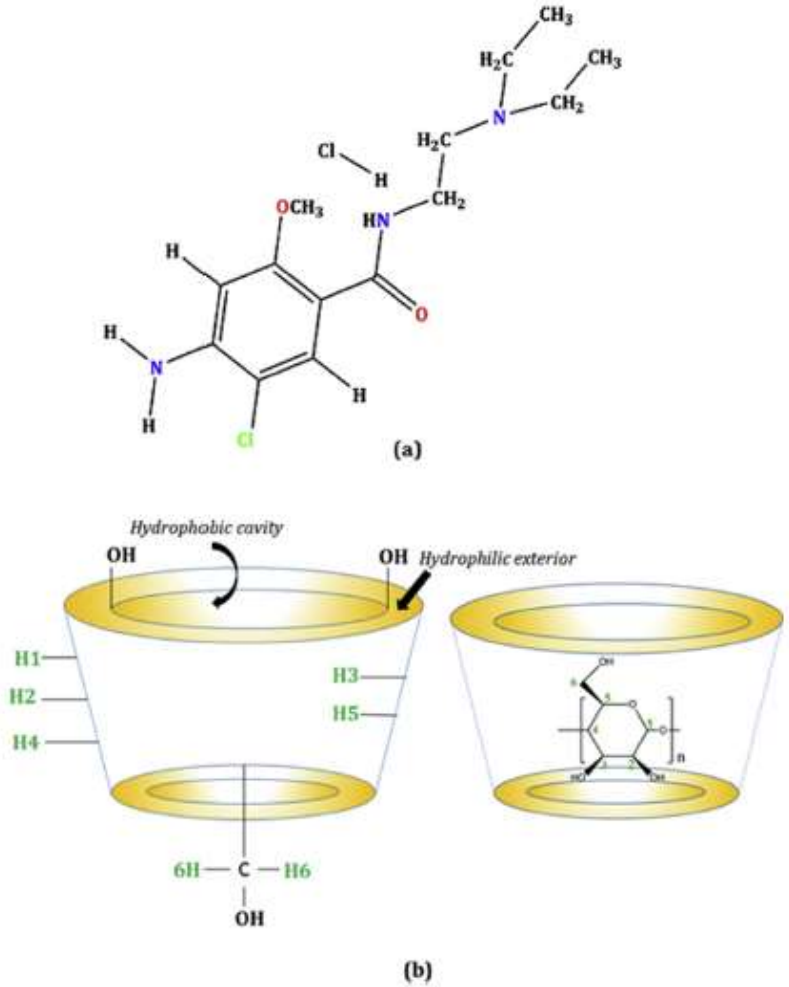
Preparation, characterization and binding behaviors of host-guest inclusion complexes of metoclopramide hydrochloride with α- and β-cyclodextrin molecules

Siti Barman, Biraj Kumar Barman, Mahendra Nath Roy*



Scheme 1. Molecular structures of (a) metoclopramide hydrochloride and (b) cyclodextrin molecule with interior and exterior protons (n = 6, 7 for α-CD and β-CD respectively).

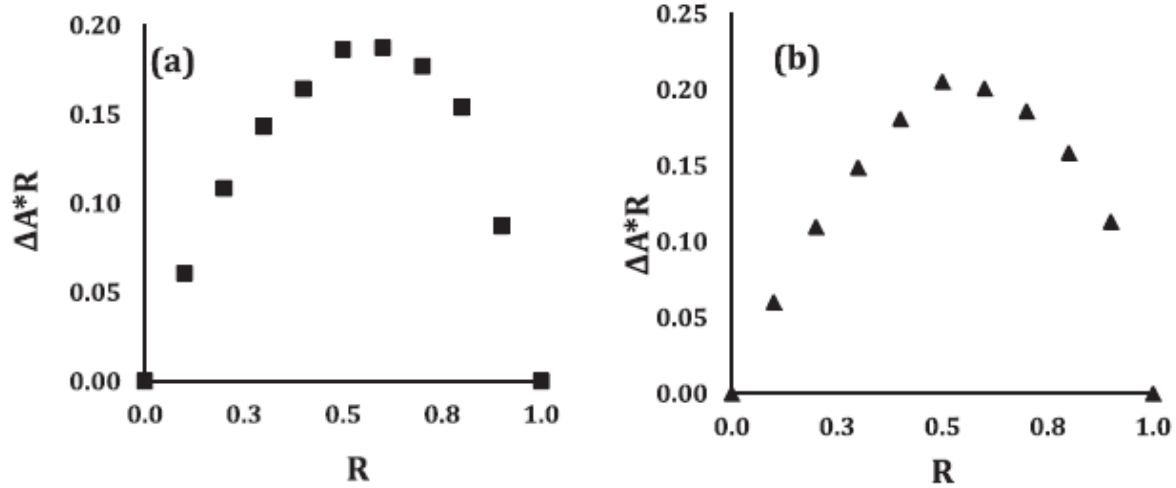


Fig. 1. Job plots of (a) MP:α-CD system and (b) MP:β-CD system at λ_{max} = 272 nm at 298.15 K. R = [MP]/([MP] + [CD]), ΔA = absorbance difference of MP with and without CD.

Association constants from UV-vis spectroscopy

Spectrophotometric titration is carried out to determine the molecular encapsulation behavior of MP with CDs in aqueous solution. The absorption intensity of MP gradually increased with the stepwise addition of CDs [Figs]. This change might be partly attributed to the shielding of chromophore groups of MP in the CD cavity.

Benesi-Hildebrand equation:

$$\frac{1}{A - A_0} = \frac{1}{\Delta\varepsilon[MP]K_a} \cdot \frac{1}{[CD]} + \frac{1}{\Delta\varepsilon[MP]}$$

where [CD] and [MP] refer to the total concentration of cyclodextrin and metoclopramide drug respectively, Δε is the change in molar extinction coefficient of the chromophore MP as the MP molecules go from the polar aqueous environment to the apolar cavity of α or β-CD making the ICs. A-A_0 denotes the absorption changes of MP on the addition of CDs.

The values of Ka for each of the complexes were evaluated by dividing the intercept by the slope of the straight line of the double reciprocal plot. The change of absorbance (A-A_0) was measured as a function of concentration of α and β-CD molecule to find out the association constant (Ka). The good linearity of the plot shows the formation of a 1:1 complex between MP and CDs.

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Preparation, characterization and binding behaviors of host-guest inclusion complexes of metoclopramide hydrochloride with α- and β-cyclodextrin molecules

Siti Barman, Biraj Kumar Barman, Mahendra Nath Roy*

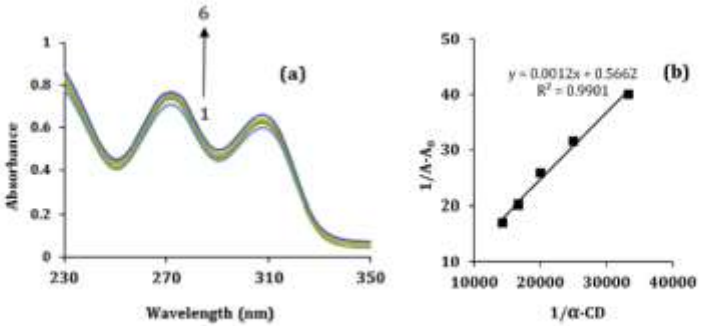

Fig. 4. (a) Absorption spectra of MP (50 μM) in different α-CD concentrations (μM): 1) without α-CD, 2) 30 μM, 3) 40 μM, 4) 50 μM, 5) 60 μM, 6) 70 μM. (b) Benesi–Hildebrand plot of 1/A–A_0 vs. 1/[α-CD] for 1:1 complexation of MP with α-CD.

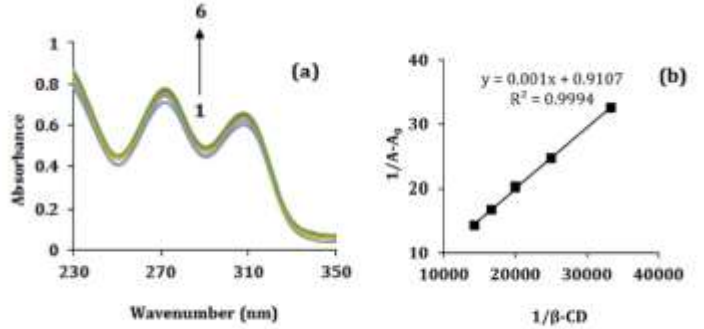

Fig. 5. (a) Absorption spectra of MP (50 μM) in different β-CD concentrations (μM): 1) without β-CD, 2) 30 μM, 3) 40 μM, 4) 50 μM, 5) 60 μM, 6) 70 μM. (b) Benesi–Hildebrand plot of 1/A–A_0 vs. 1/[β-CD] for 1:1 complexation of MP with β-CD.

Values of Association constants (K_a) obtained by Benesi–Hildebrand method both from UV–vis spectroscopy and Fluorescence spectroscopy and corresponding free energy change (ΔG^0) of the MP:CD inclusion complexes at 298.15 K[a].

	$K_a \times 10^{-2}/M^{-1}$[b]	ΔG^0/KJ mol^{-1}[b]	$K_a \times 10^{-2}/M^{-1}$[b]	ΔG^0/KJ mol^{-1}[b]
	UV–vis spectroscopy		Fluorescence spectroscopy	
MP:α-CD	4.72	−3.85	3.33	−2.98
MP:β-CD	9.11	−5.48	8.00	−5.15

[a] Standard uncertainties in temperature u are: u(T) = ±0.01 K.
[b] Mean errors in K_a = ±2 M^{-1}; ΔG^0 = ±0.01 kJ mol^{-1}.

Fluorescence spectroscopy

- Fluorescence spectroscopy is a simple, fast and very sensitive method, particularly useful for investigating the formation in solution of CD inclusion complexes of fluorescent guests.
- ***An enhancement in fluorescence is generally observed upon inclusion of a fluorescent guest molecule into the CD cavity***, due to shielding from quenching and non-radioactive decay processes.
- The fluorimetric method benefits of a high sensitivity, but its **application field is limited to fluorescent molecules**. Moreover, the preparation of samples for fluorimetry is tedious and time-consuming, because a very strict care is required to avoid spurious interferences

Fluorescence spectroscopy

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Preparation, characterization and binding behaviors of host-guest inclusion complexes of metoclopramide hydrochloride with α- and β-cyclodextrin molecules

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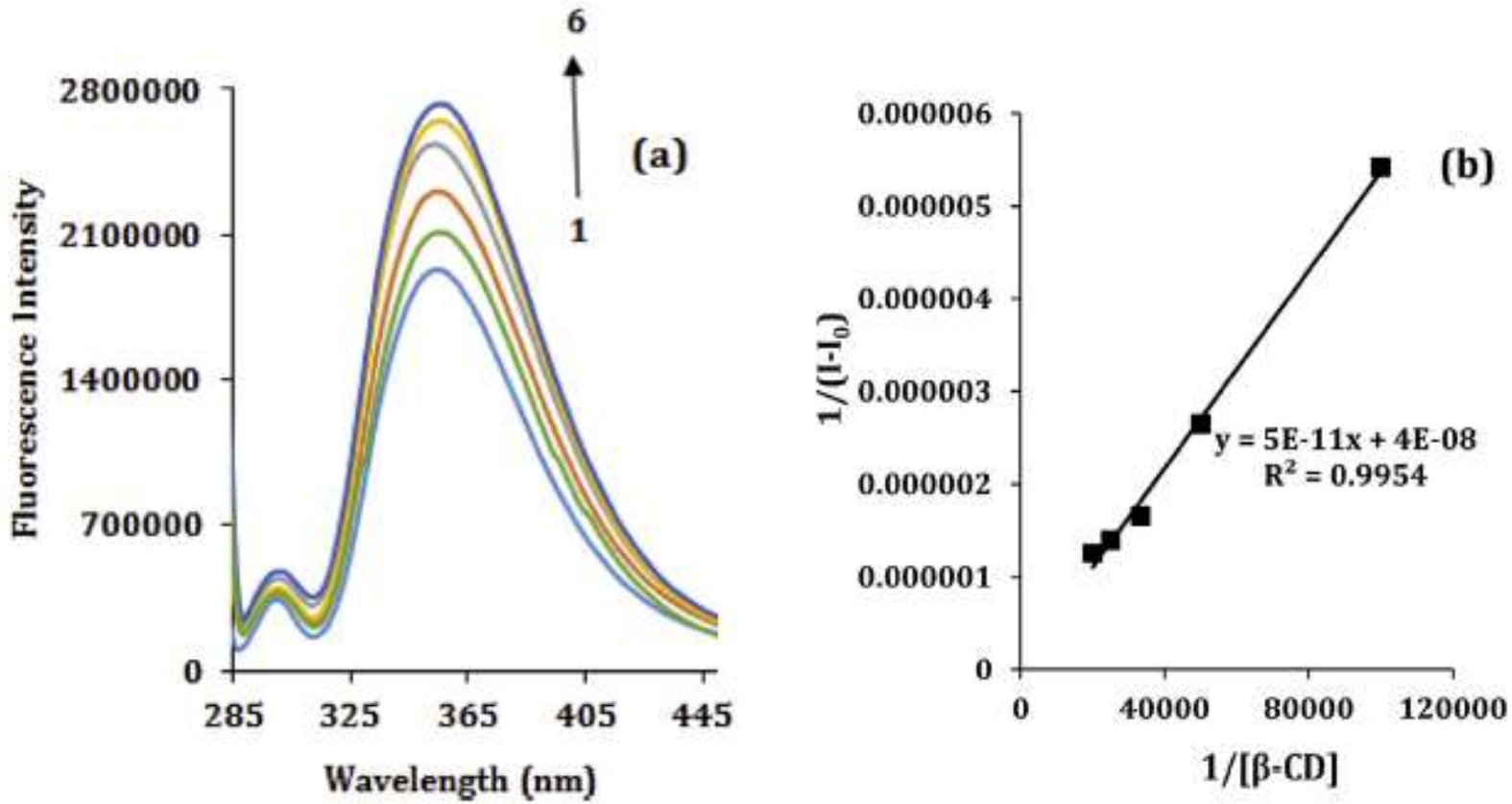


Fig. 7. (a) Fluorescence emission spectra of MP (5 μM) in different β-CD concentrations (μM): 1) without β-CD, 2) 10 μM, 3) 20 μM, 4) 30 μM, 5) 40 μM, 6) 50 μM. (b) Benesi–Hildebrand plot of $1/(I-I_0)$ vs. $1/[\beta\text{-CD}]$ for 1:1 complexation of MP with β-CD.

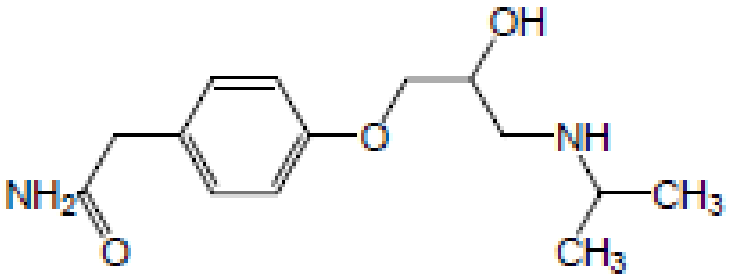
Circular dichroism spectroscopy

- Circular dichroism can represent a powerful technique to prove the CD inclusion complexation of both chiral and non chiral guest molecules and obtain information about the structure of the complex in aqueous solution. **In the case of chiral guest molecules, changes in their circular dichroism spectra may be detected, attributed to the increased optical activity induced by the formation of inclusion complexes with CDs**. The effect is only observed when the chromophore moiety of the guest molecule is actually included in the CD cavity.
- **Induction of circular dichroism in an achiral chromophore by complexation with a chiral receptor is a well known phenomenon**. Accordingly, due to the chiral environment of CDs, when an achiral guest molecule is included within their cavity, induced circular dichroism bands can be observed also in the spectrum of optically inactive guests, as a consequence of their inclusion complexation. Inclusion of an optically inactive compound within the CD cavity generates an extrinsic Cotton effect in the wavelength region of the drug chromophore.

Circular dichroism spectroscopy

- Atenolol

a beta-blocker drug used in the treatment of cardiovascular diseases. Is a very flexible molecule, consisting of fragments with different features: an amido-substituted aromatic ring, a flexible three-carbon chain and a dimethyl-substituted amino group.



While in absence of CD no signal is recorded for atenolol, upon CD addition a negative dichroic signal appears at 275 nm. The corresponding calculated transition moment is located in the plane of the aromatic ring, perpendicular to the long molecular axis (*vide infra*). The appearance of an induced circular dichroism signal of atenolol confirms its inclusion into the asymmetric CD cavity and indicates that the aromatic ring is perturbed by CD incorporation. Moreover, its negative sign indicates the perpendicular orientation of the transition moment with respect to the symmetry axis of β-CD.

The data for the system atenolol/β-CD were fitted with eq. 3, revealing the formation of complexes of 1:1 stoichiometry. One can observe the greater scattering of the experimental points, characteristic to the measurements by circular dichroism. The data were also analyzed using the Scott model (eq. 2), which yielded a K11 value in good accordance with the results of the nonlinear model, although the fit was of somewhat lower quality. The value of the association constant also correlates to that obtained from UV-vis absorption data. (110±22 M^{-1}, Scott's model).

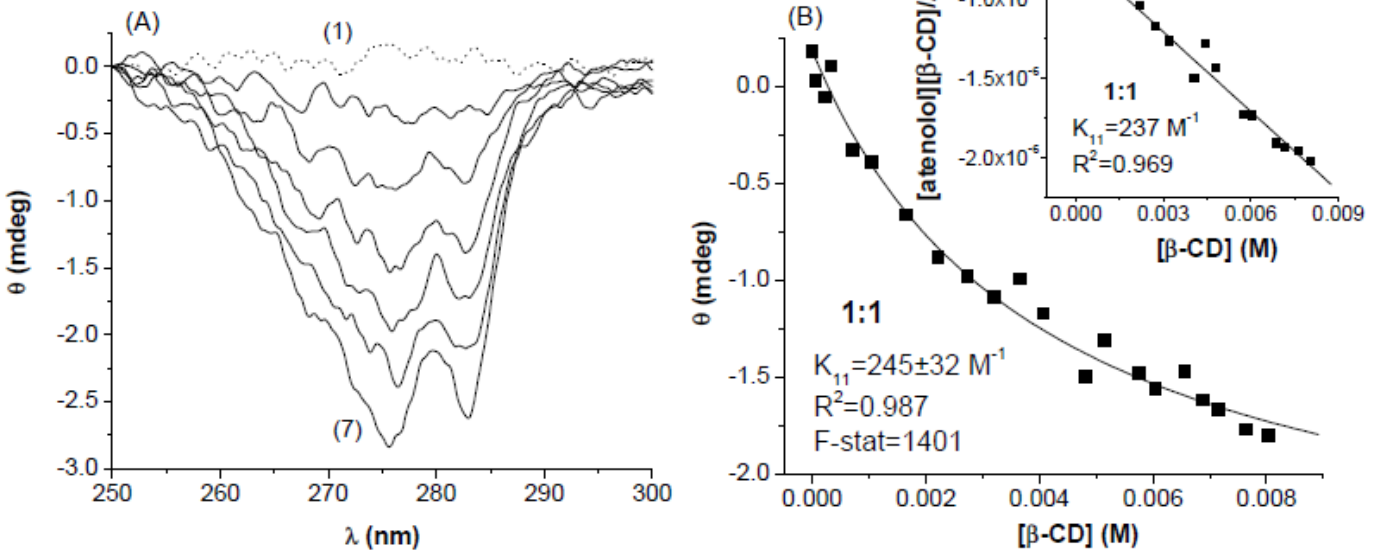
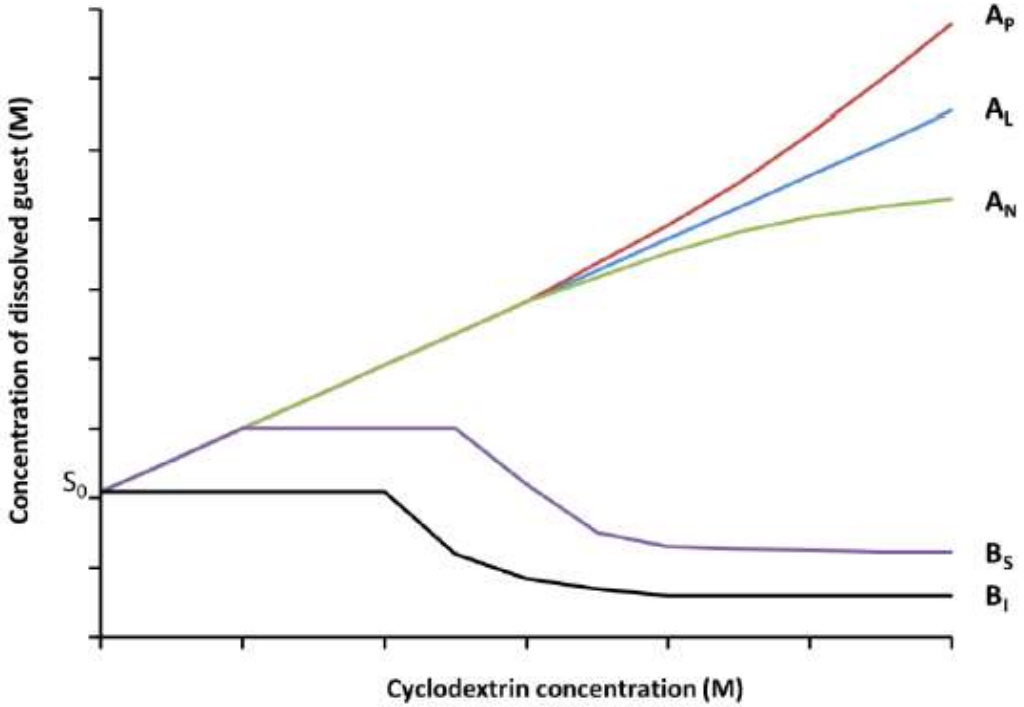


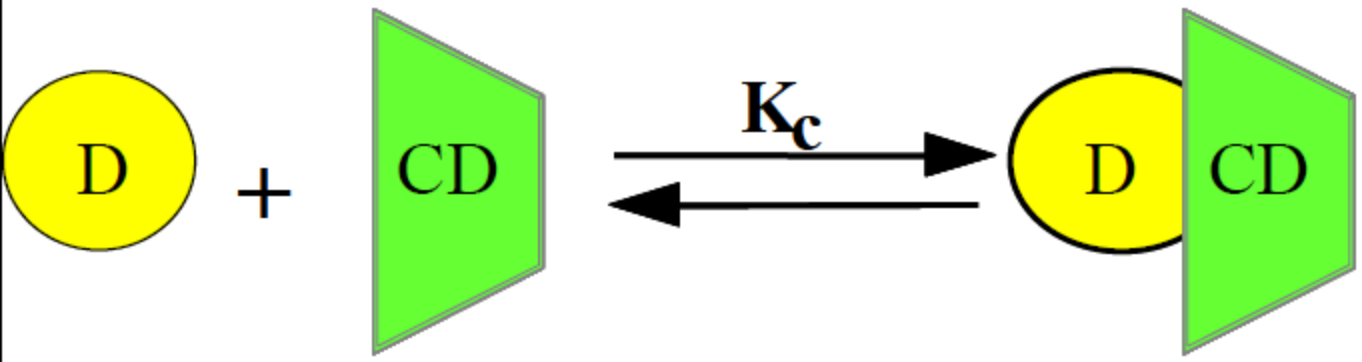
Fig. 15. (A) The circular dichroism spectra of atenolol (5×10^{-4} M in pH 7.4 phosphate buffer) in absence (1) and presence (2–7) of increasing concentrations of β-CD (up to 2×10^{-2} M). (B) Determination of the stoichiometry and association constant of the atenolol-β-CD complex.

Phase Solubility Studies

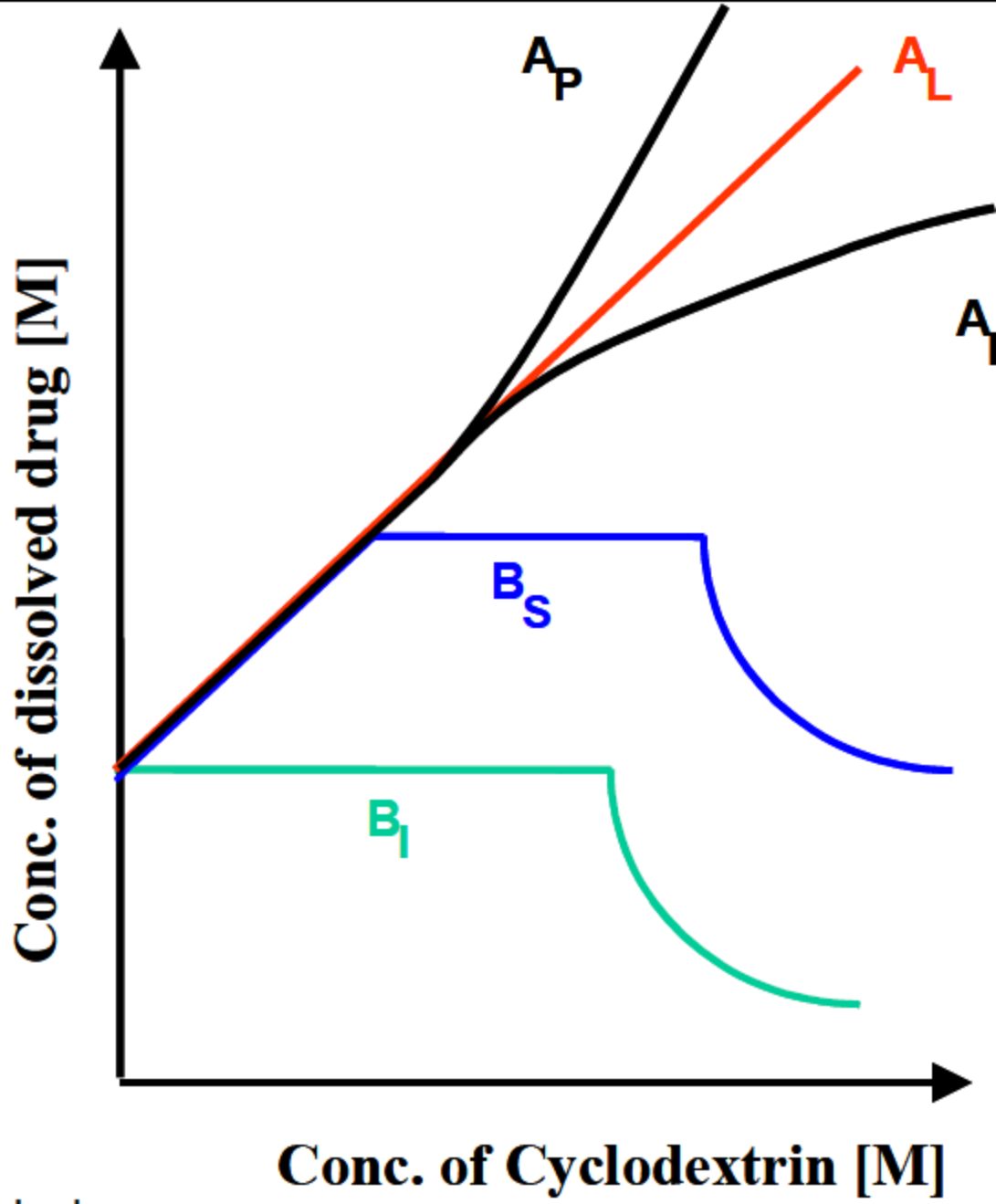
- Solubility measurements are performed according to the method developed by Higuchi and Connors, 1965.
- Excess amounts of guest are added to aqueous solutions containing various concentrations of CD and agitated until equilibrium. Thereafter, the solutions are filtered and the amount of the solubilized guest could be determined using various analytical methods (HPLC, UV-Visible, etc.). Phase solubility diagrams are obtained by plotting the solubility of the guest as a function of the CD concentration.



If one drug molecule forms a complex with one cyclodextrin molecule then:



$$K_c = \frac{[D\text{-}CD]}{[D]\,[CD]}$$



T. Higuchi and K.A. Connors: Phase-solubility techniques. *Adv. Anal. Chem. Instrum.* **4**, 117-212, 1965.

Phase Solubility Studies

Phase–solubility diagrams fall into two major types: A and B

- **A-type profiles**

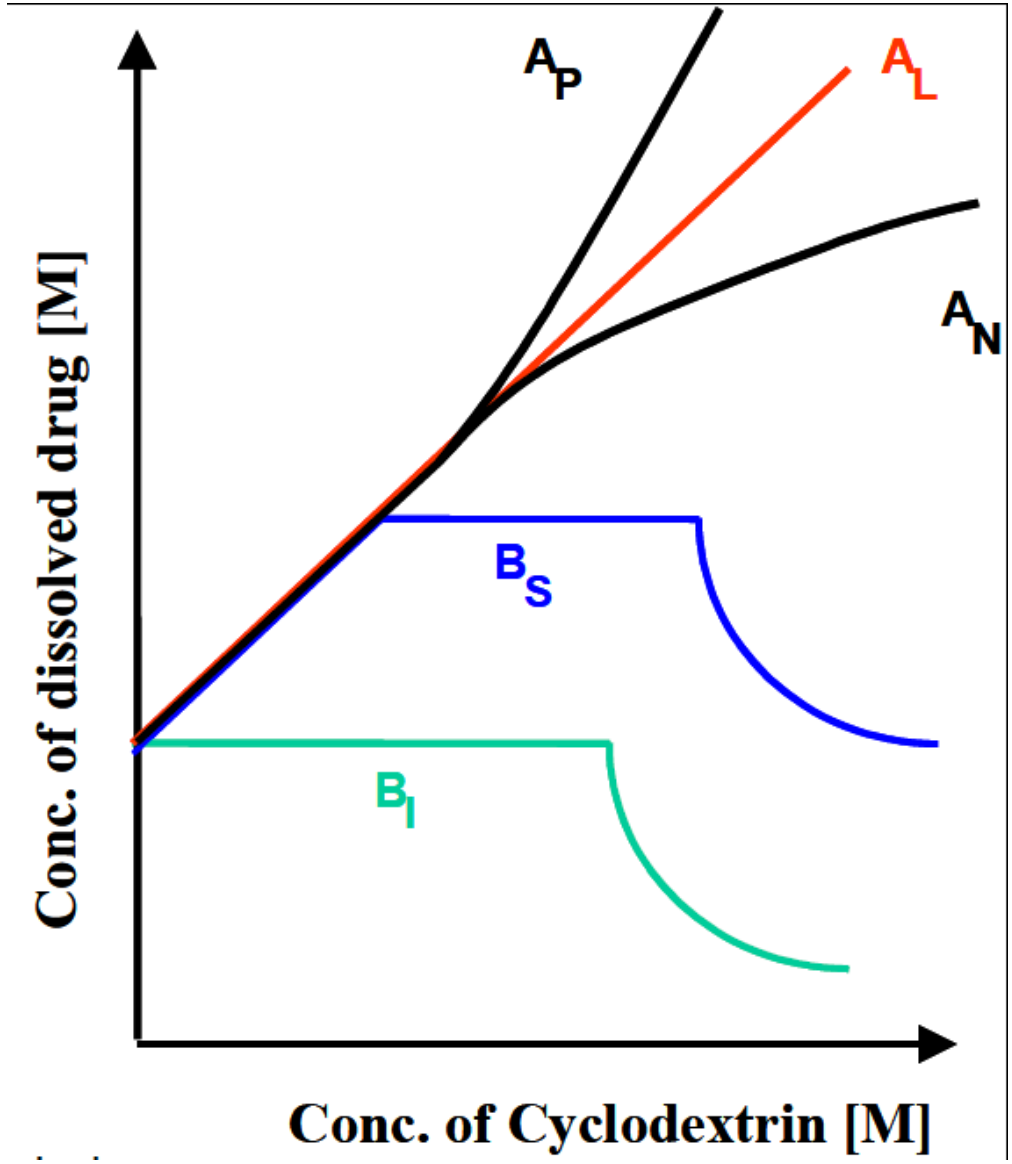
In A systems, the apparent solubility of the substrate increase as a function of CD concentration.

Three subtypes have been defined:

- A_L profiles indicate a linear increase in solubility as a function of solubilizer concentration,

- A_P systems indicate an isotherm wherein the curve deviates in a positive direction from linearity (i.e. the solubilizer is proportionally more effective at higher concentrations) and

- A_N relationships indicate a negative deviation from linearity (i.e. the CD is proportionally less effective at higher concentrations).

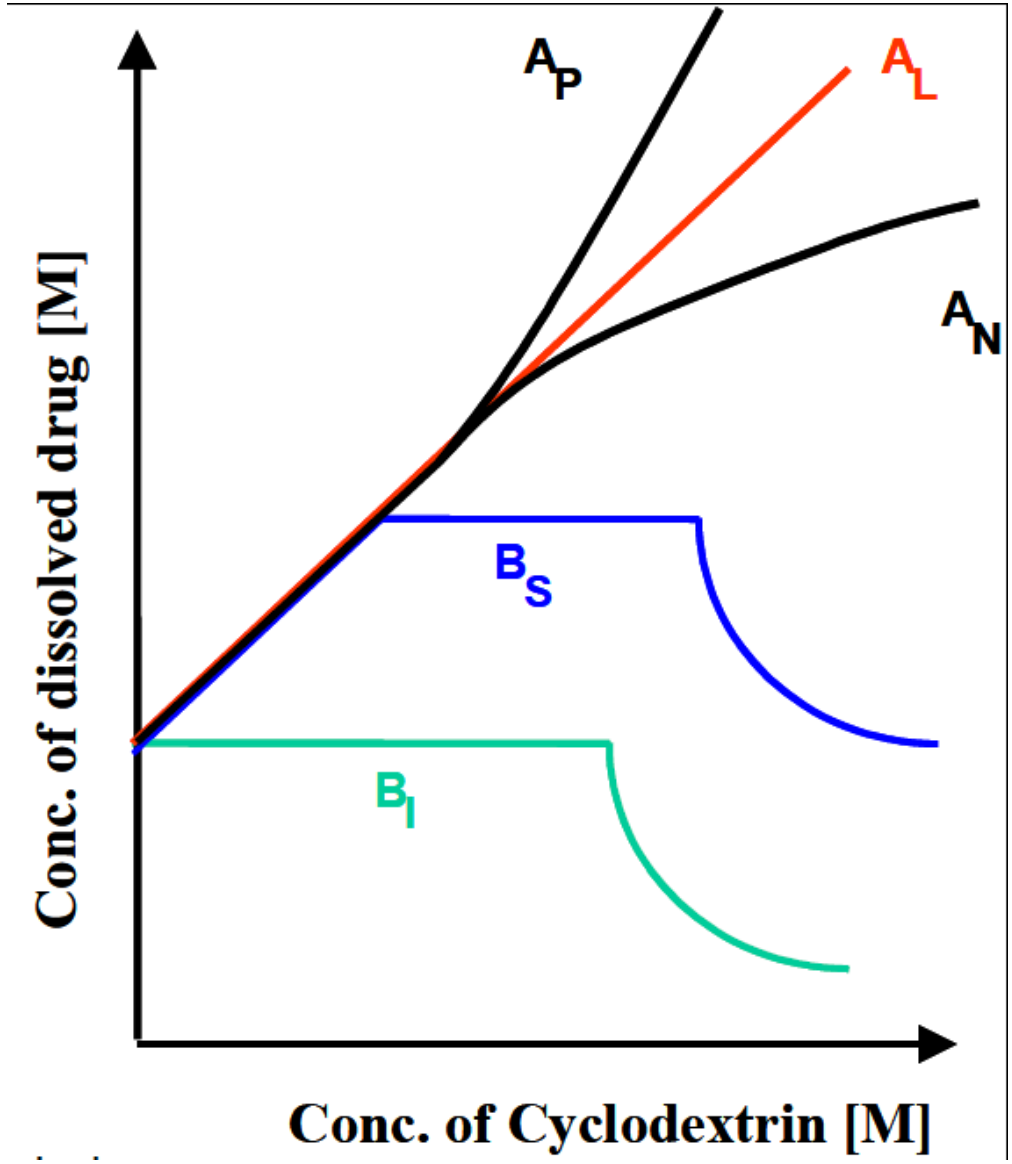


Phase Solubility Studies

- A_L-type relationships

are first order with respect to the CD and may be first of higher order with respect to the drug (i.e. *D*•CD, D_2•CD, D_3•CD, etc). If the slope of the A_L isotherm is greater than unity, higher order complexes are assumed to be involved in the solubilization.

Although a slope of less than one does not exclude the occurrence of higher order complexes, a one-to-one complex is often assumed in the absence of other information.



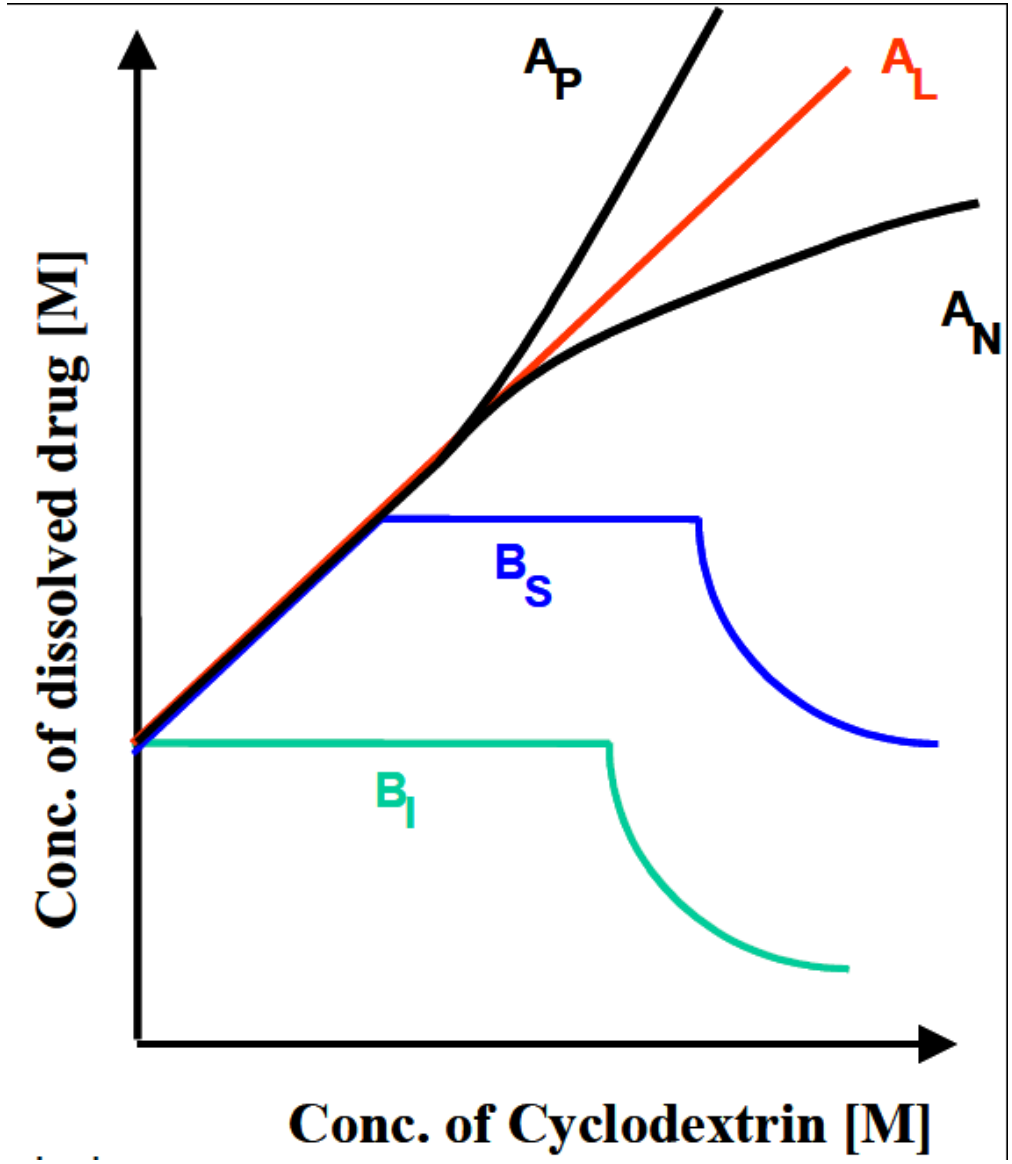
Phase Solubility Studies

- A_P systems

suggest the formation of higher order complexes with respect to the CD at higher CD concentrations (i.e. D•CD, D•CD_2, D•CD_3, etc). The stoichiometry of the formed complexes has historically been implied by the extent of curvature of the phase–solubility profile. Thus, an isotherm best fit to a quadratic function suggest the formation of a one-to-two (D•CD_2) complex, one best fit to a cubic function suggests a one-to-three complex (D•CD_3), and so forth.

- A_N profiles

have several explanations including bulk changes imparted to the solvent by the solubilizer at various concentrations and/or self-association of the solubilizer at high concentrations.



Phase Solubility Studies

$$D + CD \underset{}{\overset{K_{1:1}}{\rightleftharpoons}} D/CD \qquad (1)$$

Under such conditions an A_L-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):

$$K_{1:1} = \frac{Slope}{S_0(1 - Slope)} \qquad (2)$$

The value of $K_{1:1}$ is most often between 50 and 2000 M^{-1} with a mean value of 129, 490 and 355 M^{-1} for α-, β- and γ-cyclodextrin, respectively (Connors KA. The stability of cyclodextrin complexes in solution. *Chem. Rev.* **97**: 1325-1357 (1997)). For 1:1 drug/cyclodextrin complexes the complexation efficiency (CE) can be calculated from the slope of the phase-solubility diagram:

$$CE = \frac{[D/CD]}{[CD]} = S_0 \cdot K_{1:1} = \frac{slope}{(1 - slope))} \qquad (3)$$

Phase Solubility Studies

In the general case where the intrinsic drug solubility is given as Do and a formed complex is represented by D•CD.

$$[D] = D_o \quad (4)$$

$$D_t = D_o + m[D_m \bullet CD_n] \quad (5)$$

$$CD_t = CD + n[D_m \bullet CD_n] \quad (6)$$

then the values for $[D_m \bullet CD_n]$, $[D]$ and [CD] can be derived as:

$$[D_m \bullet CD_n] = \frac{D_t - D_o}{m} \quad (7)$$

$$[CD] = CD_t - n[D_m \bullet CD_n] \quad (8)$$

where D_o is the equilibrium solubility of the drug in the absence of the CD, D_t is the total concentration of the drug (i.e. the sum of the complexed and uncomplexed forms) and CD_t is the total concentration of the solubilizer. For equilibria that are first order with respect to the solubilizer ($n = 1$), the following equation can be obtained:

$$D_t = \frac{mKD_o^m CD_t}{1 + KD_o^m} + D_o \quad (9)$$

For A_P-defined profiles, the equilibrium constants can also be calculated. For a system in which a drug interacts with two CD species:

$$K_{1:1} = \frac{[D \bullet CD]}{[D][CD]} \quad (13)$$

$$K_{1:2} = \frac{[D \bullet CD_2]}{[D \bullet CD][CD]} \text{ or } \frac{[D \bullet CD_2]}{[D][CD]^2} \quad (14)$$

where the mass balance equations are given by:

$$D_t = [D] + [D \bullet CD] + [D \bullet CD_2] \quad (15)$$

$$[D] = D_o \quad (16)$$

$$CD_t = [CD] + [D \bullet CD] + 2[D \bullet CD_2] \quad (17)$$

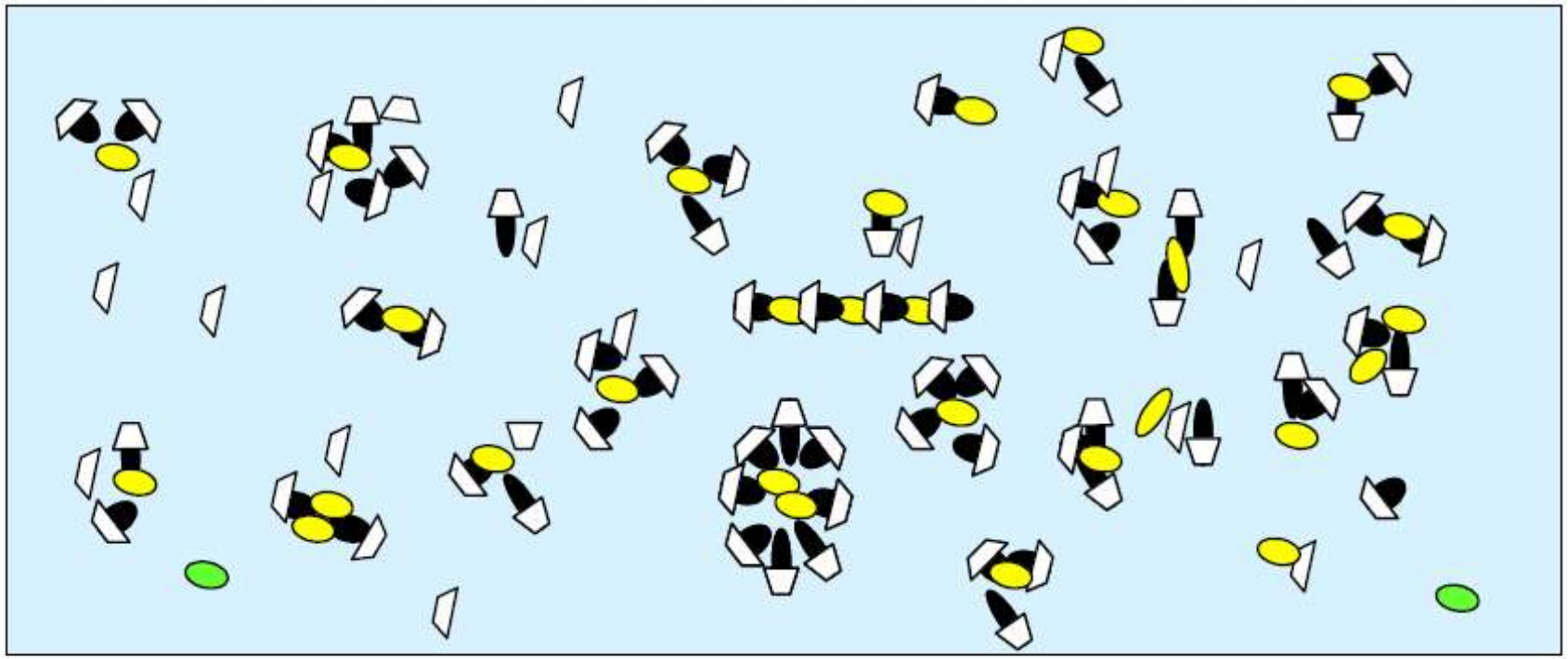
which when combined gives:

$$D_t = \frac{CD_t[K_{1:1}D_o + K_{1:1}K_{1:2}D_o[CD]]}{1 + K_{1:1}D_o + 2K_{1:1}K_{1:2}D_o[CD]} + D_o \quad (18)$$

This equation indicates that a plot of D_t versus CD_t will give a graph with an y-intercept of D_o and a slope which is increasing as function of [CD] yielding the observed curvilinear

Phase Solubility Studies

An overall binding constant estimation



Free drug molecule

Drug in a non-inclusion complex

Drug in an inclusion complex

"Empty" cyclodextrin molecule

Drug/cyclodextrin inclusion complex

Some methods that can be applied to enhance the complexation efficiency

Effect	Consequences
Dug 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 sometime 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 complexes increasing the observed stability constant of the drug/cyclodextrin complex. This observed increase in the value of the constant increases the complexation efficiency.

Effect	Consequences
Solubilization of cyclodextrin aggregates	Organic cations and anions are known to solubilize uncharged drug/cyclodextrin complexes that have limited aqueous solubility. This will enhance the complexation efficiency during preparation of, for example, solid drug/cyclodextrin complex powder.
Combination of two or more methods	Frequently the complexation efficiency can be enhanced even further my 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.

NMR*

Classic ^{1}H NMR or ^{13}C NMR experiments

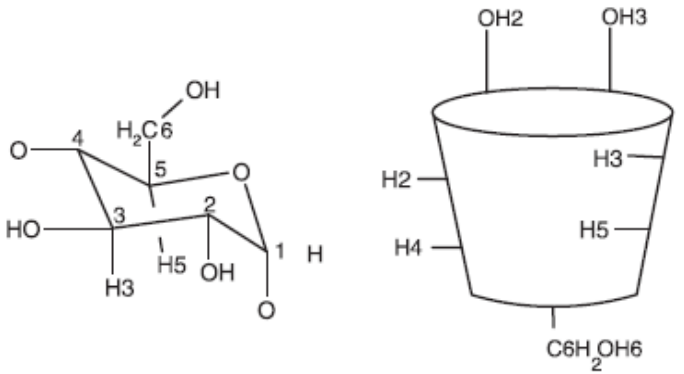
The simplest NMR experiment to fast obtain direct evidence of the inclusion of a guest into the CD cavity is the observation of the difference in the proton (1H NMR) or carbon (13C NMR) ***chemical shifts (ι) between the free guest and host species and the presumed complex.***

Analysis of chemical shift changes of both host and guest molecules can not only give evidence of the complex formation but also supply useful **information about the stoichiometry, stability, mechanism and geometry of the complex**.

Measurements of chemical shift changes of the guest as a function of increasing CD concentration (NMR titrations) allow the evaluation of the complex association constant, providing at the same time insight into the stoichiometry and conformation of the formed complex.

The main drawback of this technique is the poor solubility of the samples in deuterated water (1H NMR) or in water (13CNMR), requiring often the use of other solvents, which could modify the host–guest interactions with respect to the simple aqueous medium. Moreover, the induced shifts are sometimes too small and may suffer from signal broadening.

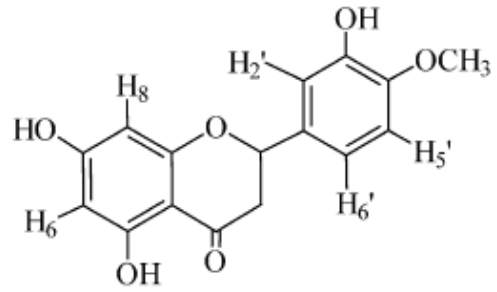
As a consequence of a guest inclusion into their cavity, the 1HNMR spectra of CDs exhibit an upfield shift of their H-3 and H-5 protons, directed toward the interior of the cavity, indicative of the complex formation.



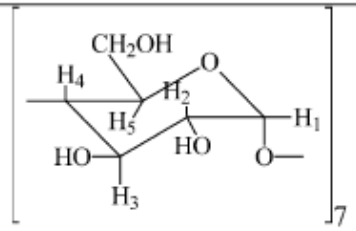
* There is also *Solid-state NMR spectroscopy*. This technique complements X-ray crystallography in that it is frequently applicable to molecules in an amorphous or liquid-crystalline state, whereas crystallography, as the name implies, is performed on molecules in a crystalline phase.

1H-NMR (1D)

Chemical shifts (ppm) for the protons of hesperetin and of *β*-CD in the free state and in the pure complex (complex 1:1)



H hesperetin	δ_{free}	δ_c	Δδ (ppm)
H_6 e H_8	6,311	6,189	0,122
$H_{2'}$, $H_{5'}$ e $H_{6'}$	6,931	6,746	0,185



H β-CD	δ_{free}	δ_γ	Δδ (ppm)
H_1	5.060	5.053	0.007
H_2	3.638	3.632	0.006
H_3	3.956	3.946	0.010
H_4	3.575	3.570	0.005
H_5	3.838	3.816	0.022
H_6	3.870	3.851	0.019

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Study of flavonoids/β-cyclodextrins inclusion complexes by NMR, FT-IR, DSC, X-ray investigation☆

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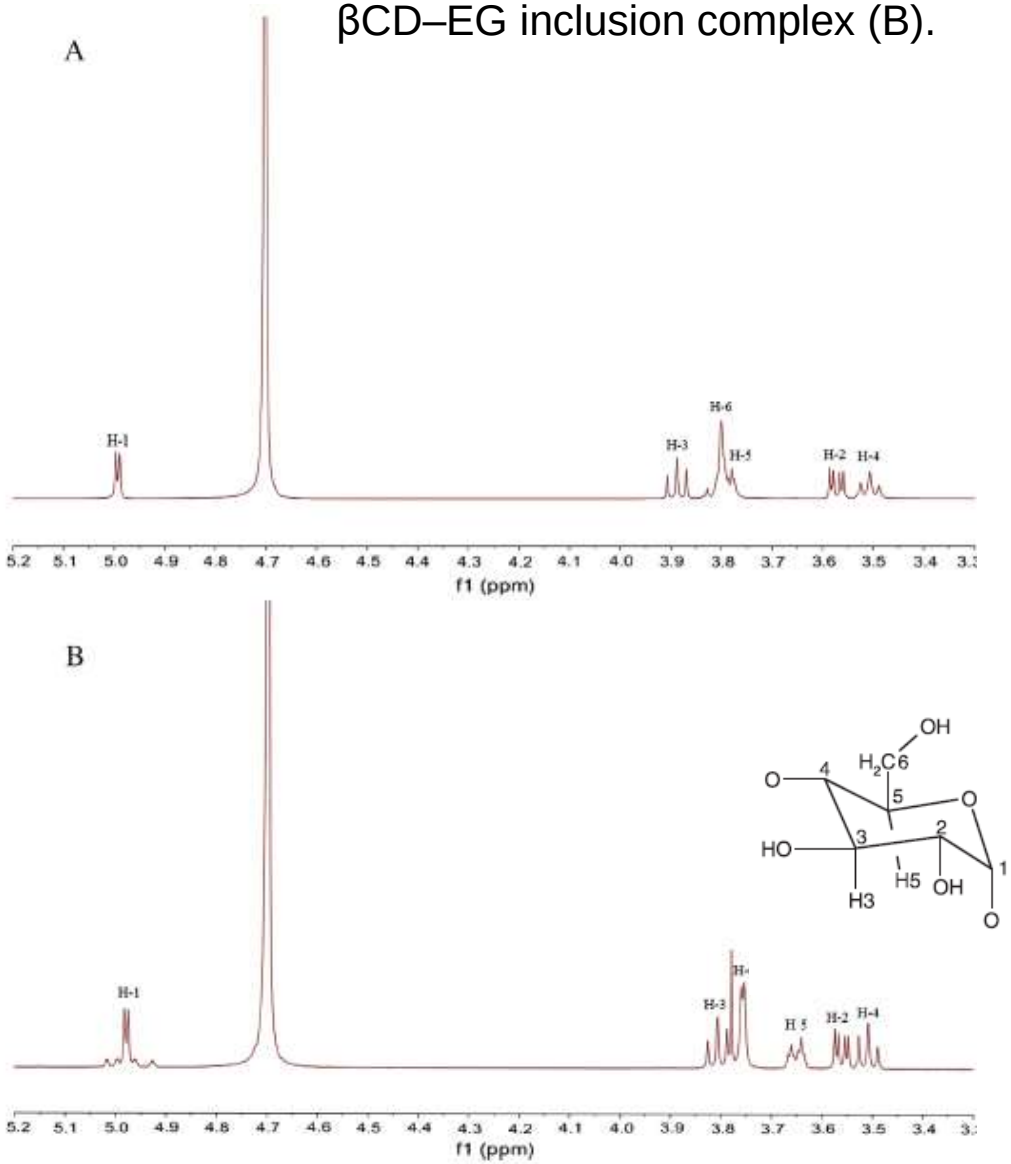
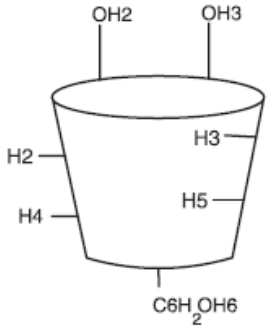
- The spectra for the individual components are compared to the spectrum of the solution of hesperitin in the presence of *β*-CD.
- Clear differences are observed.
- There is a significant shift of the signals referred to the phenilic moiety of hesperetin, that indicates the interaction of this portion of the molecules with the CD.
- Only the H3 and H5 protons, located inside the cavity, and the H6 proton, located on the narrow rim are appreciably shifted.
- 1H-NMR spectra show upfield shifts, due to the diamagnetic anisotropy of the included guest, of the H3 and H5 proton signal joined to little shifts of H6 signal, while H1, H2 and H4 signals, located outside the cavity, are relatively unaffected.
- These results indicate the interaction of part of the molecule with the cavity of the *β*-CD and consequently the formation of an inclusion complex between each flavonoid and the CD.

1H-NMR (1D)

1H nuclear magnetic resonance spectra of βCD (A) and βCD–EG inclusion complex (B).





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Food Chemistry

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An inclusion complex of eugenol into β-cyclodextrin: Preparation, and physicochemical and antifungal characterization

Liang Gong [a], Taotao Li [a], Feng Chen [b], Xuewu Duan [a], Yunfei Yuan [a], Dandan Zhang [a], Yueming Jiang [a,*]

^{1}H and ^{13}C nuclear magnetic resonance shifts of the βCD–EG inclusion complex and βCD free in D_2O. The corresponding shifts (Δδ) represent the chemical shift differences (ppm) between the two states. Negative values indicate shift to high field.

Proton	βCD	βCD–EG	Δδ	Proton	βCD	βCD–EG	Δδ
H1	4.993	4.979	−0.014	C1	102.50	102.59	0.09
H2	3.572	3.560	−0.012	C2	72.69	72.70	0.01
H3	3.888	3.807	−0.081	C3	73.73	73.87	0.14
H4	3.507	3.508	0.001	C4	81.74	81.73	−0.01
H5	3.783	3.651	−0.132	C5	72.47	72.58	0.11
H6	3.800	3.772	−0.028	C6	60.87	60.76	−0.11

The formation of the βCD–EG inclusion complex by insertion of the aromatic ring of EG into the βCD lipophilic cavity was clearly demonstrated by the chemical shifts of H-3 and H-5 resonances of the βCD.

Significantly high field shifts (Δδ) of H-3 and H-5 of βCD proton resonance in the βCD–EG inclusion complex were indicated by -0.081 and -0.132, respectively.

Another NMR-1D example

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Preparation, characterization and binding behaviors of host-guest inclusion complexes of metoclopramide hydrochloride with α- and β-cyclodextrin molecules

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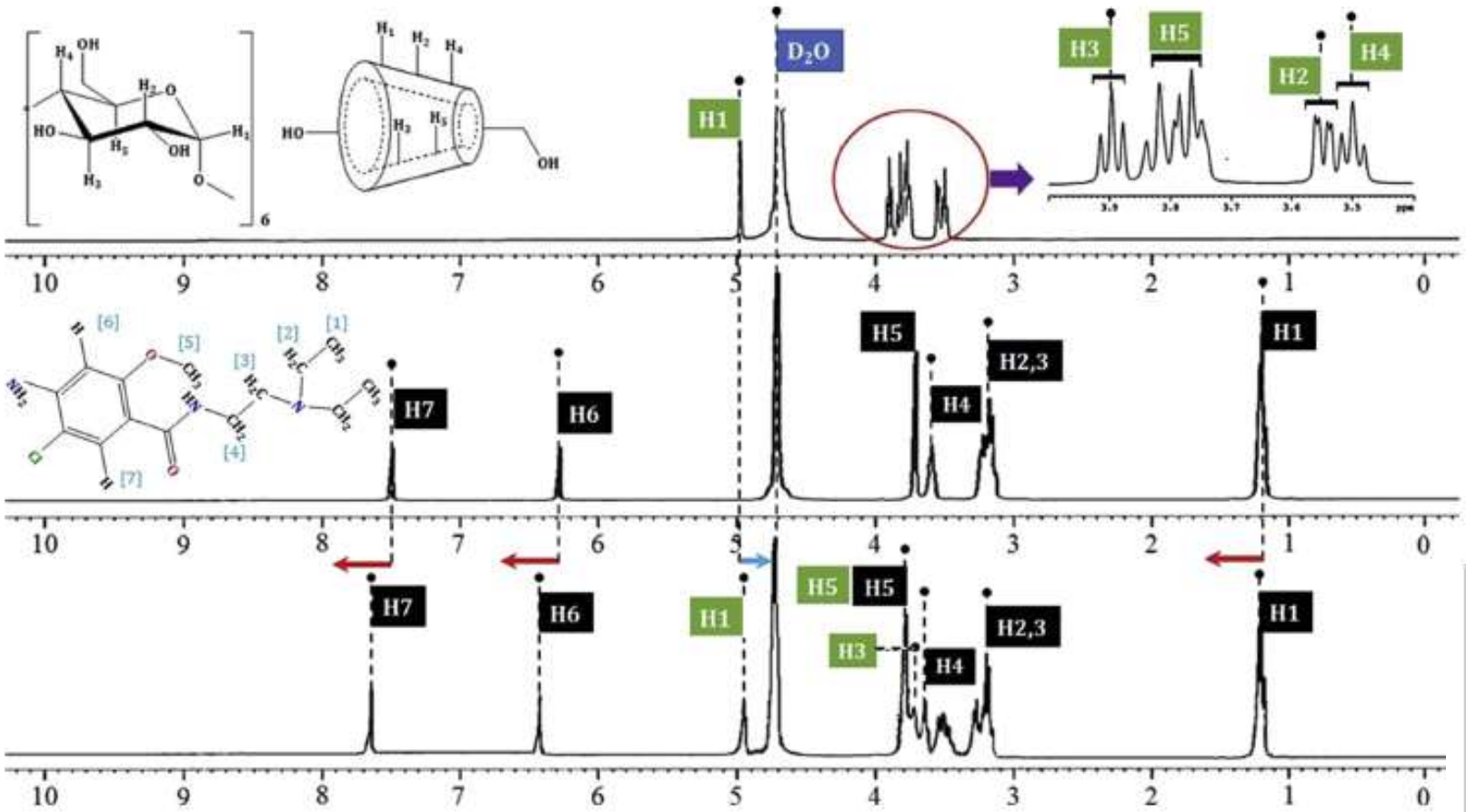
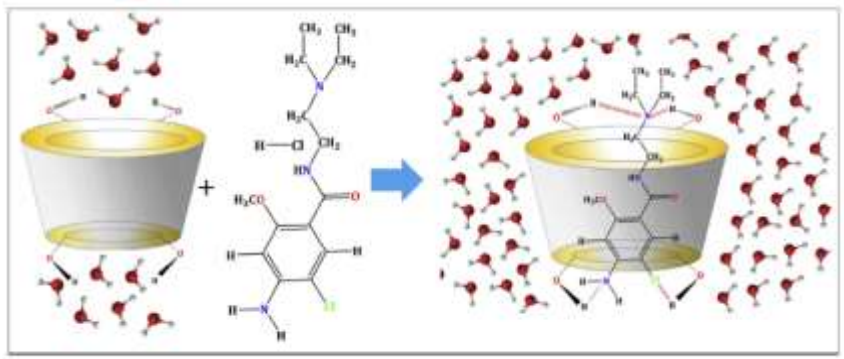


Fig. 8. ^{1}H NMR spectra of α-CD, MP and their 1:1 inclusion complex (MP:α-CD) in D_2O at 298.15 K.



Scheme 2. Plausible schematic presentation of mechanism for formation of 1:1 inclusion complex between metoclopramide hydrochloride and cyclodextrin.

NMR – 2D

NOE (Nuclear overhauser effect) is a common phenomenon observed in NMR spectroscopy, consisting in the transfer of the spin polarization from one population of nuclear spins to another, ***occurring between atoms in close proximity to each other***. The inter-atomic distances derived from the observed NOE are particularly useful to clarify the three-dimensional structure of a molecule or of a complex.

NOE-based experiments done in two-dimensions (2D), allow for evidencing in the spectrum all cross-peak correlations, making easier the data interpretation (*).

The most common NMR techniques exploiting the NOE are:

- NOESY (Nuclear Overhauser Effect SpectroscopY) and
- ROESY(Rotational Overhauser Effect SpectroscopY).

Both NOESY and ROESY experiments have been widely applied for the structural elucidation of guest:CD inclusion complexes; they are done through the NOE enhancement measurements between the guest nuclei and the CD inner cavity nuclei H-3 and H-5. Besides, NOE cross-peaks can be correlated to their respective inter-molecular distances, providing more detailed information about the supramolecular organization of these systems.

(*) In the monodimensional (1D) version, the experiments must be done separately, by applying selective pulses foreach nucleus, which turn this technique more time requiring. However 1D experiments have higher sensibility, which can be necessary in case of weak NOE interactions or poor solubility of the complex.

2D-NOESY NMR

Food Chemistry 196 (2016) 324–330

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

An inclusion complex of eugenol into β-cyclodextrin: Preparation, and physicochemical and antifungal characterization

Liang Gong [a], Taotao Li [a], Feng Chen [b], Xuewu Duan [a], Yunfei Yuan [a], Dandan Zhang [a], Yueming Jiang [a,*]

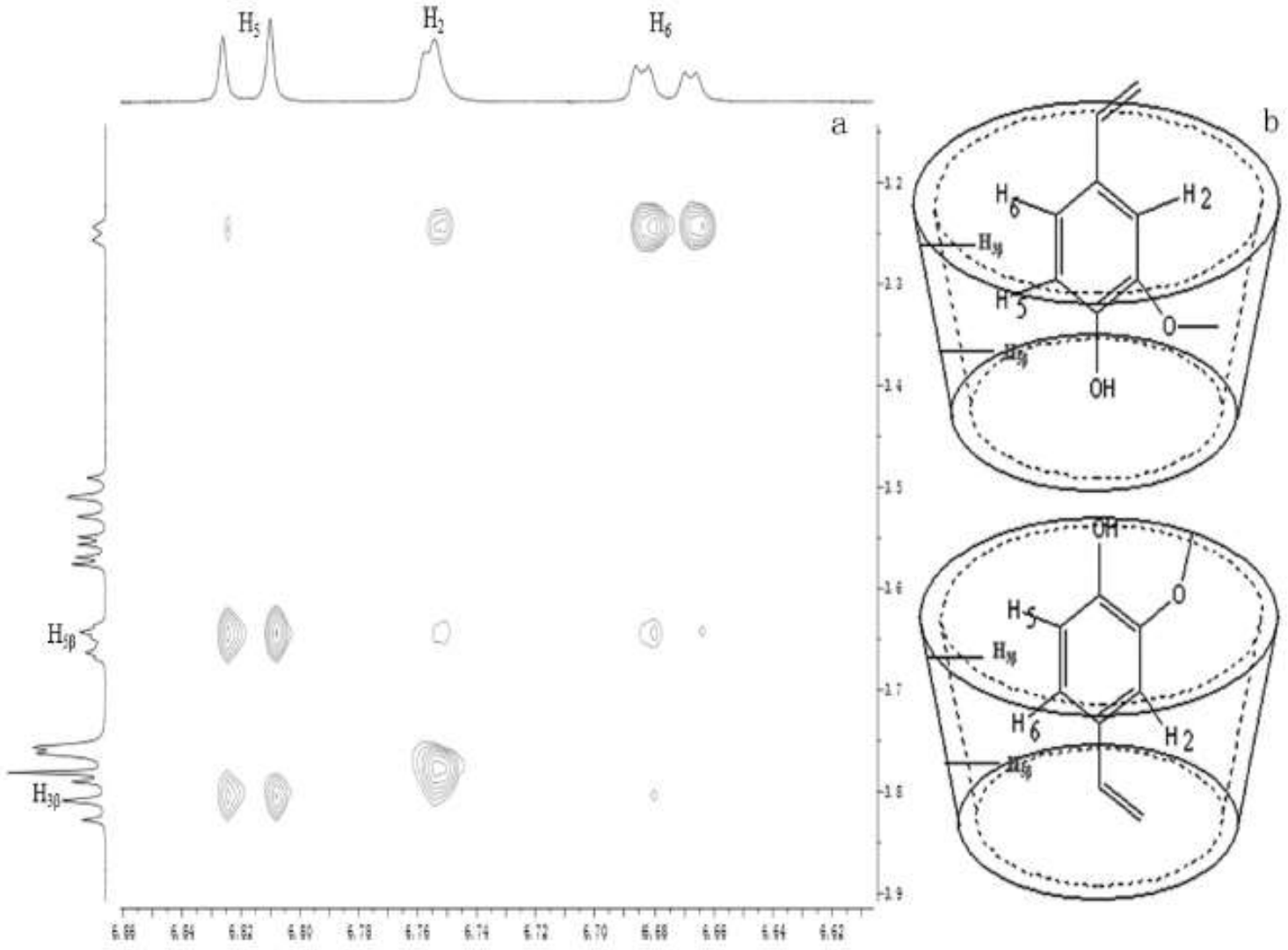
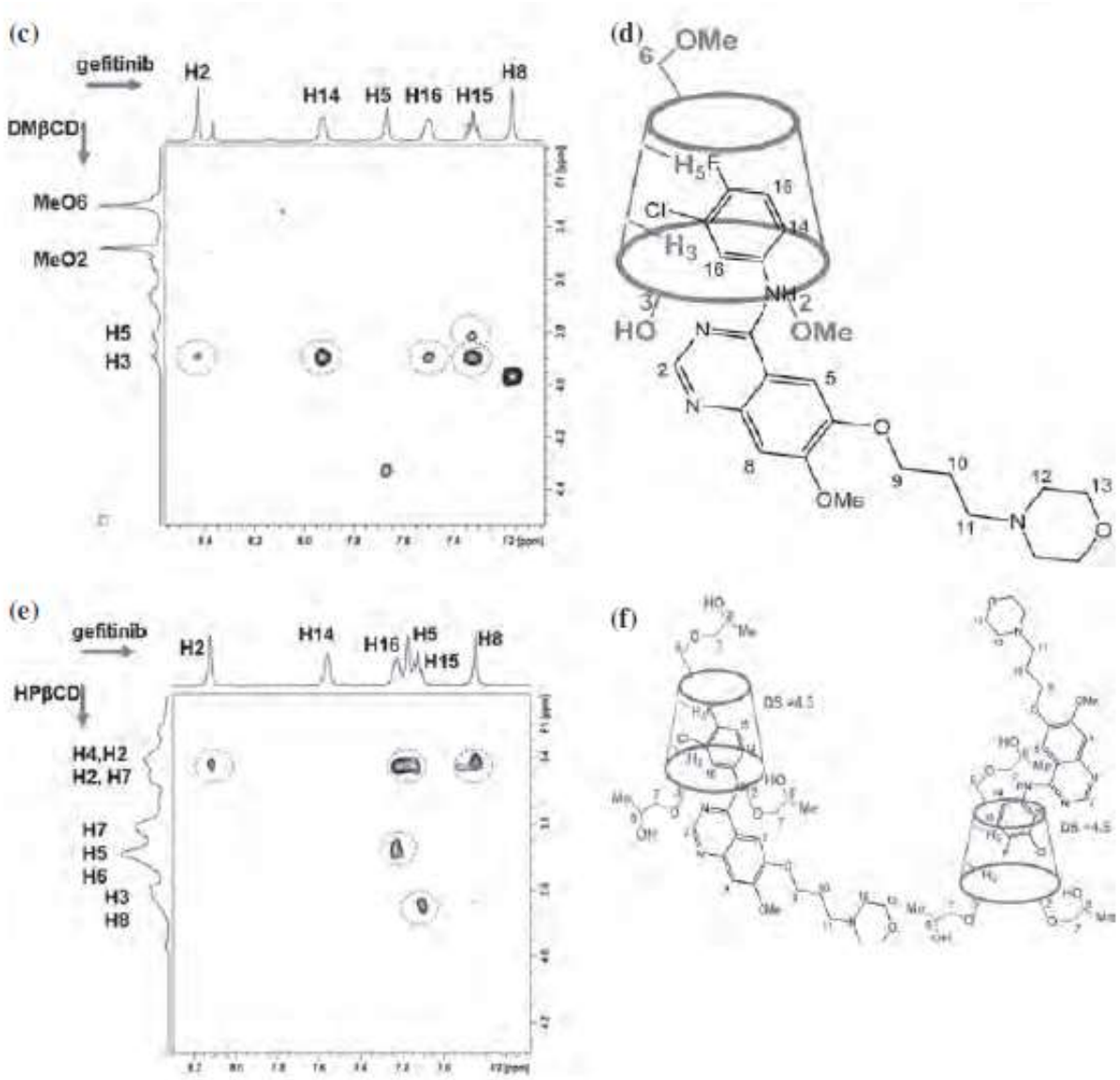


Fig. 2. NOESY spectrum of the βCD–EG inclusion complex (A) and the possible inclusion mode (B).

2D-NOESY NMR spectra of the *β*CD–EG inclusion complex showed that cross-peaks obtained between H-2 and H-5 of EG and H-5 and H-3 of *β* CD, are in accordance with the $_1$H NMR spectra. Thus, it can be deduced that the formation of the *β* CD–EG inclusion complex involved interaction of the hydrophobic aromatic ring side of EG with the lipophilic cavity of *β*CD.

2D-ROESY NMR



Journal of
Biomedical Nanotechnology
Vol. 13, 1–12, 2017
www.aspbs.com/jbn

Enhanced Gefitinib Cytotoxicity in the Presence of Cyclodextrins: *In-Vitro* and Biophysical Studies Towards Potential Therapeutic Interventions for Cancer

Kyriaki Hatziagapiou[1,†], Konstantinos Bethanis[2,*,†], George I. Lambrou[1,*,†], Konstantina Yannakopoulou[3], Michael Karpusas[2], Maria Braoudaki[1], Elias Christoforides[2], Frantzeska Tsorteki[2], Vasilis Milionis[4], Nikolaos Kavantzas[4], Fotini Tzortzatou-Stathopoulou[1], and Vasiliki Gemou-Engesaeth[1]

X-Ray Crystallography vs NMR

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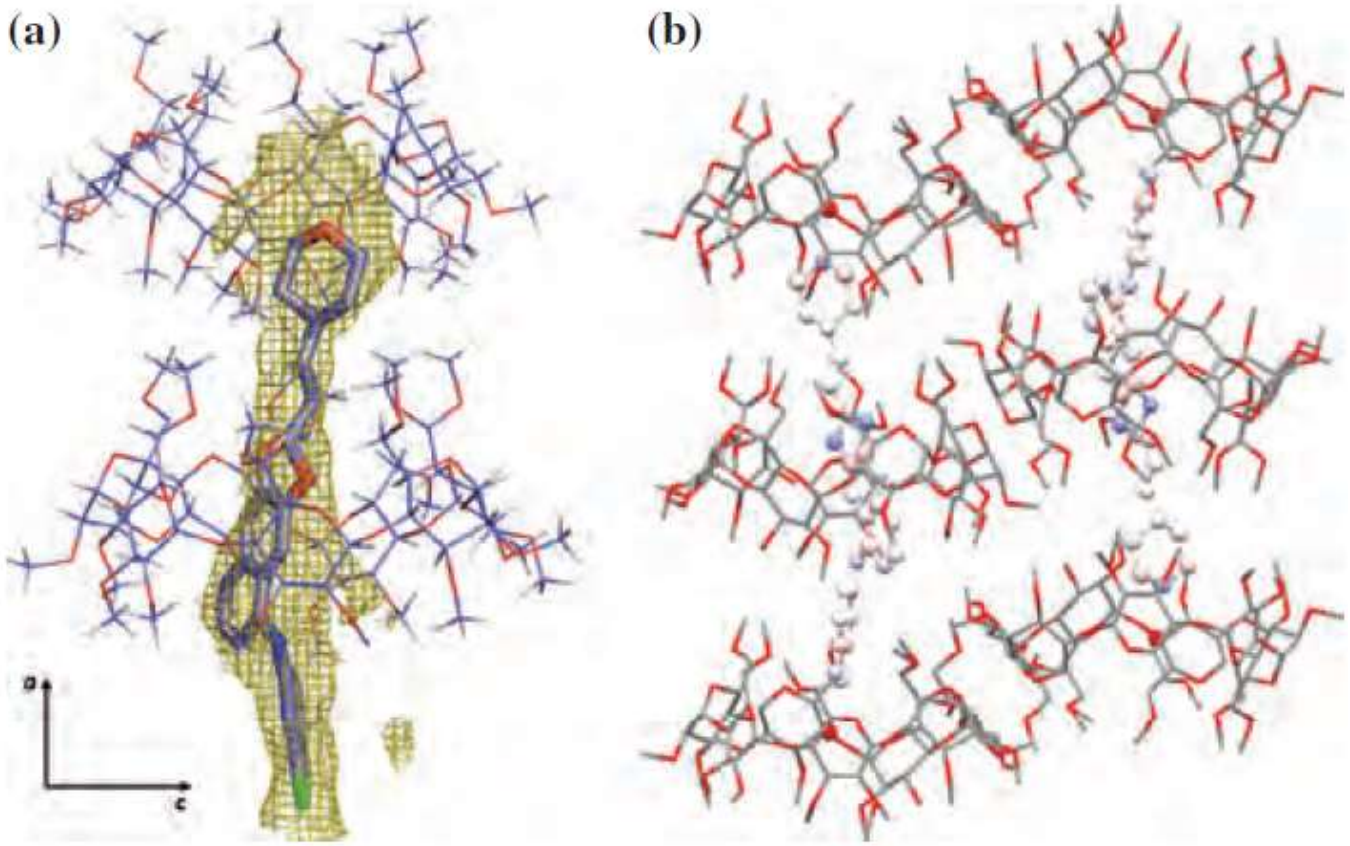

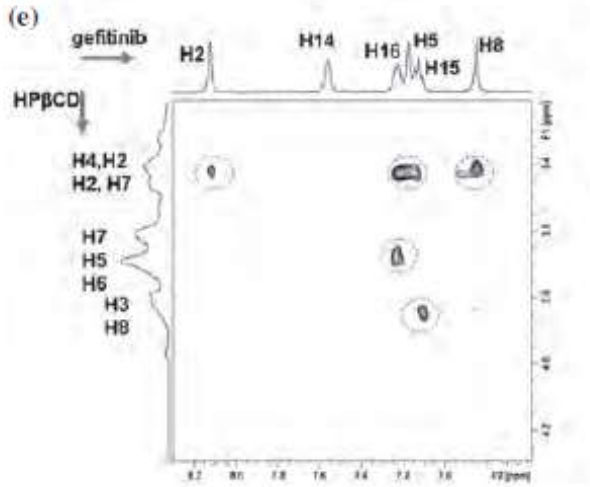

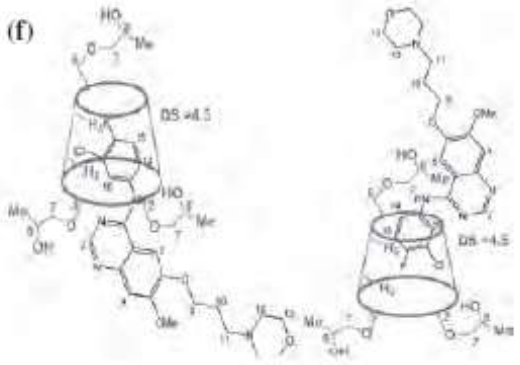

Combining NMR and Molecular Dynamics Studies

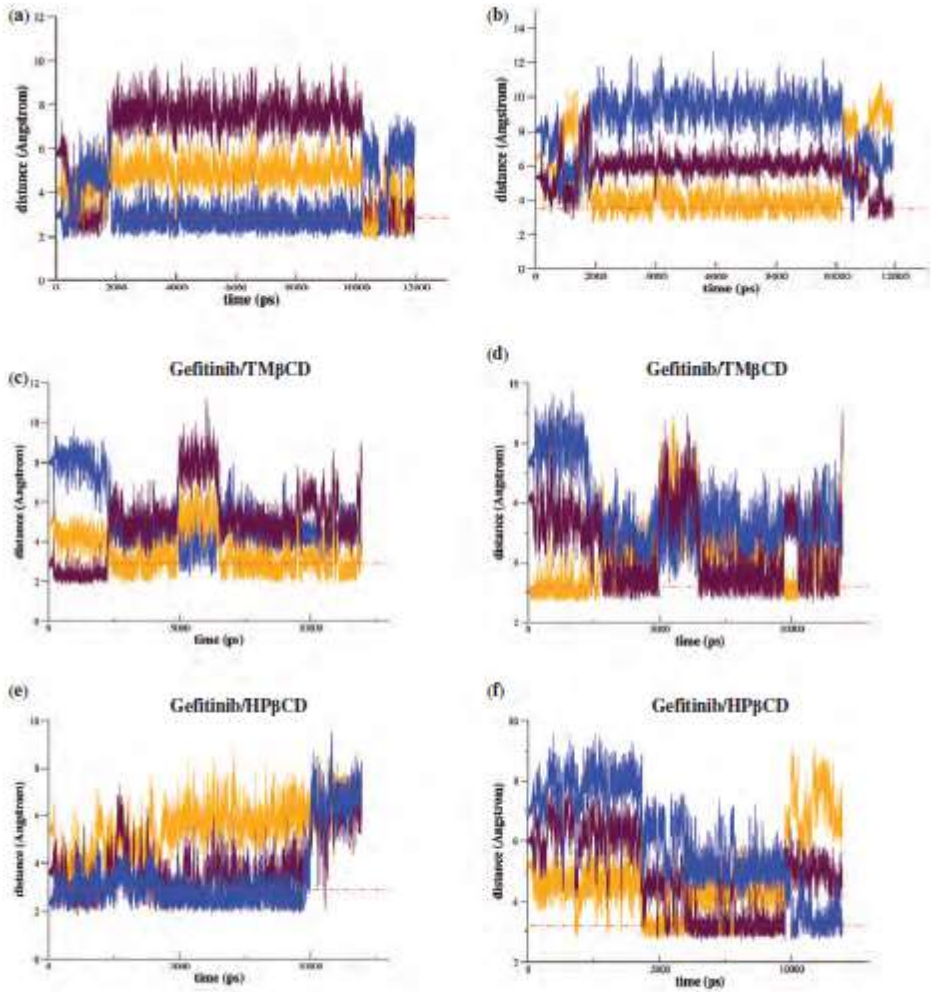

Figure 6. Molecular dynamics simulations: (a) Plot of distances between atom H14 (gefitinib) and H3 atoms located in three CD glucopyranose units of DMβCD. The presence of a distance of around 2.9 Å most of the time (near the horizontal dotted line), implies maintenance of the interaction through time. Similar graphs for TMβCD (c) and HPβCD (e). (b) Plot of distance between NH (gefitinib) and three different ether oxygen atoms of the host DMβCD. The presence of a distance of around 3.2 Å most of the time (near the horizontal dotted line), suggests presence of a hydrogen bond. Similar graphs for TMβCD (d) and HPβCD (f).

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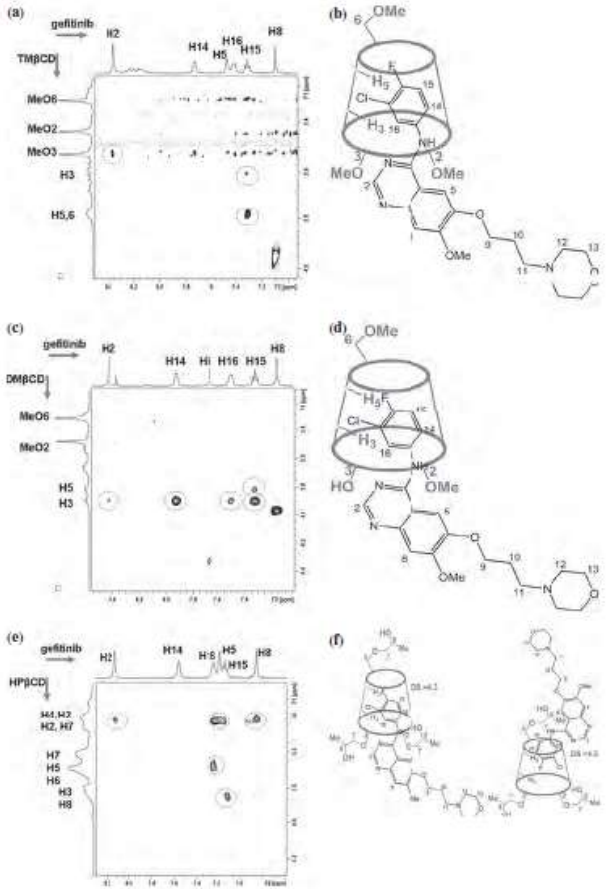
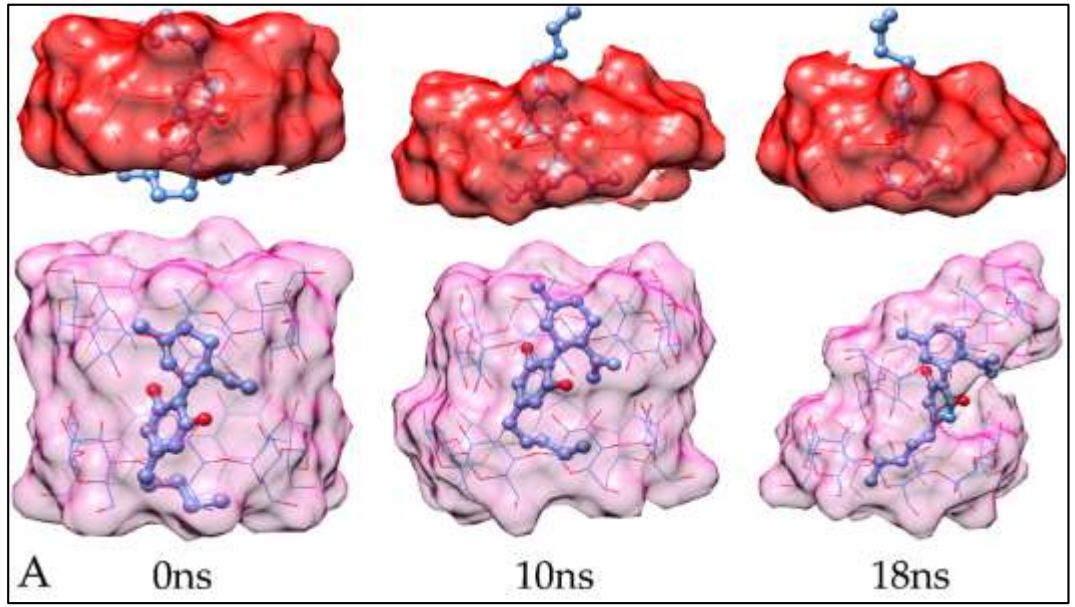
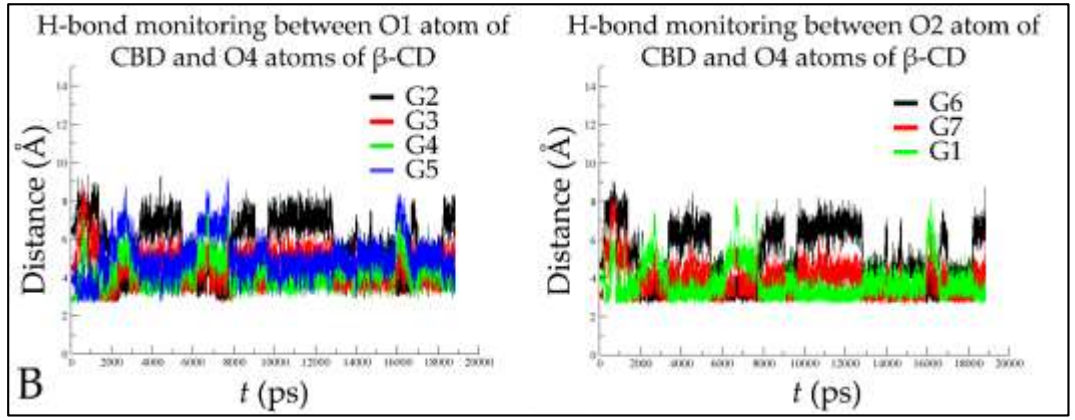


Molecular Dynamics Studies

https://www.youtube.com/watch?v=bOR5G8cqAZA





	CBD/ β-CD (1:1)	CBD/ β-CD (1:2)	CBD/ DM-β-CD (1:1)	CBD/ TM-β-CD (1:2)	CBD/ HP-β-CD (1:1)
ΔE_{vdW}	-34.59±2.13	-51.86±2.94	-31.84±5.49	-47.89±7.91	-30.30±2.30
ΔE_{ele}	-4.86±3.26	-7.39±4.96	-7.20±3.20	-5.25±2.93	-2.36±3.02
ΔE_{GB}	19.06±2.96	33.14±4.55	19.21±5.00	29.24±5.97	26.04±3.85
ΔE_{surf}	-3.67±0.19	-5.57±0.25	-3.95±0.46	-5.95±0.66	-3.83±0.27
ΔG_{gas}	-39.45±3.7	-59.25±5.6	-39.04±7.54	-53.14±9.62	-32.66±3.63
ΔG_{solv}	15.38±2.89	27.58±4.48	15.27±4.64	23.29±5.42	22.21±3.77
$\Delta G_{(GB)}$[a]	-24.07±2.51	-31.68±3.15	-23.78 ±3.83	-29.85±5.21	-10.45±2.36
$T \cdot \Delta S$	-19.42±1.43	-21.88±3.41	-19.26±1.73	-20.15±4.53	-17.40±1.49
ΔG_{all}[b]	-4.65±2.89	-9.79±4.64	-4.52±4.21	-9.70±6.90	+6.95±2.80

Binding free energies and their standard deviations (kcal/mole) resulting from MM/GBSA analysis of the inclusion compounds of CBD/*β*-CD (host guest ratio 1:1), CBD/*β*-CD dimer (2:1), CBD/DM-*β*-CD (1:1), CBD/TM-*β*-CD dimer (2:1) and CBD/HP-*β*-CD (1:1) (Legend: ΔE_{vdW}=van der Waals contribution from molecular mechanics, ΔE_{ele}=electrostatic energy as calculated by the molecular mechanics force field, ΔE_{GB}=the electrostatic contribution to the solvation free energy, calculated by G_B model, ΔE_{surf}=nonpolar contribution to the solvation free energy, calculated by an empirical model [a]$\Delta G_{(GB)}=\Delta G_{solv}+ \Delta G_{gas}$, [b]$\Delta G_{(all)} = \Delta G_{(GB)}+ (T \cdot \Delta S)$).

FT-IR spectra of solid inclusion complexes

- FT-IR spectrum is used to confirm the formation of the solid inclusion complex by considering the deviation of peak shape position and intensity

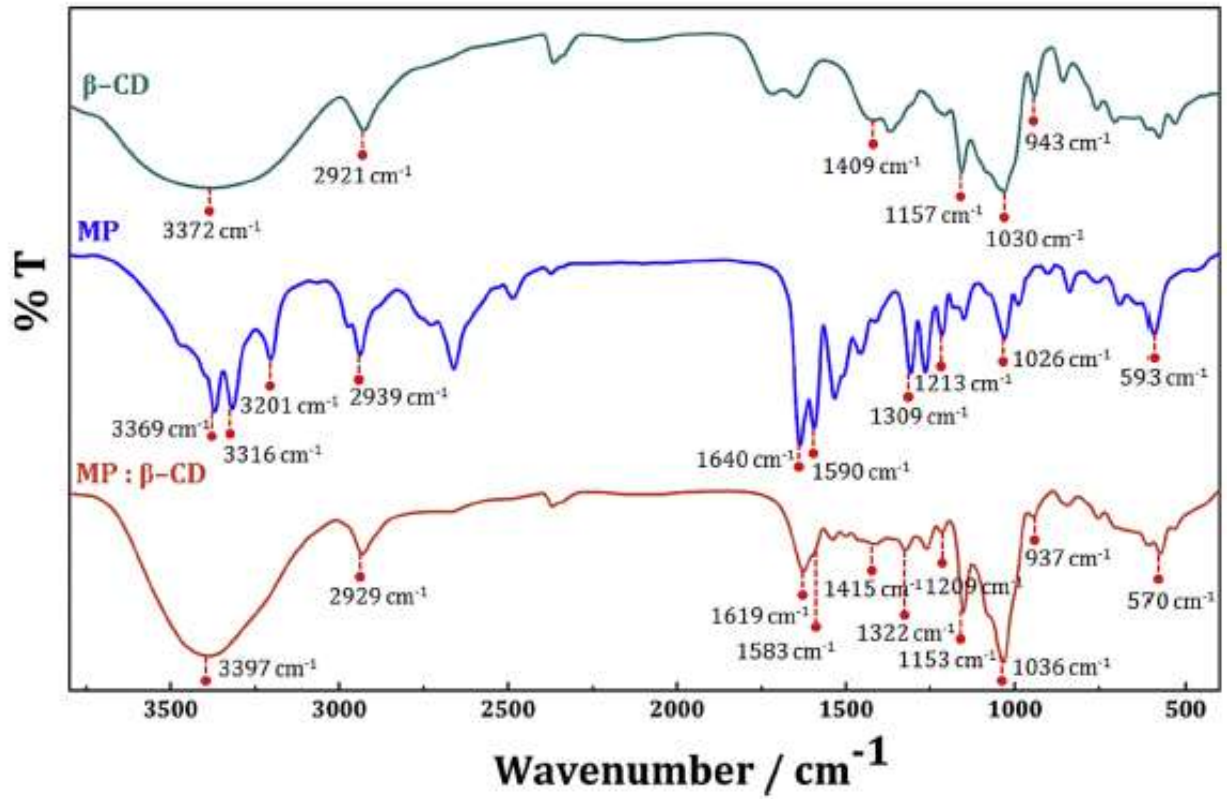


Fig. 11. FTIR spectra of free β-CD, MP and their 1:1 inclusion complex (MP:β-CD).

Journal of Molecular Structure 1155 (2018) 503–512

Contents lists available at ScienceDirect

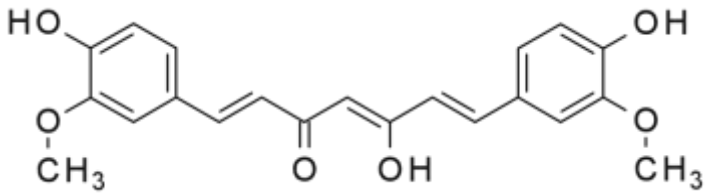
Journal of Molecular Structure

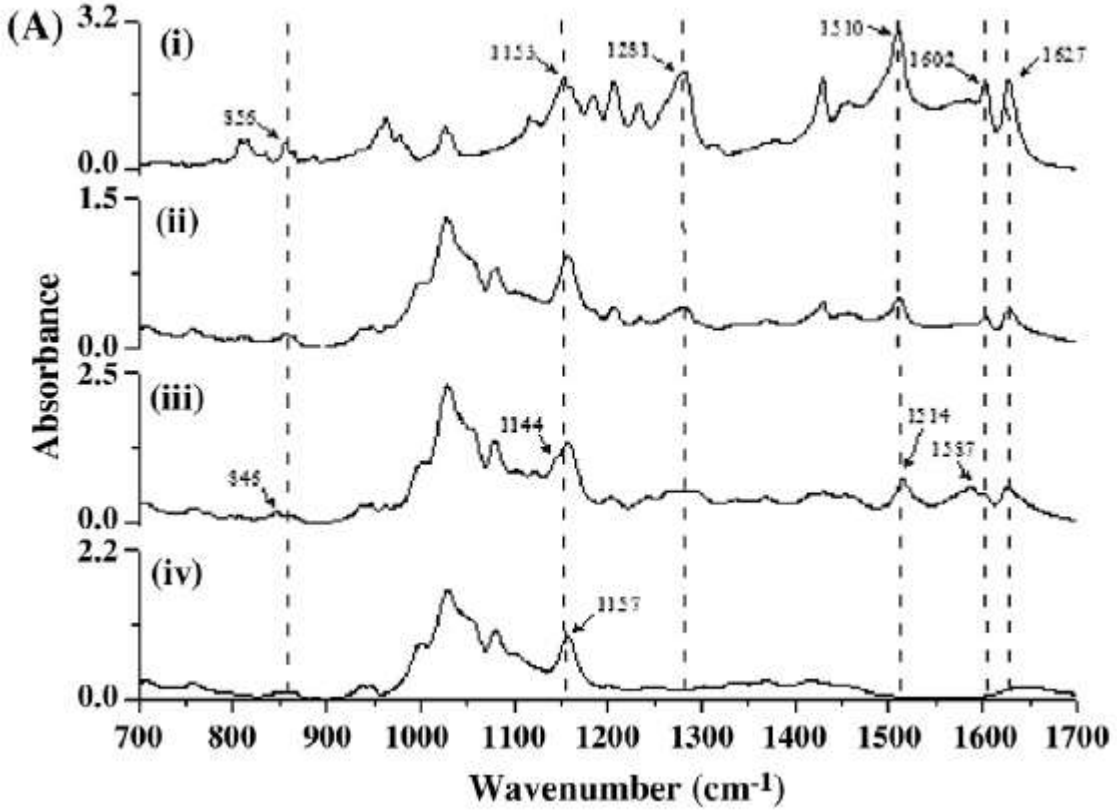
journal homepage: http://www.elsevier.com/locate/molstruc

Preparation, characterization and binding behaviors of host-guest inclusion complexes of metoclopramide hydrochloride with α- and β-cyclodextrin molecules

Siti Barman, Biraj Kumar Barman, Mahendra Nath Roy*

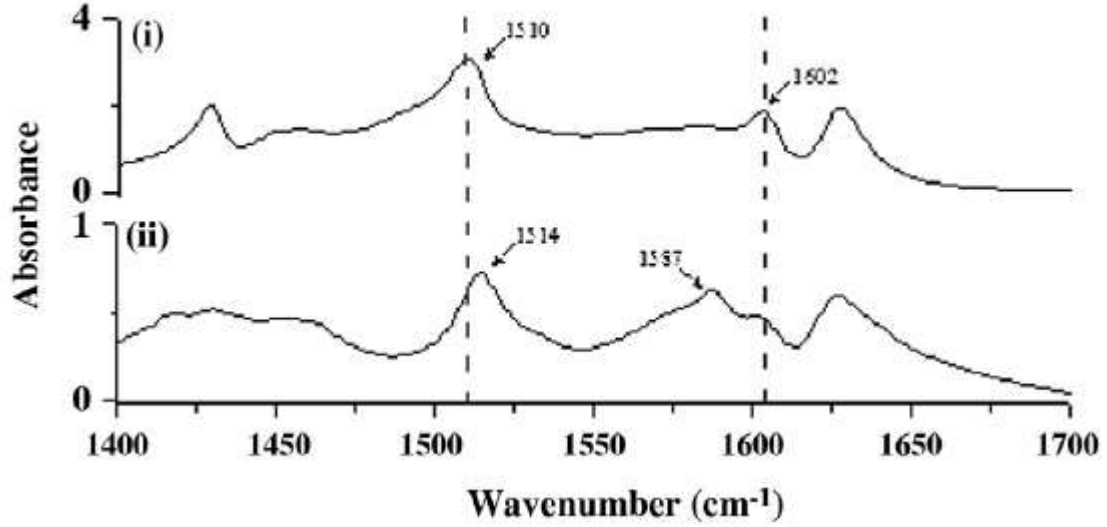
FT-IR





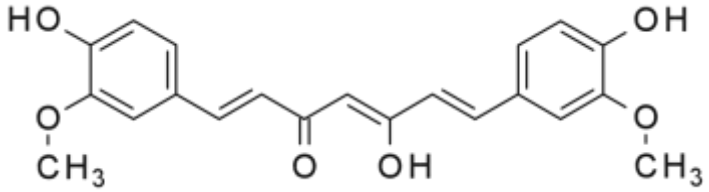
FT-IR spectra of
(i) curcumin,
(ii) simple mixture of curcumin with b-cyclodextrin in a 1:2 molar ratio
(iii) curcumin–b-cyclodextrin complex from co-precipitation and
(iv) b-cyclodextrin


Food Chemistry
journal homepage: www.elsevier.com/locate/foodchem
Curcumin–β-cyclodextrin inclusion complex: Stability, solubility, characterisation by FT-IR, FT-Raman, X-ray diffraction and photoacoustic spectroscopy, and food application



zoom in the 1400–1700 cm^{-1} region of
(i) curcumin and
(iii) curcumin–β-cyclodextrin complex from co-precipitation spectra

FT-IR

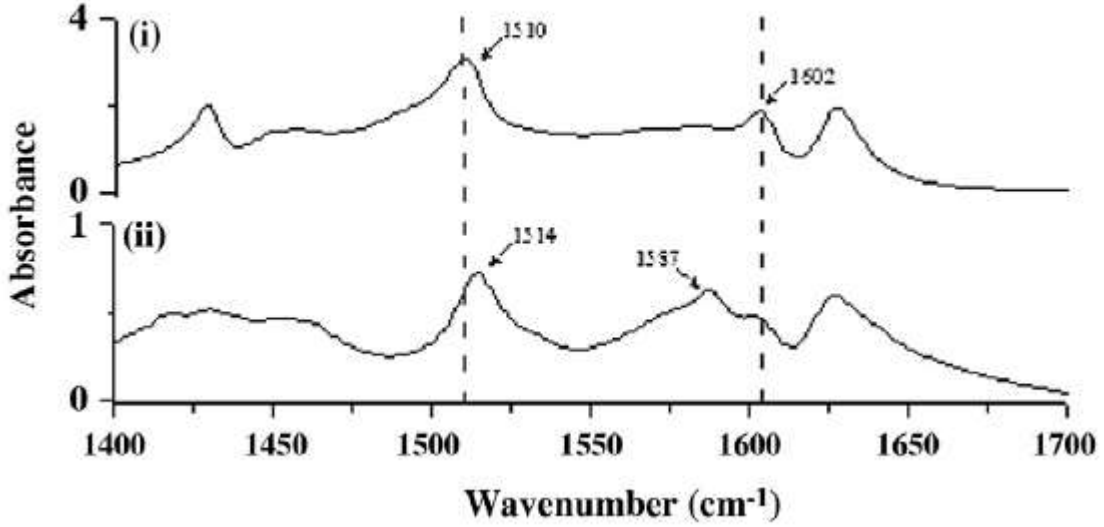



Food Chemistry
journal homepage: www.elsevier.com/locate/foodchem
Curcumin–β-cyclodextrin inclusion complex: Stability, solubility, characterisation by FT-IR, FT-Raman, X-ray diffraction and photoacoustic spectroscopy, and food application
Camila Sampaio Mangolim, Cristiane Moriwaki, Ana Claudia Nogueira, Francielle Sato, Mauro Luciano Baesso, Antônio Medina Neto, Graciette Matioli

The spectra region with the most significant variations shows that the peak at 1510 cm^{-1}, which is due to the C=O stretching and CCC and CC=O bending, **undergoes a shift to 1514 cm^{-1} for the curcumin–β-CD complex from co-precipitation**, which is good evidence of complex formation. The same phenomenon occurs for the peak at 1602 cm^{-1}, which corresponds to the C=C stretching of the aromatic rings, and could be observed in the simple mixture spectrum.

In the spectrum of the curcumin–β-CD complex from co-precipitation, the peak at 1602 cm^{-1} showed a shoulder at 1587 cm^{-1}.

Therefore, the **FT-IR technique enabled good evidence to be obtained for complex formation between β-CD and curcumin using the co-precipitation method**. The interactions appeared to occur due to the entry of one or both of the aromatic rings of curcumin into the CD cavity.

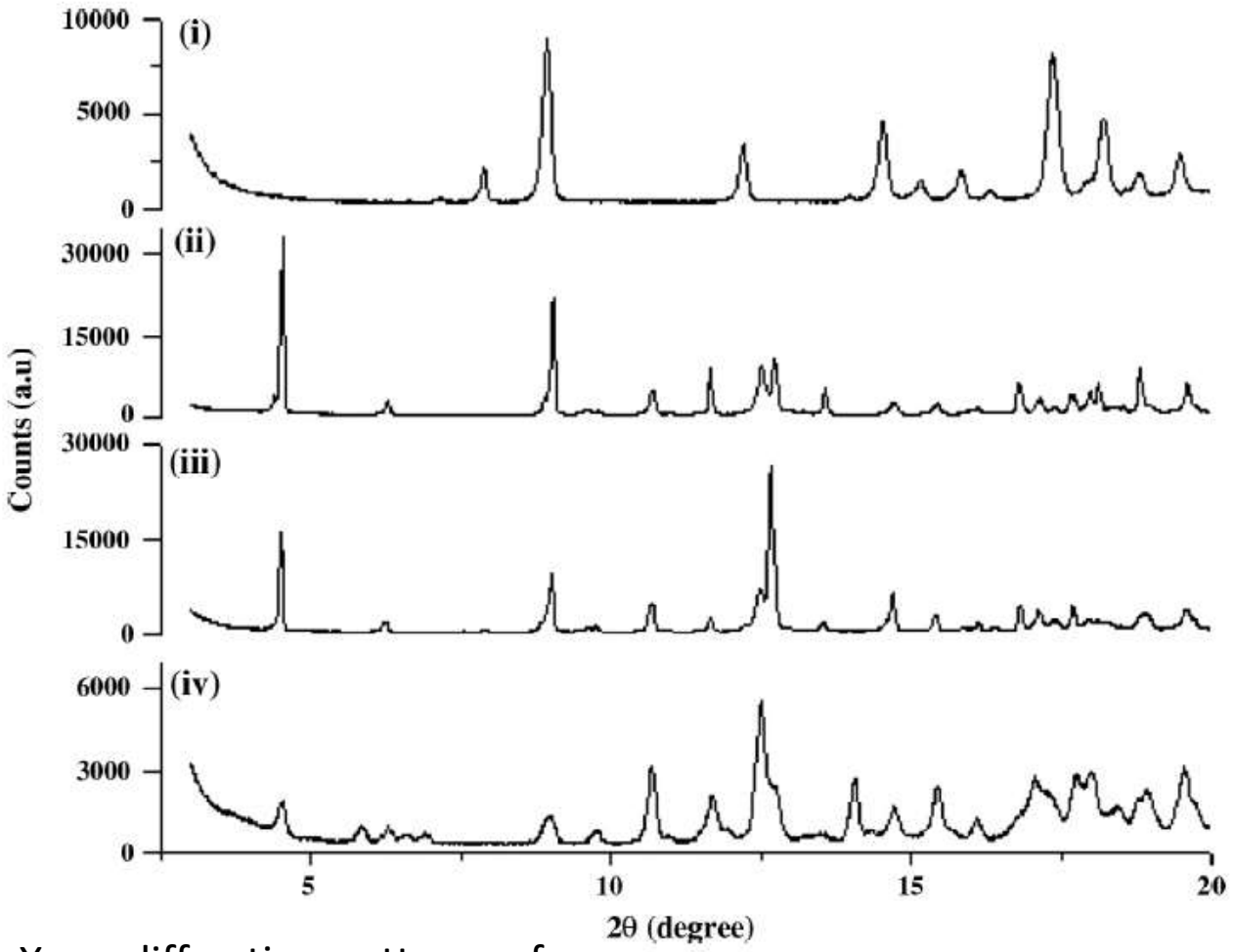


zoom in the 1400–1700 cm^{-1} region of

(i) curcumin and

(iii) curcumin–β-cyclodextrin complex from co-precipitation spectra

Powder XRD courcoumin/βCD



X-ray diffraction patterns of
(i) curcumin, (ii) β-cyclodextrin,
(iii) the simple mixture of curcumin with β-cyclodextrin in a 1:2 molar ratio and
(iv) the curcumin–β-CD complex from co-precipitation.


Food Chemistry
journal homepage: www.elsevier.com/locate/foodchem

Curcumin–β-cyclodextrin inclusion complex: Stability, solubility, characterisation by FT-IR, FT-Raman, X-ray diffraction and photoacoustic spectroscopy, and food application

Camila Sampaio Mangolim, Cristiane Moriwaki, Ana Claudia Nogueira, Francielle Sato, Mauro Luciano Baesso, Antônio Medina Neto, Graciette Matioli

The diffractogram of the simple mixture was the sum of the spectral lines of both of the components that were present, as expected.
However, the diffractogram of the curcumin–β-CD complex from co-precipitation exhibited the disappearance of some of the curcumin spectral lines at 7.90, 14.5, 15.2, 15.8 and 18.2 (2h). Additionally, the appearance of new lines was observed,
including weak lines at 5.83, 6.58 and 6.91 (2h) and an intense line at 14.1 (2h), indicating the presence of new solid crystalline phases that correspond to an inclusion complex of the same nature.
Thus, the X-ray diffraction corroborated the results that were obtained from FT-IR spectroscopy for the curcumin–β-CD complex that was prepared by co-precipitation.

Single crystal XRD

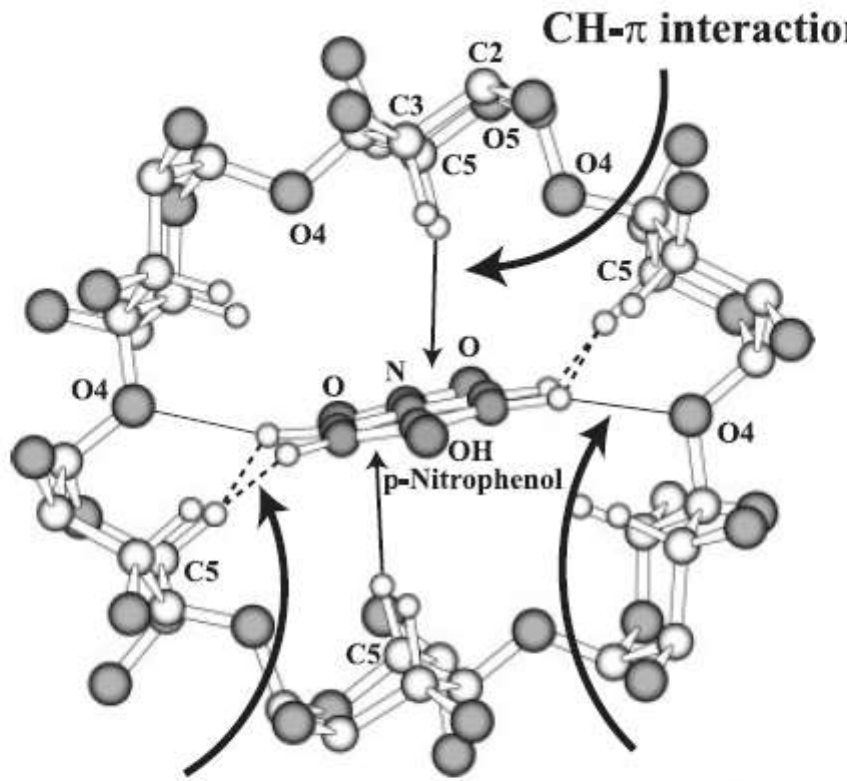


Fig. 7.12. Host–guest interaction observed in the α-CyD complex with *p*-nitrophenol. Hydrogen atoms of the phenylene ring are in van der Waals contact with hydrogen atoms of C-5H methine groups (dashed lines) and are also hydrogen bonded to O4 atoms of α-CyD (thin lines). The hydrogen atoms of C-5H methine groups of the two pyranose rings facing to the benzene ring are in the C–H–π contact as shown by arrows.

Single crystal XRD

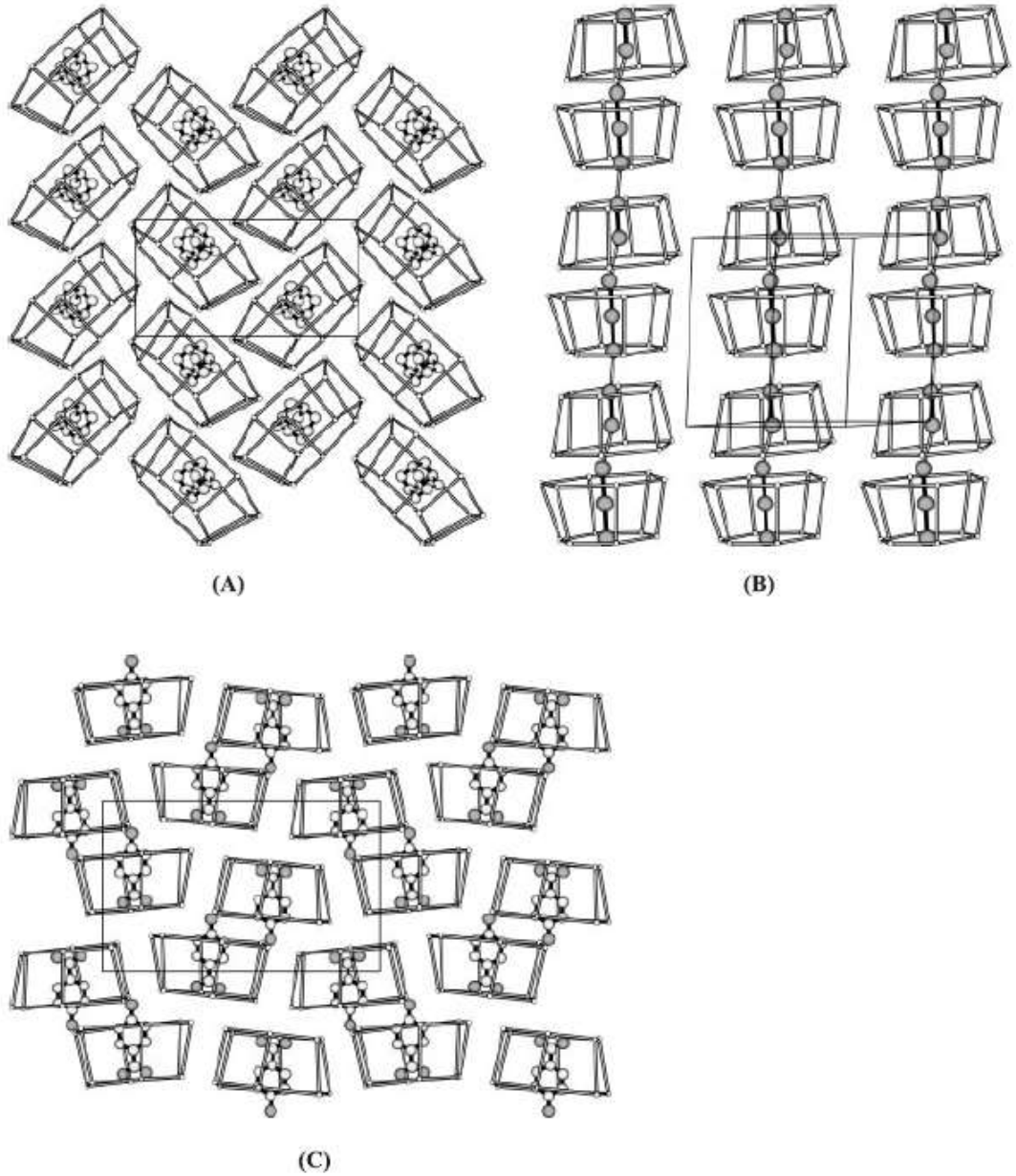


Fig. 7.13. Schematic drawing of crystal packings: cage-type observed in the β-CyD complex with hexamethylenetetramine (A), head-to-head channel-type observed in the α-CyD complex with iodine-iodide (B), and layer-type observed in the α-CyD complex with *p*-nitrophenol (C).

Single crystal XRD

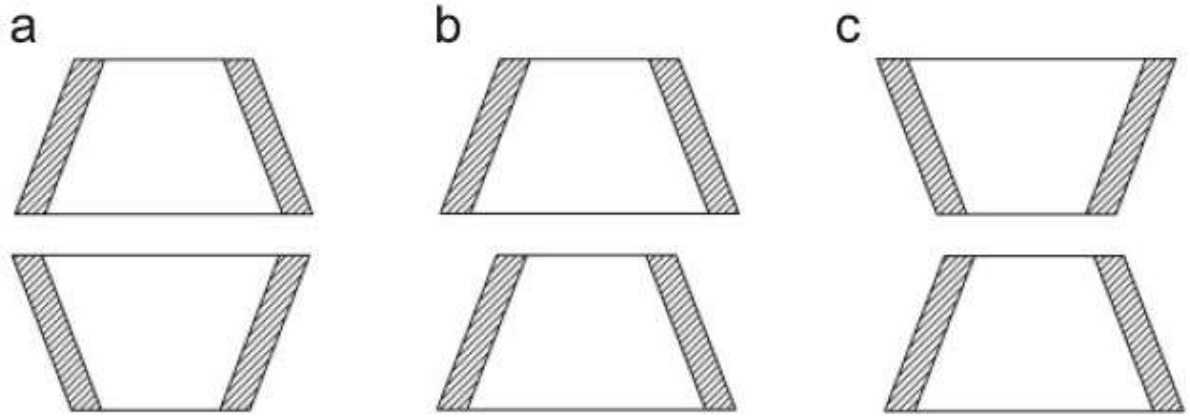


Fig. 1.9. Head-to-head (a), head-to-tail (b) and tail-to-tail (c) orientations in a CyD dimer.

Single crystal XRD

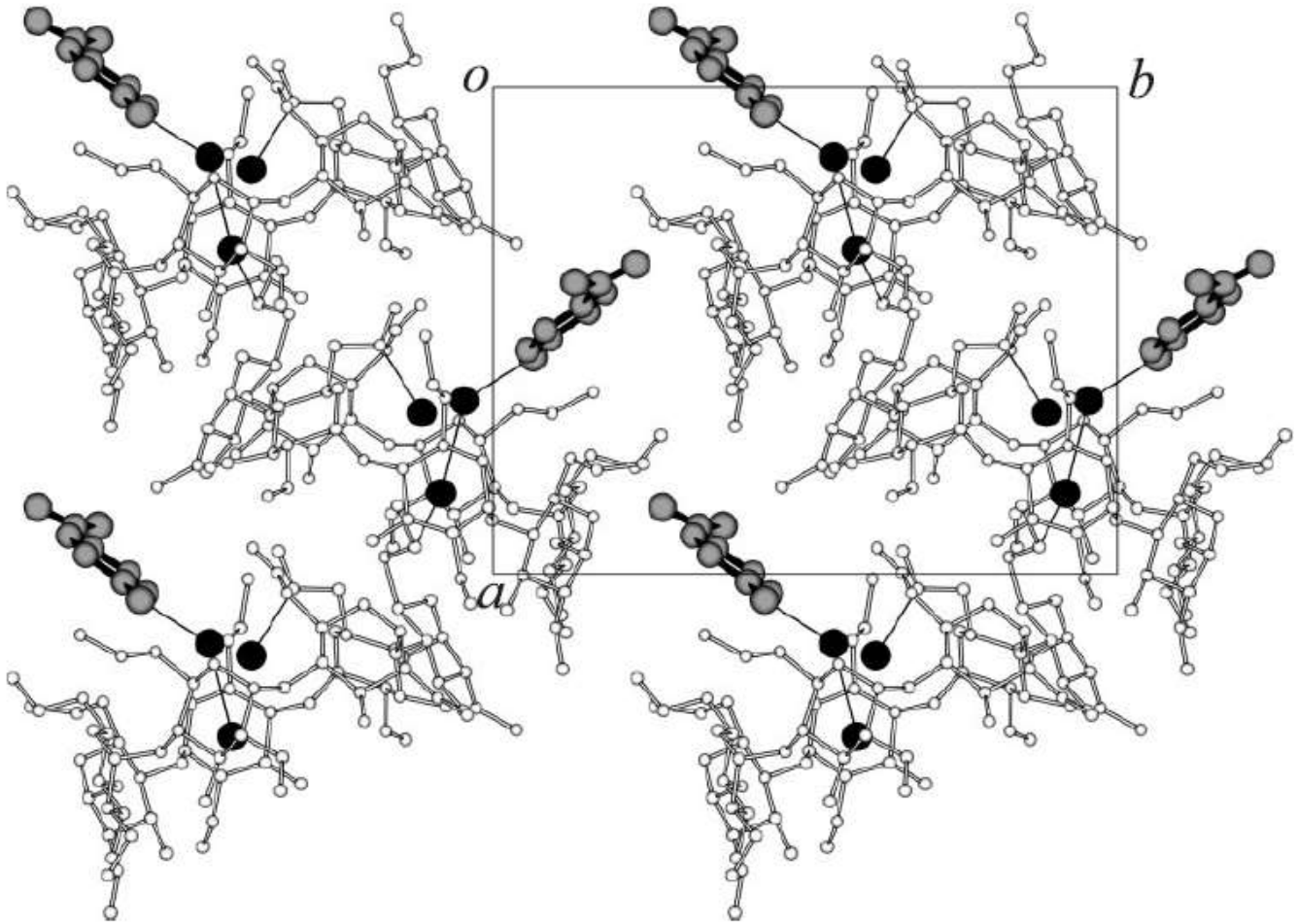


Fig. 7.20. Crystal structure of the heptakis(2,6-di-*O*-methyl)-β-CyD complex with *p*-nitrophenol. Water molecules are shown with filled circles and thin lines denote hydrogen bonds.

SEM

AAPS PharmSciTech, Vol. 14, No. 4, December 2013 (© 2013)
DOI: 10.1208/s12249-013-0023-5

Research Article

Molecular Inclusion Complex of Curcumin–β-Cyclodextrin Nanoparticle to Enhance Curcumin Skin Permeability from Hydrophilic Matrix Gel

Heni Rachmawati,[1,2] Citra Ariani Edityaningrum,[1] and Rachmat Mauludin[1]

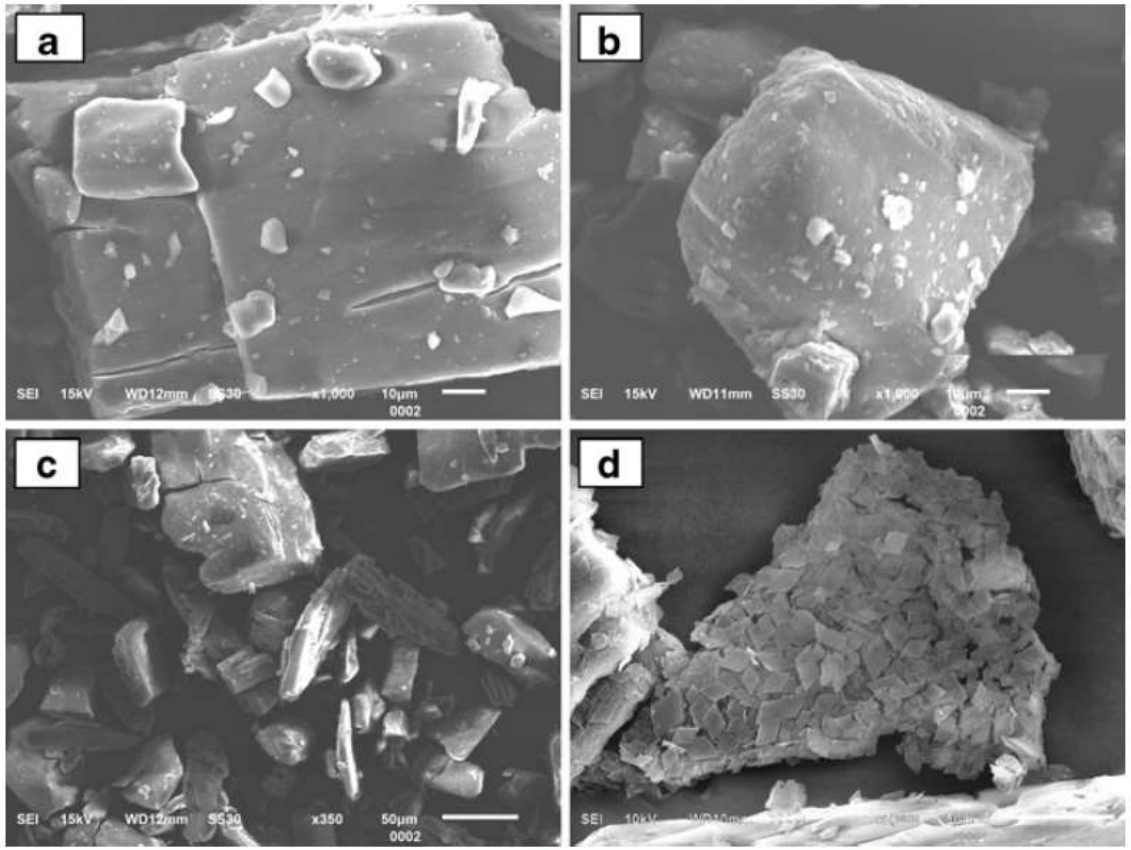


Fig. 6. Scanning electron microscope images of **a** β-cyclodextrin (×1,000), **b** curcumin (×1,000), **c** physical mixture (×350) and **d** β-cyclodextrin–curcumin inclusion complex nanoparticle (×5,000)

Figure illustrates the surface morphology of BCD, CUR, PM and CUR20 (BCD–CUR-N) under different SEM magnifications. BCD was observed to be irregular in shape (Fig. a), while CUR was rather spherical (Fig. b). PM was seen as a combination of the morphology of the parent compounds (Fig. c). In the case of BCD–CUR-N, the morphology was parallelogram, in which the original morphology of both the components had disappeared (Fig. d). These changes suggested the formation of BCD–CUR-N inclusion complexes. Moreover, the SEM of BCD–CUR-N showed a uniform particle size distribution with no aggregation, and there was a gap between the particles, thus suggesting good redispersibility.

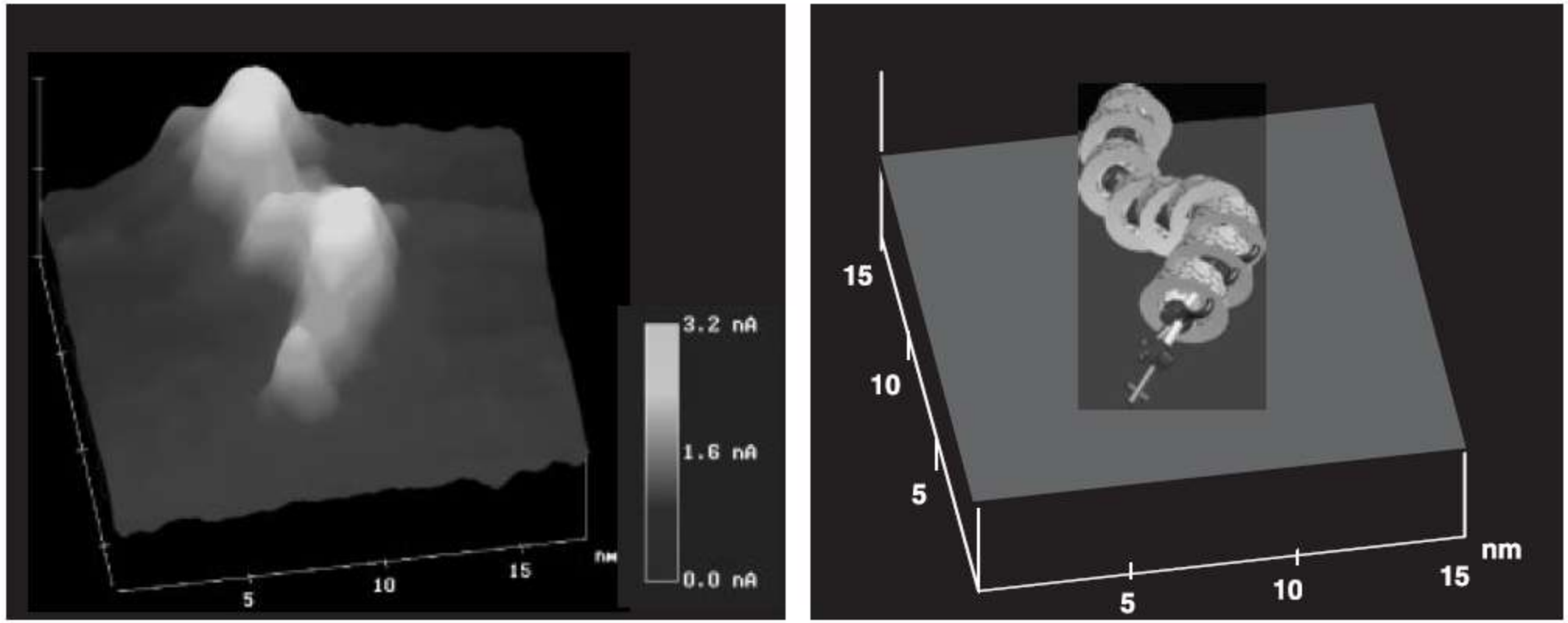


Fig. 3.25. STM image of 3-*p*-^{t}BocCiNH-α-CD from concentrated aqueous solution on a MoS_2 substrate (a) and its schematic structure (b).

Differential scanning calorimetry (DSC)

ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 46 (1998) 355–360

European Journal of Pharmaceutics and Biopharmaceutics

Research paper

Characterization of an inclusion complex of cholesterol and hydroxypropyl-β-cyclodextrin

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College of Pharmacy, The University of Texas at Austin, Austin, TX, USA

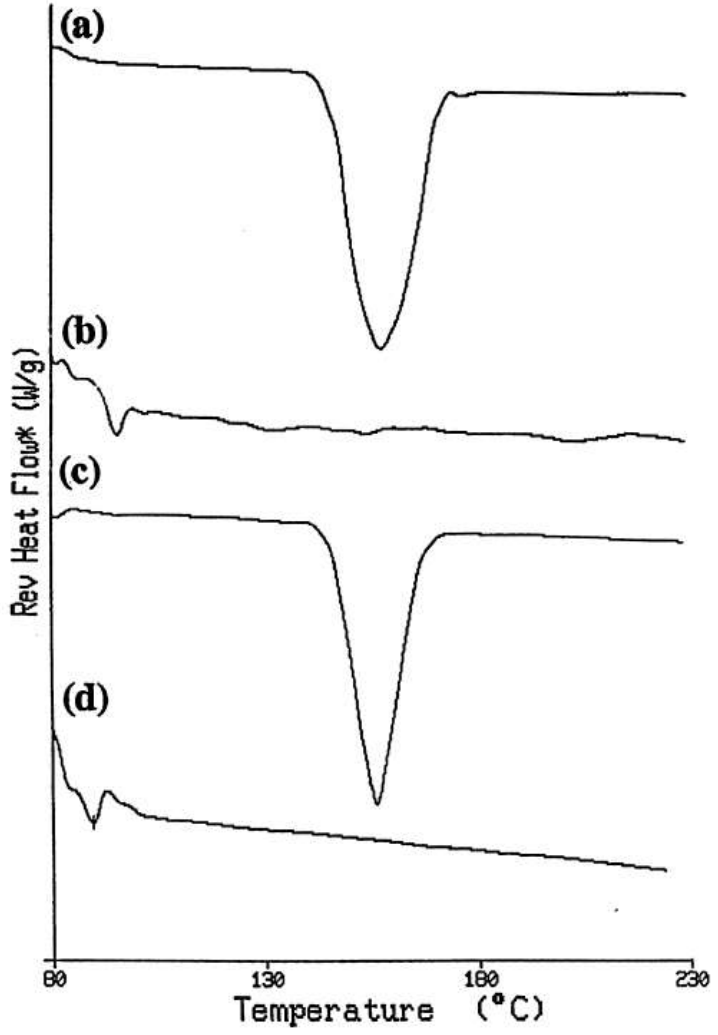

Fig. 2. DSC thermograms of (a) cholesterol, (b) HPβCD, (c) physical mixture, (d) inclusion complex.

The DSC results presented in figure demonstrate **an endothermic peak for cholesterol at 152°C, which corresponded to the melting point**. Similar results were seen for the physical mixture of cholesterol and HPβCD in Fig. c. The physical mixture thermogram was nearly identical to that of pure cholesterol, and showed a strong endothermic peak at approximately 152°C. The DSC thermogram of HPβCD observed in this study was similar to previously published studies. As can be seen in Fig. 2b,d, **the thermogram of HPβCD and of the inclusion complex did not show any sharp endothermic peak in the temperature range investigated. This indicated the amorphous character of both samples**. No cholesterol peak was detected. **The disappearance of the endothermic peak from the thermogram obtained for cholesterol compared with the thermogram obtained for the complex indicated that the freeze drying technique produced an inclusion complex between cholesterol and HPbCD, not a simple physical mixture**. Therefore, the endothermic peak of cholesterol was not detected since the crystalline cholesterol molecule was contained within the cavity of the HPβCD ring molecule.

Thermogravimetric analysis (TGA)

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Food Chemistry

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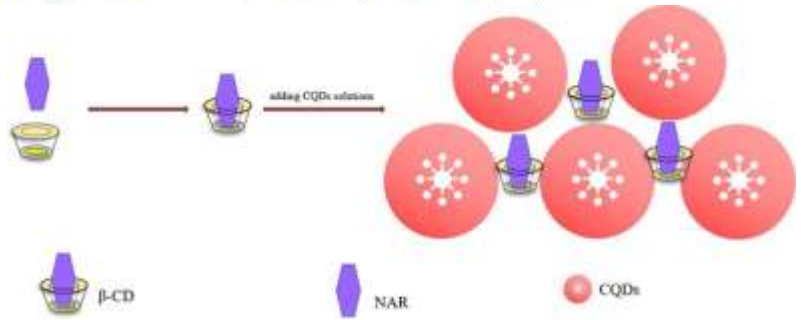
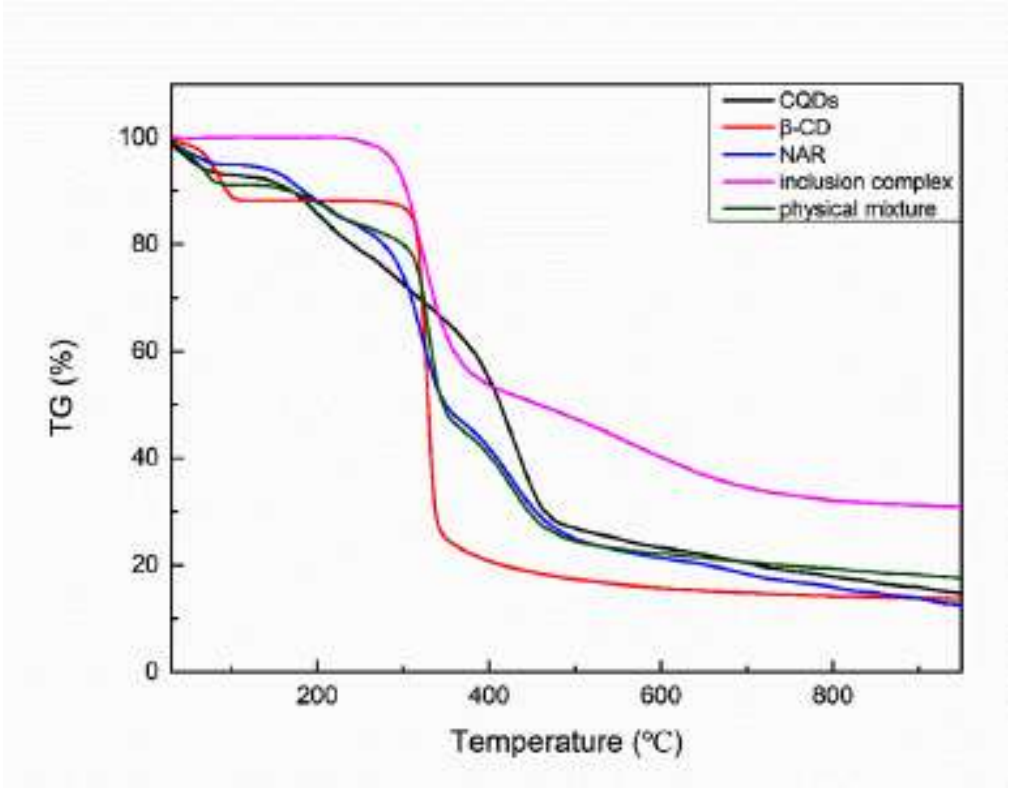
Preparation, characterization of naringenin, β-cyclodextrin and carbon quantum dot antioxidant nanocomposites

Yan Liang [a,2], Dongyuan Hou [b,c,d,1,2], Zan Ni [e,*], Minjie Cao [f], Luyun Cai [b,c,d,*]





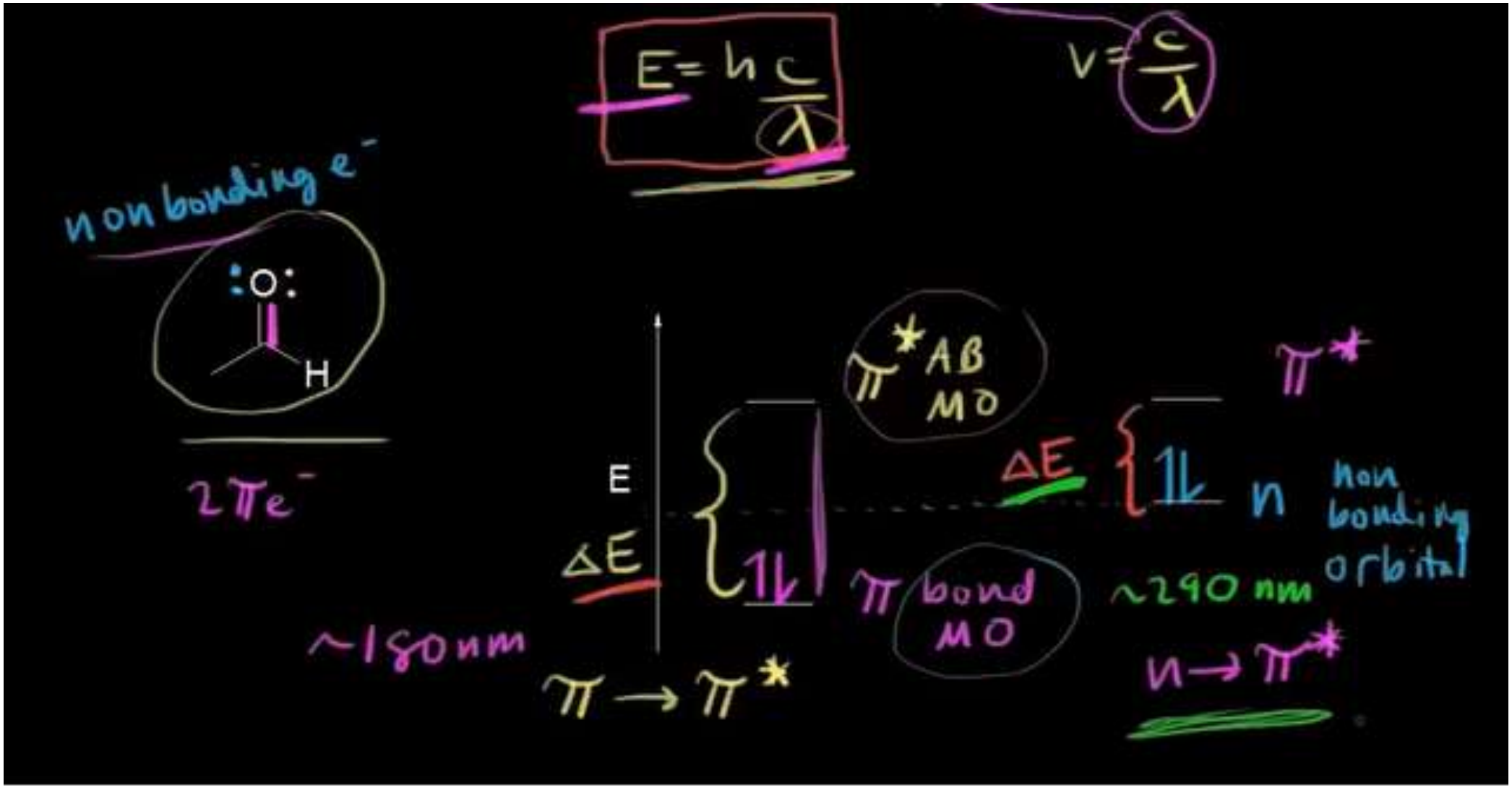
- CQDs have only one weight loss zone (72%) from 30 °C (initial temperature) to about 476 °C, associating with the water evaporation.
- For β-CD, three stages can be distinguished during the heating process of the sample.

  - The first stage (between 30 °C to 105 °C): a slight weight loss (mass loss 11.47% of total weight) due to the loss of adsorbed water and water of crystallization.

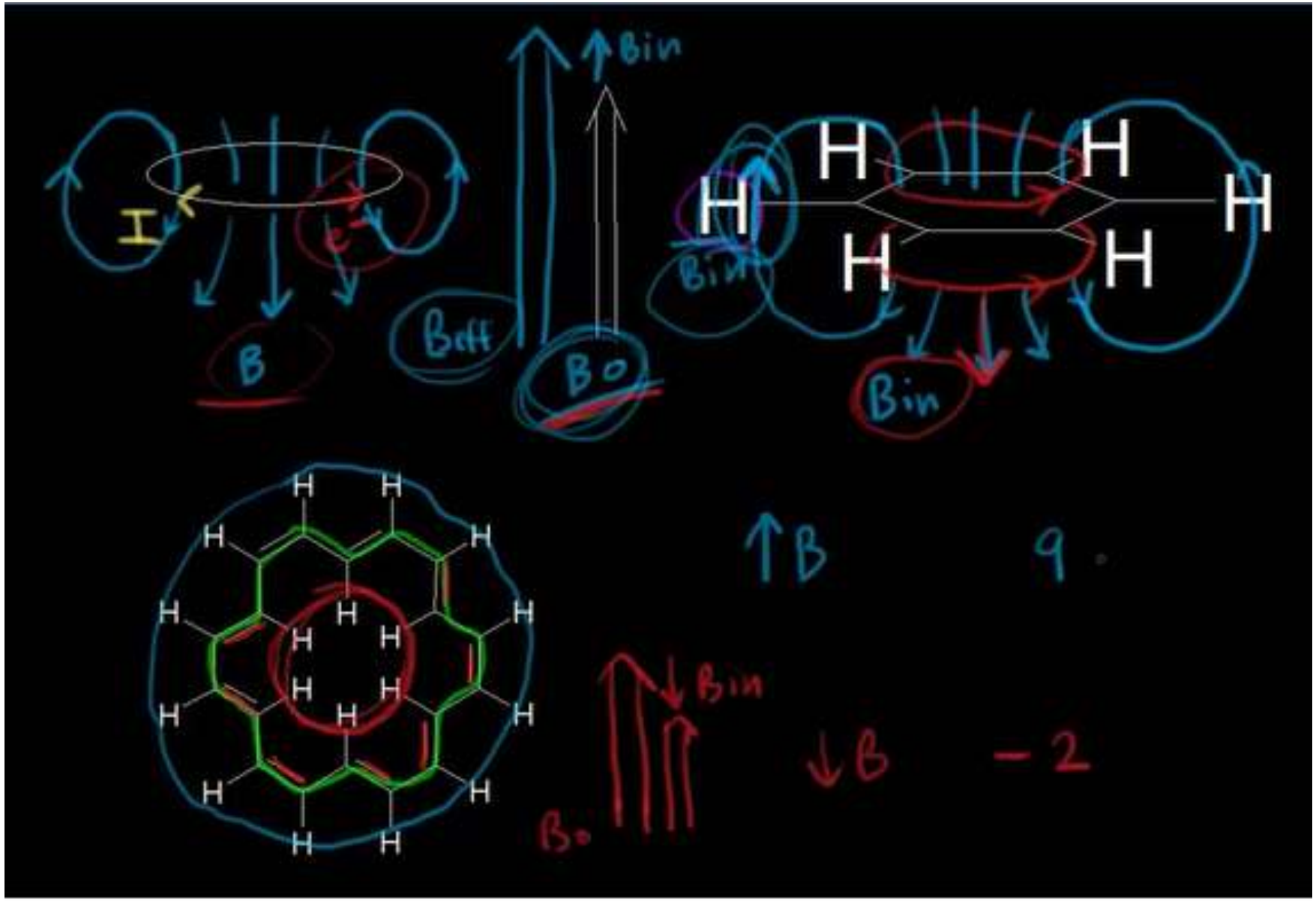
  - The second stage (from 105 to 350 °C): a stable curve in the range of approximately 105–300 °C and a rapid curve fall-off in the range of 300–350 °C. The latter indicates a major weight loss, which corresponds to the β-CD thermal degradation (mass loss 64.3% of total weight).

  - The third stage (from 350 °C to the final temperature 950 °C): The solid residuals continuously decompose at a very slow rate as it is indicated by the slowly continuous loss of weight shown in the β-CD TG curve (mass loss 10.6% of total weight).
- The curve of NAR has weight reduction between 30 °C to 460 °C and the mass loss is 71.17%.
- The curve of the physical mixture follows that of NAR indicating a similar weight loss behavior.
- The thermogram of the inclusion complex indicates that it is relatively stable between 30 and 300 °C, whereas a weight loss of 45.42% appears from 300 to 395 ◦C. It is obvious that the thermal stability of NAR is significantly enhanced in the inclusion complex.

ΠΑΡΑΡΤΗΜΑ



UV/Vis spectroscopy



Diamagnetic anisotropy

SPECTRAL METHODS

SPECIFIC REQUIREMENTS

Absorption

Experimental data must be analysed:

- In the spectral region free from band overlap
- On the band showing the largest change in intensity

Fluorescence

- A very careful study of the photophysical properties of the free guest is a must (emission wavelength, quantum yield, presence of several species, influence of solvent, temperature, *etc.*)
- Perform experiments using several excitation wavelengths if the presence of several species is assumed

Circular dichroism

- Spectra must be recorded with a large number of accumulations
- Identify the position of the dichroic signals, their sign and intensity

Correlation with the theoretical results:

- The sign of the band indicates the orientation of the guest transition moment
- Comparison of the experimental and simulated spectra

GENERAL REQUIREMENTS

- Use several fitting models
- Examine carefully the statistical parameters of the fits
- Compare when possible with theoretical simulations